ELSEVIER

Thermochimica Acta 302 (1997) 47–52

thermochimica
acta

# Thermal stability of modified caseins

N. Somanathan*, V. Subramanian, A.B. Mandal

*Polymer Laboratory, Central Leather Research Institute, Adyar, Madras-600020, India*

Received 17 February 1997

**Abstract**

Casein was modified by techniques like crosslinking with formaldehyde and grafting with monomers. Thermal stability of modified caseins was studied using viscosity and thermogravimetric analysis (TGA). Thermodynamic activation parameters were calculated from these results. Viscosity results suggest that there are transitions around 30 and 70°C for casein grafted with acrylonitrile (AN) and *n*-Butyl methacrylate (*n*-BMA). DSC studies also show transition around 30°C. DSC studies show that formaldehyde crosslinked casein irreversibly changes at around 120°C. © 1997 Elsevier Science B.V.

*Keywords:* Casein; Degradation; Graft copolymer; Transition

## 1. Introduction

Casein, a milk protein is used in food, paper, textile and leather industries. In leather, casein is used as a material for finishing. Casein forms a discontinuous film and the film is hydrophilic in nature. Apart from these drawbacks, casein has very good properties like machinability and glazability. During the glazing process, enormous amount of heat is produced. Therefore, studies on the thermal stability of casein at various temperatures is essential.

In order to improve these properties, casein can be modified in structure. Methods like crosslinking with formaldehyde [1–4], grafting with monomers [5–8] were used for modifying casein to improve its properties.

The stability of a polymer to heat, the rate of its thermal decomposition and the nature of the resulting product depend on the chemical structure [9] of the polymer. Change at the molecular level is directly reflected in the viscosity of the solution. Temperature dependence study of the viscosity of polymers helps to understand the mechanism of flow process. The temperature dependence of the viscosity of polymers has a substantial effect on their processibility also.

Eyring's theory [10] of absolute reaction rates was extended to the diffusion process and to the flow of liquids. In this theory, the temperature and viscosity are used in the determination of the number of possible jumps of molecular-kinetic units over the potential barrier at various temperatures. The kinetic parameters give an idea about the stability and the disorder of the system during transition.

In polymer pyrolysis, thermogravimetric analysis (TGA) follows a relatively simple sigmoidal path. The shape of the curve depends primarily upon reaction order, frequency factor and activation energy. The values of these parameters can be of major importance in the elucidation of the mechanism involved in degradation [11]. The activation energy is one of

---

*Corresponding author. Fax: 00-91-44-491-1589.

*PII* S0040-6031(97)00107-X

the factors affecting the thermal stability of the polymers. Many methods [12,13] were reported in the literature in calculating the activation energies of degradation.

Thermal degradation of natural polymers like collagen, and the evaluation of kinetic parameters have been studied by many workers [14–16]. Thermal decomposition of copolymers has also been studied in detail [17,18]. Thermal decomposition of polyacrylates was reported by Klempner and Frisch [19]. The kinetics of the thermal degradation of polystyrene and polyethylene was reported by Anderson and Freeman [20]. Degradation of the grafted system depends on the constituents present in it. Generally, the degree to which a graft copolymer retains the desirable properties is affected by the co-monomer ratio of its chemical structure [9].

In the present investigation, the viscosity and TGA studies were undertaken for pure casein, alkali-treated casein, formaldehyde crosslinked casein and casein grafted with acrylonitrile and for casein grafted with acrylonitrile and $n$-butyl methacrylate ($n$-BMA). Thermodynamic activation parameters were calculated from viscosity and TGA data.

## 2. Experimental

### 2.1. Pure casein

Casein was soaked overnight in water and was dissolved in 25% ammonia solution by adding it drop by drop.

### 2.2. Alkali-treated casein

Casein (5 g in 50 ml of water) was soaked overnight in water and then treated with 1 ml of triethanolamine (TEA). After complete dissolution of casein, the contents were heated at 60 ± 2°C for 3 h and immediately cooled.

### 2.3. Crosslinked casein

Casein (5 g) soaked in water and made up to 100 ml with 1 ml of TEA. 50 ml of the casein solution was mixed with 20 ml of 10% formaldehyde solution in a closed cell. The pH value of the solution was adjusted to 8. This solution was taken after 6 h of reaction and viscosity studies were undertaken. The solution was made into films, and TGA was studied for these films.

### 2.4. Grafted casein

5% casein solution was grafted with 2 mol of acrylonitrile monomer with potassium persulphate as the initiator (9.7 mmol/l). The reaction was carried out at 60 ± 2°C temperature (Ca-g-AN).

In another reaction, 5% casein solution was grafted with a binary mixture of acrylonitrile and $n$-BMA (mole fraction 0.9 : 0.1). The concentration of the binary mixtures in the reaction was 1.2 mol/l. Potassium persulphate was used as the initiator (9.7 mmol/l) and reaction was performed at 60 ± 2°C (Ca-g-AN-Co-$n$BMA).

### 2.5. Viscosity studies

Viscosity of the treated caseins and pure casein solutions was measured by a Ubbelohde-type dilution viscometer in water at various temperatures using a thermostatic water bath. The relative viscosity was calculated from the flow times of the solution and water. Thermodynamic parameters were calculated using the plot log $\eta$ vs. $(1000/T)$.

### 2.6. TGA studies

Pure and modified casein were subjected to TGA using a Mettler TA 3000 thermobalance. All experiments were carried out in a nitrogen atmosphere with a flow rate of about 200 ml/min and using a heating rate of 20°C/min. In the case of grafted samples, homopolymers were totally removed by soxhlet extractions using dimethyl acetamide. The grafted samples were washed with water and dried in vacuum at 50°C for 24 h. TGA was taken on these dry samples which contain less than 1% water.

### 2.7. DSC studies

A Mettler DSC 7 instrument was used for differential scanning calorimetric studies. The analysis was done at the heating rate of 10°C $\text{min}^{-1}$ on dry samples (containing less than 1% of water). The temperature and enthalpy values were standardised by using pure indium as a calibration substance. Base-line correction

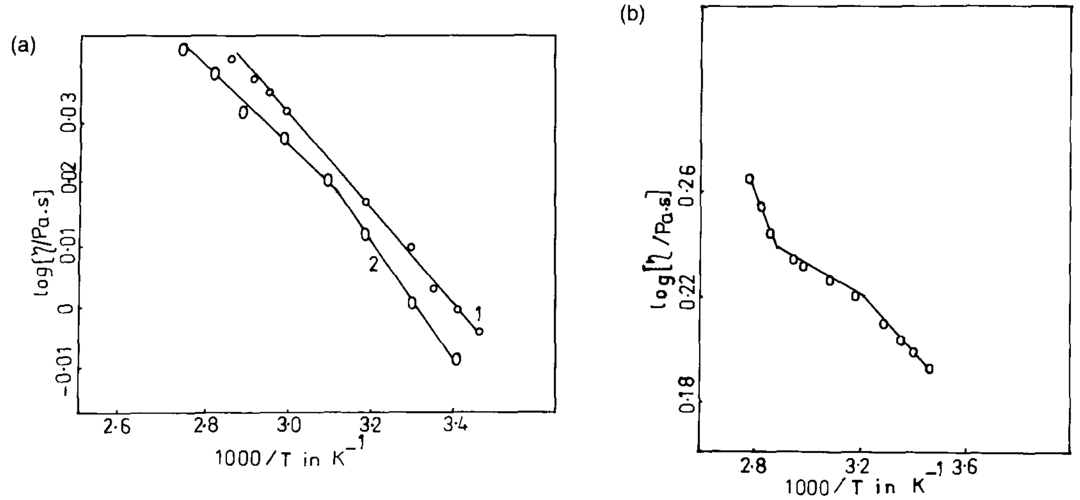

Fig. 1. (a) Effect of temperature on viscosity: (1) pure casein (2) alkali treated casein (b) Effect of temperature on viscosity for Ca-g-AN-Co-*n*BMA

was carried as follows: The experiment was performed with empty pans identical to the experiments with the sample. The data obtained were subtracted from the DSC curves obtained for the sample.

## 3. Results and discussion

### 3.1. *Viscosity studies*

The effects of temperature on the viscosity for casein systems are presented in Fig. 1. Thermodynamic parameters were calculated using the following Eyring equation.

$$\log\eta = \log[\{(hN/V) - (\Delta S^{*}/2.303R)\} + (\Delta H^{*}/2.303R)](1/T) \qquad (1)$$

where $\eta$: dynamic viscosity; $h$: Planck's constant; $N$: Avogadro's number; $V$: molar volume of the solvent; $R$: universal gas constant; $T$: absolute temperature.

The enthalpy of activation ($\Delta H$) values calculated from the slope are given in Table 1.

The Gibb's free energy of activation ($\Delta G$) was calculated using the following equation

$$\Delta G^{*} = RT\ln(\eta V/hN) \qquad (2)$$

The entropy of activation $\Delta S^{*}$ has been calculated using the following Gibb's equation

$$\Delta G^{*} = \Delta H^{*} - T\Delta S^{*} \qquad (3)$$

In this regard, it must be pointed out that it is possible to calculate the value of $S$ from the intercept of the plot of $\log\eta$ vs. $(1000/T)$ using Eq. (1). But the intercept is not sharp enough and therefore, we have avoided such calculations. Regarding details in calculating thermodynamic activation parameters from viscosity measurements, reference is made to our earlier publication [21].

The thermodynamic activation parameters calculated from $\log\eta$ vs. $(1000/T)$ graph for all casein systems are presented in Table 1. Pure casein does not show any transition in the worked out temperature range (Fig. 1(a)) whereas alkali-treated casein shows a transition around 50°C. The results of temperature effect on stress–strain and stress relaxation experiments also suggest that there is a transition around 50°C [22].

It can be seen from Table 1 that over the entire range of investigated temperatures, Gibb's free energy of activation, $\Delta G^{*}$ decreased with the increase in temperature. The decrease of $\Delta G^{*}$ clearly indicates that the work done by the system decreases with the increase in temperature. $H^{*}$ and $\Delta S^{*}$ values were higher in the case of alkali-treated casein when compared to a pure casein solution. The results suggest that thermal stability of alkali-treated casein is initially lowered due to alkali treatment and was improved over pure casein above 50°C.

Table 1

(a) Activation parameters calculated from viscosity data for pure and alkali treated casein

| Temp. in K | Pure casein | | | Alkali-treated casein | | |
|---|---|---|---|---|---|---|
| | $\Delta G$ | $\Delta H$ | $\Delta S$ | $\Delta G$ | $\Delta H$ | $\Delta S$ |
| 293 | 9.26 | | 18.18 | 40.62 | | |
| 303 | 9.01 | | 18.43 | 39.82 | | |
| 313 | 9.01 | | 17.81 | 38.28 | 6.73 | 122.22 |
| 323 | 8.63 | 14.60 | 18.51 | 37.36 | | |
| 333 | 8.55 | | 18.10 | 36.65 | | |
| 343 | 8.42 | | 18.01 | 36.82 | 6.21 | 45.12 |
| 353 | 8.21 | | 18.10 | 36.11 | | |
| 363 | 8.17 | | 17.89 | 35.95 | | |

(b) Thermodynamic activation parameters calculated from viscosity data for crosslinked casein and Ca-g-AN

| Temp. in K | Crosslinked casein | | | Ca-g-AN | | |
|---|---|---|---|---|---|---|
| | $\Delta G$ | $\Delta H$ | $\Delta S$ | $\Delta G$ | $\Delta H$ | $\Delta S$ |
| 293 | 20.80 | | | 40.36 | | 35.28 |
| 303 | 20.39 | | | 39.66 | | 31.78 |
| 313 | 20.01 | | | 39.24 | | 29.44 |
| 323 | 19.77 | 29.89 | 31.98 | 37.70 | 29.94 | 23.81 |
| 333 | 19.60 | | | 36.28 | | 18.85 |
| 343 | 18.89 | | | 36.61 | | 17.97 |
| 353 | 18.39 | | | 35.57 | | 15.68 |
| 358 | 18.01 | | | | | |

(c) Thermodynamic activation parameters calculated from viscosity data for Ca-g-AN-Co-nBMA

| Temp. in K | $\Delta G$ | $\Delta H$ | $\Delta S$ |
|---|---|---|---|
| 288 | 9.47 | | |
| 293 | 9.22 | | |
| 297 | 9.09 | 20.98 | 39.82 |
| 303 | 8.97 | | |
| 313 | 8.67 | | 5.34 |
| 323 | 8.59 | | |
| 333 | 8.55 | | |
| 338 | 8.38 | 10.26 | 53.38 |
| 343 | 8.38 | | |
| 348 | 8.29 | | |
| 353 | 8.21 | 50.08 | 118.42 |
| 358 | 8.26 | | |

$\Delta G$ and $\Delta H$ in kJ mol$^{-1}$, $\Delta S$ in J mol$^{-1}$ K$^{-1}$.

Crosslinked casein and acrylonitrile-grafted casein does not show transition in the temperature range worked out here. Both the systems are more stable when compared to alkali-treated casein, even though they are less stable when compared to pure casein. Higher values of $d\Delta S^{*}/dT$ (Ca-g-AN) suggest that the system is probably more irreversible around 50°C.

Viscosity data obtained for Ca-g-AN-C0-*n*BMA suggest that there are two possible transitions around 30°C and 70°C respectively. The randomness in the system was minimal between 30 and 70°C. After that level there is an increase in values of $\Delta S^{*}$. It is interesting to note that stress–strain and stress relaxation experiments also suggest that there are two transitions in the above-mentioned temperature range

[23]. These transitions probably arise due to the mutual influence of acrylonitrile and *n*BMA (shift in glass transitions), the components of the grafted side chain.

### 3.2. TGA studies

The TGA curves of pure casein show that decomposition takes place at various stages. The first decomposition may be due to loss of water (either adsorbed or bound). The other peaks obtained above 300°C are due to peptide degradation. In general, the degradation of casein system takes place in four stages. However in the case of crosslinked casein, it took place in five stages. The amount of polymer degraded at 100°C for the five casein systems were 6%, 1.6%, 2.5%, 0.5% and 1.9% respectively. At 350°C, the degraded amounts for the above mentioned casein systems were 48%, 42%, 26%, 23.3% and 46.2% respectively.

These results suggest that Ca-g-AN is more stable when compared to other casein systems. The activation energies at different degradation stages are determined using the equation suggested by Doyle [24].

$$\ln(1 - C) = [-2.315(A/B)] + [0.4567(A/B)](E_a/R)(1/T) \quad (4)$$

where $C$ is the amount of polymer degraded, $E_a$ is the energy of activation and A and B are constants.

The activation energy of various degradation stages was calculated by plotting $\ln(1 - C)$ against $(1000/T)$ (Fig. 2). The results are given in Table 2. The $E_a$ values for alkali-treated casein are slightly higher than that of unmodified casein except in the last stage. Ca-g-AN-Co-*n*BMA has higher $E_a$ values, when compared to Ca-g-AN, except in the last degradation stage. In the first stage, $E_a$ value is double for Ca-g-AN-Co-*n*BMA compared to Ca-g-AN. It may possibly be due to the cyclization [25] of the acrylonitrile side chain in this temperature range and therefore, more stable than that of Ca-g-AN-Co-*n*BMA.

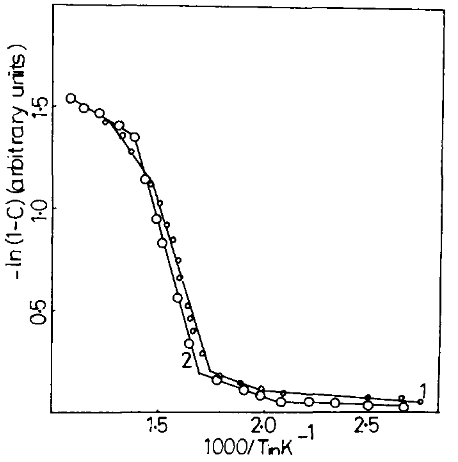

Fig. 2. Effect of temperature on the concentration of the polymer degraded ($\ln(1 - C)$ vs. $1/T$) (1) – pure casein (2) – alkali-treated casein.

It was reported [10] that for polymers of sufficiently high molecular masses, the activation energy is not dependent on molecular mass. The content of polar

Table 2
Energy of activation values calculated from TGA curves

| Temp. in K | $E_a$ | Temp. in K | $E_a$ | | |
|---|---|---|---|---|---|
| Pure casein | | Alkali-treated casein | | | |
| 333–476 | 13.34 | 373–478 | 13.97 | | |
| 476–573 | 17.93 | 478–585 | 19.56 | | |
| 573–666 | 23.35 | 585–725 | 23.56 | | |
| 666–800 | 19.60 | 725–973 | 11.97 | | |
| Crosslinked casein | | Ca-g-AN | | Ca-g-An-Co-nBMA | |
| 378–478 | 13.47 | 378–546 | 9.09 | 425–538 | 16.93 |
| 478–553 | 12.51 | 546–714 | 21.02 | 538–671 | 21.98 |
| 553–598 | 22.02 | 714–909 | 17.14 | 671–855 | 16.93 |
| 598–763 | 20.43 | 909–1075 | 33.61 | 855–1087 | 11.93 |
| 763–870 | 26.81 | | | | |

$E_a$: Energy of activation in kJ mol$^{-1}$.

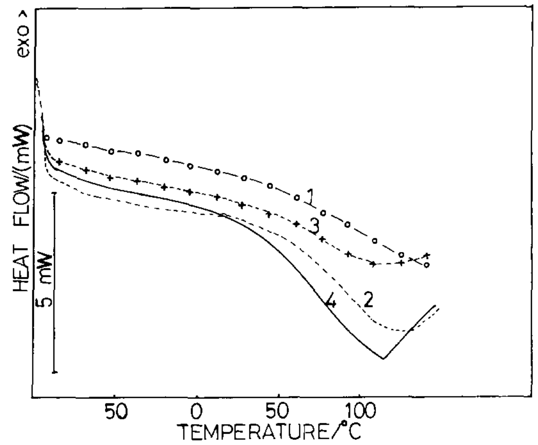

Fig. 3. Measured DSC curves obtained for different modified caseins (1) – Pure; (2) – crosslinked; (3) – Ca-g-AN (4) – Ca-g-AN-Co-*n*BMA.

groups, the regularly or randomly distributed side branches affect the energy of activation.

### *3.3. DSC studies*

The results obtained from DSC studies in the temperature range of −100°C to +140°C are given in Fig. 3. The DSC curve Ca-g-AN-Co-*n*BMA suggests that there are possible transitions around 30°C and 100°C, whereas it is seen at 100°C and 120°C in the case of Ca-g-AN and crosslinked casein respectively. The transitions for Ca-g-AN-Co-*n*BMA and Ca-g-AN were also seen in the cooling curve, whereas the peak obtained for crosslinked casein is not seen in the cooling curve. This suggests that there is an irreversible change taking place around 120°C in aldehyde crosslinked casein.

## 4. Conclusions

The above-mentioned studies show the temperature dependence of the systems and the effect of grafting and crosslinking of casein on its stability at various temperature ranges. It has been shown that in the case of caseins grafted with acrylonitrile and *n*-BMA, property changes around 30°C and 70°C due to thermal transitions. Properties of formaldehyde-crosslinked casein irreversibly change after 120°C.

## Acknowledgements

The authors are thankful to Miss Petra Eller, Max Planck Institut für Polymer Forschung, Mainz for her help during the analysis.

## References

[1] P.T. Davey, D.R. Willams and G. Winter, J. Appl. Biochem., 2 (1980) 60.
[2] P.T. Devey, M.R. Houchin and G. Winter, J. Chem. Tech. Biotech., 33A (1983) 164.
[3] A. Takayoshi and K. Yoshitaka, Agri. Biol. Chem., 48 (1984) 1017.
[4] J. Korolezk, J. Dairy Sci., 19 (1984) 107.
[5] D. Mohan, G. Radhakrishnan, S. Rajadurai, K.V. Rao and G.G. Cameron, J. Poly. Sci. Poly. Chem. edn., 27 (1989) 2123.
[6] D. Mohan, G. Radhakrishnan, S. Rajadurai, T. Nagabhushanam and K.T. Joseph, J. Appl. Poly. Sci., 29 (1984) 329.
[7] D. Mohan, G. Radhakrishnan and S. Rajadurai, J. Macromol Sci. chem., 22A (1985) 75.
[8] Y. Lakshminarayana, N. Radhakrishnan, K. Parthasarathy, K.S.V. Srinivasan and K.T. Joseph, Leather Sci., 32 (1985) 134.
[9] J.F. Kenny, Poly. Engg. Sci., 8 (1968) 216.
[10] G.V. Vinogradov and Y.A. Malkin, Rheology of Polymers, Mir, Moscow (1980) Chap. 2.
[11] D.V. Lewi, L. Reich and H.T. Lee, Poly. Engg. Sci., 5 (1965) 135.
[12] H.C. Anderson, J. Poly. Sci., Part B, 2 (1964) 115.
[13] L. Reich, J. Appl. Poly. Sci., 9 (1965) 3033.
[14] C. Rose, M. Kumar and A.B. Mandal, Biochem. J., 249 (1988) 127.
[15] A.B. Mandal, V. Ramesh and S.C. Dhar, Eur. J. Biochem., 169 (1987) 617.
[16] V. Ramesh, A.B. Mandal, P.K. Sehgal and S.C. Dhar, Leather Sci., 33 (1986) 1.
[17] J.J. Lim and M.H. Shamos, Biopolymers, 13 (1974) 1791.
[18] A.T. Atkinson and J.R. MacCallum, Eur. Poly. J., 8 (1972) 809.
[19] D. Klempner and H.C. Frisch, J. Poly. Sci. Polymer letter edn., 8 (1970) 525.
[20] D.A. Anderson and E.S. Freeman, J. Poly. Sci., 54 (1961) 253.
[21] A.B. Mandal, S. Ray, A.M. Biswas and S.P. Moulik, J. Phy. Chem., 84 (1980) 856.
[22] N. Somanathan, V. Arumugam, M.D. Naresh, R. Sanjeevi and T.S. Ranganathan, Polymer J., 24 (1992) 603.
[23] N. Somanathan, V. Arumugam and R. Sanjeevi, Eur. Poly. J., 23 (1987) 803.
[24] C.D. Doyle, J. Appl. Poly. Sci., 5 (1961) 285.
[25] K.C. Smeltz and K. Dyer, J. Am. Chem. Soc., 74 (1952) 263.