

GLASS TRANSITION OF HYDRATED WHEAT GLIADIN POWDERS*

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Abstract Modulated-temperature differential scanning calorimetric and dynamic mechanical analyses and dielectric spectroscopy were used to investigate the glass transition of hydrated wheat gliadin powders with moisture absorption ranged from 2.30 db% to 18.21 db%. Glass transition temperature (T_g) of dry wheat gliadin was estimated according to the Gordon-Taylor equation. Structural heterogeneity at high degrees of hydration was revealed in dielectric temperature and frequency spectra. The activation energies (E_a) of the two relaxations were calculated from Arrhenius equation.

Keywords: Hydrated gliadin; Glass transition; MDSC; DMA; Dielectric spectra.

INTRODUCTION

Petrol resources have been over-consumed with the rapid economic development, and white pollution arises accordingly from petrol-based synthetic polymers. Therefore, some low cost, environment-friendly and reproducible biopolymers like polysaccharides (such as starch and cellulose) and plant (such as soy, corn and wheat) proteins have been attracting worlds' attention for development of edible films or biodegradable bioplastics. Due to excellent biodegradable properties, low price and abundant resources, wheat proteins have been extensively studied as biodegradable resources^[1–3]. We have prepared biodegradable wheat gluten plastics through thermo-molding methods and investigated factors influencing the properties of the gluten plastics^[4–7]. Wheat gliadins, as major component of wheat gluten, have been used to prepare degradable films by casting^[8, 9] and transparent bioplastics by thermo-molding^[10]. Water plays an important role in determining the final properties of wheat protein-based materials. Our previous studies^[6, 11, 12] showed that water absorption and tensile properties of wheat protein-based plastics and films were strongly dependent on relative humidity (RH).

The presence of water would affect molecular relaxation processes in wheat proteins. There were some research works on glass transition of wheat gluten systems^[13–15]. Glass transition temperature (T_g) of wheat protein powders has been determined at 397–418 K using differential scanning calorimetry (DSC)^[13, 14, 16]. However, there is little work on T_g of wheat gliadin powders. In this article, we took modulated-temperature differential scanning calorimetry (MDSC), dynamic mechanical analysis (DMA) and dielectric spectra to study the glass transition of hydrated wheat gliadin powders.

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MATERIALS AND METHODS

Materials

Wheat gluten with a protein content $\geq 75\%$, a moisture content $\leq 9\%$ and an ash content $\leq 0.95\%$ was supplied by Shanghai Wangwei Food Co. Ltd, China. All chemicals were of analytical grade.

Sample Preparation

Dry powders of wheat gliadins with a molecular weight from 30 kDa to 100 kDa^[17] were prepared following the procedure described elsewhere^[10]. The powders were hydrated in RH chambers controlled by saturated salt solutions for at least one week to give rise to a moisture absorption (MA) that ranged from 2.30 db% (dry basis, water as a percentage of total solids) to 18.21 db%. Bacteria growth was observed in less than a week for the samples with MA higher than 18.21 db%.

Modulated-temperature Differential Scanning Calorimetry (MDSC)

The calorimetric properties of hydrated gliadins were measured using a differential scanning calorimeter (Q100, TA, USA) with a modulated mode. Nitrogen was used as a purge gas at a flow rate of 50 mL min⁻¹. The hydrated gliadin powders were accurately weighed (10–13 mg) with aluminum pans for liquid sample. Samples were heated from -60°C to 120°C at a heating rate of 2 K min⁻¹ with the amplitude modulation of $\pm 0.32^{\circ}\text{C}$ per minute.

Dynamic Mechanical Analysis (DMA)

A metal pocket technique has been used in DMA for determining glass transitions of powder samples which are difficult to be molded into a certain shape^[18,19]. The modulus measured from DMA reflects the combination of both sample and metal pocket. This method is useful for identifying thermal transitions of the sample from loss factor $\tan\delta$ curve only if the metal pocket does not show any thermal transition within the tested temperature range. The hydrated gliadin powders were distributed evenly inside the pockets and the pockets were shut by folding. The pockets were further covered with paraffin oil to minimize moisture loss during the tests. DMA was conducted at 1 Hz and 10 Hz with a dynamic mechanical thermal analyzer (Q800, TA, USA) in single cantilever geometry at a heating rate of 2 K min⁻¹.

Dielectric Spectra

Dielectric spectra of the hydrated gliadin powders were measured using a Novocontrol Alpha high resolution dielectric analyzer (Novocontrol GmbH Concept 40, Novocontrol Technology, Germany), equipped with a Novocool cryogenic system for temperature control. The gliadin powders were placed into a metal cell BDS 1308 (for liquid samples), and press-shaped into a cylinder of about 1 mm in thickness and 20 mm in diameter. Alpha all calibration, alpha reference calibration, load short calibration and low capacity open calibration were conducted before measurements. Frequency sweeps were conducted from 10^{-2} Hz to 10^7 Hz with an applied voltage of 1 V_{rms} at 5°C , 15°C , 25°C and 35°C , respectively. Temperature sweeps were performed from -50°C to 100°C at 0.1, 1, 10, 100, 1000 and 5000 Hz, respectively, with an equilibrium time of 1 min.

RESULTS AND DISCUSSION

Figure 1 shows MA of gliadin powders under different relative humidities at 25°C . MA of samples is strongly dependent on RH, increasing from 8.93 db% at RH = 35% to 18.21 db% at RH = 83%. The strong dependence of MA on RH is due to the hydrophilic amino acid residues in gliadins. The MA values of wheat gliadins are quite close to the hydrated wheat gluten^[15] under the same relative humidities.

Figure 2 shows the reverse heat flow of hydrated gliadin powders from MDSC thermograms. The onset, inflection and end temperatures of the glass transition are denoted on the sample with MA = 8.93 db%. T_g defined as the inflection temperature is shown in Fig. 3 as a function of water mass fraction. T_g shifts to low temperatures with increasing water content due to the plasticization effect. There is no transition in heat flow curve for the sample with MA = 2.3 db% in the test temperature range. No heat flow peaks could be observed around 0°C in the samples with different degrees of hydration, indicating that there is no melting or freezing of ice water. Therefore, all water could be bonded to the hydrophilic region of gliadin molecules at MA ≤ 18.21 db%.

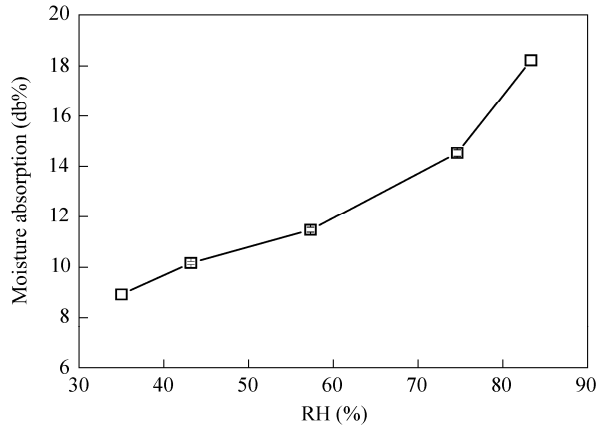


Fig. 1 Moisture absorption (dry basis) versus relative humidity (RH) for wheat gliadin powders at 25°C. The error bar is given.

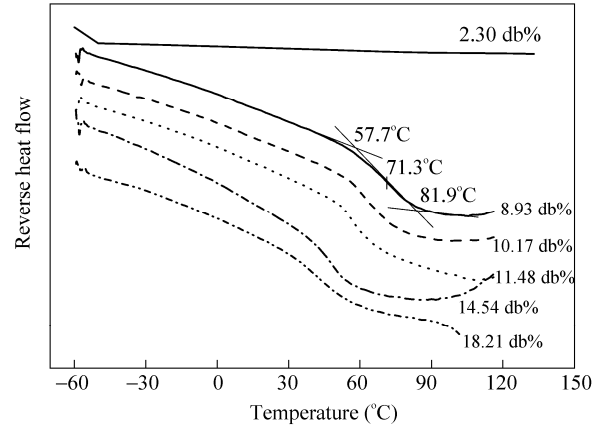


Fig. 2 Reverse heat flow curves of hydrated gliadins recorded by MDSC during first heating

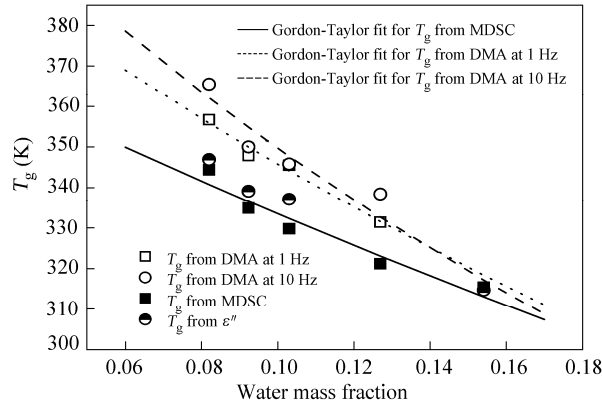


Fig. 3 T_g determined from MDSC, DMA and dielectric spectra as a function of water mass fraction for hydrated gliadins and the Gordon-Taylor fit for hydrated gliadins

Figure 4 shows temperature dependence of mechanical loss factor $\tan\delta$ for hydrated gliadins at 1 Hz. The broad transition peaks are associated with glass transition. The wide transition breadth may reflect the high heterogeneity of gliadins with a wide distribution of molecular weights^[17]. The origin of the broadening has also been related to a large distribution of relaxation times within the protein-water system, or a size distribution of water clusters on the protein surface^[20–22]. T_g measured at 10 Hz is higher than that at 1 Hz, as shown in Fig. 3. T_g from DMA is higher than that from DSC, due to different test methods.

The Gordon-Taylor equation (Eq. (1))^[23] was used to estimate glass transition temperature (T_{g1}) of dry gliadins from that (T_g) of hydrated gliadins

$$T_g = (W_1 T_{g1} + kW_2 T_{g2}) / (W_1 + kW_2) \quad (1)$$

Here, T_{g2} at about -139°C from literature^[14, 24] is glass transition temperature of water. W_1 and W_2 are the weight fractions of gliadins and water, respectively. k is a constant proportional to the plasticizing effect. T_{g1} is estimated at 377 K from MDSC and 410 K at 1 Hz and 433 K at 10 Hz from DMA.

Dielectric spectra have been frequently used in detecting glass transition^[25–27] and to investigate the nature of water-protein interactions^[28–31]. Figure 5 shows the temperature dependence of dielectric loss factor $\tan\delta$ at frequency (f) of 1 Hz for hydrated gliadins with different MAs. Two peaks centered at 38.9°C and 80.1°C are observed in the sample with MA = 11.48 db%. The corresponding temperature at low temperature peak is close

to T_g calculated from DMA, suggesting the low temperature peak is associated with glass transition of hydrated gliadins. The high temperature peak would probably be related to the relaxation of the weak network formed in hydrated gliadins through hydrogen bonds and other physical interactions.

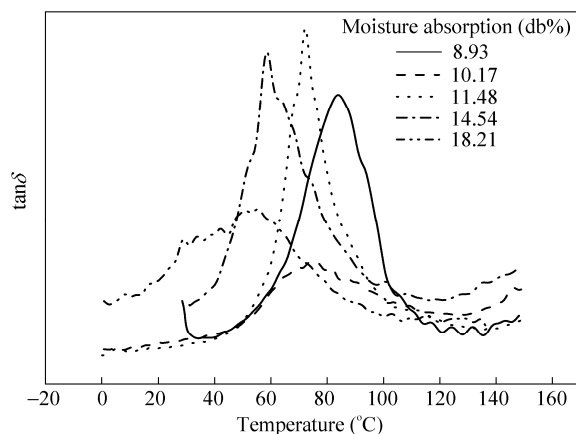


Fig. 4 Mechanical loss factor $\tan\delta$ determined from DMA at 1 Hz as a function of temperature for hydrated gliadins

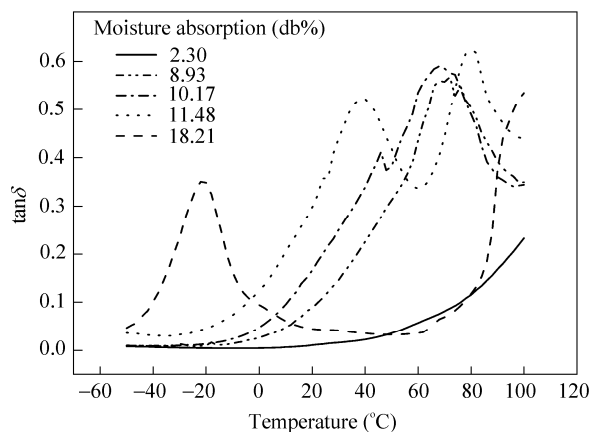


Fig. 5 Dielectric loss factor $\tan\delta$ measured at 1 Hz as a function of temperature for hydrated gliadins

The sample with MA = 18.21% shows a peak at -22°C while another strong relaxation peak could be only partially observed at high temperatures. The other samples at low degrees of hydration exhibit only one relaxation peak within the test temperature range. The temperature sweep dielectric spectra reveal the formation of heterogeneous structure of gliadins at high degrees of hydration, which could not be detected in DSC and DMA measurements and was not revealed in previous studies^[13, 14, 16]. Khodadadi *et al.* used dielectric spectroscopy to identify several relaxation processes of hydrated lysozyme powders to follow protein dynamics at different hydration levels over broad frequency and temperature ranges^[29]. Table 1 shows peak (the low-temperature peak for the sample with MA = 11.48 db%) temperature of hydrated gliadins at various frequencies. For the samples with MA ≤ 11.48 db%, the peak temperature from dielectric spectra increases first and then levels off with increasing frequency. The leveled value of peak temperature is close to that from DSC, revealing that the relaxation peak (the low-temperature peak for the sample with MA = 11.48 db%) is related to the glass transition.

Table 1. T_g of hydrated gliadins from dielectric spectra

Water content (db%)	T_g from dielectric spectra ($^\circ\text{C}$)					
	0.1 Hz	1 Hz	10 Hz	100 Hz	1000 Hz	5000 Hz
8.93	56	72	71	73.8	—	—
10.17	38	68	66	66	—	—
11.48	20	39	64	60	64	64
18.21	—	-22	-12	0	—	28

Figure 6 shows f dependences of real and imaginary dielectric permittivity (ϵ' and ϵ'') and $\tan\delta$ for hydrated gliadins at 25°C . Two major relaxation peaks could be observed on ϵ'' and $\tan\delta$ curves for the sample of MA = 18.21 db% while only one relaxation peak could be observed for the sample of MA = 11.48 db%. For samples of MA = 10.17 db% and lower, the major relaxation peak could only be partially obscured on ϵ'' and $\tan\delta$ curves. It seems that this relaxation moves to lower frequencies with decreasing MA. The sample of MA = 2.30 db% exhibits a weak wide relaxation at frequencies from 10^3 Hz to 10^6 Hz with a ϵ'' maximum at 32.8 kHz. The relaxation peak moves to higher frequencies beyond the test range with increasing degree of

hydration.

MA significantly increases local molecular mobility and the associated rate/magnitude of dielectric relaxations, and also enhances charge carrier mobility/concentration in gliadins. The first derivative of ϵ' has been shown to be a good approximation of the Kramers-Kronig transform of ϵ' to ϵ'' , $\epsilon''_{\text{der}} = -\frac{\pi}{2} \frac{\partial \epsilon'(\omega)}{\partial (\ln \omega)}$, and to provide an “ohmic conductive-free” representation of dielectric loss^[32]. Interestingly, ϵ''_{der} and ϵ'' have been demonstrated to exhibit the same peak frequencies as long as the relaxations are relatively broad^[33]. The Kramers-Kronig approximation allows to pick up the dielectric loss from the polarization part only and the conduction loss is eliminated in this calculated loss since electrical conduction does not contribute to ϵ' . This offers the possibility to study polarization losses usually overshadowed by the high conduction losses. ϵ''_{der} is presented as solid curve in Fig. 6. The contribution from polarization effects is dominant, since a marked difference in ϵ''_{der} and ϵ'' is observed only at $f < 0.1$ Hz for gliadins with more than 11.48 db%.

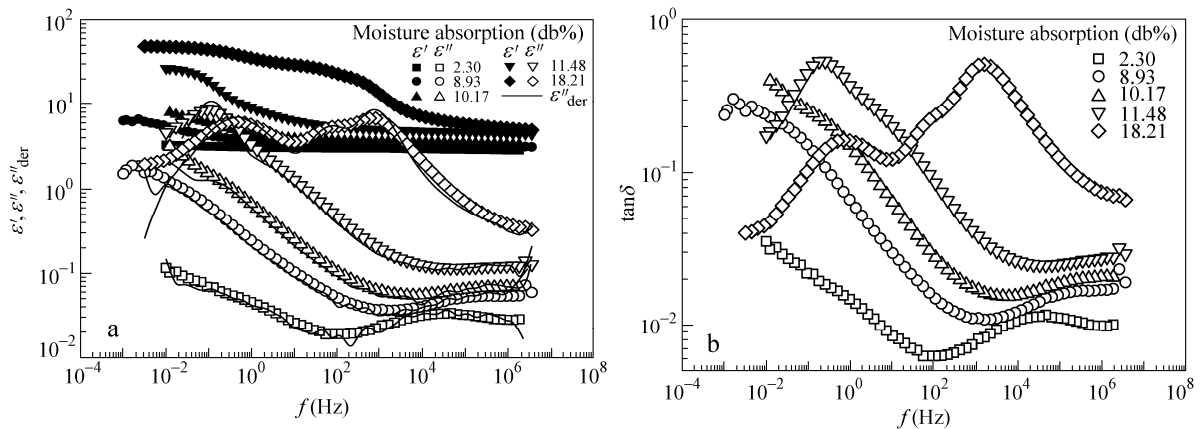


Fig. 6 Real and imaginary permittivities (ϵ' and ϵ'') (a) and loss factor $\tan \delta$ (b) as a function of frequency (f) for hydrated gliadins at 25°C

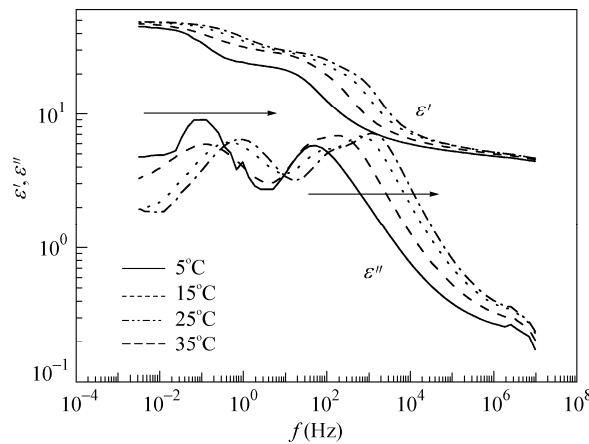


Fig. 7 Real and imaginary permittivities (ϵ' and ϵ'') as a function of frequency (f) for hydrated gliadin with 18.21 db% water at various temperatures

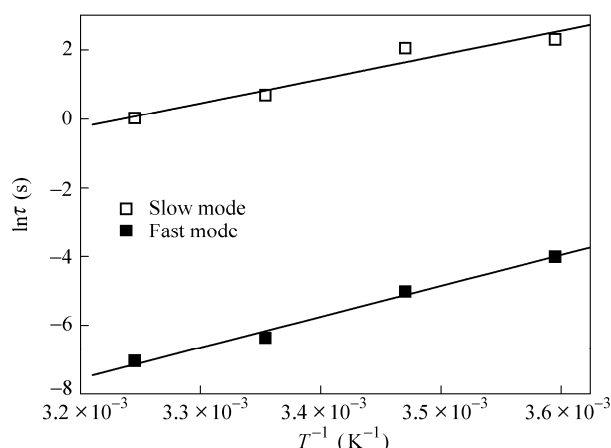


Fig. 8 Arrhenius plots of characteristic relaxation times for hydrated gliadins with 18.21 db% MA

Figure 7 gives f -dependence of ϵ' and ϵ'' for hydrated gliadins with MA = 18.21 db% at four different temperatures. The two major ϵ'' peaks reflecting the structural heterogeneity due to hydration shift to higher frequencies with increasing temperature. Characteristic relaxation times $\tau = (2\pi f_{\max})^{-1}$ of the fast and slow processes were determined at 0.3–4.6 ms and 0.3–1.6 s, respectively. Here, $f_{\max}$ is the frequency at ϵ'' maximum. τ is plotted as a function of reciprocal temperature in Fig. 8. Both processes exhibit Arrhenius type of dependence on temperature as described by $\tau = \tau_0 \exp(-E_a/RT)$ ^[34, 35]. Here, R is gas constant and τ_0 is a constant corresponding to quasi-lattice or molecular vibration time. Apparent molar activation energy E_a is determined as 74.44 kJ mol⁻¹ and 57.87 kJ mol⁻¹, respectively, for the two relaxations.

CONCLUSIONS

Glass transitions of hydrated gliadins with 2.3 db% to 18.21 db% MA were investigated using MDSC, DMA and dielectric spectroscopy. Depending on the test method, T_g of dry gliadin was estimated at about 377–433 K from Gordon-Taylor equation. Hydration significantly affects glass transition and molecular relaxation of gliadins, even though no free water could be detected. Dielectric spectroscopy studies reveal the formation of heterogeneous structure of gliadins at high degrees of hydration. The relaxation heterogeneity in hydrated gliadins has not been detected in previous studies focusing on thermal analyzing of hydrated wheat gluten.

REFERENCES

- 1 Jerez, A., Partal, P., Martinez, I., Gallegos, C. and Guerrero, A., *Biochem. Eng. J.*, 2005, 26(2-3): 131
- 2 Kunanopparat, T., Menut, P., Morel, M.H. and Guilbert, S., *Compos. Part A-Appl. Sci. Manuf.*, 2008, 39(5): 777
- 3 Hernandez-Munoz, P., Kanavouras, A., Ng, P.K.W. and Gavara, R., *J. Agric. Food Chem.*, 2003, 51: 7647
- 4 Song, Y.H., Zheng, Q. and Lai, Z.Z., *Chinese J. Polym. Sci.*, 2008, 26(5): 631
- 5 Song, Y.H. and Zheng, Q., *Ind. Crop. Prod.*, 2009, 29(2-3): 446
- 6 Sun, S.M., Song, Y.H. and Zheng, Q., *Food Hydrocolloids*, 2008, 22(6): 1006
- 7 Sun, S., Song, Y. and Zheng, Q., *Food Hydrocolloids*, 2007, 21(7): 1005
- 8 Hernandez-Munoz, P., Kanavouras, A., Villalobos, R. and Chiralt, A., *J. Agric. Food Chem.*, 2004, 52: 7897
- 9 Sanchez, A.C., Popineau, Y., Mangavel, C., Larre, C. and Gueguen, J., *J. Agric. Food Chem.*, 1998, 46: 4539
- 10 Sun, S.M., Song, Y.H. and Zheng, Q., *J. Cereal Sci.*, 2008, 48(3): 613
- 11 Sun, S.M., Song, Y.H., Zhang, Q.B. and Zheng, Q., *Gongneng Cailiao/Journal of Functional Materials*, 2006, 37(7): 1094

- 12 Sun, S.M., Song, Y.H. and Zheng, Q., *Food Hydrocolloids*, 2007, 21(7): 1005
- 13 Noel, T.R., Parker, R., Ring, S.G. and Tatham, A.S., *Int. J. Biol. Macromol.*, 1995, 17(2): 81
- 14 Micard, V. and Guilbert, S., *Int. J. Biol. Macromol.*, 2000, 27(3): 229
- 15 Almutawah, A., Barker, S.A. and Belton, P.S., *Biomacromolecules*, 2007, 8(5): 1601
- 16 Degraaf, E.M., Madeka, H., Cocero, A.M. and Kokini, J.L., *Biotechnol. Prog.*, 1993, 9(2): 210
- 17 Sun, S., Song, Y. and Zheng, Q., *Food Hydrocolloids*, 2008, 22(6): 1090
- 18 Pinheiro, A. and Mano, J.F., *Polym. Test*, 2009, 28(1): 89
- 19 Royall, P.G., Huang, C.Y., Tang, S.W.J., Duncan, J., Van-de-Velde, G. and Brown, M.B., *Int. J. Pharm.*, 2005, 301(1-2): 181
- 20 Jansson, H. and Swenson, J., *BBA-Proteins Proteomics*, 2010, 1804(1): 20
- 21 Sartor, G., Mayer, E. and Johari, G.P., *Biophys. J.*, 1994, 66(1): 249
- 22 Doster, W., Bachleitner, A., Dunau, R., Hiebl, M. and Luscher, E., *Biophys. J.*, 1986, 50(2): 213
- 23 Gordon, M. and Taylor, J.S., *J. Appl. Chem.*, 1952, 2(9): 493
- 24 Kalichevsky, M.T., Jaroszkiewicz, E.M. and Blanshard, J.M.V., *Int. J. Biol. Macromol.*, 1992, 14(5): 257
- 25 Gonzalez-Campos, J.B., Prokhorov, E., Luna-Barcenas, G., Fonseca-Garcia, A. and Sanchez, I.C., *J. Polym. Sci. Part B-Polym. Phys.*, 2009, 47(22): 2259
- 26 Fukao, K., Nihon Reorji Gakkaishi, 2008, 36(2): 73
- 27 D'Angelo, P., Barra, M., Cassinese, A., Guido, S. and Tomaiuolo, G., *Macromol. Symposia*, 2007, 247: 43
- 28 Suhrman, P.M., Taylor, P. and Smith, G., *J. Non-Cryst. Solids*, 2002, 305(1-3): 317
- 29 Khodadadi, S., Pawlus, S. and Sokolov, A.P., *J. Phys. Chem. B*, 2008, 112(45): 14273
- 30 Smith, G., Duffy, A.P., Shen, J. and Olliff, C.J., *J. Pharm. Sci.*, 1995, 84(9): 1029
- 31 Pethig, R., *Annu. Rev. Phys. Chem.*, 1992, 43: 177
- 32 Steeman, P.A.M. and Vanturnhout, J., *Macromolecules*, 1994, 27(19): 5421
- 33 Wubbenhorst, M. and van Turnhout, J., *J. Non-Cryst. Solids*, 2002, 305(1-3): 40
- 34 Jansson, H., Bergman, R. and Swenson, J., *J. Phys. Chem. B*, 2005, 109(50): 24134
- 35 Shinyashiki, N., Shinohara, M., Iwata, Y., Goto, T., Oyama, M., Suzuki, S., Yamamoto, W., Yagihara, S., Inoue, T., Oyaizu, S., Yamamoto, S., Ngai, K.L. and Capaccioli, S., *J. Phys. Chem. B*, 2008, 112(48): 15470