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Polymer 42 (2001) 7343–7347

polymer

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Ca^{2+}-induced complexation between thermally sensitive spherical poly(*N*-vinyl-caprolactam-*co*-sodium acrylate) microgels and linear gelatin chains in water

Shufu Peng[a], Chi Wu[b,*]

[a]*Department of Chemical Physics, The Open Laboratory of Bond-selective Chemistry, University of Science and Technology of China, Hefei, Anhui, People's Republic of China*

[b]*Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, People's Republic of China*

Received 27 November 2000; received in revised form 14 February 2001; accepted 16 February 2001

Abstract

The temperature dependence of the complexation between thermally sensitive spherical poly(*N*-vinylcaprolactam-*co*-sodium acrylate) microgels and linear gelatin chains without and with the addition of Ca^{2+} was studied by a combination of static and dynamic laser light scattering over a wide range of the gelatin/microgel ratios ([G]/[M]) in terms of the complex's average hydrodynamic radius ($\langle R_h \rangle$), weight average molar mass (M_w), average aggregation number (N_{agg}), and average chain density ($\langle \rho \rangle$). Without Ca^{2+}, the complexation is weak and each complex, on an average, contains not more than two microgels. In the presence of Ca^{2+}, the complexation occurred at ~32°C is much stronger and the onset of complexation is independent of [G]/[M], but both N_{agg} and $\langle \rho \rangle$ decrease as [G]/[M] increases because the adsorption of too many hydrophilic gelatin chains on the microgels hinders the complexation and reduces the shrinking of the complexes. The complexation is essentially reversible but there exists a slight hysteresis on cooling. On the basis of this study, a gel composite made of microgel and gelatin was prepared and its biomedical applications have been considered. © 2001 Published by Elsevier Science Ltd.

Keywords: Complexation; Microgel; Gelatin

1. Introduction

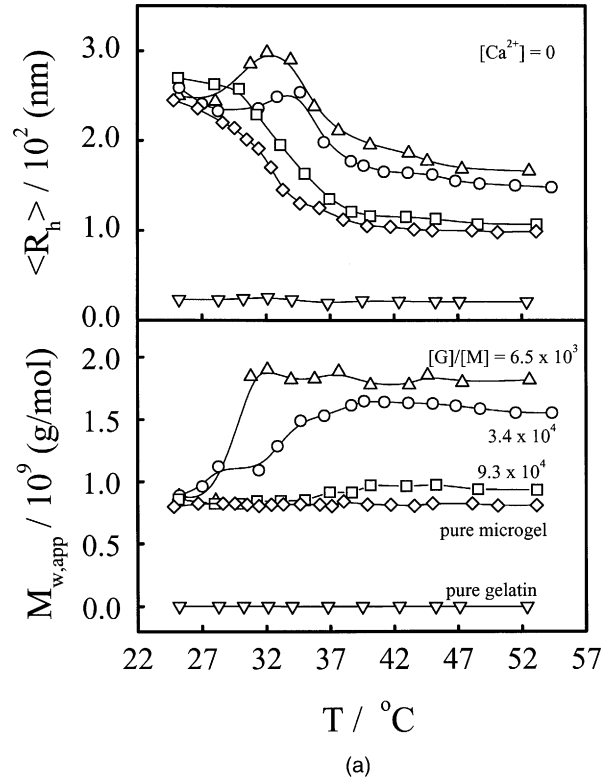
The complexation of a polyelectrolyte and a protein can provide a range of physicochemical properties, including biocompatibility. Special attention has been drawn to their biomedical applications [1,2]. Moreover, the complexation of synthetic polyelectrolytes and proteins presents a convenient model for various biological processes [3]. It is also known that certain metal ions as well as biphillic low molar mass compounds can bind to polyelectrolytes and lead to the complexation of polymer chains with a complementary surface [4–6]. Bowman et al. [7] and Hara et al. [8] studied the effects of various parameters on the structure of soluble complexes. Carboxylic groups (COO^-) have a tendency to interact with Ca^{2+}, leading to distinct conformational changes or intermolecular bridges between polyelectrolyte chains. In biopolymers, this complexation can modify their biological activities, such as blood clotting and muscular contraction [9,10]. In spite of a great deal of effort spent on this subject, the details of the complexation and stabilization are still missing because of its complex nature.

On the other hand, thermally sensitive poly(*N*-vinylcaprolactam) (PVCL) has a convenient low critical solution temperature of ~32°C in aqueous solution [11]; changing from hydrophilic to hydrophobic when the temperatures are raised beyond ~32°C. This special property leads to a range of potential applications, such as the immobilization of enzymes, cells and drugs. PVCL can interact with a range of compounds with different structures in aqueous solutions [12,13]. Its interaction with gelatin is particularly interesting to us because we found that a hybrid hydrogel made of gelatin and PVCL is also thermally sensitive and has potential biomedical applications. In this study, we intend to find how Ca^{2+} can affect the interaction between PVCL gels and gelatin, which serves as a model for the study of the interaction between PVCL and protein. Instead of using a bulk gel, we prepared PVCL microgels and incorporated carboxylic groups on the gel surface. The complexation

* Corresponding author. Address: Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, People's Republic of China. Tel.: +852-2609-6106; fax: +852-2603-5057.

E-mail address: chiwu@cuhk.edu.hk (C. Wu).

0032-3861/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0032-3861(01)00176-8



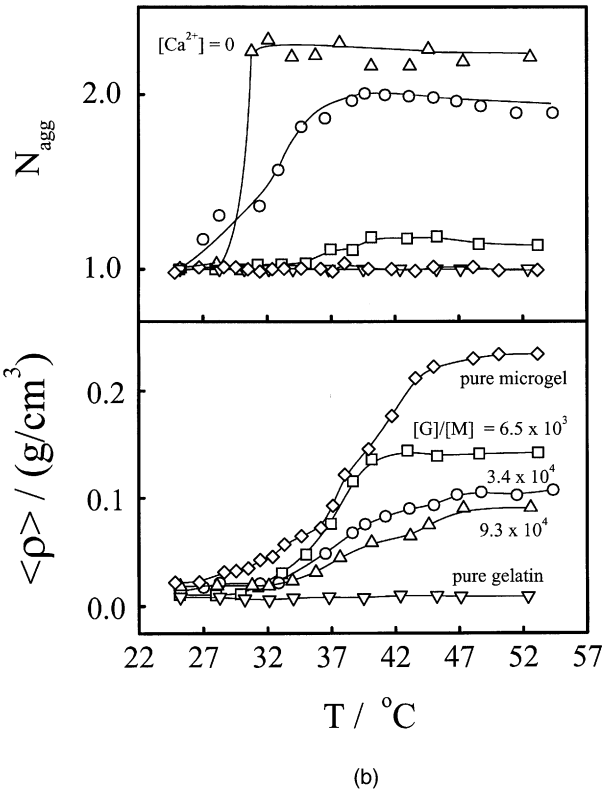
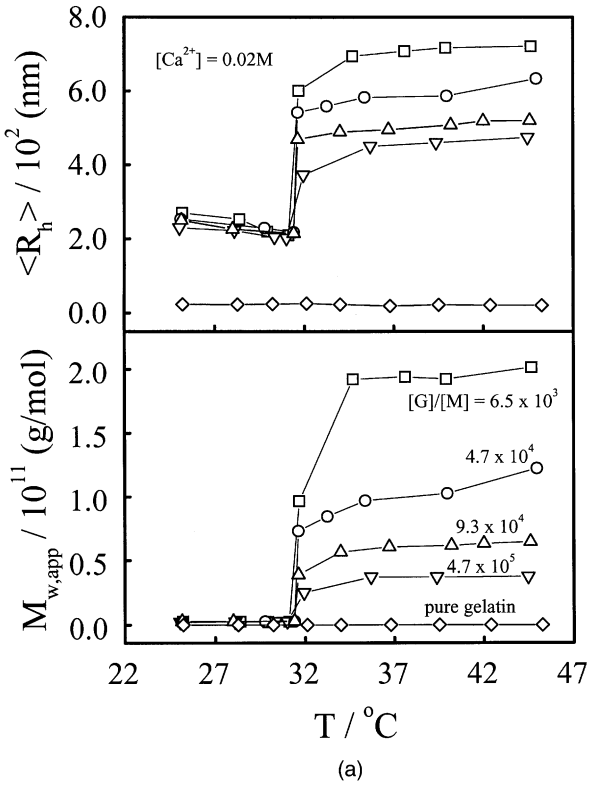


Fig. 1. Temperature dependence of (a) average hydrodynamic radius $\langle R_h \rangle$; (b) apparent weight average molar mass ($M_{w,app}$); (c) average aggregation number (N_{agg}); and (d) average chain density $\langle \rho \rangle$ of the microgel/gelatin complexes, where [G]/[M] is the initial gelatin/microgel molar ratio and $\langle \rho \rangle$ is defined as $M_w/[(4/3)\pi\langle R_h \rangle^3]$.



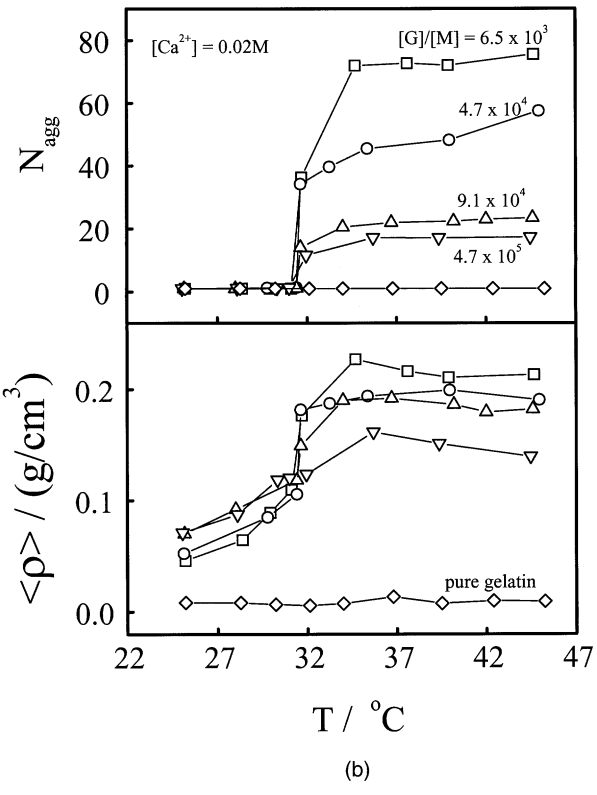


Fig. 2. Temperature dependence of (a) average hydrodynamic radius $\langle R_h \rangle$; (b) apparent weight average molar mass ($M_{w,app}$); (c) average aggregation number (N_{agg}); and (d) average chain density $\langle \rho \rangle$ of the microgel/gelatin complexes formed in the presence of Ca^{2+}, where [G]/[M] is the initial gelatin/microgel molar ratio and $\langle \rho \rangle$ is defined as $M_w/[(4/3)\pi\langle R_h \rangle^3]$.

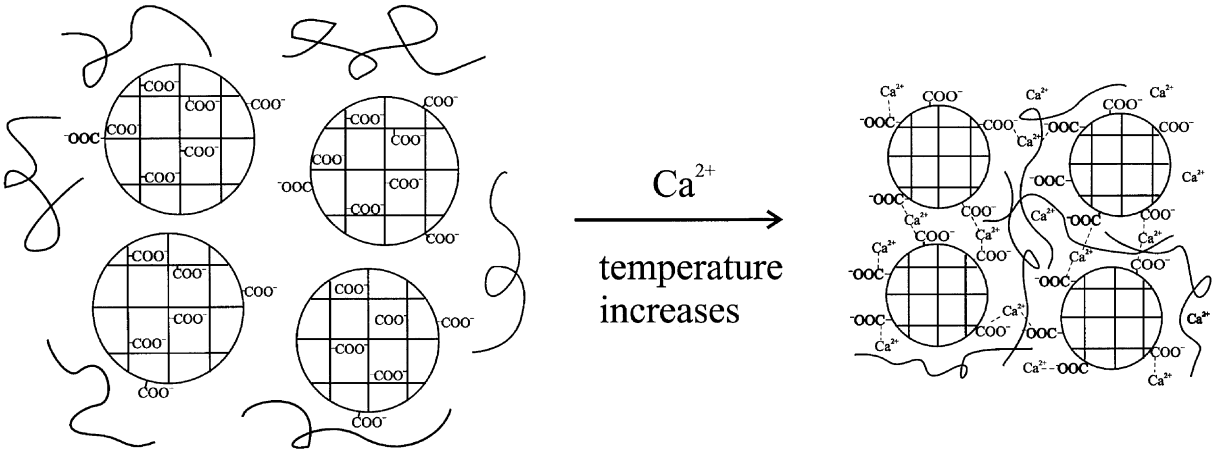


Fig. 3. Schematic of the temperature dependence of the microgel/gelatin complexation in the presence of Ca^{2+}.

was investigated by using a combination of static and dynamic laser light scattering.

2. Experimental

2.1. Materials

N-vinylcaprolactam monomer (VCL, courtesy of BASF) was purified by a reduced pressure distillation. Sodium acrylate monomer (NaA, from Lancaster) was used without further purification. Potassium persulfate as an initiator (KPS, from Aldrich) and *N*,*N*′-methylenebisacrylamide as a cross-linking agent (MBAA, from Aldrich) were recrystallized three times in methanol. The gelatin sample (B-type, B74397, courtesy of BASF) with a weight-average molar mass (M_w) of 2.24×10^5 g/mol was used. Spherical poly(*N*-vinylcaprolactam-*co*-sodium acrylate) (P(VCL-*co*-NaA)) microgel was prepared by precipitation polymerization. Into a 150 ml three-necked flask equipped with a reflux condenser, a thermometer and a nitrogen-bubbling tube, were added VCL monomer (7.3 mmol), NaA comonomer (0.37 mmol), MBAA crosslinking (0.18 mmol) and deionized water (40 ml). The solution was stirred and nitrogen bubbled through it for 1 h to remove oxygen before adding a KPS aqueous solution (0.05 mmol) to start the polymerization at 60°C for 24 h. The resultant P(VCL-*co*-NaA) microgel was purified by four successive cycle centrifugation (Sigma 2K15 ultracentrifuge, at 15,300 rpm and 40°C), decantation and redispersion in deionized water to remove unreacted low molar mass molecules. On an average, each microgel contains 4.3 mol% of acrylic groups. In order to completely dissolve gelatin in water, it was first dissolved in a small amount of formamide and the formamide solution was added into a large amount of water. The final formamide concentration in the gelatin solution was only 2%. The complexation among the microgels and gelatin chains was studied with and without the addition of 0.02 M $CaCl_2$ aqueous solution.

2.2. Laser light scattering

The detail of our laser light scattering spectrometer can be found elsewhere [14]. In static LLS, the angular dependence of the absolute excess time-averaged scattered intensity, known as the Rayleigh ratio $R_{vv}(q)$, can lead to the weight-average molar mass (M_w), the *z*-average root-mean-square radius of gyration ($\langle R_g^2 \rangle_z^{1/2}$, or written as $\langle R_g \rangle$) and the second virial coefficient (A_2), where **q** is the scattering vector. In dynamic LLS, the cumulant analysis or Laplace inversion of the measured intensity–intensity time correlation function $G^{(2)}(q,t)$ in the self-beating mode can result in an average line width ($\langle \Gamma \rangle$) or a line width distribution ($G(\Gamma)$) [15,16]. For a pure diffusive relaxation, Γ is related to the translational diffusion coefficient D by $(\Gamma/q^2)_{C\to 0,q\to 0} = D$ and the hydrodynamic radius (R_h) via the Stokes–Einstein equation, $D = k_B T/(6\pi\eta R_h)$, where k_B, T and η are the Boltzmann constant, the absolute temperature and the solvent viscosity, respectively [17].

3. Results and discussion

Fig. 1 shows that when the gelatin/microgel molar ratio is less than $\sim 3.4 \times 10^4$ and in the absence of Ca^{2+}, both $\langle R_h \rangle$ and $M_{w,app}$ of the microgel/gelatin complexes only slightly increase at the temperature around 32°C, revealing that on an average, each complex contains only ~2 microgels or less. A further increase of the temperature leads to a gradual decrease of $\langle R_h \rangle$, but $M_{w,app}$ is nearly independent of the temperature in the same range, indicating the shrinking of the microgels, but no further interparticle association. For the mixture with a higher ratio [G]/[M], $\langle R_h \rangle$ decreases monotonously as the temperature increases in the whole temperature range studied. The increase of the average chain density ($\langle \rho \rangle$) of the microgel/gelatin complexes as the temperature increases in the range ~32–45°C reflects the shrinking of the microgels inside the complex. Note that at high temperatures, $\langle \rho \rangle$ increases as the ratio of [G]/[M]

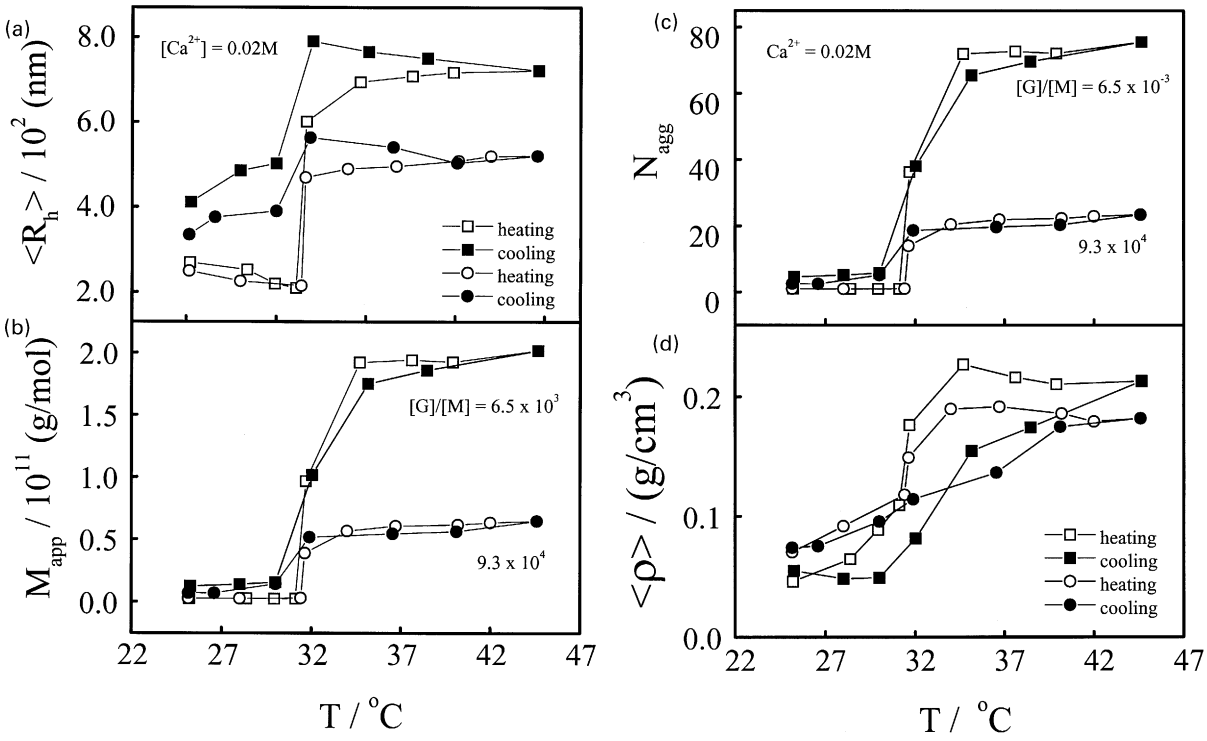


Fig. 4. Heating and cooling dependence of (a) average hydrodynamic radius $\langle R_h \rangle$; (b) apparent weight average molar mass ($M_{w,app}$); (c) average aggregation number (N_{agg}); and (d) average chain density $\langle \rho \rangle$ of the microgel/gelatin complexes formed in the presence of Ca^{2+}, where [G]/[M] is the initial gelatin/microgel molar ratio and $\langle \rho \rangle$ is defined as $M_w/[(4/3)\pi\langle R_h \rangle^3]$.

decreases, further reflecting that the shrinking is related to the microgels. More microgels inside each complex can provide a stronger shrinking force and lead to a more compact structure at high temperatures.

Fig. 2 shows that in the presence of Ca^{2+}, the complexation is more profound in comparison with that shown in Fig. 1. Both $\langle R_h \rangle$ and $M_{w,app}$ increase as the [G]/[M] ratio decreases, indicating that gelatin chains act as a stabilizer,

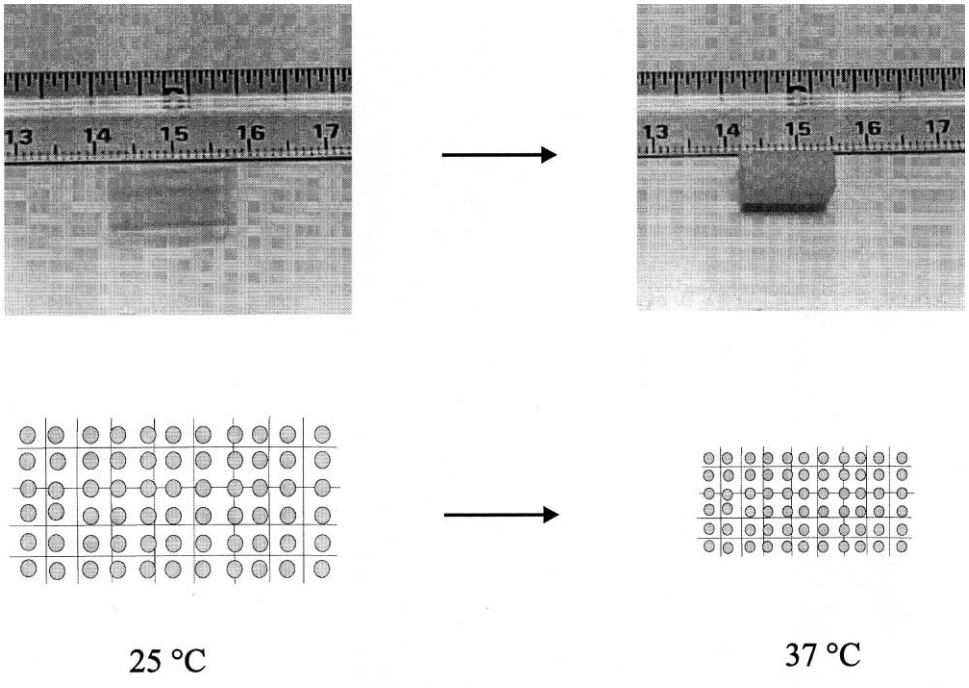


Fig. 5. Swelling and shrinking of a novel microgel/gelatin composite at two different temperatures and schematic of the corresponding structures of the composite.

presumably by the adsorption of gelatin chains on the microgel surface. Note that the complexation occurs at a similar temperature in spite of a big difference in the gelatin concentration. This clearly shows that when the microgel is in its shrunk state (hydrophobic), most of the carboxylic groups are forced to locate on the surface of the microgel so that Ca^{2+} can bind the microgels and gelatin chains through the carboxylic groups. On an average, each complex contains 20–70 microgels and the average density of the complexes is in the range 0.13–0.22 g/cm^3 at temperatures higher than ~32°C. The decrease of N_{agg} [$\equiv M_{w,aggregate}/M_{w,microgel}$] and $\langle\rho\rangle$ [$\equiv M_{w,aggregate}/[(4/3)\pi\langle R_h\rangle^3]$] as [G]/[M] increases further indicate that the adsorption of gelatin chains on the microgel surface reduces the complexation of the microgels. Fig. 3 shows a schematic diagram of the temperature dependence of the microgel/gelatin complexation in the presence of Ca^{2+}.

Fig. 4 shows that in the presence of Ca^{2+}, the temperature dependence of $\langle R_h\rangle$, $M_{w,app}$, N_{agg} and $\langle\rho\rangle$ of the complexes during heating and cooling are slightly different. Generally, there is a hysteresis during cooling. The slight increase of $\langle R_h\rangle$ during cooling before it drops at ~32°C and a lower $\langle\rho\rangle$ reveals that the complexes swell before their dissociation at lower temperatures. This indicates that the complexation of the microgels and gelatin chains formed via Ca^{2+} at high temperatures is relatively strong. When $T < \sim 32$°C, the swollen microgels become so hydrophilic that the interaction between the COO^- groups and Ca^{2+} is not able to hold the microgel/gelatin complexes together. The swelling of the microgels is also evidenced by the fact that at a temperature higher than ~32°C, the complexes are larger during cooling, but there is no change in $M_{w,app}$. The swelling is due to the gel network's elasticity, which was absent when linear poly(*N*-isopropylacrylamide) or its copolymer chains were used [18,19]. Note that for a given temperature, there is nearly no difference between N_{agg} during heating and cooling. However, N_{agg} becomes larger at 25°C after the first heating-and-cooling cycle, indicating that the complexation induced at higher temperature is not completely reversible.

Fig. 5 shows a gel composite in which the microgels are embedded in a chemically crosslinked gelatin network. This clearly shows that the shrinking of each microgel inside leads to the shrinking of the bulk gel. The degree of shrinking can be well controlled by the amount of microgels embedded inside and by the crosslinking density of the gelatin network. More important, both the gelatin solutions before its gelation and the microgel dispersion are injectable. The gel formation inside the body makes such a gelatin/microgel composite a potential biomedical material. Its application in surgery is undergoing.

4. Conclusions

Our results reveal that when the P(VCL-*co*-NaA) microgel becomes hydrophobic at temperatures higher than ~32°C, most of the carboxylic groups are located on its surface such that the microgel can complex with gelatin in the presence of Ca^{2+}, suggesting that the microgel/gelatin complexation is induced by both hydrophobic interaction and electrostatic attraction. However, if an excess of gelatin chains is used, the adsorption of gelatin chains on the microgel surface prevents the complexation. The shrinking of each microgel inside can lead to the shrinking of a bulk microgel/gelatin gel composite at temperatures higher than ~32°C. The complexation is essentially reversible. The shrinking of each microgel inside a gel composite leads to the shrinking of the bulk gel. This study leads to a novel and injectable thermally sensitive gel composition. It can never repair the broken bone tissue without any surgical stitch. Its swelling and shrinking temperature can be adjusted by the amount of carboxylic group incorporated inside the microgel and its swelling and shrinking extent can be controlled by the microgel/gelatin ratio.

Acknowledgements

Financial support of the Research Grants Council of the Hong Kong Special Administration Region Earmarked Grant 1999/2000 (CUHK 4209/99P, 2160122) and of NNSFC 29974027 is gratefully acknowledged.

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