Carbohydrate Polymers 86 (2011) 1145–1150

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Rehydration of dried alginate gel beads: Effect of the presence of gelatin and gum arabic

Yapeng Fang[a,b,*], Liangbin Li[c], Rob Vreeker[d], Xiaolin Yao[a], Jianguo Wang[a], Qing Ma[a], Fatang Jiang[a], Glyn O. Phillips[a,b]

[a] Glyn O. Phillips Hydrocolloid Research Center at HUT, Hubei University of Technology, Wuhan 430068, China
[b] Glyn O. Phillips Hydrocolloid Research Center, Glyndwr University, Plas Coch, Mold Road, Wrexham LL11 2AW, UK
[c] National Synchrotron Radiation Laboratory and Department of Polymer Science and Engineering, University of Science and Technology of China, Anhui 230026, China
[d] Unilever Food and Health Research Institute, Olivier van Noortlaan 120, 3133AT Vlaardingen, The Netherlands

ARTICLE INFO

Article history:
Received 18 February 2011
Received in revised form 21 May 2011
Accepted 5 June 2011
Available online 13 June 2011

Keywords:
Rehydration
Swelling
Alginate gel beads
Biopolymer mixture
Phase separation

ABSTRACT

This study investigates how the phase separation induced by the biopolymers gelatin and gum arabic influences the microstructure of alginate beads prepared in $CaCl_2$ solutions and the subsequent rehydration of the air-dried beads. The extent of associative phase separation in mixture gel beads can be controlled via pH. Compared with control beads, alginate/gelatin mixture beads swelled faster at the initial stage of rehydration while slowed down at the late stage, reaching a lower equilibrium swelling ratio. The faster initial swelling kinetics can be attributed to the presence of gelatin which prevents the side-by-side aggregation of egg-box junctions. This conclusion was confirmed using wide angle X-ray diffraction (WAXD) measurements. The lower equilibrium swelling ratio was due to the gelatin network restricting alginate from further swelling. This was evidenced by temperature dependence swelling experiments and comparison with alginate/gum arabic beads where no additional network was formed on top of the alginate network. The varying of pH, correspondingly the change of phase separation extent, had a significant influence on the rehydration of mixture gel beads. The best rehydratability was observed at higher pHs where no phase separation occurred and the components were homogenously mixed. With decreasing pH, the associative phase separation between alginate and gelatin was promoted, and led to local over-concentration of alginate, which gave rise to poor rehydratability.

1. Introduction

The controlled rehydratability of dried biopolymers either cross-linked or non-crosslinked is crucially important to many applications such as the preparation of instant foods and drug delivery (Vreeker, Li, Fang, Appelqvist, & Mendes, 2008). How to control and improve the rehydratability of dried biopolymer materials and understanding the physics behind it could add much value to the related applications. Actually, nature gives us many clues of which we could take advantage to design rehydratable structures. For example, resurrection plants can lose almost all of their waters, and still spring to life when the rains come. Sugars and polymeric components in the plants are considered to have a major role in preventing irreversible structural damages during dehydration that would otherwise lead to incomplete rehydration and hence the death of the plants (Pammenter & Berjak, 1999). Then, a question arises: can we imitate the natural strategy to control and improve the rehydratability of a dried biopolymer by blending them with another type of biopolymer? In this work, we tested the nature-inspired strategy using Ca^{2+}-crosslinked alginate bead as a model system and examining the effect of adding gelatin or gum arabic on the rehydration behavior of the beads after air-drying. The difference in rehydration behavior was interpreted in terms of the difference in structures formed during drying.

Alginate is a linear polysaccharide isolated from brown seaweeds and certain bacteria. It has a wide range of applications in food, drug delivery, tissue engineering, cell encapsulation and transplantation (Draget, Smidsrod, & Skjak-brak 2005; Soon-Shiong et al., 1993, 1994), thanks to the ready gel-forming ability at mild conditions, i.e. by the addition of calcium ions or by acidification. The success in design of improved rehydratability of alginate could add values in such applications. Alginate is a (1–4)-linked block copolymer of beta-D-mannuronate (M) and its C-5 epimer alpha-L-guluronate (G), with residues arranged in homopolymeric sequences of both types and in regions which approximate to the disaccharide repeating structure (MG) (Rees & Samuel, 1967). The gelation in the presence of calcium ions is considered to arise from

---

[*] Corresponding author at: Glyn O. Phillips Hydrocolloid Research Center at HUT, Hubei University of Technology, Wuhan 430068, China.
E-mail addresses: y.fang@glyndwr.ac.uk, fangyp@mail.hbut.edu.cn (Y. Fang).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.06.003

Ca^{2+}-mediated chain-chain associations between G blocks (Donati, Holtan, Morch, Borgogna, Dentini, & Skjak-Braek, 2005). The mechanism has been described as the ‘egg-box’ model (Grant, Morris, Rees, Smith, & Thom, 1973; Morris, Rees, Thom, & Boyd, 1978). If calcium alginate gel is dried in air, the gel network will collapse, and the egg-box junctions will form side-by-side aggregations under the mediation of free Ca^{2+}, giving rise to poor swelling ability when rehydrated (Vreeker et al., 2008).

2. Materials and methods

2.1. Materials

Sodium alginate with ∼70% guluronic residues was purchased from FMC BioPolymer (Norway). Type B gelatin, extracted from pigskin, was purchased from GELITA EUROPE. It had an isoelectric point of 4.9 as determined by Nano-ZS ZetaSizer (Malvern Instruments, UK). Gum arabic was obtained from Sigma–Aldrich. The other chemicals used in the work, calcium chloride ($CaCl_2$), sodium hydroxide (NaOH) and 1 mol/L hydrochloric acid (HCl) were purchased from Sigma–Aldrich and were of analytical grades.

2.2. Preparation of alginate gel beads

2.0% (wt%) alginate solution was prepared by dispersing alginate powder into 90 °C hot Millipore water with continuous stirring for 30 min. 1.0% gelatin or gum arabic solutions were similarly prepared, but at ≤60 °C in order to avoid thermal degradation of the polymer chains at higher temperatures. The alginate solution was mixed in equal quantity with either the gelatin or the gum arabic solution to obtain alginate (1.0%)/gelatin (0.5%) or alginate (1.0%)/gum arabic (0.5%) mixture solutions (wt%). The biopolymer concentrations and mixing ratio were chosen such that they could exhibit different phase behaviors upon changing pH and could form gel beads strong enough to maintain integrity during preparation by Ca^{2+}-crosslinking. The mixture solutions were adjusted to different pHs with concentrated NaOH or HCl to achieve different extents of phase separation. To prepare Ca^{2+}-crosslinked gel beads, the mixture solutions were dropped into a 1.0% $CaCl_2$ solution at room temperature, and equilibrated for 1 h before being fished out. The as-prepared gel beads have a diameter of ∼5 mm in wet state. The drying of the beads was carried out in a humidity-controlling cabinet (RH = 33%, 20 °C) until no weight change was recorded. The use of the relatively high humidity allowed a slow drying process which was favorable for the formation of structures of high crystallinity (Li, Fang, Vreeker, & Appelqvist, 2007). For the purpose of comparison, the control alginate beads without adding gelatin or gum arabic were also prepared using alginate solutions at a concentration of 1.0%.

2.3. Swelling measurements

The swelling measurements were performed by immersing dried alginate beads into an excessive amount of 0, 0.05%, or 0.5% NaCl solution (wt%). The beads were taken out at regular time intervals, and gently wiped off surface water by tissue paper, and then weighed. The swelling ratio at time t (R_t) was calculated as follows:

$$R_t = \frac{W_t - W_0}{W_0}$$

where W_t and W_0 are weights at time t and 0, respectively. All the swelling experiments were done at room temperature. One exception is the temperature dependence measurements of the swelling ratio for alginate/gelatin gel beads. In these experiments, alginate/gelatin dried gel beads were allowed to swell at temperatures of 5, 10, 20 25, 30, 35, and 40 °C, below and above the melting temperature of gelatin network. Temperature was maintained by external water bath. The swelling ratio at 700 min R_{700} was measured. It was confirmed that no significant disintegration of gel beads occurred at temperatures higher than the melting temperature of gelatin network. The weight of gel beads dried after swelling experiments were slightly lower than that of the initial ones, indicating that a small number of gelatin chains disentangled from the network and diffused out to the external solutions.

2.4. Confocal scanning laser microscope (CSLM)

To characterize the extent of phase separation, the wet alginate/gelatin mixture gel beads as freshly prepared were loaded onto a BioRad MRC 600 confocal scanning laser microscope for observation. The CSLM was equipped with an Ar/Kr mixed gas laser emitting at 488 and 568 nm, and was connected to a Zeiss inverted microscope. Gelatin was stained with a drop of 0.05% Rodamine B solution. Gelatin-rich domains would appear as bright regions in CSLM images.

2.5. Wide angle X-ray diffraction

Wide angle X-ray diffraction (WAXD) technique was used to examine the structural difference of the dried gel beads. The instrument used was a Bruker D8 Discovery equipped with a Cu Kα source (0.154 nm) and a two-dimensional gas filled multi-wire detector. The two-dimensional scattering patterns were integrated along the azimuthal angle to give one-dimensional intensity curves as function of 2θ. Here θ is the scattering angle. All the measurements were conducted in transmission mode.

3. Results

3.1. Phase separation in mixture gel beads

Alginate contains a considerable number of negatively charged carboxyl groups. Gelatin has both positively and negatively charged groups, and its overall charge depends on pH. When the two are mixed, an ‘associative phase separation’ occurs through electrostatic attraction (Piculell, Bergfeldt, & Nilsson, 1995). It leads to the enrichment of the two polymers in the same phase (always in the dispersed droplets). Fig. 1 shows the CSLM images of alginate (1.0%)/gelatin (0.5%) mixture gel beads before drying. Apart from the sample at pH 10.5, the other three samples all showed phase separations, with phase separation extent increased with decreasing pH. Because of the nearly complete deprotonation of the positively charged groups of gelatin at pH 10.5 (>pK_a of most positive groups), the interaction between the two polymers at this pH is predominated by negative–negative electrostatic repulsion, which disfavours the associative phase separation and leads to a homogenous system. Although the overall charge of gelatin at pH 7 is negative (>pI of gelatin, 4.9), a phase separation was still observed. This is due to the uneven distribution of cationic and anionic groups along gelatin chain that produces some positive patches electrostatically attracting with alginate (Bowman, Rubinstein, & Tan, 1997). At pH 4 and 3.5, since gelatin turns overall positively charged, the attraction with alginate leads to intensive phase separations.

Since the change of pH generated different microstructures of alginate/gelatin beads, it was supposed to exert influence on the swelling behavior of air-dried beads.

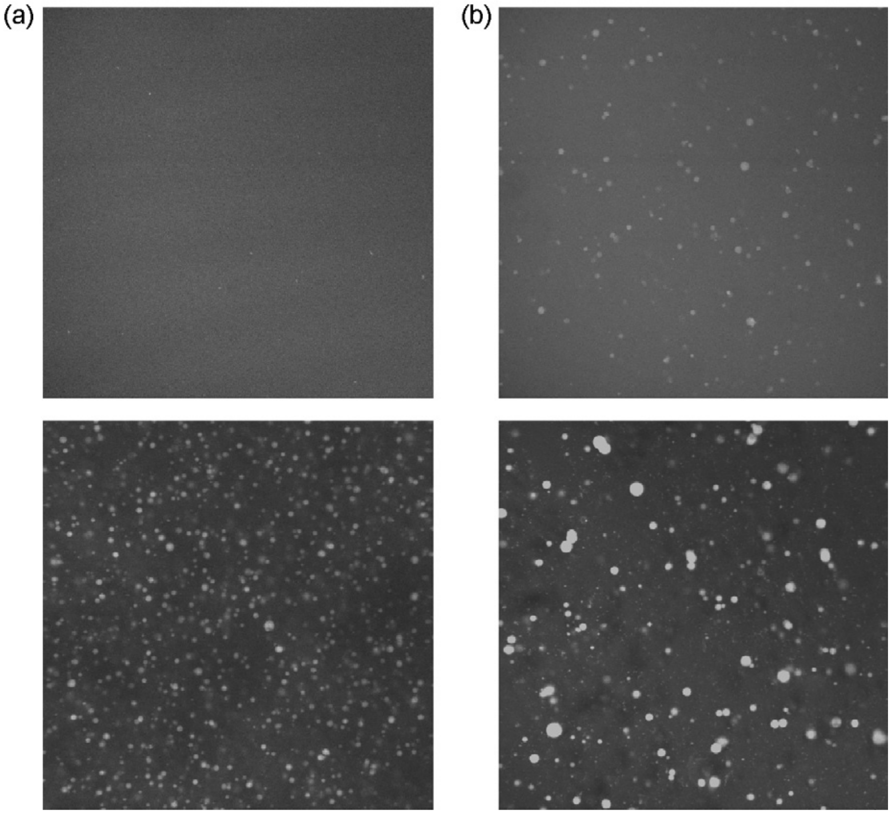

Fig. 1. The CSLM images obtained for alginate (1.0%)/gelatin (0.5%) mixture gel beads at different pHs: 10.5 (a); 7 (b); 4 (c); and 3.5 (d). The images were taken before the beads were dried. All the images have the size of 262 μm × 262 μm.

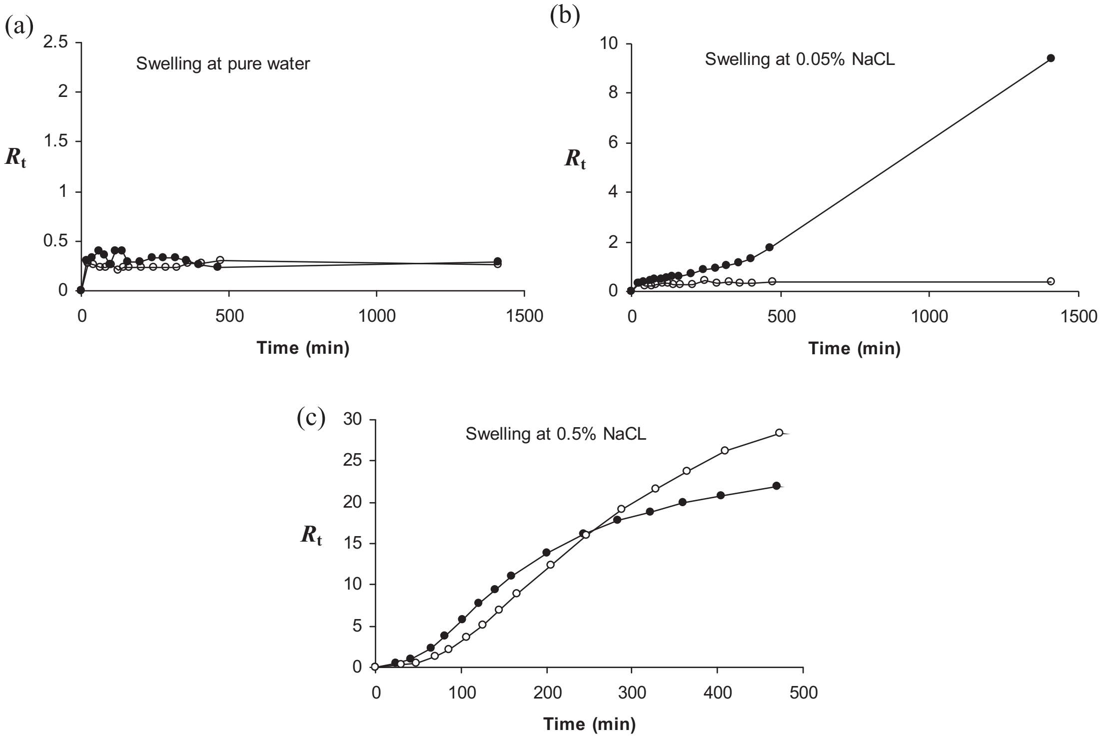

Fig. 2. The plot of swelling ratio against time for alginate (1.0%)/gelatin (0.5%) gel beads (prepared at pH 10.5) swollen at different NaCl concentrations: control beads (○); mixture beads (●).

3.2. Swelling behavior

The swelling behavior of alginate/gelatin mixture beads after air-drying is exemplified in Fig. 2. The data shown is for the beads prepared at pH 10.5. The beads prepared at pH 7, pH 4, and pH 3.5 showed the same dependence of swelling on NaCl concentration. Neither the control beads nor the alginate (1.0%)/gelatin (0.5%) beads swelled in pure water, apart from a wetting process at very beginning of the rehydration. The increase of NaCl concentration up to 0.05% was insufficient for the control beads to swell, whereas it led to a considerable swelling of the mixture beads. It manifested that the presence of gelatin helps and improves the swelling of Ca^{2+}-crosslinked alginate beads. At a further higher concentration of NaCl of 0.5%, both the control beads and the mixture beads swelled significantly. The mixture beads swelled relatively faster at initial stage, while slowed down at late stage, in comparison with the control beads. The control beads however reached a higher plateau of equilibrium swelling.

The effect of preparation pH on the swelling of alginate/gelatin mixture beads is shown in Fig. 3. For pH 10.5 and 7, the mixture beads had almost the same swelling profile. However, with the preparation pH decreasing to 4 and 3.5, the swelling kinetics was significantly slowed down, and the equilibrium swelling ratio was also reduced. Compared with the mixture gel beads, the swelling of control beads had little dependence on preparation pH (data not shown).

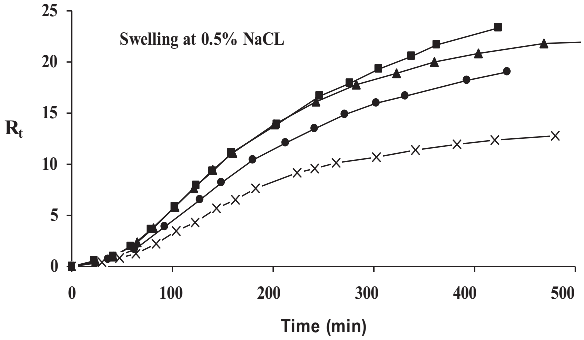

Fig. 3. The comparison of the swelling of alginate (1.0%)/gelatin (0.5%) beads prepared at different pHs: 10.5 (▲); 7 (■); 4 (●); and pH 3.5 (×).

4. Discussion

It has been widely accepted that the gelation of alginate in the presence of calcium ions is due to the formation of egg-box junctions that bridge between different alginate chains (Atkins, Nieduszynski, Mackie, Parker, & Smolko, 1973a, 1973b; Grant et al., 1973; Mackie, Perez, Rizzo, Taravel, & Vignon, 1983; Morris et al., 1978). Calcium ions are not only involved in egg-box junction zones, but also present in large amount as free ions in the aqueous medium of the gel network. When dried, alginate network shrinks and collapses and egg-box junctions get close enough to form side-by-side aggregations under the mediation of free calcium ions. This is equal to increasing cross-linking density and makes the dried alginate gel structure extremely dense. This is the reason why alginate gel beads barely swelled in pure water as shown in Fig. 2a. It is worthy of noting that the binding of calcium ions in both junction zone and aggregation regions are mainly via coordination and electrostatic interactions. These interactions are not as strong as chemical covalent bonds. When swelling in the presence of NaCl, Na^+ could

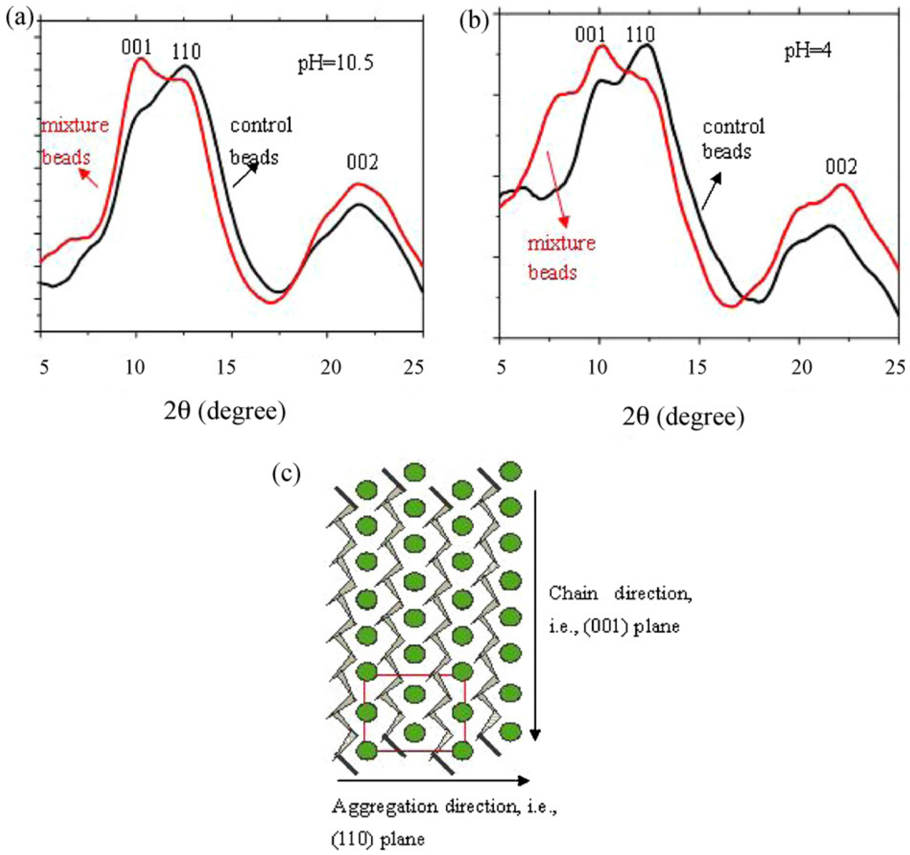

Fig. 4. WAXD patterns for dried alginate (1.0%)/gelatin (0.5%) mixture beads and control beads prepared at different pHs: 10.5 (a) and 4 (b). The assignment of WAXD diffraction peaks by schematic drawings is given in (c): the square indicates a primary crystal unit cell of aggregated egg-box junctions.

diffuse into alginate gel beads and substitute Ca^{2+} out of the network. This leads to the dismantling of the side-by-side aggregation, and the swelling of network (Vreeker et al., 2008). The increase in NaCl concentration accelerates the ion exchange between Na^+ and Ca^{2+}, and speeds up the swelling process. It explains the results shown in Fig. 2b and c. Note that for the control beads swelling in 0.05% NaCl (Fig. 2b), the salt concentration might be too low to promote swelling.

The addition of biopolymers, i.e. gelatin, is expected to alter structure formation during drying, and hence influence the subsequent swelling behavior. As shown in Fig. 2, the presence of gelatin improved the swelling ability of alginate gel beads in 0.05% NaCl, and speeded up the swelling at initial stage in 0.5% NaCl. The role of gelatin might be identified as reducing/preventing the side-by-side aggregation of alginate egg-box junctions. This is readily understandable as there is strong affinity between alginate and gelatin through electrostatic attraction that may hinder the aggregations between different alginate chains. The hypothesis was verified by WAXD investigation into the structures of dried alginate beads. Fig. 4 shows the WAXD patterns recorded for dried alginate/gelatin and control beads, prepared at pH 10.5 and 4, respectively. For both the mixture beads and control beads, three diffraction peaks at $2\theta \approx 10.6^\circ$, 13°, and 21.5° were clearly identified. Although the presence of gelatin slightly altered the overall shape of WAXD profiles, these characteristic diffraction peaks did not shift considerably. Following Atkins et al. (1973b), the peaks were assigned to (0 0 1), (1 1 0), and (0 0 2) planes, respectively. Among them, (0 0 1) and (1 1 0) reflections are of particular relevance, as they stand for the regularity of the crystal structure in egg-box regions along the alginate chain direction and chain-aggregating direction. The ratio of peak intensity of (1 1 0) to (0 0 1) planes, $I_{(1\,1\,0)}/I_{(0\,0\,1)}$, is therefore characteristic of the degree of aggregation of egg-box junction zones in dried alginate gels, with higher ratio representing higher degree of aggregation. It is apparent that at each of the pHs the mixture beads have a lower value of $I_{(1\,1\,0)}/I_{(0\,0\,1)}$. This supported our speculation that the mixture beads have less side-by-side aggregations due to the presence of gelatin.

The addition of gelatin on the one hand led to less aggregated and less dense structure of alginate beads; however, on the other hand it decreased the equilibrium swelling ratio, as is shown in Fig. 2c. This can be interpreted by the superimposition of another gelatin network on alginate network that restricts the latter from further swelling when the swelling reaches a certain level. It is well-known that gelatin can form a physical gel network via the formation of triple helix at room temperature (Joly-Duhamel, Hellio, & Djabourov, 2002). For the present gelatin sample, it has an onset coil-helix transition temperature of ~26 °C. To test this, we carried out swelling experiments at different temperatures for alginate (1.0%)/gelatin (0.5%) and control beads (Fig. 5). Roughly when temperature is below the onset melting temperature of gelatin helices, the swelling ratio measured at 700 min R_{700} of the control beads was higher than that of mixture beads. However, above this particular temperature, R_{700} of the mixture beads exceeded over that of the control beads. This transition is due to the disappearance of restriction of gelatin network as it is destroyed by increasing temperatures. Fig. 5 also implies that if the restriction of gelatin network is absent, the mixture beads would have a higher potential to swell than the control beads.

We also tested the swelling behavior of alginate/gum arabic mixture gel beads. Gum arabic and gelatin are similar in the sense that they are both ampholytic polyelectrolytes, but differ in that the former is incapable of forming gel network by itself. As shown in Fig. 6, the alginate/gum arabic gel beads swelled relatively faster in the whole process of rehydration, approaching the same equilibrium swelling ratio with the control beads. It indicates that the presence of gum arabic was also effective in reducing/preventing the side-by-side aggregation of alginate egg-box junction zones. The contrast between the swellings of alginate/gelatin and alginate/gum arabic highlighted the importance of additional network that could be present and restrict equilibrium swelling.

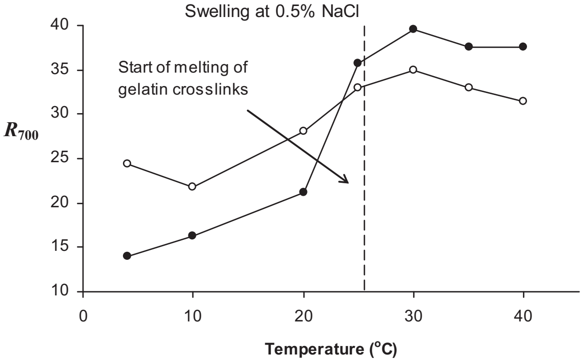

Fig. 5. The swelling ratio at 700 min R_{700} as a function of swelling temperature for alginate (1.0%)/gelatin (0.5%) beads (●) and control beads (○): NaCl concentration is 0.5%; the dashed line represents the temperature above which gelatin crosslinks, i.e. helices, start to melt.

As for the effect of preparation pH, maximum swelling was found for beads prepared at pH 10.5 and 7 (Fig. 3). These are the pHs where associative phase separation was absent or negligible, and the two polymers mixed to the largest extent of homogeneity (Fig. 1a and b). The compatible or nearly compatible mixing of the two polymers is likely to be more efficient in hindering the formation of side-by-side aggregations of egg-box junction zones during drying, consequently giving better swelling ability. For mixture beads prepared at lower pH, i.e. 4 and 3.5, they showed extensive phase separations, and majority of the alginate was enriched in the dispersed droplets (white spots in Fig. 1c and d). The local over-concentration should be hold responsible for poor swelling ability of the beads prepared at these pHs, since the over-concentration regions have higher cross-linking density and are less accessible for water to diffuse in. Another possible reason for the poor swelling ability is that alginate forms acidic gels at low pH (Mackie et al., 1983). The formation of acidic gel adds to the cross-linking density. The effect of preparation pH suggested that, to obtain better rehydratability or swelling ability, the added biopolymer ideally should have molecular-level miscibility with alginate, so that the side-by-side aggregation of egg-box junction could be suppressed to the largest extent.

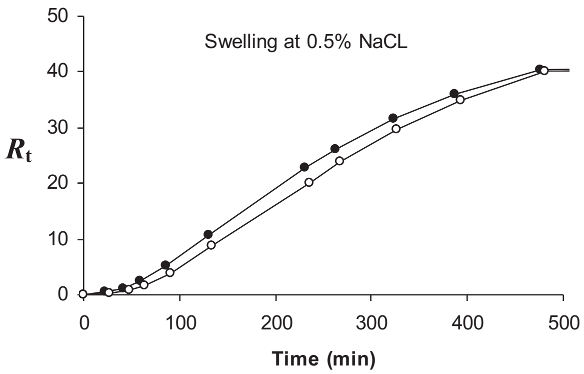

Fig. 6. The plot of swelling ratio against time for alginate (1.0%)/gum arabic (0.5%) gel beads (prepared at pH 7) swollen at 0.5% NaCl: control beads (○); mixture beads (●).

5. Conclusions

In conclusion, the addition of gelatin or gum arabic improved the swelling of dried Ca^{2+}-alginate gel beads upon rehydration. The underlying mechanism is that the presence of either of the two biopolymers can reduce/prevent the formation of side-by-side aggregations of egg-box junction zones during drying. However, a problem might exist that the equilibrium swelling ratio of alginate beads will be restricted, if the added biopolymer forms an additional physical gel network superimposed on the alginate network. With regard to the effect of miscibility, the addition of biopolymer into alginate solutions ideally should cause no phase separation so that it can to the largest extent hinder the formation of side-by-side aggregations of egg-box junction zones and lead to a maximum swelling.

Acknowledgements

Y.F. thanks Unilever R&D Vlaardingen for support of the work and Drs. Ingrid Appelqvist and Leif Lundin for helpful discussions.

References

Atkins, E. D. T., Nieduszynski, I. A., Mackie, W., Parker, K. D., & Smolko, E. E. (1973a). Structural components of alginic acid. I. The crystalline structure of poly-beta-D-mannuronic acid: Results of x-ray diffraction and polarized infrared studies. *Biopolymers*, *12*, 1865–1878.

Atkins, E. D. T., Nieduszynski, I. A., Mackie, W., Parker, K. D., & Smolko, E. E. (1973b). Structural components of alginic acid. II. The crystalline structure of poly-alpha-L-guluronic acid: Results of x-ray diffraction and polarized infrared studies. *Biopolymers*, *12*, 1879–1887.

Bowman, W. A., Rubinstein, M., & Tan, J. S. (1997). Polyelectrolyte-gelatin complexation: Light-scattering study. *Macromolecules*, *30*, 3262–3270.

Donati, I., Holtan, S., Morch, Y. A., Borgogna, M., Dentini, M., & Skjak-Braek, G. (2005). New hypothesis on the role of alternating sequences in calcium-alginate gels. *Biomacromolecules*, *6*, 1031–1040.

Draget, K. I., Smidsrod, O., & Skjak-brak, G. (2005). Alginates from Algae. In A. Steinbuchel, & S. K. Rhee (Eds.), *Polysaccharides and polyamides in the food industry, properties, production, and patent* (pp. 1–30). Weinheim: Wiley-VCH.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations. *FEBS Letters*, *32*, 195–198.

Joly-Duhamel, C., Hellio, D., & Djabourov, M. (2002). All gelatin networks: 1. Biodiversity and physical chemistry. *Langmuir*, *18*, 7208–7217.

Li, L., Fang, Y., Vreeker, R., & Appelqvist, I. (2007). Reexamining the egg-box model in calcium-alginate gels with X-ray diffraction. *Biomacromolecules*, *8*, 464–468.

Mackie, W., Perez, S., Rizzo, R., Taravel, F., & Vignon, M. (1983). Aspects of the conformation of polyguluronate in the solid-state and solution. *International Journal of Biological Macromolecules*, *5*, 329–341.

Morris, E. R., Rees, D. A., Thom, D., & Boyd, J. (1978). Chiroptical and stoichiometric evidence of a specific, primary, dimerization process in alginate gelation. *Carbohydrate Research*, *66*, 145–154.

Pammenter, N. W., & Berjak, P. (1999). A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research*, *9*, 13–37.

Piculell, I., Bergfeldt, K., & Nilsson, S. (1995). Factors determining phase behavior of multi-component polymer system. In S. E. Harding, S. E. Hill, & J. R. Mitchell (Eds.), *Biopolymer mixtures* (pp. 13–35). UK: Nottingham University Press.

Rees, D. A., & Samuel, J. W. B. (1967). The structure of alginic acid. Part VI. Minor features and structural variations. *Journal of the Chemical Society*, *C*, 2295–2298.

Soon-Shiong, P., Feldman, E., Nelson, R., Heintz, R., Yao, Q., Yao, Z., Zheng, T., Merideth, N., Skjak-Braek, G., Espevik, T., Smidsrod, O., & Sandford, P. (1993). Long-term reversal of diabetes by the injection of immunoprotected islets. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 5843–5847.

Soon-Shiong, P., Heintz, R. E., Merideth, N., Yao, Q. X., Yao, Z., Zheng, T., Murphy, M., Moloney, M. K., Schmehl, M., Harris, M., Mendez, R., Mendez, R., & Sandford, P. A. (1994). Insulin independence in a type-1 diabetic patient after encapsulated islet transplantation. *Lancet*, *343*, 950–951.

Vreeker, R., Li, L., Fang, Y., Appelqvist, I., & Mendes, E. (2008). Drying and rehydration of calcium alginate gels. *Food Biophysics*, *3*, 361–369.