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Swelling behaviour of alginate–chitosan microcapsules prepared by external gelation or internal gelation technology

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Abstract

Swelling behaviour is one of the important properties for microcapsules made by hydrogels, which always affects the diffusion and release of drugs when the microcapsules are applied in drug delivery systems. In this paper, alginate–chitosan microcapsules were prepared by different technologies called external or internal gelation process respectively. With the volume swelling degree (S_w) as an index, the effect of properties of chitosan on the swelling behaviour of both microcapsules was investigated. It was demonstrated that the microcapsules with low molecular weight and high concentration of chitosan gave rise to low S_w. Considering the need of maintaining drug activity and drug loading, neutral pH and short gelation time were favorable. It was also noticed that S_w of internal gelation microcapsules was lower than that of external gelation microcapsules, which was interpreted by the structure analysis of internal or external gelation Ca–alginate beads with the aid of confocal laser scanning microscope.

Keywords: Swelling behaviour; Alginate–chitosan microcapsule; External gelation; Internal gelation

1. Introduction

There has been increasing interest in the study on alginate–chitosan microcapsules as carriers for controlled release of proteins and drugs (Hari, Chandy, & Sharma, 1996; Huguet, Groboillot, Neufeld, Poncelet, & Dellacherie, 1994; Mi, Sung, & Shyu, 2002; Ramadas, Paul, Dileep, Anitha, & Sharma, 2000; Vandenberg & De La Noüe, 2001; Wheatley, Chang, Park, & Langer, 1991). However, most of the reports focused on preparation methods of microcapsules and characteristics of drug release. Swelling behaviour, one of the important properties of alginate–chitosan microcapsules used in drug delivery system, was seldom involved. Because the core of microcapsules is Ca–alginate hydrogel, when it encounters electrolyte solution such as sodium chloride and sodium citrate, the ionotropy will occur between Ca^{2+} and Na^{+} resulting in the conversion to liquid with the trend of volume expansion (Martinsen, Skjåk-Bræk, & Smidsrød, 1989; Smidsrød & Skjåk-Bræk, 1990), which is called 'liquefaction'. When drug-loaded alginate–chitosan microcapsules were administered, volume swelling usually occurs under the environment in vivo, and the rupture of microcapsules even takes place on some conditions, which results in the 'burst release' of entrapped drugs. On one hand, a series of pharmacological side effect, including drug intoxication will be caused to threaten the life of patients; on the other hand, the 'burst release' will lead to the instant exposure of drugs to in vivo severe environment such as low pH and more enzymes, which will increase the chance of drug inactivation especially for proteins, so that the expected therapeutic effect can not be realized. Therefore, it is the prerequisite to have the knowledge of swelling behaviour for microcapsules applied in drug delivery systems.

In our previous study, Ca–alginate beads with external or internal calcium sources were successfully obtained, and the differences in structure and properties were observed (Liu et al., 2002). In the present paper, alginate–chitosan microcapsules were prepared with external or internal calcium sources, so called external gelation microcapsule

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(EGM) or internal gelation microcapsule (IGM), respectively. The effects of properties of chitosan, including molecular weight (M_w), concentration (C) and pH, as well as membrane formation time on the swelling behaviour of these microcapsules were investigated in order to provide guidance for the preparation and application of alginate–chitosan microcapsules.

2. Experimental

2.1. Materials

Sodium alginate was purchased from Chemical Reagent Corporation (Shanghai, China), the viscosity of 1% (w/v) aqueous solution at 20 °C is over 0.02 Pa s, and the powder is smaller than 200 mesh. Chitosan was purchased from Zhida Chemical Industry Factory (Sichuan, China) and modified by our lab. (DICP, CAS, China). $CaCO_3$ powder (40 nm in size) was kindly provided by Beijing University of Chemical Technology. The surfactant Sorbitan monooleate (Span 80) was from Lüshun Chemical Factory (Dalian, China). Others are analytical reagents.

2.2. Preparation of alginate–chitosan microcapsules

EGM. Sodium alginate was dissolved in 0.9% w/v NaCl solution to form final concentration of 1.5% w/v. The solution was stored overnight in refrigerator (4 °C) before use in order to facilitate deaeration. Then the solution was extruded dropwise into 100 mM $CaCl_2$ solution. After being hardened for period of time, the beads were rinsed with distilled water and stored in distilled water. The beads were shifted in a graduated test tube. The chitosan solution formed by dissolved in acetic buffer was added at the volume ratio of 1:5–1:10 (bead: solution) to form alginate–chitosan microcapsules followed by rinsing with distilled water to remove the excess chitosan.

IGM. 1.5% w/v sodium alginate solution, as above, was mixed with $CaCO_3$ powder (100 mM Ca^{2+} equivalent) to form finely dispersed suspension. The suspension was extruded dropwise into liquid paraffin containing 1.5% v/v Span80 and 0.2% v/v glacial acetic acid, after being reacted for period of time, the beads were rinsed with 1% v/v Tween 80 solution and distilled water successively and stored in water. Then the alginate–chitosan microcapsules were formed as the same procedure above.

2.3. Determination of the volume swelling degree (S_w) of microcapsules

The S_w of microcapsules is defined as (Ma, Vacek, & Sun, 1994; Ma et al., 1995):

$$S_w(\%) = 100\left[\frac{(V_t - V_0)}{V_0}\right]$$

V_0 and V_t represent the volume of bead and microcapsule, respectively. When the beads are spherical, the formulation is rewritten as:

$$S_w(\%) = 100\left[\left(\frac{D_t}{D_0}\right)^3 - 1\right]$$

D_0 and D_t represent the diameter of bead and microcapsule, respectively. The high S_w suggests severe volume swelling of microcapsules.

Ca–alginate gel beads (0.5 ml) were sampled in a graduated test tube. The diameters of 50 beads were randomly measured under optical microscope (XDS-1 Inverted Biological Microscope, Chongqing Optical Instrument Factory, China) and averaged as D_0. The chitosan solution was added to the beads to form alginate–chitosan microcapsules, followed by immersing the microcapsules in 0.055 M sodium citrate solution for period of time (e.g. 5, 15, 30, 60, 90, and 120 min). The diameters of 50 microcapsules were randomly measured under optical microscope and averaged as D_t. Thus, the S_w can be calculated according to the definition. The effects of some parameters including M_w, C, pH of chitosan and membrane formation time on the swelling behaviour of alginate–chitosan microcapsules by external or internal gelation were investigated in this paper.

2.4. Measurement of thickness of microcapsule membrane

Both the beads were sampled in a graduated test tube, respectively. Under optical microscope (XDS-1 Inverted Biological Microscope, Chongqing Optical Instrument Factory, China), the chitosan solution was added to form alginate–chitosan microcapsules and simultaneously to observe and measure the thickness changes of the membrane with the time.

2.5. Confocal laser scanning microscopy (CLSM)

Ca–alginate beads were placed on a microscope slide with aqueous surroundings, coated with coverslip and sealed with gum. The structures were observed using CLSM (Leica TCS NT, Leica Instruments Ltd, Germany) with reflection mode.

3. Results and discussion

3.1. Effect of M_w of chitosan on swelling behaviour

The Ca–alginate beads, whose initial size is about 650 μm in diameter, were formed using external gelation or internal gelation process respectively. The solutions of chitosan with different M_w in the range from 33,000 to 436,000 Da were added to the Ca–alginate beads to form alginate–chitosan microcapsules. The changes of S_w of

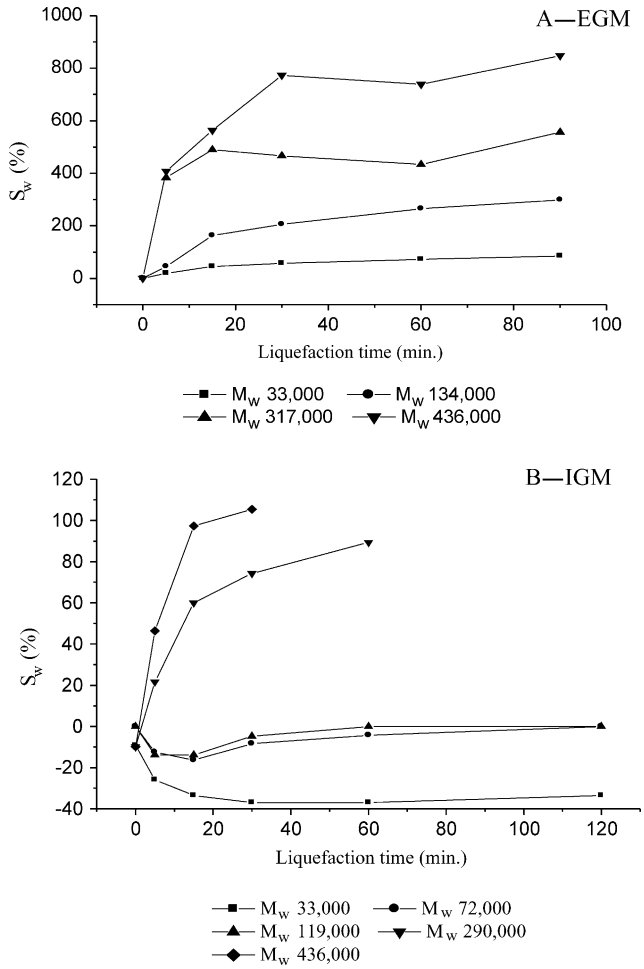

Fig. 1. Changes of S_w of microcapsules affected by variation of M_w of chitosan with liquefaction time. A—EGM, B—IGM.

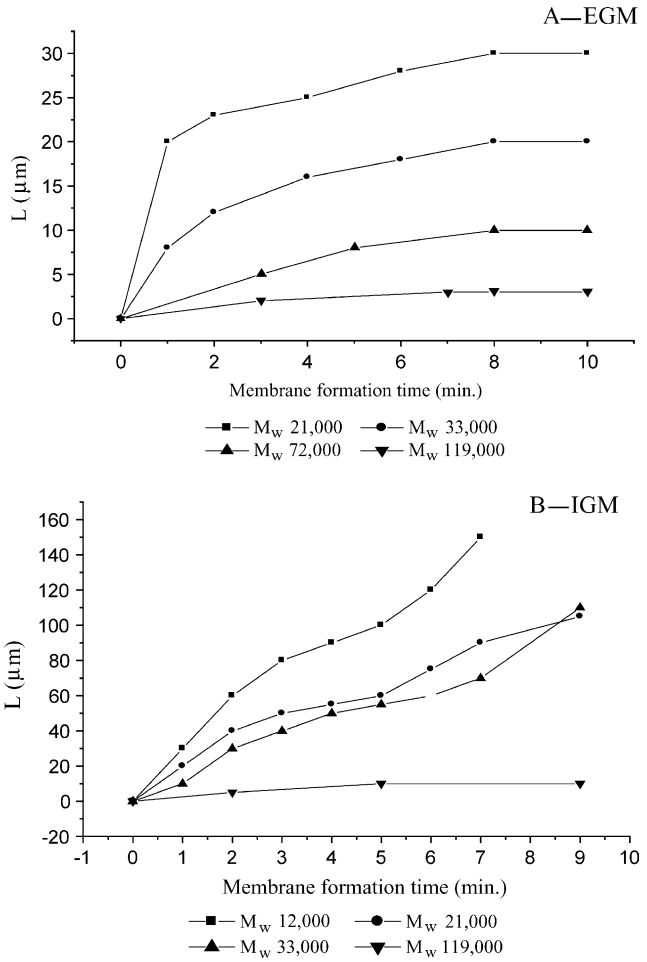

Fig. 2. Changes of membrane thickness of microcapsules (L) affected by variation of M_w of chitosan with membrane formation time. A—EGM, B—IGM.

these microcapsules with liquefaction time were shown in Fig. 1. With the decrease of M_w of chitosan from 436,000 to 33,000 Da, S_w of both EGM and IGM decreased accordingly during liquefaction course. It can be interpreted according to the diffusion rate and extent of chitosan molecule to the three-dimensional network of Ca–alginate gel beads, and the extent of reaction between chitosan and sodium alginate molecules.

In general, the larger the M_w of chitosan is, the more the positive-charged amino of chitosan chain is, which means more binding sites with alginate. However, the large spatial size of chains for large chitosan molecules results in high diffusion resistance, and causes low diffusion rate and less diffusion extent. The reaction occurs mainly at the surface of Ca–alginate beads to form thin membrane (Fig. 2), which has weak anti-swelling ability while being liquefied. On the contrary, when chitosan with low M_w was used, the small steric hindrance results in thick membrane formation (Fig. 2), with strong anti-swelling ability. Therefore, the low M_w of chitosan results in microcapsule with thick and strong membrane.

Furthermore, it was noticed that there was obvious differences in S_w and thickness between EGM and IGM with the same M_w of chitosan. With the decrease of M_w from 436,000 to 33,000 Da, the S_w of EGM after 30 min liquefaction decreased from 800% to less than 100%, whereas the S_w of IGM decreased from 100% to zero, which suggested the shrinkage occurs (Fig. 1). The membrane thickness of EGM and IGM after 10 min reaction is 3 μm versus 10 μm with M_w of 119,000, and that is 30 μm versus 100 μm with M_w of 21,000. From the above analysis, it was shown that the swelling extent of IGM was lower than that of EGM using the same M_w chitosan.

Fig. 3 is the CLSM photographs of the cross section of Ca–alginate beads made by external gelation (EGB) or internal gelation (IGB) technology. The beads were scanned from top to bottom by laser at one section every 6 μm. By comparing the sections of EGB (Fig. 3A) and IGB (Fig. 3B), it was found that the pore size of the former was smaller and the gel structure of the former was denser than that of the latter. It is the structure difference of the beads that contributes to the difference in the swelling behaviour

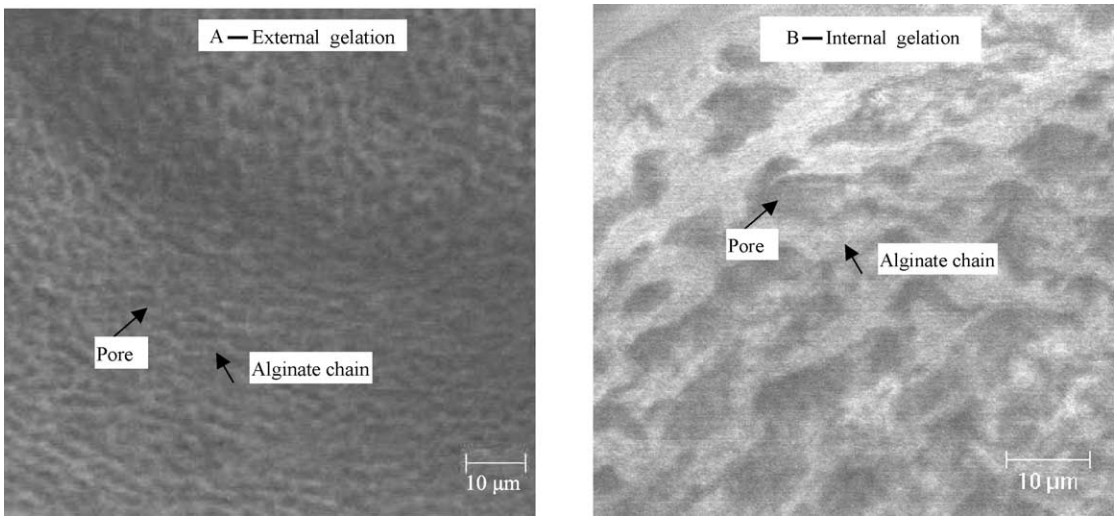

Fig. 3. CLSM photographs of the cross-section of Ca–alginate beads with external gelation (A) or internal gelation (B).

between EGM and IGM. During the formation of microcapsules, chitosan molecules can diffuse easily into internal gelation beads to form thick membrane, so the gel core volume of IGM is smaller than that of EGM under the same microcapsule size. When liquefaction begins, on one hand, the swelling force of IGM resulting from the ionotropy of gel core and subsequently phase change is less than that of EGM; on the other hand, the elastic shrink force of IGM is larger than that of EGM for thicker membrane. Therefore, swelling extent of IGM is lower than that of EGM under these two forces.

3.2. Effect of C of chitosan on swelling behaviour

Fig. 4 shows the changes of S_w of microcapsules affected by variation of C of chitosan solution with liquefaction time. The initial size of Ca–alginate beads is about 600 μm. With the increase in C of chitosan, S_w of both EGM and IGM decreased accordingly. When C of chitosan increases, the concentration gradient formed between the solution and the beads increases, that is, the driven force of chitosan molecules into the beads increases, which means that the extent of molecular diffusion and reaction increases resulting in thick membrane and subsequent high swelling extent.

During the liquefaction course, both EGM and IGM swelled and the EGM broke up at 60 min when C is less than 2 mg/ml; and the shrinkage of IGM occurred when C is more than 4 mg/ml. It demonstrated that the swelling extent of IGM was lower than that of EGM, which is also due to the structure difference of beads.

3.3. Effect of pH of chitosan on swelling behaviour

Because biopharmaceuticals such as proteins and polypeptides are usually sensitive to pH variation of environment, pH control during preparation process is very important to maintain their bioactivity.

Fig. 5 shows the changes of S_w of microcapsules affected by pH variation of chitosan solution with liquefaction time. The initial size of Ca–alginate beads is about 600 μm. It was noticed that the S_w of EGM was as follow: S_w (pH 3.5)

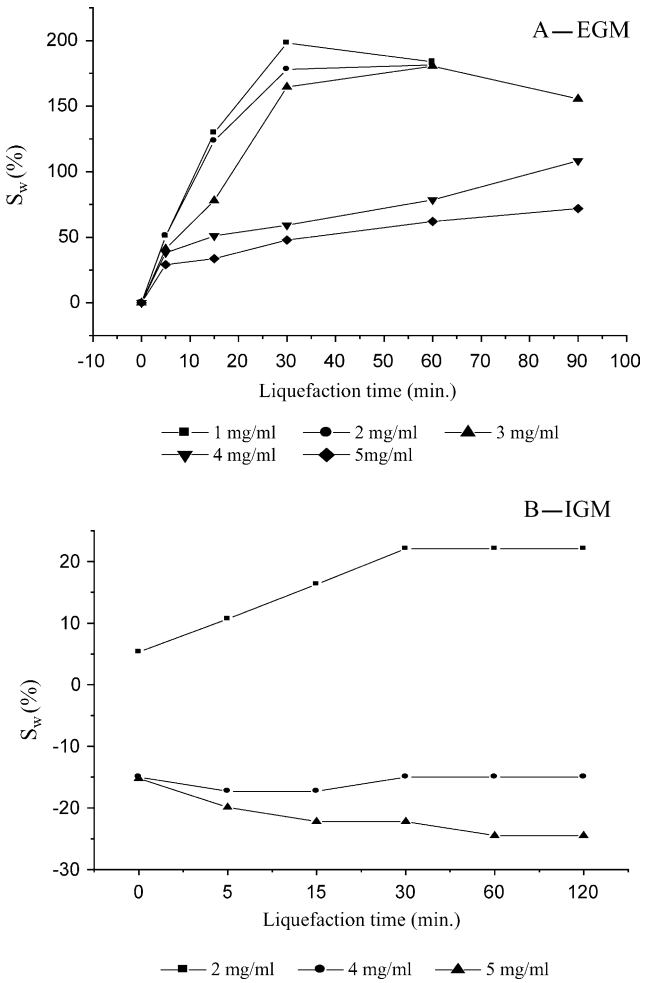

Fig. 4. Changes of S_w of microcapsules affected by variation of C of chitosan solution with liquefaction time. A—EGM, B—IGM.

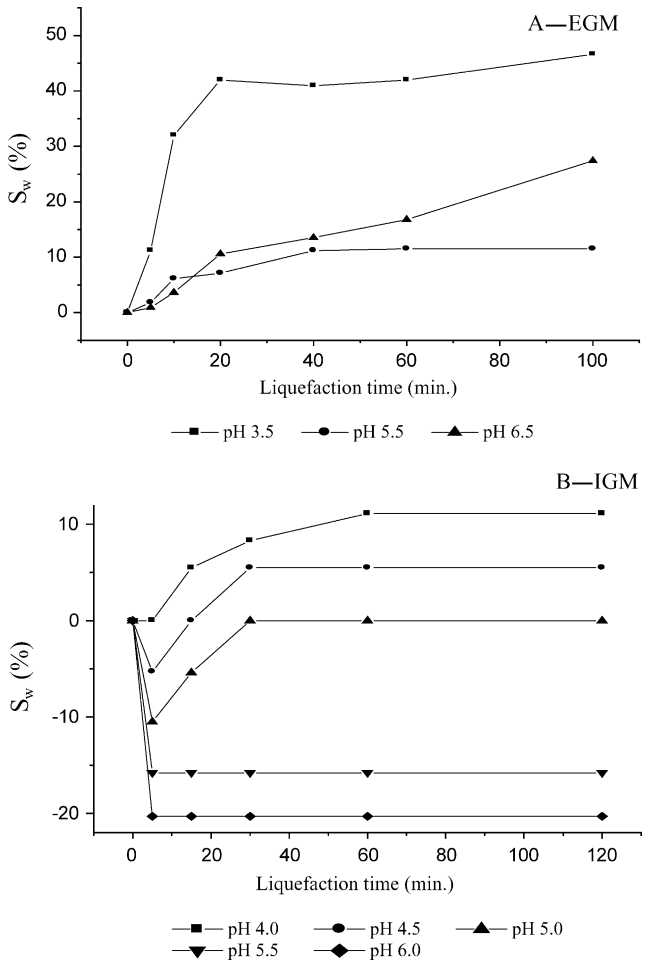

Fig. 5. Changes of S_w of microcapsules affected by pH variation of chitosan solution with liquefaction time. A—EGM, B—IGM.

$>S_w$ (pH 6.5) $>S_w$ (pH 5.5). For IGM, the S_w decreased with the increase of pH of chitosan solution from 4.0 to 6.0, accompanying with the shrinkage and subsequent swelling of microcapsules. Moreover, the S_w of IGM was also less than that of EGM.

Both sodium alginate and chitosan are polyelectrolytes. It has been reported that the pK values of mannuronic acid (M) and guluronic acid (G) of alginate chain are $pK_M = 3.38$ and $pK_G = 3.65$ (Haug, 1964), respectively. The pK value of chitosan is $pK_\chi = 6.3$ (Yalpani & Hall, 1984). It has also been found that it is the carboxyl of M unit that reacts with amino base during the membrane formation between alginate and polylysine (Dupuy, Arien, & Minnot, 1994). Thus, among the range of pH 3.5 to 5.5, it means $pK_M \langle pH \langle pK_\chi$. According to Lewis law of acid–base equilibrium, with pH increasing from 3.5 to 5.5, COO^- of M unit of alginate and NH_3^+ of chitosan increase so that there is more reaction sites to take part in the membrane formation, which means the increase of anti-swelling force. At pH 6.5, which means $pK_M \langle pK_\chi \langle pH, NH_3^+$ of chitosan is less than that at pH 5.5. Therefore, the reaction extent is reduced, and the swelling extent is higher than that at pH 5.5. For IGM, there is similar trend at the pH range from 4.0 to 6.0. Especially at pH 6.0, which is close to pK_χ, the charge density of chitosan chains remarkably reduces, and the molecular diffusivity of chitosan increases resulting in lowest swelling extent among investigated pH range. Therefore, alginate–chitosan microcapsule membrane should be formed under the condition close to neutrality, which will benefit to maintain the stability of biopharmaceuticals with low swelling extent.

3.4. Effect of membrane formation time on swelling behaviour

Membrane formation time is another important parameter for the maintenance of the biopharmaceutical activity and drug loading. Generally, the longer the membrane formation time, the lower the drug loading because of the diffusion of the drugs from the inside to the outside of the microcapsule.

The membrane formation time also affects the swelling behaviour of microcapsules. Fig. 6 shows the changes of S_w of microcapsules affected by variation of membrane

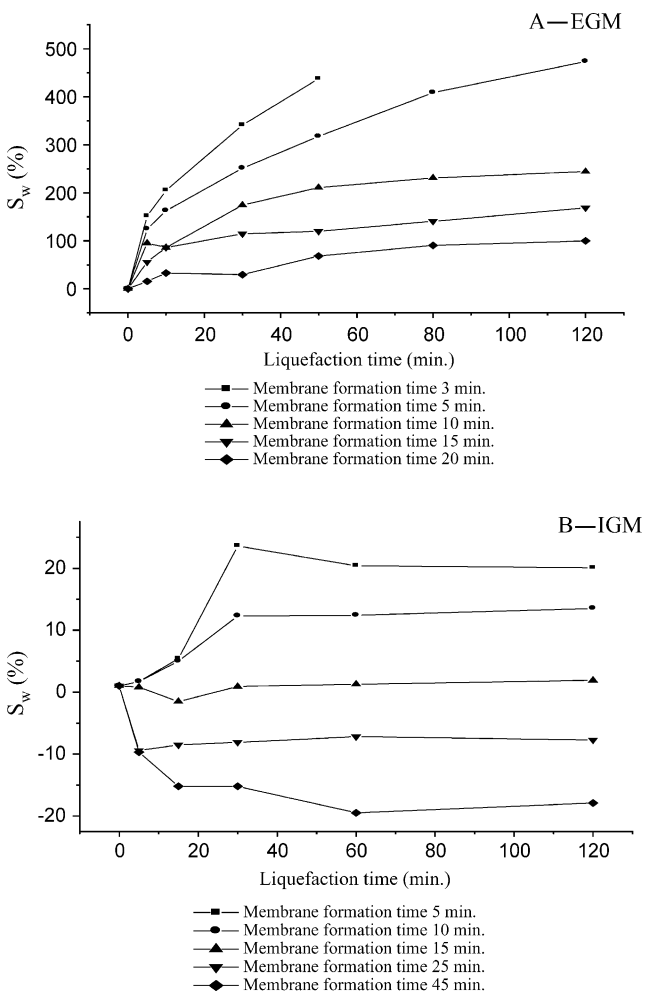

Fig. 6. Changes of S_w of microcapsules affected by variation of membrane formation time between alginate and chitosan with liquefaction time. A—EGM, B—IGM.

formation time between alginate and chitosan with liquefaction time. The initial size of Ca–alginate beads is about 400 μm. It was shown that S_w decreased during liquefaction course with the increase of membrane formation time. It can be interpreted that the longer the membrane formation time is, the deeper the chitosan molecule into the three-dimensional network of Ca–alginate gel beads is. Therefore, the extent of reaction between chitosan and sodium alginate molecules is high resulting in the increase of membrane thickness and subsequent decrease of swelling extent.

Since the increased membrane formation time is related to the low drug retaining, the adequate membrane formation time should be determined according to the compromise between the swelling behaviour of microcapsules and drug loading.

4. Conclusion

Alginate–chitosan microcapsules were prepared by different technologies called external or internal gelation process respectively, and the swelling behaviour of both microcapsules was investigated. It was demonstrated that the swelling behaviour of these alginate–chitosan microcapsules was affected by the properties of chitosan, including the M_w, C, and pH, as well as the membrane formation time. Compared to external gelation microcapsule, the S_w of internal gelation microcapsule is lower suggesting the low swelling extent. Considering the ease of scale-up, internal gelation technology may be a better way for large-scale production of alginate–chitosan microcapsuels as drug delivery carriers.

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