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pH-Dependent Release Property of Agar Beads Containing Chitosan Particles

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Agar beads containing chitosan particles were prepared by dropping hot suspension of agar/chitosan (2/1, w/w) into cold distilled water. The bead size was 2–3 mm and the cross section of the bead revealed a porous structure on FE-SEM (Field Emission Scanning electron microscopy) photos. The pH-dependent release property of the bead was observed using either blue dextran or FITC-dextran (Fluorescein isothiocyanate-dextran) as a dye. The % releases of blue dextran for 6 h were 42% at pH 3.5, 48% at pH 5.5 and 52% at pH 8.0. Obviously, the release was significantly suppressed at acidic pHs. This is possibly because the positive charge intensity of chitosan becomes stronger at an acidic pH. As a result, it tends to interact with negatively charged blue dextran, leading to a suppressed release at acidic pHs. On the other hand, the % releases of FITC-dextran for 6 h were 88% at pH 4.5, 80% at pH 5.5 and 76% at pH 8.0. The release was somewhat promoted at acidic pHs. At an acidic pH such as pH 3.5, chitosan could be dissolved within agar beads, giving rise to cavities. In addition, there might be no electrostatic interaction between positively charged chitosan and neutral FITC-dextran. Therefore, FITC-dextran would readily release through the cavities, resulting in a higher release at an acidic pH.

Keywords: Agar, chitosan, bead, blue dextran, FITC-dextran, pH-sensitive release

1 Introduction

Extensive studies have been done on stimuli-sensitive drug carriers, which release their contents in response to external stimuli, such as changes in pH, ionic strength, temperature and electromagnetic radiation (1,2,3,4). Among them, pH-sensitive carrier is one of the most widely studied carriers (5). Several types of pH-sensitive carriers have been developed to deliver anti-cancer agents (paclitaxel (6), doxorubicin (7), mastoparan (8)) and polypeptide drugs (insulin (9), vancomycin (10)). If carriers were designed to achieve the improved activity of an anticancer agent or the higher transfection activity of a genetic material, they should release extensively their therapeutic agents at acidic pHs since the pH around the tumor and endosomal pH are acidic. On the contrary, if carriers were designed to administer peptide drugs orally, they should release extensively the drugs not at acidic pHs but at neutral pH. That is because stomach fluid is acidic, and the small intestine and blood, which are the sites of the drug release, are neutral. Representative types of pH-sensitive carriers are polymeric nanoparticles (11), micelles (12), liposomes (13) and dendrimers (14). The pH-dependent releases from the carriers could be obtained successfully due to sol-gel transition of a caprolactone copolymer, coil-to-globule transition of an *N*-isopropylacrylamide copolymer, hydrazone bond cleavage, membrane fusion between lipsomal and biological membrane, etc (15–17). However, most of the carriers take advantage of synthetic polymers which are known to be toxic in the human body. For the last decades, polysaccharides, naturally occurring polymers, have attracted many scientists' interests in the area of drug delivery. Alginate is one of frequently employed polysaccharides due to its biocompatibility, good morphological and mechanical properties (18). Recently, alginate beads were coated with chitosan to control the release in response to pH (19). At acidic pHs, chitosan has a strong positive charge and it forms a complex with negatively charged alginate. As a result, the release was suppressed when the release medium was acidic. On the other hand, microparticles of calcium carbonate were included in alginate beads to obtain pH-sensitive release properties (20). When the release medium was acidic, the calcium carbonate particles contained in the alginate beads were leached out, leaving void volumes within the beads. As a result, the release was accelerated at acidic pHs. In addition, beads composed of alginate

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and copolymer of NIPAM and MAA were prepared to achieve pH-sensitive release property (21). The copolymers take a contracted form at acidic pHs and they block the pore of alginate matrix. Even though there wasn't much difference detected in the release with pH change, the release was obviously suppressed at acidic conditions. In this study, the pH-dependent release property was investigated with agar beads. Agar is a gel-forming polysaccharide derived from seaweed. It is made up of subunits of the sugar galactose and it is a major structural component of algae's cell walls. Since agar has biocompatibility and good morphological property, it can be used as a material for the drug carrier. Unlike alginate, it has the property of gelation in an aqueous solution without divalent cations. Furthermore, the agar bead is stable in the presence of surfactants and ions. Despite the fact that there are advantages of agar over alginate, few studies has been done on the development of agar bead which releases its contents in response to pH variation. To achieve the pH-sensitive release property, chitosan particles were included in the matrix of agar beads. Chitosan is protonated and becomes soluble at acidic pHs. Accordingly, it could play a role in regulating the release in response to pH. For the pH-dependent release test, loaded in the agar beads were blue dextran (avg. M.W. 2,000,000) and FITC-dextran (avg. M.W. 4,000), which are a negatively charged dye and a neutral one, respectively.

2 Experimental

2.1 Materials

Agar, chitosan (low molecular weight), blue dextran (avg. M.W. 2,000,000), FITC-dextran (avg. M.W. 4,000) were purchased from Sigma Chemical Co (St. Louis, MO). Mineral oil was purchased from Acros Organics (New Jersey). All other reagents were analytical grade.

2.2 Preparation of Agar Beads Containing Chitosan Particles

Agar was added to distilled water so that the content of agar is 2%. The mixture was then heated up to 90°C to dissolve agar. 0.6 g of chitosan suspension (1%) at the same temperature was added to 1.2 g of agar solution. The ratio of agar to chitosan was 2:1. Then, 360 mg of blue dextran or 36 mg of FITC-dextran was dissolved in the suspension containing agar and chitosan particles. The mixture was dropped into 600 ml of cold distilled water covered with 40 ml of mineral oil through a syringe needle. The cold distilled water was kept below 10°C during the bead preparation. To remove unloaded dye, the beads were washed several times with distilled water and they were freeze-dried.

2.3 Field Emission Scanning Electron Microscopy

Immediately after preparation, beads were freeze-dried and were cross-sectioned using a blade. The cross-sectioned beads were then mounted on metal stubs with double-sided tape, sputtered with gold and viewed in a Field Emission scanning electron microscope (Hitachi S4300, Japan).

2.4 pH-Dependent Release of Dye from Agar Beads Containing Chitosan Particles

The release of dye was observed at three different pHs, namely pH 3.5, pH 4.5, pH 5.5 and pH 8.0. Dry agar beads with or without chitosan particles, 0.3 g, were contained in a round mesh cage (diameter 3.5 cm) and it was immersed into 100 ml of distilled water contained in a 100 ml-beaker, of which pH was adjusted to 3.5, 4.5, 5.5 and 8.0 with 1.0 N HCl or 1.0 N NaOH. It was then gently stirred on a magnetic stirrer. 1.0 ml of the release mediums were taken at predetermined time intervals and the amount of dye released was determined using a UV spectrophotometer (JENWAY 6505, UK) or a fluorospectrometer (Hitachi F2500, Japan). The absorbance of blue dextran was measured at 630 nm. The fluorescence intensity of FITC-dextran was measured at 495 nm with excitation wavelength of 520 nm. Since the fluorescence intensity of FITC-dextran strongly depends on the pH of release medium, standard curves were established at each pH, where a release experiment was performed. Every time the release medium was removed, distilled water of 1.0 ml was added to compensate for the amount of the release medium taken for the measurements. The % of release is defined as the percentage of the released amount on the basis of the total amount entrapped in agar beads.

2.5 Swelling Ratio of Agar Beads

The swelling property of agar beads, with or without chitosan particles, were investigated with time and pH (22,23). Freeze-dried beads free of dye, 0.15 g, was put into a round mesh cage and it was immersed in 200 ml of distilled water, which was pre-adjusted to pH 3.5, pH 5.5 and pH 8.0. They were gently stirred on a magnetic stirrer for predetermined time intervals. The weight was measured after removing the excess free water with a filter paper. The swelling ratio is defined as follows.

$$\text{Swelling ratio (\%)} = [(\text{wet weight} - \text{dry weight})/\text{dry weight}] \times 100$$

3 Results and Discussion

3.1 Field Emission Scanning Electron Microscopy

Figure 1a shows electron micrographs of as-received chitosan particles. The shape was irregular and the size was

tens of μm to hundreds ofμm . Figure 1b shows electron micrographs of cross sections of freeze-dried agar beads containing no chitosan particles. The size was 2–3 mm and a porous structure was observed. The porous structure is a typical one obtained by freeze-drying (21). During the freeze-drying process, the sublimation of ice leaves void volumes. Figure 1c shows electron micrographs of cross sections of freeze-dried agar beads containing chitosan

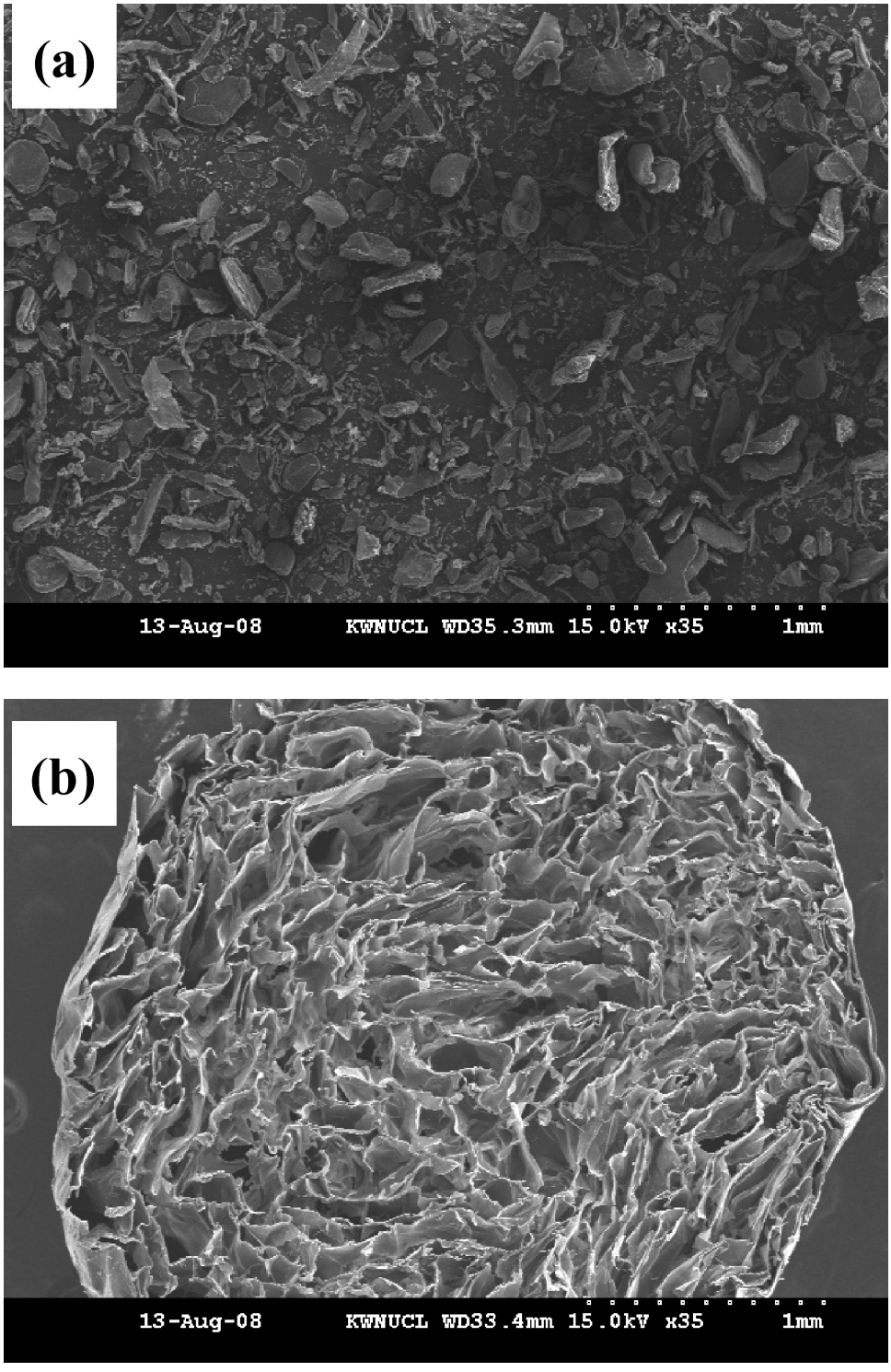

Fig. 1. Field Emission Scanning electron micrographs of chitosan particles (a), cross section of freeze-dried agar bead containing no chitosan particles (b), and cross section of freeze-dried agar bead containing chitosan particles (c). Bar in each panel represents 1 mm. (Continued)

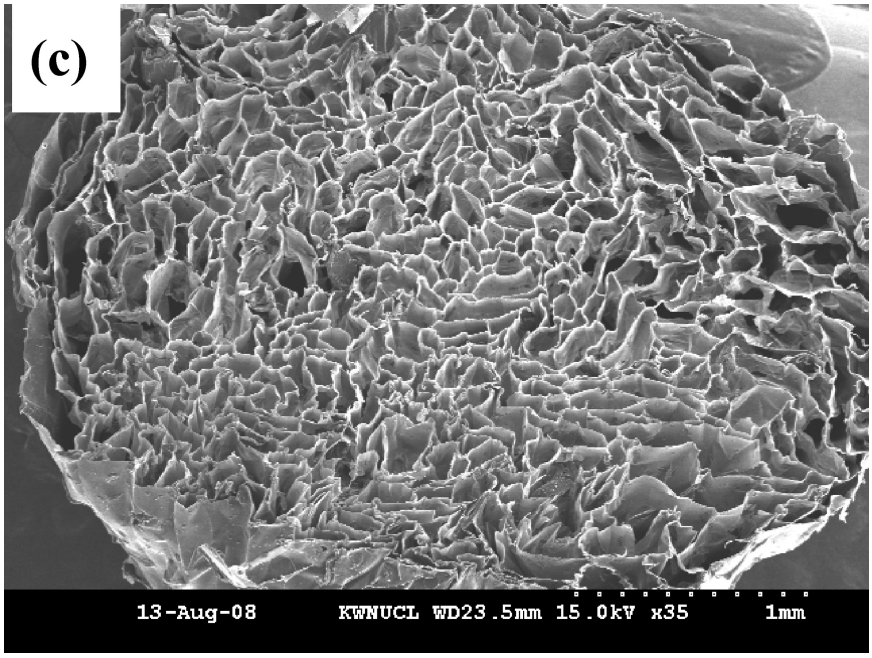

Fig. 1. (Continued)

particles. The size and structures were almost the same as those of agar beads containing no chitosan particles. It was hard to find chitosan particles in the porous structure of the beads. However, the bead containing chitosan particles was much whiter and opaque than the bead free of chitosan.

3.2 Release of Blue Dextran

Figure 2 shows release of blue dextran from agar beads at pH 3.5, pH 5.5 and pH 8.0. Whether chitosan particles were included in agar beads or not, the degree of release increased in a saturation manner with time. When the beads contained no chitosan, the % of release was almost invariable with respect to pH and the values for 6 h were 42–46% (Figure 2a). Since agar is made up of subunits of the sugar galactose and it has no titrable groups, the agar bead seemed to exhibit pH-independent release. When agar beads contained chitosan particles, the % of releases for 6 h were 42% at pH 3.5, 48% at pH 5.5 and 52% at pH 8.0 (Figure 2b). Obviously, the release was significantly suppressed at acidic pHs. At pH 3.5, most of amino groups in chitosan are protonated and chitosan has strong positive charges. Hence, chitosan could be dissolved at the strong acidic pH due to an electrostatic intramolecular repulsion. In this circumstance, chitosan might leach out of the bead, or it may be distributed throughout the matrix. In any case, cavities would be formed, leading to a promoted release. In a previous report, alginate beads containing calcium carbonate particles were prepared as a pH-sensitive carrier (20). According to the result, calcium carbonate particles were dissolved at acidic pHs, leaving cavities. As a result, the release of blue dextran was accelerated. However, the release of blue dextran in this study was suppressed at acidic pH. This is possibly because the positive charge intensity of chitosan becomes stronger at acidic pH and it tends to interact with negatively charged blue dextran. Even though chitosan becomes soluble at acidic pHs, it seems not to leach out of agar beads. Instead, it would be entangled with agar because both chitosan and agar are polymers. Unlike chitosan, calcium carbonate is a low molecular weight inorganic compound and it is decomposed at acidic pHs. Therefore, there would be no interaction between blue dextran and calcium carbonate. Whether chitosan particles were included in the bead or not, and whatever the medium pH was, the maximum releases for 6 h were only 42–52%. The molecular weight of blue dextran used in this study is 2,000,000. Due to its high molecular weight, the interaction (e.g. physical entanglement) of blue dextran with agar would be strong, leading to a low release.

3.3 Release of FITC-Dextran

Figure 3 shows release of FITC-dextran from agar beads at pH 4.5, pH 5.5 and pH 8.0. Like the release of blue dextran, the % of release increased in a saturation manner with time regardless of the presence of chitosan. Without chitosan, the % of release was almost constant with respect to pH and the values for 6 h were about 40% (Figure 3a). The % of release in the saturated stage was almost the same as that of blue dextran, but the initial release rate of FITC-dextran was higher than that of blue dextran. For example, the % of FITC-dextran release for the first 30 min was about 30% and the % of blue dextran release for the same period

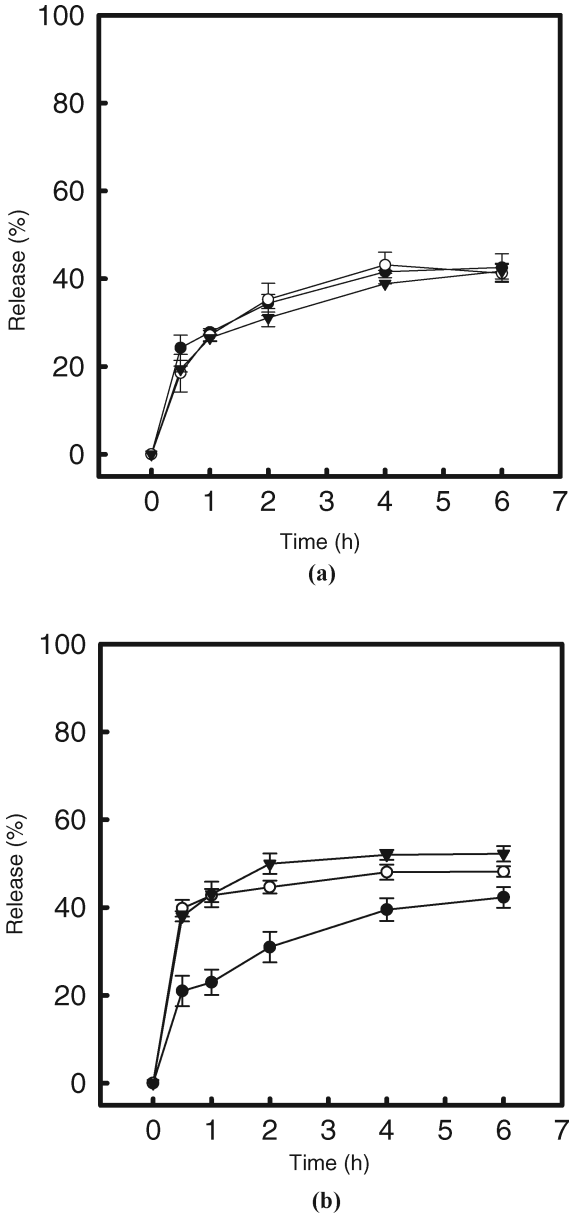

Fig. 2. Release of blue dextran from agar beads, free of chitosan particles (a) and containing chitosan particles (b), at pH 3.5 (●), pH 5.5 (○) and pH 8.0 (▼).

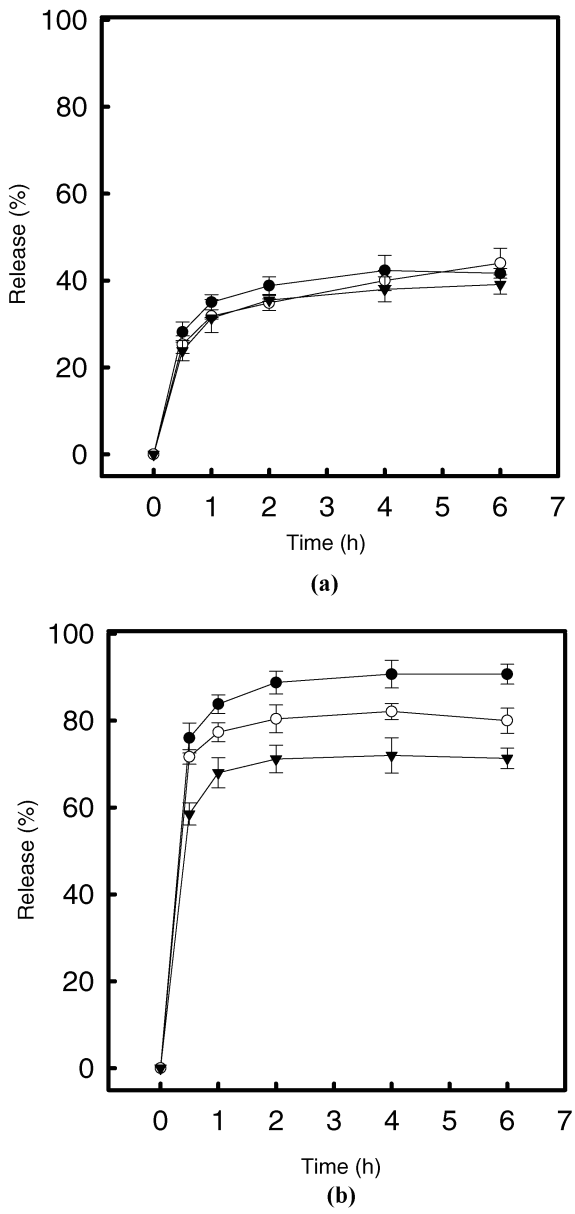

Fig. 3. Release of FITC dextran from agar beads, free of chitosan particles (a) and containing chitosan particles (b), at pH 4.5 (●), pH 5.5 (○) and pH 8.0 (▼).

was about 20%. This is because molecular weight of FITC-dextran, 4,000, is much smaller than that of blue dextran, 2,000,000. When agar beads contained chitosan particles, the % of releases for 6 h were 88% at pH 4.5, 80% at pH 5.5 and 76% at pH 8.0 (Figure 3b). Obviously, the release was promoted at all pHs tested, compared with the releases from agar beads containing no chitosan particles, and the promotion was outstanding especially at acidic pHs. When chitosan particles were embedded in the matrix of agar beads, the domains of chitosan seems to be main pathways for the dye release. Especially at acidic pHs, such as pH 4.5 and 5.5, chitosan has strong positive charges and it could be dissolved within agar beads, giving rise to cavities. Since there may be no strong electrostatic interaction between positively charged chitosan and neutral FITC-dextran, the dye would release through the cavities. This may be the reason why the release of FITC-dextran was promoted to a great extent at acidic pHs.

3.4 Swelling Ratio of Agar Beads

Figure 4 shows swelling ratio of agar beads with or without chitosan particles at pH 3.5, pH 5.5 and pH 8.0. Whether the beads contained chitosan particles, the swelling was

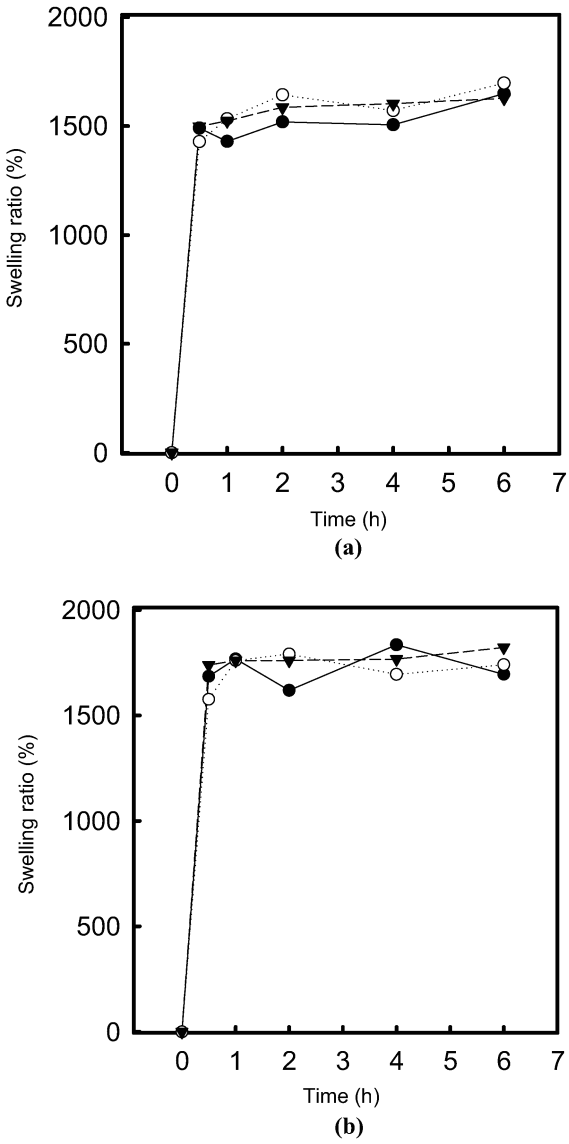

Fig. 4. Swelling ratio of agar beads, free of chitosan particles (a) and containing chitosan particles (b), at pH 3.5 (•), pH 5.5 (○) and pH 8.0 (▼).

almost completed for the first 30 min. The early completion of the swelling is due to the fact that the beads were porous and they would readily uptake water. This may explain the reason the initial rate of release was so high as in Figures 2 and 3. Furthermore, the swelling ratios were independent on the media pH, even when chitosan particles were included. Accordingly, the swelling ratios would have little effect on the pH-responsive release characteristics of the beads containing chitosan particles. As described in the section of release of dextran, either the electrostatic interaction with chitosan or the free pass through chitosan domains could be responsible for the pH-responsive release characteristics. On the other hand, the swelling ratios of agar beads were 1500–1700% in Figure 4 and the values were about 8 times higher than that of the alginate bead. The agar beads were prepared by dropping the hot solution into mineral oil and by freezing chains of the polysaccharide in cold water. Therefore, there would be no crosslinking sites within agar beads. Whereas, the alginate beads were prepared by dropping the solution into an aqueous solution containing a multivalent ion, and by crosslinking chains of the polysaccharide with aid of the ion (18). Therefore, crosslinking sites exist within alginate beads. The uptake of water by beads would be suppressed in case the matrix of the bead is crosslinked, since the mobility of polysaccharide chains is restricted by the crosslinking. The marked difference in the swelling ratio between the agar bead and the alginate is thought to be closely related to whether polysaccharides constituting beads are crosslinked.

4 Conclusions

Agar beads containing chitosan particles released their contents in a controlled manner. The release of blue dextran, a negatively charged dye, was suppressed at acidic pHs. The release of FITC dextran, an electrostatically neutral dye, was enhanced at acidic pHs. It is believed that the agar bead developed in this study could be used as a carrier for an oral delivery of a therapeutic agent.

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