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Novel pH-sensitive microgels prepared using salt bridge

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ABSTRACT

pH-sensitive microgels were prepared by crosslinking carboxymethylcellulose (CMC) and polymeric β-cyclodextrin (PβCD) using (2-hydroxyethyl)trimethylammonium chloride benzoate (TMACB) as a crosslinker. PβCD was prepared by reacting epichlorohydrin and β-CD in an aqueous phase (NaOH solution, 30% (w/w)). TMACB will interact with CMC by an electrostatic interaction and it will also interact with PβCD by a hydrophobic interaction. The size of microgel was tens of nanometers to several micrometers. The degree of calcein release in 24 h from the microgels was as low as 23% at pH 8.0. The degree of release at pH 3.0 was almost 100%. The carboxyl groups of CMC will lose their charge in an acidic condition and they would lose their ability to form salt bridges with TMACB, leading to the disintegration of microgels. The degree of release at pH 11, about 47%, was less than the value at pH 3.0 but it was greater than the value at pH 8.0. The CMC will be strongly electrostatically charged in the alkali condition, so the microgels would swell due to the electrostatic repulsion among CMC molecules, which could promote the release of their contents.

1. Introduction

Microgels are crosslinked hydrogel microparticles and they could swell in an aqueous phase and imbibe lots of water (Dong et al., 2008). Recently, polymer microgels are attracting much attention for their use as drug carriers, since they exhibit high water-retaining ability, biocompatibility, biodegradability, and stimuli-sensitivity (Oh et al., 2008). The stimuli-sensitivity of microgels can be obtained by introducing specific functional groups to the polymer network or using polymers which are sensitive to stimuli such as the changes in temperature, pH, or light intensity (Liu et al., 2008; Zha et al., 2002; Zhang et al., 2006; Garcia et al., 2007). Polysaccharides (e.g. chitosan, cellulose, carrageenan, and dextran) are the most commonly used natural polymer in the preparation of microgels (Krishna Rao et al., 2006; Lu et al., 2000; Raemdonck et al., 2008). They can be crosslinked by non-covalent interaction, including electrostatic interaction, and the preparation methods for physically crosslinked microgel are relatively simple. For instance, polyelectrolyte complex microgel was reported to be prepared successfully by simply mixing the solutions of negatively charged dextran sulfate and positively charged chitosan (Sakiyama et al., 1999). This kind of microgel prepared by electrostatic interaction could show a great pH-sensitivity in terms of swelling ratios due to the ionizable groups in the polymer (Kratz et al., 2000).

Cyclodextrins are cyclic oligosaccharides with internal hydrophobic cavities and hydrophilic outer surfaces (Challa et al., 2005). They can form inclusion complex with many kinds of molecules. β-Cyclodextrin (β-CD) with seven glucose monomers in a ring was reported to be suitable for the widest range of drugs (Salmaso et al., 2007; Mura et al., 2002; Hussein et al., 2008). Besides, the hydrogel based on β-CD was also reported to show a high drug loading efficiency and local sustained drug release ability (Rodriguez-Tenreiro et al., 2006). According to the previous researches, water soluble polymer β-CD (PβCD) can be prepared by using epichlorohydrin (EPI) as a crosslinker (Renard et al., 1996; Gao and Zhao, 2004). The polymerized β-CD is thought to be a useful candidate for the preparation of microgels, since the hydrophobic cavities still exist after polymerization. Until now, many researches have focused on β-CD based microgels by taking the advantages of host–guest interaction. It has been reported that hydrophobically modified materials, such as dextran with lauryl side chain, could form self-assembled nanogels with PβCD and be further used in sustained drug delivery system (Gref et al., 2006; Daoud-Mahammed et al., 2007a,b).

Up to the present, no research has been done on the preparations of microgels using both a hydrophobic interaction and an electrostatic interaction. In this study, the microgels were prepared by using (2-hydroxyethyl)trimethylammonium chloride benzoate (TMACB), with a hydrophobic benzene ring and a hydrophilic amino group, as a crosslinker. As shown in Fig. 1, the benzene ring would be included in CD cavity of PβCD, while the cationic amino group was thought to conjugate with carboxyl group of CMC by salt bridge.

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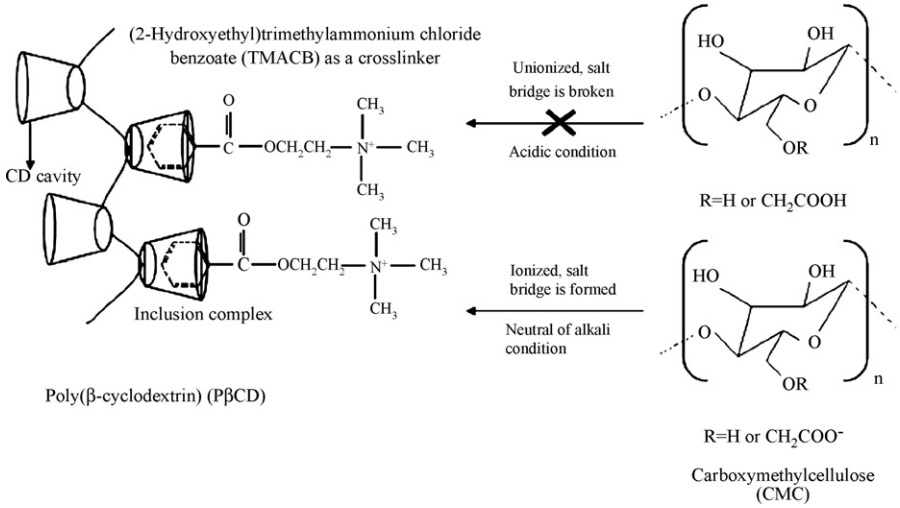

Fig. 1. Schematic representation of the formation of PβCD/CMC microgels. TMACB interacts with PβCD through a hydrophobic interaction and it interacts with CMC through an electrostatic interaction. TMACB act as a crosslinker of PβCD and CMC.

Hence, with the aid of TMACB, the network of the microgel could be formed by bridging CMC and PβCD through two kinds of non-covalent interactions. Moreover, the microgels could be sensitive to the change in pH, because the ionizable carboxyl group of CMC may influence the formation and the swelling ratio of the microgels. In our experiments, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used for observing the morphology of the microgels. The pH-sensitivity was investigated by measuring the release of TMACB and calcein from microgels at various pHs for 24 h.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD, M.W. 1135) was purchased from Wako pure chemical industries. Ltd (Osaka, Japan), Carboxymethylcellulose (CMC, Medium viscosity), calcein (M.W. 622.5), phenolphthalein (M.W. 318.3) and epichlorohydrin (EPI, M.W. 92.5) were purchased from Sigma (St. Louis, MO, USA). (2-Hydroxyethyl) trimethylammonium chloride benzoate (TMACB, W.M. 243.7) was purchased from TCI (Tokyo, Japan). Water was doubly distilled in a Milli-Q water purification system (Millipore Corp.) until the resistivity was 18 MΩ/cm. All other reagents were in analytical grade.

2.2. Preparation of PβCD

The water soluble cyclodextrin polymer was synthesized by reacting β-CD with EPI under strong alkaline conditions (Renard et al., 1996). In brief, 5 g of β-CD in 8 ml of 30% NaOH (w/w) aqueous solution was stirred overnight in a 100-ml beaker. Then, 3.34 ml of EPI was added dropwise into β-CD solution slowly at 30 °C so that the molar ratio of CD/EPI was 1/10. The reaction was done at the same temperature for 3 h and 40 min and it was stopped by adding 20 ml of acetone to the reaction mixture and stirring it for 10 min. After standing the reaction mixture at room temperature for 30 min, it separated into two phases. Acetone in the upper layer was removed using a pipette and the residual solvent was removed in a rotary evaporator under a reduced pressure. The pH of the mixture was adjusted to 12 with 6N HCl, and then kept at 50 °C overnight. After cooling down to room temperature, the mixture was neutralized with 6N HCl and it was dialyzed using a dialysis bag (MWCO 1000, Spectra/Por, Cole-Parmer, USA) in 1000 ml of distilled water for 48 h with 6 time exchanges of water. The dialyzed reaction was precipitated in 1000 ml of acetone. White product was collected by filtration using a filter paper (24.0 cm cycles, Watman, England), and then dried at 40 °C under vacuum.

2.3. Preparation of TMACB crosslinked PβCD/CMC microgels

Microgels were obtained by mixing the solution of TMACB-PβCD inclusion complex and the solution of CMC at room temperature. Specifically, 60 mg of TMACB was first dissolved in 1 ml of PβCD solution in distilled water (180 mg/ml, pH 8.0, adjusted using 0.1 N NaOH). Subsequently, in order to obtain microgel suspension, the solution of TMACB-PβCD inclusion complex was added to 2 ml of CMC solution in distilled water (20 mg/ml, pH 8.0), and then stirred for 1 h. To determine unbound TMACB in the microgel suspension, the microgel suspension was dialyzed using a dialysis bag (MWCO 1000) against 1000 ml of distilled water (pH 8, adjusted using 0.1N NaOH) for 24 h, and the amount of TMACB released out of the dialysis bag was measured with time on UV/Vis spectrophotometer (6505 UV/Vis. Spectrophotometer, JENWAY, UK) at 275 nm. And, the percent of unbound TMACB in microgel suspension was calculated as follows:

$$\%\ \text{unbound TMACB} = \left(\frac{\text{amount of TMACB released out of dialysis bag (mg)}}{\text{total amount added TMACB (mg)}}\right) \times 100$$

In parallel, TMACB solution, TMACB in CMC solution, TMACB in PβCD solution were also dialyzed under the same condition and the amount of TMACB released out of the dialysis bag was determined.

For the preparation of calcein-containing microgel, 9 mg of calcein was dissolved in 2 ml of CMC solution and the pH was adjusted to 8 before mixing with the solution of TMACB-PβCD inclusion. The unloaded calcein and the unloaded and unbound TMACB were removed by dialysis (MWCO 1000) against 1000 ml of distilled water for 24 h with 6 time exchanges of distilled water.

2.4. Characterization of PβCD and microgels

^{13}C NMR of PβCD was taken on a Bruker Avance 600 (Karlsruhe, Germany) spectrometer using D_2O as a solvent. In addition, the content of CD in PβCD was determined by a colorimetric method using phenolphthalein as reported previously (Basappa et al., 1998). The FT-IR spectra of βCD, PβCD, TMACB, CMC, and TMACB/PβCD/CMC microgel were observed on a Fourier Transformed Infrared spectrophotometer (FT-IR, FT-3000, MX, Excalibur).

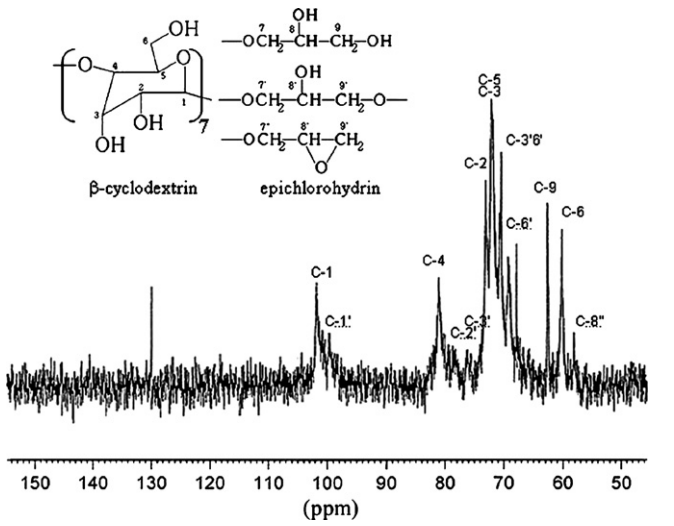

Fig. 2. Nomenclature of carbons of β-CD and EPI residues in PβCD, and ^{13}C NMR spectrum of PβCD in D_2O.

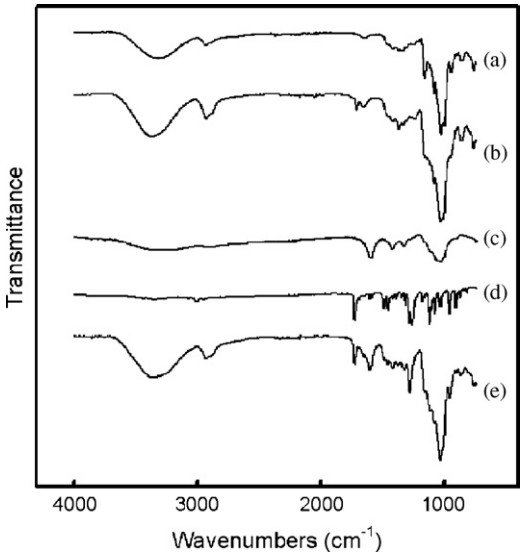

Fig. 3. FT-IR spectra of β-CD (a), PβCD (b), CMC (c), TMACB (d), and microgel (e).

2.5. Observation of size distribution and electron microscopy

The size of microgel was determined using a particle size analyzer (Plus 90, Brookhaven, USA) at 23 °C at pH 8. And the morphology of microgels were observed on scanning electron microscope (SEM, Jeol JSM-840A), for freeze-dried microgels, or transmission electron microscope (TEM, LEO-912AB OMEGA, LEO, Germany), for the microgel suspension. When observed on SEM, the freeze-dried microgels were mounted on metal stubs with double-sided tape, sputtered with gold. When observe on TEM, the microgel suspension was stained using a negative staining technique (Harris et al., 1999).

2.6. pH-dependent releases of TMACB and calcein from microgels

The releases of TMACB and calcein were carried out by a dialysis method. 1 ml of microgel suspension was put into dialysis bag (MWCO 1000), and then the dialysis bag was put into 100 ml distilled water in a 120 ml beaker, which was pre-adjusted to pH 3, 8 or 11. 1 ml of distilled water was taken for the assay of the amount of TMACD and calcein released out at predetermined time intervals (0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h). Immediately after taking 1 ml for the assay, the same amount of fresh distilled water (pH 3, 8 or 11) was added in order to keep the total volume constant. The amount of TMACB released out was determined on UV/Vis spectrophotometer (6505 UV/Vis. Spectrophotometer, JENWAY, UK) at 275 nm, and the amount of calcein release out was determined on fluorescence spectrometer (F-2500, HITACHI, Tokyo, Japan) at 515 nm with excitation of 495 nm. The % of release is defined as the percentage of the released amount on the basis of the total amount entrapped in the microgel.

3. Results and discussion

3.1. Characterization of PβCD and microgels

The ^{13}C NMR signals of C-1, C-2, C-3, C-4, C-5 and C-6 in the nomenclatures of β-CD residue in PβCD depicted in Fig. 2 were found around 101, 73, 71, 81, 70, and 60 ppm, respectively. The peak labeled as C-3′6′ is ascribed to the result of the substitutions of C-3 and C-6. The signal of C-6 substitution was downfield shifted and it was indicated as C-6′. According to previous reports, the signals of C-1′, C-2′ and C-3′ were the results of C-2 and C-3 substitutions. Following the above results, the reactions took place at all three positions on the primary surface (C-6) and the secondary one of the CD. Since a strong alkaline condition (30% NaOH solution) was employed for the preparation of PβCD, most of the substitution occurred on the primary surfaces of β-CD. As a result, the signals of C-1′, C-2′ and C-3′ were relatively weak, while the signals of C-6′ were strong (Renard et al., 1996). The signals of C-8″ and C-9 of EPI residue were observed around 58 and 63 ppm, respectively.

In addition, FT-IR spectra provided additional information on the formation of PβCD and microgels, and the results were shown in Fig. 3. The characteristic peaks of CD were observed at 1028–1159 cm^{-1} (C–O and C–O–C of stretching) (spectrum (a)). The peaks of the PβCD were wider than those of β-CD monomer (spectrum (b)). The peaks at 1640 cm^{-1} (plane bending) and 3340 cm^{-1} (stretching) were from the OH groups of CD and PβCD (spectrum (a) and spectrum (b)). The spectrum of PβCD was similar to that of β-CD, suggesting that PβCD had the characteristic structure of β-CD. On the other hand, the peak at 1596 cm^{-1} corresponds to the C=C stretching vibrations of the benzoate ring of TMACB (spectrum (d)), and the peak intensity became weaker after included in PβCD (spectrum (e)) (Karabacak et al., 2008).

The molar ratio of β-CD to EPI for the preparation of PβCD was 1:10 and the molar ratio of β-CD to EPI residue in PβCD, determined by a colorimetric method using phenolphthalein, was 1:12.2. The content of EPI residue in PβCD was somewhat higher than that of EPI in the feed. It was reported that the contents of EPI in the polymer was higher than that of EPI in the feed when NaOH concentration was higher than 22% (Renard et al., 1996). It means that the reactivity of EPI is higher in a stronger alkali condition.

3.2. Size distribution and electron microscopy

The size distributions of the microgels were shown in Fig. 4. Three populations were observed, ranging from 19 nm to 107 nm, 378 nm to 1137 nm, and 2839 nm to 7774 nm. The microgels observed on SEM were shown in Fig. 5(a). Most of microgels were around 1000 nm in diameter and they are thought to be the population of 294–1137 nm in Fig. 4. Much smaller gel particles were also found with the microgels according to the result of size distribution, but they were hard to be identified on SEM. Fig. 5(b) shows the TEM photo of microgel, which was obtained by focusing on the smaller particles. The particles were less than 100 nm and they would be the population of 19 nm to 107 nm in Fig. 4. The popula-

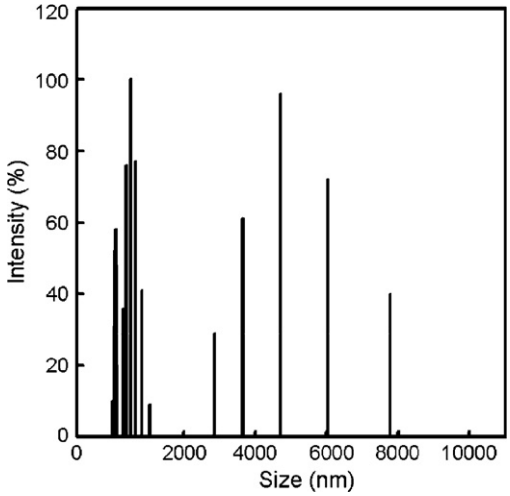

Fig. 4. Size distributions of microgels.

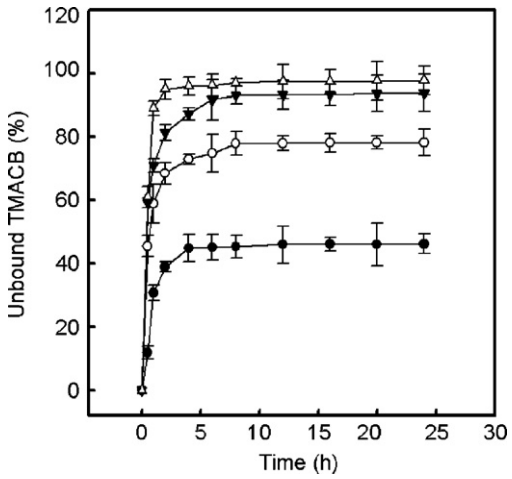

Fig. 6. Removal of unbound TMACB from dialysis bags containing TMACB solution (△), TMACB/CMC solution (▼), PβCD/TMACB solution (○), or PβCD/CMC microgels crosslinked by TMACB (●).

tion ranging from 2839 nm to 7774 nm in Fig. 4 might be due to the agglomeration of the microgels.

3.3. Determination of unbound TMACB

Fig. 6 shows the degrees of removal of TMACB from dialysis bags containing four kinds of preparations. Almost 100% of removal was obtained for 2 h with TMACB solution. Therefore, it was confirmed that the dialysis membrane (MWCO 1000) could hardly prevent TMACB from being evacuated from bag, once the solution was dialyzed for more than 2 h. In fact, the molecular weight of TMACB, 243.7, is much smaller than the MWCO of the dialysis membrane. On the other hand, the removal was somewhat suppressed and retarded when it was with CMC. The electrostatic interaction between the positively charged TMACB and the negatively charged CMC would be responsible for the suppressed removal. When TMACB coexisted with PβCD, the degree of removal was about 78% in equilibrium state and the value was less than in case of TMACB plus CMC. Hydrophobic benzene residues of TMACB will be included in the hydrophobic cavities of β-CD residues of PβCD, so the removal could be suppressed by the hydrophobic interaction. It was reported that the inclusion complex of β-CD and benzene residue was readily formed (Challa et al., 2005). Much more reduction in the removal was observed when TMACB coexisted with PβCD and CMC. The degree of removal in equilibrium was about 47%. The electrostatic interaction of TMACB with CMC along with the hydrophobic interaction with PβCD could give a rise to a marked decrease in the removal. Accordingly, TMACB seemed to bridge CMC and PβCD, leading to the formation of microgels.

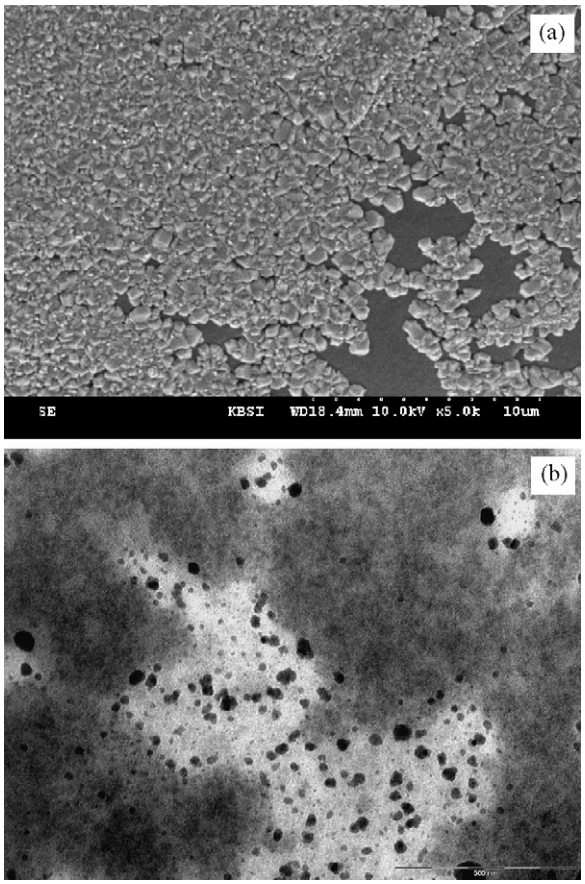

Fig. 5. SEM (a) and TEM (b) photos of microgels. Bars represent 10 μm and 500 nm, respectively.

3.4. pH-dependent releases of TMACB from microgels

Fig. 7 shows pH-dependent releases of TMACB from microgels after removing unbound TMACB. The degrees of release increased with time in a saturation manner and the release rates seemed to be a first order. At pH 3.0, the degree of release in 24 h was about 73%. In acidic condition, the carboxyl group of CMC will be unionized so the electrostatic interaction between CMC and TMACB would disappear. As a result, TMACB could hardly act as a crosslinker to bridge CMC and PβCD. In fact, microgels were disintegrated upon acidification. The disintegration caused by the lack of intermolecular electrostatic interaction could account for the extensive release of TMACB in the acidic condition. At pH 8.0, the degree of release in 24 h was about 18%. Since pH 8.0 is above the pK value (pK_a = 4) of the carboxyl group, more than 50% of the carboxyl groups will be ionized and an intermolecular electrostatic interaction could form salt bridges between CMC and TMACB (Girard et al., 2006). As a result, microgels were stable at pH 8.0, leading to the lower degree of release. At pH 11, the degree of release in 24 h was about 44%. In alkali condition, most of carboxyl groups of CMC will be ionized so the electrostatic interaction between CMC and TMACB will become stronger. As a result, microgels were stable in terms of their integrity. The microgels, however, readily swelled in the alkali condition possibly due to the intermolecular electrostatic repulsion

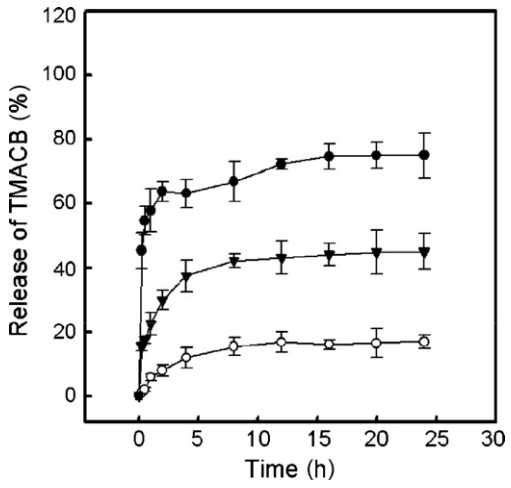

Fig. 7. Releases of TMACB from microgels at pH 3.0 (●), pH 8.0 (○), and pH 11 (▼) after removing unbound TMACB.

among CMC molecules. The swelling behaviors may explain the significant release in the alkali condition.

3.5. pH-dependent calcein release from microgels

Fig. 8 shows the release of fluorescence dye, calcein, from microgels. The degrees of calcein release increased with time in a saturation manner and the rates of release seem to be a first order. At pH 3.0, the degree of release in 24 h was almost 100%. Upon acidification, the number of ionized carboxyl groups of CMC will decrease dramatically and the salt bridges between CMC and TMACB could hardly be formed, leading to the disintegration of the microgels. This could account for the extensive release of calcein in the acidic condition. At pH 8.0, the salt bridges between CMC and TMACB could be formed, since a significant portion of the carboxyl groups will be ionized. As a result, the microgels were stable in terms of the integrity and the release. Hence, the degree of the release in 24 h was as low as 23% in neutral condition. At pH 11, the salt bridges between CMC and TMACB will be more robust because of the higher degree ionization of the carboxyl group. However, the degrees of release, about 47% in 24 h, were much more than in case of pH 8.0. This is possibly due to the microgels swelling properties, which were caused by a strong electrostatic repulsion among CMC molecules.

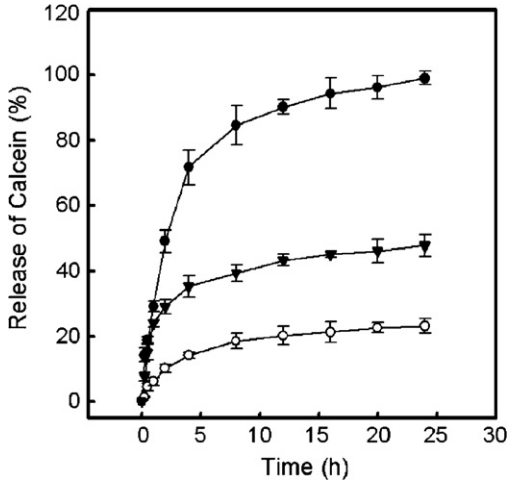

Fig. 8. Release of calcein from microgels at pH 3.0 (●), pH 8.0 (○), and pH 11 (▼) after removing unbound TMACB and unloaded calcein.

4. Conclusions

pH-sensitive microgels were successfully prepared by bridging CMC and PβCD using a crosslinker, TMACB. The microgels were relatively stable in neutral condition (e.g. pH 8.0) for 24 h in terms of the integrity and the release. However, they were unstable in acidic (e.g. pH 3) and alkali condition (e.g. pH 11.0). In acidic condition, the microgels were disintegrated possibly due to the loss of an electrostatic interaction between CMC and TMACB. As a result, almost 100% of release was observed. In alkali conditions, the marked increase in the release from the microgels was observed possible due to the swelling by an intermolecular repulsion among CMC molecules. This kind of microgel, which could be prepared by a simple method, was thought to have a great potential in pH-sensitive drug delivery system.

Acknowledgements

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