pH-Responsive Release from Polypeptide Microcapsules

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ABSTRACT: Microcapsules were prepared from $[Glu(OMe)]_m(Sar)_n$ (m = 21, n = 19) and $[Lys(Z)]_m(Sar)_n$ (m = 27, n = 15), and were chemically modified to obtain a pH-responsive releasing membranes. One membrane was prepared by partially deprotecting the ester groups of $[Glu(OMe)]_m(Sar)_n$. The other membrane was prepared by connecting of poly(Glu) to side chain amino groups that were generated by a partial deprotection of $[Lys(Z)]_m(Sar)_n$. Consequently, two types of polypeptidic microcapsules were prepared; Glu residues in the main chain, and Glu residues in the graft chains on the positively charged main chain. Both microcapsules showed pH-responsive release of FITC-dextran encapsulated in the microcapsules. The release rate became slower in the medium at pH 3.0 than pH 7.5. Optical microscope observation revealed that partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules swelled more at pH 7.5 than at pH 3.0; hence, enhanced permeation through the polypeptide membrane at pH 7.5. However, the shape of poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsules changed a little by changing pH of the medium. It is suggested that ion-pairing between carboxylate groups of poly(Glu) and ammonium groups of Lys acts as crosslinking to give the shape stability. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **63:** 453–458, 1997

Key words: polypeptide microcapsules; pH-responsive release; biodegradation; block polypeptides; chemical modification

INTRODUCTION

The microencapsulation technique is very useful for a drug deliver system in terms of protection of drugs, sustained release, targeting, etc. Poly(α-hydroxy acid)s such as poly(lactide)[1,2] and poly(lactide-*co*-glycolide),[3,4] have been most intensively studied because of their biodegradability. However, chemical modification of polyesters are, in general, difficult, and the difficulty obstructs the development of polyesters for functional microcapsules. However, the chemical modification of polypeptides is easy according to amino acid components.

A number of synthetic polypeptides have been investigated for medical applications such as biodegradable suture,[5] artificial skin substitutes,[6,7] and sustained release devices.[8–10] The rate of degradation of synthetic polypeptides can be controlled by a proper selection of amino acid components.[11,12]

The purpose of the present study is to prepare functional polypeptide microcapsules. The block polypeptides, $[Lys(Z)]_m(Sar)_n$ and $[Glu(OMe)]_m(Sar)_n$, were synthesized by the N-carboxyanhydride (NCA) method, and they were fabricated to microcapsules by a solvent evaporation technique of W/O/W emulsion. After preparation of the microcapsules, the protecting groups were partially removed to produce amino and carboxyl groups. The permeability of these polypeptide membrane should depend on the degree of ionization of these groups.

Several types of pH-responsive membranes have been reported. When ionic gel polymers are used for the membrane materials, the permeabil-

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ity changes in response to swelling/shrinkage of the gel according to the ionization state of the polymers.[13] However, ionic polymers grafted on the surface of porous membranes are shown to act as a chemical valve to regulate the permeability, depending on the conformation of the ionic polymers.[14] The latter type of microcapsules made of polypeptides of two kinds were prepared in the present investigation. In one of them, Glu residues are introduced in the main chain, and in the other, graft chains of poly(Glu) were connected to positively charged main chain. The release of dextran from these microcapsules in the medium of different pHs was investigated.

EXPERIMENTAL

Materials

Block polypeptides were prepared by NCA method as reported previously.[15] Fluorescein isothiocyanate (FITC)-labeled dextran (weight-average molecular weight of 72,000), poly(Glu) (weight-average molecular weight of 34,000), and gelatin (type B, from bovine skin) were purchased from Sigma Chemical Co., St. Louis, MO. Poly(vinyl alcohol) (PVA, weight-average molecular weight of 85,000–145,000, degree of saponification of 87–89 mol %) was purchased from Aldrich Chemical Co., Milwaukee, WI. Water-soluble carbodiimide (WSC) was purchased from Nacalai Tesque, Inc., Kyoto, Japan.

Microcapsules Preparation

Polypeptide microcapsules were prepared by a solvent-evaporation technique of W/O/W emulsion as reported previously.[15] Briefly, FITC-dextran was dissolved in an aqueous gelatin solution (3 wt %), and the aqueous solution was dispersed in a chloroform solution of polypeptides under magnetic stirring. The W/O emulsion was poured into a large amount of an aqueous gelatin solution (1 wt %). The W/O/W emulsion was stirred at room temperature for 30 min and at 40°C for 3 h to obtain polypeptide microcapsules. The dispersion was centrifuged at 200 $\times$ g for 5 min, and the supernatant was discarded. The sediment was washed four times with water by centrifugation at 200 $\times$ g.

Chemical Modification of Microcapsules

$[Lys(Z)]_m(Sar)_n$ (m = 27, n = 15) microcapsules (25 mg) were suspended in 5 N hydrochloric acid (2 mL), and the mixture was heated at 50°C for 3 h. The suspension was centrifuged at 200 $\times$ g for 5 min, and the supernatant was discarded. The sediment was washed four times with water. Under the conditions, 3.3 mol % of the Z groups was deblocked, which was determined from reduction of the absorption around 1720 cm^{-1} due to the urethane carbonyl group in IR spectra of the microcapsules. The sediment was dispersed in a MES buffer solution (10 mM, pH 6.2), and poly(Glu) (12.5 mg) and WSC (1.6 mg) were added. The mixture was kept at 4°C for 24 h and at 37°C for 24 h under shaking. The suspension was centrifuged at 200 $\times$ g for 5 min. The sediment was washed several times with water. The microcapsules were immediately used for FITC-dextran release experiments under various conditions. The microcapsules were also subjected to IR spectroscopy after treatment with diluted HCl aqueous solution, and 125 $\pm$ 15 μg of poly(Glu) was found to be attached onto the partially deblocked $[Lys(Z)]_m(Sar)_n$ microcapsules of dry weight of 1 mg on the basis of increase in the absorption due to the carboxyl groups.

$[Glu(OMe)]_m(Sar)_n$ (m = 21, n = 19) microcapsules (20 mg) were suspended in 0.5 N aqueous NaOH solution (4 mL) at room temperature for 7 min. The suspension was centrifuged at 200 $\times$ g for 5 min, and the sediment was washed three times of water. The microcapsules were immediately used for release experiments. The microcapsules were also subjected to IR spectroscopy after treatment with diluted HCl aqueous solution. The absorption appeared around 1740 cm^{-1} decreased upon partial conversion from the methyl ester group to the carboxyl group because of the different molecular absorption coefficients between them. Under the conditions, about 30 mol % of the methyl ester was deblocked from $[Glu(OMe)]_m(Sar)_n$ microcapsules.

In the preparation, the microcapsules containing FITC-dextran were treated with 5 N HCl or 0.5 N NaOH. The effect of these treatments on FITC-dextran was checked by measuring the molecular weight of FITC-dextran after the treatment. FITC-dextran was not degraded by the NaOH treatment under the present experimental conditions. However, the dextran was partially degraded by the HCl treatment to reduce the molecular weight from 72,000 to 25,000. Therefore, the permeants used for partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules and poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsules were different in the molecular weight.

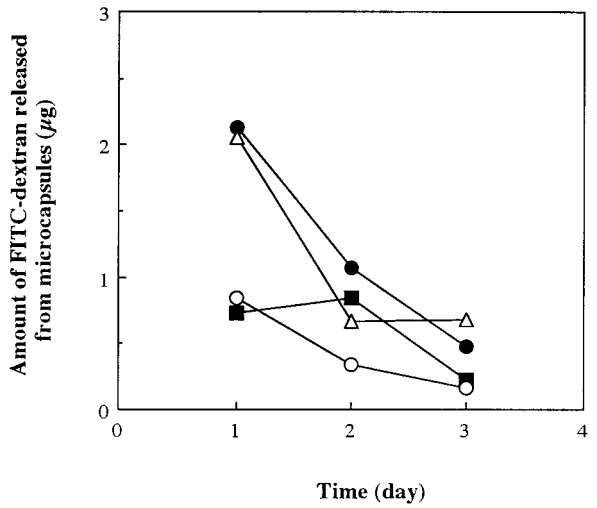

Figure 1 Release of FITC-dextran from partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules in different pH media. (●) pH 7.5; (△) the initial pH 7.5 of the medium lowered to pH 3.0 and raised to pH 7.5; (■) the initial pH 3.0 of medium raised to pH 7.5 and lowered to pH 3.0; (○) pH 3.0.

Release Study

The microcapsules (3.0 mg) were suspended in a Tris buffer solution (10 m*M,* pH 7.5, 5 mL) or a sodium acetate buffer solution (10 m*M,* pH 3.0, 5 mL), and incubated at 37°C for 24 h under shaking. The suspension was centrifuged at 200 × *g*, and the supernatant (4 mL) was subjected to fluorescence spectroscopy to determine the concentration of FITC-dextran released. Before the fluorescence spectroscopy determination, the pH of the supernatant was adjusted to pH 7.5 by adding aqueous NaOH solution. Excitation and monitoring wavelengths were 470 and 520 nm, respectively. The sediment was resuspended in other buffer solution (pH 3.0 or 7.5), and the suspension was incubated for 24 h. The amount of FITC-dextran released was determined by the same way as described above. Each experiment was done in duplicate, and the data were averaged.

Morphology Analysis

The shape and the size distribution of the microcapsules were investigated by an Olympus CH optical microscope (Tokyo, Japan).

RESULTS AND DISCUSSION

Preparation of Microcapsules

Microcapsules were prepared by a solvent–evaporation technique of W/O/W emulsion. The protection groups of polypeptide microcapsules were detached by chemical treatments. It took more than 1 h to partially remove Z groups of $[Lys(Z)]_m(Sar)_n$ microcapsules at 50°C with 5 *N* hydrochloric acid. On the other hand, $[Glu(OMe)]_m(Sar)_n$ microcapsules became water soluble when treated with 0.5 *N* aqueous NaOH solution for about 10 min.

The loading rate (the amount of FITC-dextran encapsulated per polypeptide microcapsules of 1.0 mg) of poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsules was 3.2%, which is lower than 9.5% for partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules. Poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsules were suspended in water longer than partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules for the preparation, because the former took longer time for deprotection and coupling with poly(Glu). Consequently, FITC-dextran encapsulated leaked much in the case of poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsules.

pH-Dependent Release

The amount of FITC-dextran released from partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules was studied at pH 3.0 and 7.5 (Fig. 1). The amount of FITC-dextran released for 1 day at pH 7.5 is significantly larger than that at pH 3.0. When the microcapsules were kept in the same conditions, the amount of release decreased mo-

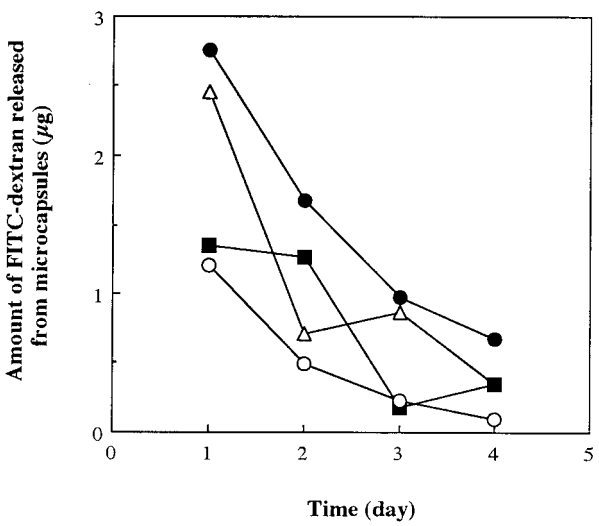

Figure 2 Release of FITC-dextran from poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsules in different pH media. (●) pH 7.5; (△) the initial pH 7.5 of the medium lowered to pH 3.0, raised to pH 7.5, and lowered to pH 3.0; (■) the initial pH 3.0 of medium raised to pH 7.5, lowered to pH 3.0, and raised to pH 7.5; (○) pH 3.0.

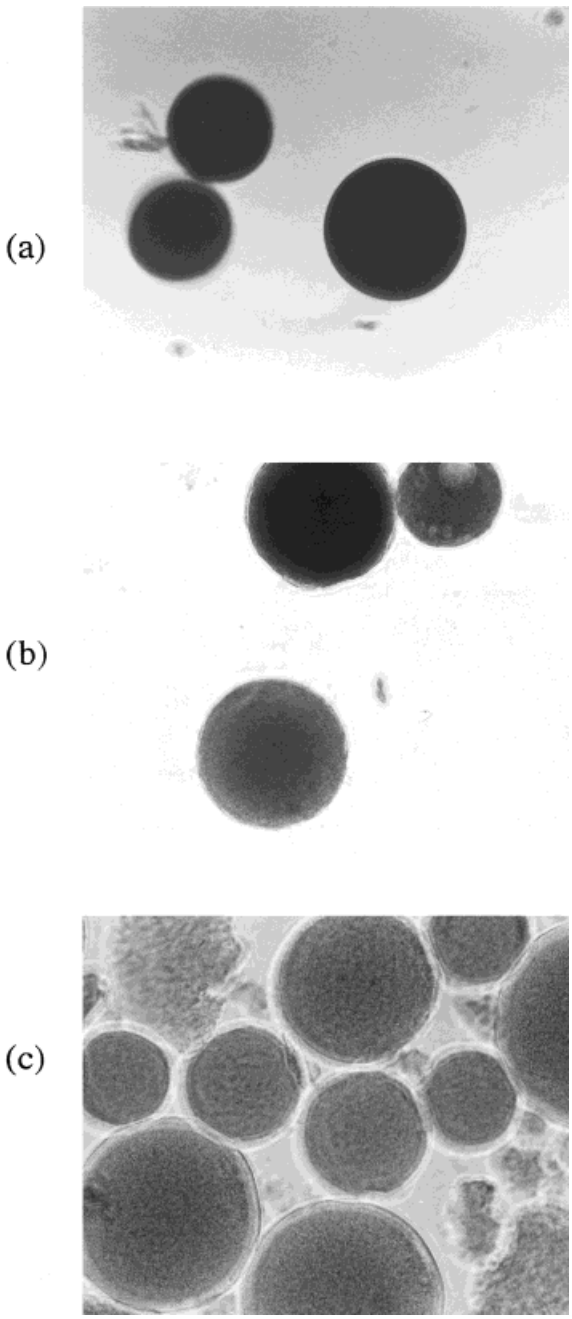

Figure 3 Optical micrographs of $[Glu(OMe)]_m(Sar)_n$ microcapsules. (a) Before deblocking and suspended in the medium of pH 7.5, and after partially deblocking and suspended in the medium of (b) pH 3.0 and (c) pH 7.5. Magnification; ×100.

notonously with time due to decreasing amount of FITC-dextran remaining in the microcapsules. When the pH of the medium was lowered to 3.0 after a 1-day release at pH 7.5, the amount of release decreased drastically. However, when the pH of the medium was raised to 7.5 after a 1-day release at pH 3.0, the amount of release on the second day kept nearly the same level as that on the first day. The pH sensitive release was reversible and reproducible.

The release pattern of FITC-dextran from poly(Glu)-grafted $[Lys(Z)]_m(Sar)_n$ microcapsulesis the same as that from partially deblocked $[Glu(OMe)]_m(Sar)_n$ microcapsules (Fig. 2). The amount of release at pH 7.5 is higher than that at pH 3.0, and decreased monotonously with time when the conditions were kept unchanged. The release rate became slower by changing pH of the medium from 7.5 to 3.0, or became faster by changing pH from 3.0 to 7.5. The mechanism of the pH-dependent release will be discussed later.

Morphology of Microcapsules

$[Glu(OMe)]_m(Sar)_n$ microcapsules after partial removal of the methyl ester groups swelled in aqueous medium, especially at pH 7.5 (Fig. 3). This change is explained by increasing hydrophilicity of the partially deprotected surface (at pH 3.0) and by electrostatic repulsion between polypeptide carrying carboxylate groups (at pH 7.5). When the microcapsules were transferred in the medium of pH 7.5 from the medium of pH 3.0 [Fig. 3(b) and (c)], the microcapsules swelled due to increase of electrostatic repulsion accompanied by deprotonation of carboxyl groups. The changes

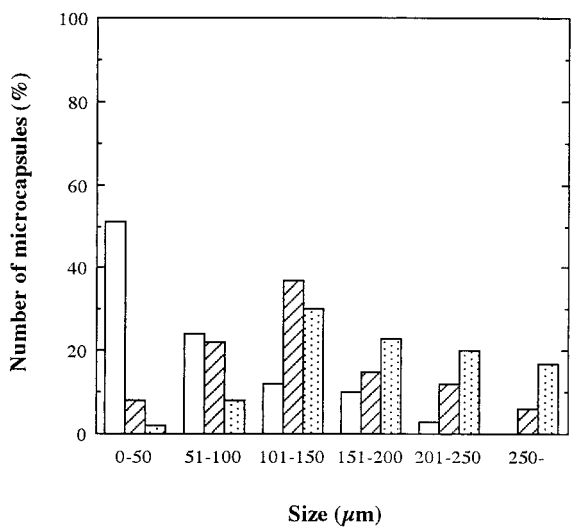

Figure 4 Changes of size distribution of $[Glu(OMe)]_m(Sar)_n$ microcapsules; before deblocking and suspended in the medium of pH 7.5 (open column), and after partially deblocking and suspended in the medium of pH 3.0 (stripped column) and pH 7.5 (dotted column).

of size distribution of microcapsules with varying pH of the medium are summarized in Figure 4, which shows that deprotection of ester groups and change from pH 3.0 to pH 7.5 caused swelling of microcapsules. Therefore, the enhanced release of FITC-dextran at pH 7.5 should be due to the increase of hydration in the polypeptide membrane. Not only the surface but also the matrix part of the microcapsules may become hydrophilic at pH 7.5 from the following points: a) the deblocking rate of the methyl ester groups in the microcapsules was high, suggesting the ester groups in the matrix part should be deblocked, b) the large change in the diameter of the microcapsules upon pH change (Fig. 4).

$[Lys(Z)]_m(Sar)_n$ microcapsules in the medium of pH 7.5 swelled slightly after partial removal of the Z protecting groups [Fig. 5(a) and (b)]. The swelling can be explained by increasing hydrophilicity of the microcapsules and by electrostatic repulsion between ammonium groups at pH 7.5. The slight swelling rate should be due to the small deblocking rate of the urethane groups in the microcapsules. The connection of poly(Glu) graft chains to the microcapsules brought about a slight shrinkage of the microcapsules, suggesting the neutralization of the ammonium groups in the main chain by carboxylate groups in the grafted chain [Fig. 5 (d)]. Notably, the size of poly(Glu)-grafted microcapsules was similar in the medium

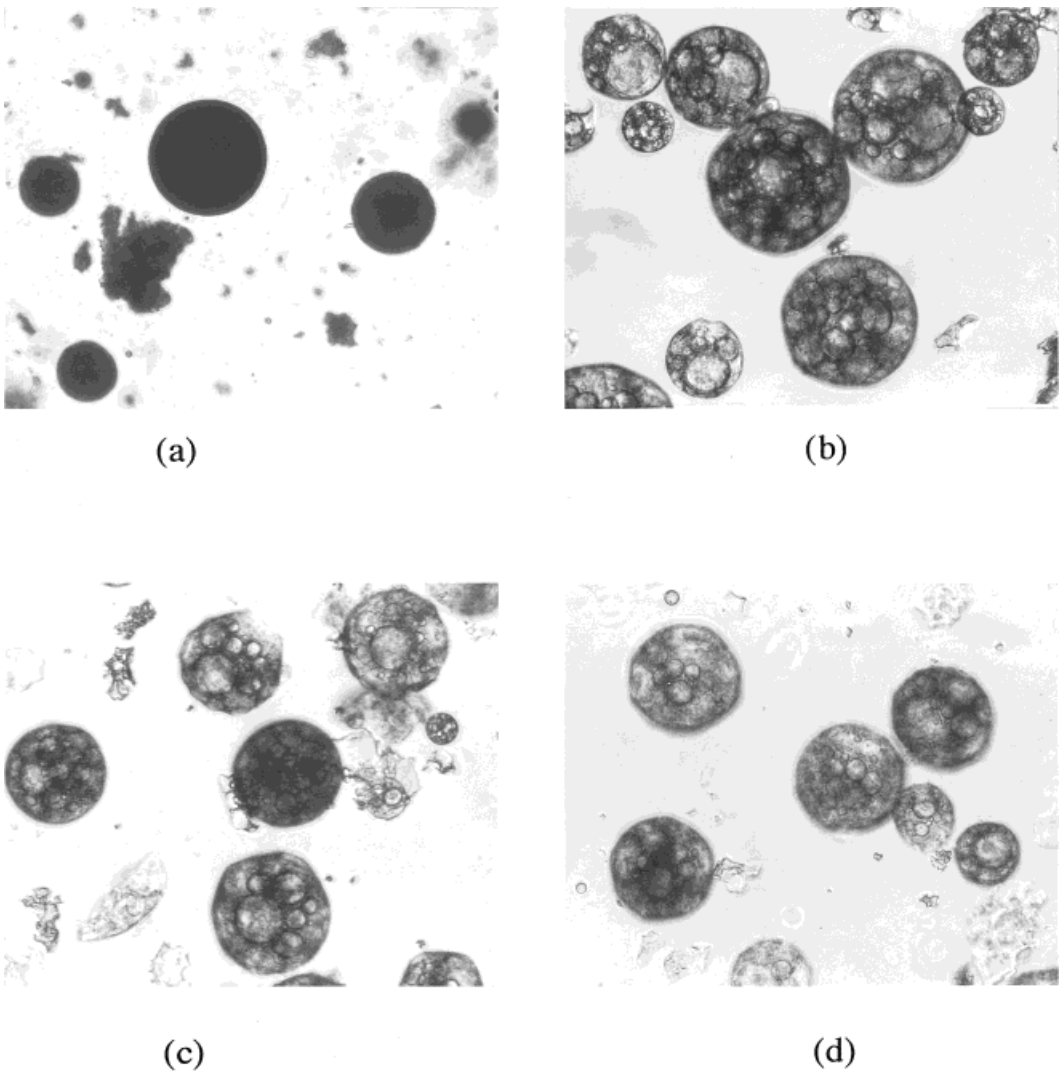

Figure 5 Optical micrographs of $[Lys(Z)]_m(Sar)_n$ microcapsules. (a) Before deblocking and suspended in the medium of pH 7.5, (b) after deblocking and suspended in the medium of pH 7.5, and poly(Glu)-grafted and suspended in the medium of (c) pH 3.0 and (d) pH 7.5. Magnification: ×40.

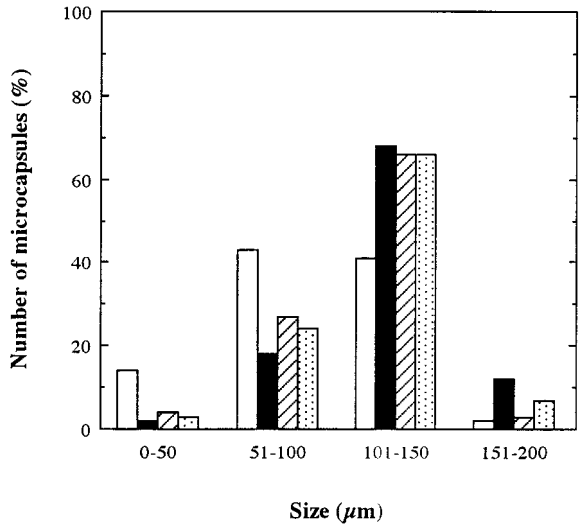

Figure 6 Changes of size distribution of $[Lys(Z)]_m$-$(Sar)_n$ microcapsules before deblocking and suspended in the medium of pH 7.5 (open column), after deblocking and suspended in the medium of pH 7.5 (solid column), and poly(Glu)-grafted and suspended in the medium of pH 3.0 (stripped column) and pH 7.5 (dotted column).

of pH 7.5 and 3.0 [Fig. 5(c) and (d)]. The changes of size distribution of microcapsules with varying pH of the medium are summarized in Figure 6. This result suggests that the ammonium groups and the carboxylate groups form ion pairs in the polypeptide membrane, acting as crosslinking to suppress shape change, which would be induced by pH change in this region. However, carboxylate groups exist in excess over ammonium groups. The carboxylate groups in excess are protonated at pH 3.0, decreasing the hydrophilicity of the membrane to reduce the permeability of dextran.

CONCLUSION

pH-sensitive permeation was attained by polypeptide microcapsules. The membrane permeability increased by ionization of carboxyl groups, which brought increasing hydration of the matrix peptide membrane. Polypeptide microcapsules carrying only carboxyl groups or ammonium groups are transformed by swelling upon pH change. However, polypeptide microcapsules carrying carboxylate groups in addition to ammonium groups in the matrix are neither swelled nor transformed. This is probably due to formation of ion pairs, acting as crosslinking points of polypeptide chains.

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