pH-Responsive Hydrogel Microparticles as Intelligent Delivery Carriers for α-MSH Antagonists

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For a first step in the development of an intelligent delivery system for a nonapeptide as an α-MSH antagonist, pH-responsive P(MAA-co-EGMA) hydrogel microparticles were prepared and their feasibility as intelligent delivery carriers was evaluated. There was a drastic change in the swelling ratio of P(MAA-co-EGMA) microparticles at a pH of around 5 and as the MAA amount in the hydrogel increased, the swelling ratio increased at a pH above 5. The loading efficiency of the nonapeptide at pH 7 increased with the amount of Methacrylic acid (MAA) in the hydrogel and at pH 2, where the electrostatic attraction was greatest, a high loading efficiency was not obtained because of the low swelling ratio of the hydrogel. The P(MAA-co-EGMA) microparticles demonstrated a pH-sensitive release behavior for the nonapeptide. In addition, the P(MAA-co-EGMA) microparticles showed a protective ability for the nonapeptide and preserved the stability of the nonapeptide. © 2010 American Institute of Chemical Engineers *AIChE J,* 57: 1919–1925, 2011

Keywords: hydrogel microparticles, pH-responsive, intelligent delivery system, α-MSH antagonists, skin permeability

Introduction

Recently, considerable efforts have been made to use environmentally responsive hydrogels as novel drug delivery carriers.[1–5] Here, using pH-sensitive hydrogels, one of the environmentally responsive hydrogels, we have developed a new intelligent delivery system for cosmetic applications. In human body, melanin is primarily responsible for the color of skin and produced by the cell of melanocyte located in the bottom layer of the skin's epidermis. Most of skin lightening cosmetic products, such as lightening creams, antiaging whitening creams, and age spot corrective creams, target to inhibit the melanin formation in the skin. α-Melanocyte-stimulating hormone (α-MSH) is a class of peptide hormone having an amino acid sequence Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2 and stimulates the production and release of melanin. Binding of α-MSH to melanotropin receptors (MC1-R) on melanocytes is the first step in melanogenesis and pigment formation. The α-MSH has been shown to induce melanogenesis by increasing the

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activity of tyrosinase, an enzyme in melanogenesis.[6–10] Therefore, one of the methods to block the melanin formation in the skin is to use α-MSH antagonists in cosmetic products. The α-MSH antagonists compete against α-MSH on its specific receptor (MC1-R) leading to tyrosinase inhibition, which reduces the formation of unwanted pigmentation and makes the skin bright.[11–15] The biomimetic peptide having an amino acid sequence Met-Pro-D-Phe-Arg-D-Trp-Phe-Lys-Pro-Val-NH_2 was reported to be one of the most potent α-MSH antagonists[16] due to containing Phe-Arg-Trp residue interacting with the melanocortin receptors.[17–20]

In this study, using oligopeptides having an amino acid sequence Met-Pro-D-Phe-Arg-D-Trp-Phe-Lys-Pro-Val-NH_2 as an α-MSH antagonist (henceforth designated as nonapeptide), we have developed an intelligent delivery system of an α-MSH antagonist triggered by an external pH change for cosmetic applications. In our design of an intelligent α-MSH antagonist delivery system, the nonapeptide is incorporated within pH-sensitive hydrogel microparticles and then stored in a cosmetic container where the pH is maintained lower than the pK_a of the hydrogel. In this low pH condition, the nonapeptide cannot be released from the hydrogel particles due to the collapsed hydrogel network so that the peptide activity is protected. When the nonapeptide-loaded hydrogel particles are applied to the skin, where the pH is always around pH 6 due to human homeostasis, the surrounding pH increases leading to the release of the nonapeptide from the particles and its absorption through the skin.

As a first step in the development of an intelligent delivery system for an α-MSH antagonist, the present work describes the pH-responsive swelling and release behavior of P(MAA-co-EGMA) hydrogel microparticles, the loading efficiency of the nonapeptide in the hydrogel microparticles according to the hydrogel composition and the loading pH conditions, and the skin permeability of the nonapeptide depending on the surrounding pH. Finally, the protective ability of hydrogel particles for the nonapeptide was investigated.

Experimental

Materials

Methacrylic acid (MAA), poly(ethylene glycol) methacrylate (PEGMA, molecular weight 360), poly(ethylene glycol) dimethacrylate (PEGDMA, molecular weight 330), silicon oil, pepsin from porcine gastric mucosa, and tetrafluoroacetic acid (TFA) were purchased from Sigma-Aldrich. 1-Hydroxycyclohexyl phenyl ketone (otherwise known as Irgacure® 184) was obtained from Ciba Specialty Chemicals. Dimethicone Copolyol (DC) was obtained from Nabion (Korea). Acetonitrile (ACN) was purchased from Burdick & Jackson (HPLC grade). Nonapeptide (Met-Pro-D-Phe-Arg-D-Trp-Phe-Lys-Pro-Val-NH_2) was purchased from Peptron (Korea).

Synthesis of P(MAA-co-EGMA) hydrogel microparticles

The copolymer of MAA and PEGMA, designated P(MAA-co-EGMA), hydrogel microparticles were synthesized via suspension photopolymerization. Monomers with feed compositions (molar ratio) of 1:1, 0.8:1, and 0.6:1 MAA:EG were mixed. In each set of the monomer mixtures, the PEGDMA was added as a cross-linker in an amount of 0.75 mol% of total monomers. Irgacure®184, as a UV-light sensitive initiator, was added in an amount of 2.0 wt % of total monomers and these mixtures were then diluted with deionized water to 25% by weight of total monomers. The mixture was purged with nitrogen for 10 min to remove dissolved oxygen that would act as an inhibitor to the reaction and then added to 30 ml of silicon oil to which DC was added in an amount of 2.0 wt % of total monomers. The DC was used as a dispersion stabilizer. The hydrophilic mixture was dispersed in the silicon oil by an ultrasonic processor (VCX750, Sonics & Materials) for 2 min. The suspension solution was irradiated with UV light (intensity 1000 mW/cm^2) for 300 seconds for the polymerization. Synthesized particles were then separated from oil by several repeated cycles of washing with deionized water, centrifugation, and sonication. The washed particles were lyophilized using a freeze-dryer (Ecospin 3180C, Biotron) for 24 h at maximum vacuum. The size and shape of the particles were observed by scanning electron microscopy (SEM). The SEM sample was prepared by dropping a particle suspension onto a glass plate and drying under a vacuum condition. This plate was coated with Au and then observed with SEM (JEOL JSM-6700F, Japan).

Swelling studies of P(MAA-co-EGMA) hydrogel microparticles

To determine the pH-responsive swelling behavior of the P(MAA-co-EGMA) hydrogel microparticles, the freeze-dried microparticles were weighed and then placed in phosphate-citrate buffer solutions with pH values in the range from 2.0 to 8.0. The ionic strength of each buffer solution was adjusted to 0.5 M by the addition of potassium chloride. After swelling, the microparticles were removed from the buffer solution by centrifugation and weighed. The swelling of the microparticles was expressed as the weight swelling ratio, q, defined as the ratio of the weight of the swollen microparticles to the weight of the dried microparticles. The equilibrium weight swelling ratio was obtained when the weight of swollen microparticles reached a constant value (±1%).

Nonapeptide incorporation and release studies

Incorporation of nonapeptide was carried out by soaking 0.05 g of hydrogel microparticles in 25 ml of the nonapeptide stock solution (2.0 mg/ml) for 24 h. At specific time points, 0.5 ml of sample was withdrawn and the nonapeptide concentration was measured to calculate the nonapeptide loading efficiency, defined as the ratio of the amount of nonapeptide incorporated into the hydrogel microparticles to the amount of nonapeptide in the stock solution. After 24 h, the nonapeptide-loaded hydrogel microparticles were separated from the solution by centrifugation and then used for release experiments. To release the nonapeptide from the particles, 0.05 g of nonapeptide-loaded hydrogel microparticles were placed in 25 ml of buffer solutions with pH values of 4.0 and 6.0. At specific time points, 0.5 ml of sample was withdrawn from the solution and the released nonapeptide concentration was measured. For loading and release

experiments of the nonapeptide, HPLC was used to determine the nonapeptide concentration. The HPLC used in the analysis consisted of a Waters 600E controller, a Waters 600 pump, a Waters 717 plus autosampler, a Waters 996 photodiode array detector, and a column (RS tech Optimapak+, C18, 4.6 × 250 mm, 5 μm particle size). The mobile phase consisted of two solutions; solution A was water with 0.1% (v/v) TFA and solution B was ACN with 0.1% (v/v) TFA. The flow gradient was from 95 to 30% of solution A over 30 min and the flow rate was 1.0 ml/min. The sample injection volume was 10 μL and the UV detection wavelength was 280 nm. The calibration curve of nonapeptide concentration vs HPLC peak area was prepared to obtain quantitative information on incorporated and released nonapeptide.

Skin permeability studies of nonapeptide

The skin permeation experiments were carried out using Franz diffusion cells (FCDV-15, Labfine) and human epidermis, which was purchased from Hans Biomed (Korea). A diffusion cell consisted of a donor chamber and a receptor chamber and the skin was positioned between the chambers. The absorption surface area of the skin was 0.785 cm^2. A volume of 5 ml of the receptor chamber was filled with pH 4.0 or pH 6.0 buffer solutions. After equilibration of skin with receptor phase, 2 mg of the nonapeptide-loaded hydrogel microparticles and 1 ml of buffer solution were introduced into the donor chamber. The receptor fluid was maintained in contact with the underside of the skin from the time of application until the end of the collection of the receptor fluid. To compare the skin permeability, we prepared a control, which was the solution of nonapeptide not incorporated within the hydrogel at pH 6.0. The concentration of the nonapeptide for the control was equal to the final concentration of nonapetide that could be released from the nonapeptide loaded microparticles. The diffusion cell and skin were maintained at a constant temperature of 36°C. The receptor solution was continuously agitated with a magnetic stirrer (500 rpm). After 24 h, 0.5 ml of sample was taken from the receptor chamber and the nonapeptide concentration was measured using HPLC.

Stability studies of nonapeptide

To determine the protective ability of the P(MAA-co-EGMA) hydrogel microparticles for nonapeptide, pure nonapeptide and nonapeptide-loaded microparticles were placed in a pepsin solution. Pepsin is one of the proteolytic peptidases that break peptide bonds, resulting in the degradation of proteins. The pepsin solution was prepared by dissolving 50 mg (1.0 wt % of solvent) of pepsin in HCl and water. The pH of the pepsin solution was adjusted to 2.0, where the pepsin functions optimally. During the experiment, the temperature and stirring speed were maintained at 36°C and 500 rpm, respectively. After 24 h, the stability of the nonapeptide was determined using HPLC. To determine the stability of the nonapeptide that was incorporated within the particles, the nonapeptide-loaded microparticles were collected by centrifugation and transferred to 25 ml of pH 6.0 phosphate buffer solution to release the nonapeptide from the hydrogel microparticles.

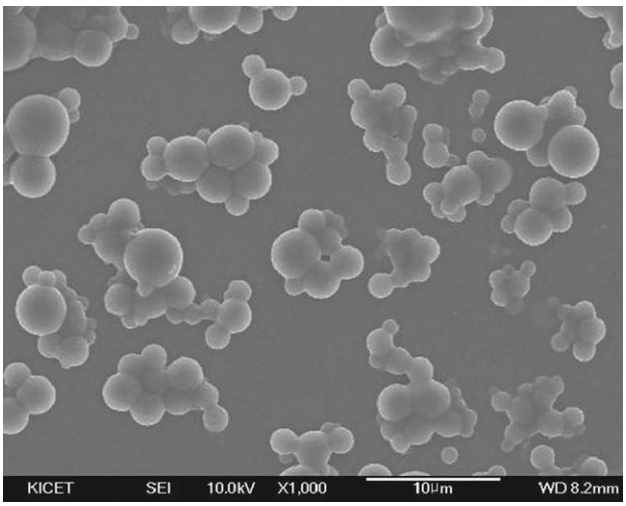

Figure 1. SEM image of P(MAA-co-EGMA) hydrogel microparticles.

Scale bar is 10 μm.

Results and Discussion

Synthesis and swelling behavior of P(MAA-co-EGMA) hydrogel microparticles

pH-Responsive P(MAA-co-EGMA) hydrogel microparticles were synthesized by suspension photopolymerization. SEM image of the synthesized microparticles is shown in Figure 1, which demonstrates that the average diameter of the particles is ~3 μm and that the particles are spherical with a uniform shape.

The pH-responsive swelling behavior of MAA-containing hydrogels results from the ionization or deionization of the carboxylic acid groups of the MAA in response to external pH changes. The carboxylic acid groups of MAA in the hydrogel become ionized as the pH of the external medium increases over the pK_a of the hydrogel. Thus, the presence of MAA in the P(MAA-co-EGMA) hydrogels resulted in a typical pH-responsive swelling behavior of the anionic hydrogel, that is, low swelling ratios at low pH and high swelling ratios at high pH. As the equilibrium weight swelling ratios of P(MAA-co-EGMA) hydrogel microparticles having various MAA and EG compositions were investigated as a function of pH in the range from 2.0 to 8.0, there was a drastic change of the equilibrium weight swelling ratio of P(MAA-co-EGMA) hydrogels at a pH of 5, which is the pK_a of PMAA. At a pH below 5, the hydrogels were in a relatively collapsed state but at a pH higher than 5, the hydrogels swelled to a high degree. In addition, as the MAA content in the hydrogel increased, the swelling ratio increased. The reason for this was that at a pH higher than the pK_a of the hydrogels, the hydrogels that had more MAA could produce more ionized carboxylic acid groups, which resulted in larger electrostatic repulsion between the charged groups, leading to the high swelling ratio. This sharp transition between the swollen and collapsed states at a specific pH indicates that the hydrogels can be used as an on-off switch with which the release of the solute from the hydrogels can be controlled by the external pH changes. In order to quantify the pH-sensitivity of the P(MAA-co-EGMA) hydrogel microparticles, the ratio of

Table 1. Ratio of Equilibrium Weight Swelling Ratios at pH 6 (q_{pH6}) to pH 4 (q_{pH4}) of P(MAA-co-EGMA) Hydrogel Microparticles Having Various MAA and EG Compositions

MAA:EG	q_{pH6}	q_{pH4}	q_{pH6}/q_{pH4}
1:1	6.36 (±0.11)	1.37 (±0.07)	4.64
0.8:1	5.69 (±0.23)	1.24 (±0.04)	4.58
0.6:1	5.29 (±0.22)	1.24 (±0.02)	4.26

Table 2. Loading Efficiency of the Nonapeptide into P(MAA-co-EGMA) Hydrogel Microparticles Having Various MAA and EG Compositions for 3 h

MAA:EG	Loading Efficiency (%)
1:1	29.0 (±1.5)
0.8:1	24.1 (±1.2)
0.6:1	23.7 (±2.1)

the equilibrium weight swelling ratio at pH 6–pH 4, depending on the MAA and EG composition of the hydrogel, was calculated and is summarized in Table 1.

Incorporation of nonapeptide within P(MAA-co-EGMA) hydrogel microparticles

To determine the effect of MAA and EG composition of the P(MAA-co-EGMA) hydrogel microparticles on the loading efficiency of the nonapeptide, the P(MAA-co-EGMA) hydrogel microparticles having various MAA and EG contents were placed in the nonapeptide stock solution with a concentration of 2.0 mg/ml and at pH 7.0. The loading efficiency of nonapeptide into the microparticles is shown in Figure 2. Most of the nonapeptide was loaded into the particles within 1 h. The loading efficiency of nonapeptide into the hydrogel microparticles having various MAA and EG contents for 3 h is listed in Table 2. The loading efficiency of the nonapeptide increased as the amount of MAA in the hydrogel increased. At pH 7.0, where the loading experiments were carried out, the hydrogel networks had negative charges due to the ionization of the carboxylic acid groups of MAA whereas the nonapeptide had positive charges. Nonapeptide contains Arg and Lys, which are basic amino acids, and has a pI (average of pK values for ionizable groups of the peptide form with zero net charge) of pH 11. Thus, the nonapeptide has a positive charge at a pH below the pI but a negative charge at a pH above the pI. The structure of the nonapeptide is presented in Figure 3 and the net charges according to pH are listed in Table 3. Therefore, there was an electrostatic attraction between negatively charged hydrogel networks and positively charged peptide at pH 7.0 and as the amount of MAA in the hydrogel increased, there was more electrostatic attraction, leading to higher loading efficiency. Since the incorporation of nonapeptide in the hydrogel was affected by the electrostatic interaction between the hydrogel and the peptide, the effect of pH at which the loading experiments were performed on the loading efficiency was investigated. The loading efficiency of nonapeptide into the P(MAA-co-EGMA) hydrogel microparticles as a function of pH is listed in Table 4. From Table 3, nonapeptide has +3 charges at pH 2, +2 charges at pH 7, zero charge at pH 11, and −1 charge at pH 13. Therefore, it was expected that the highest loading efficiency was obtained at pH 2 due to the highest electrostatic attractions and then the loading efficiency decreased as the pH increased at a pH above 5, since the hydrogel network had negative charges above pH 5. However, the highest loading efficiency was obtained at pH 7 and the loading efficiency decreased as the pH increased above 7. The reason why the loading efficiency was so low at pH 2 is that the hydrogel microparticles were in a relatively collapsed state at pH 2, thus, it was difficult for the nonapeptide to be incorporated into the hydrogel network.

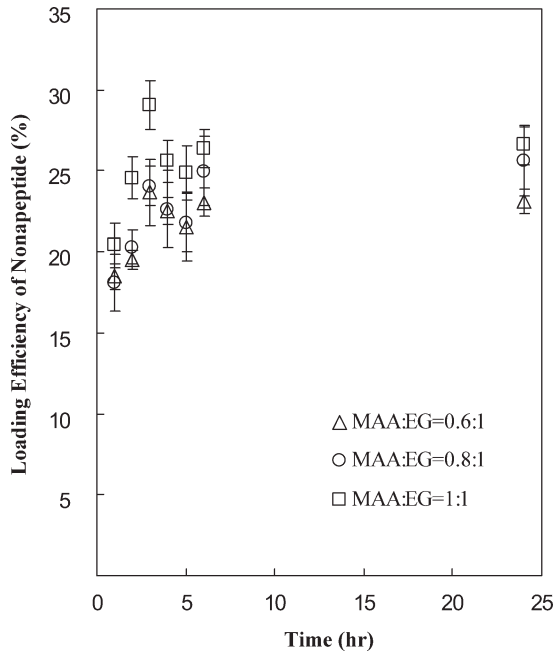

Figure 2. Loading efficiency of the nonapeptide into P(MAA-co-EGMA) hydrogel microparticles having various MAA and EG compositions; MAA:EG = 1:1(□), 0.8:1(○), and 0.6:1(△).

pH-Sensitive release behavior of P(MAA-co-EGMA) hydrogel microparticles

To investigate the pH-sensitive release behavior of the P(MAA-co-EGMA) hydrogel microparticles, the nonapeptide-loaded hydrogel particles were placed in pH 4.0 and pH 6.0 buffer solutions. The cumulative amount of nonapeptide released from the particles as a function of time is shown in

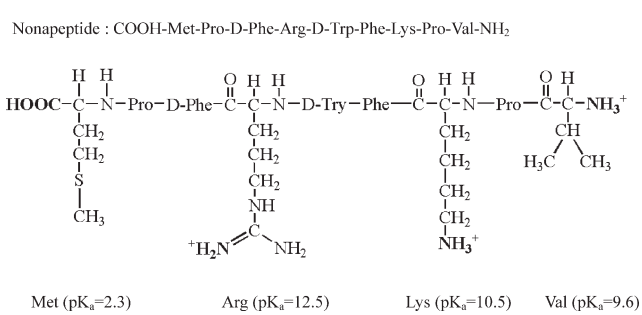

Figure 3. Structure of the nonapeptide.

Table 3. Net Charges of the Nonapeptide Depending on pH

pH	Met	Arg	Lys	Val	Net charges
2	COOH	NH_2^+	NH_3^+	NH_3^+	+3
7	COO^-	NH_2^+	NH_3^+	NH_3^+	+2
11	COO^-	NH_2^+	NH_2	NH_2	0
13	COO^-	NH	NH_2	NH_2	−1

Figure 4. The P(MAA-co-EGMA) hydrogel microparticles showed a pH-sensitive release behavior. At low pH (pH 4.0) small amounts of nonapeptide were released from the particles while at high pH (pH 6.0) relatively large amounts of nonapeptide were released from the particles. The average cumulative amounts of nonapeptide released from the microparticles for 120 min at pH 4.0 and pH 6.0 were 72.3 and 169.1 mg/g, respectively. At pH 6.0 about 2.3 times more nonapeptide was released from the particles than at pH 4.0. This pH-sensitive release behavior of P(MAA-co-EGMA) hydrogel microparticles for nonapeptide indicates that the P(MAA-co-EGMA) hydrogel microparticles can be used as a biological on-off switch for a cosmetic active ingredient like nonapeptide, triggered by an external pH change in the body. This means that the P(MAA-co-EGMA) hydrogel microparticles are able to keep the nonapeptide inside the particles when they are in the cosmetic container, where the pH is maintained to pH 4, and release the nonapeptide from the particles when they are applied to the skin, where the external pH increases to around 6. In addition, for release at pH 6.0, there was an electrostatic attraction between the positively charged nonapeptide and the negatively charged hydrogel network, inhibiting its release. However, there was also a large difference in concentration of the loaded nonapeptide between the inside and the outside of the hydrogel network. Thus, the concentration difference overcame the electrostatic attraction, resulting in the release of the loaded nonapeptide at pH 6.

Skin permeability of nonapeptide

Figure 5 shows the skin permeation of the nonapeptide from the nonapeptide-loaded hydrogel microparticles through the human epidermis as a function of pH. At pH 4.0 very little of the nonapeptide permeated through the skin, whereas at pH 6.0 relatively high skin permeability was obtained. This is because, as previously mentioned, the P(MAA-co-EGMA) hydrogel microparticles had pH-sensitive release behavior for the loaded materials, in other words, at pH 6.0 more nonapeptide was released from the particles and permeated through the skin than at pH 4.0. The skin permeability of the nonapeptide at pH 6.0 reached about 62% of the permeability of the control, which was the solution of nonapeptide not incorporated within the hydrogel at pH 6.0. This implies that the nonapeptide incorporated within P(MAA-co-EGMA) hydrogel microparticles can be released from the particles and then permeate through the skin as they are exposed to the pH condition that is a higher pH than the pK_a of the hydrogel.

Table 4. Loading Efficiency of the Nonapeptide into the P(MAA-co-EGMA) Hydrogel Microparticles as a Function of pH Using MAA:EG = 1:1 Hydrogel and for 3 h

pH	Loading Efficiency (%)
2	3.8 (± 0.2)
7	29.0 (±1.5)
11	24.2 (±0.9)
13	20.1 (±1.57)

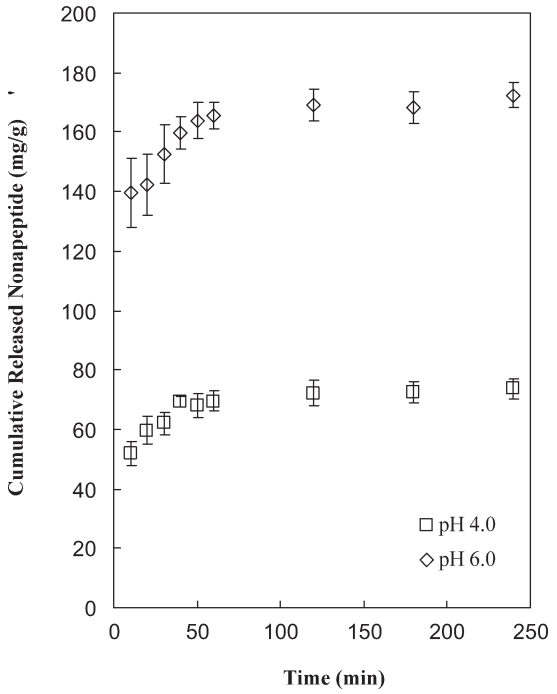

Figure 4. Cumulative amount of the nonapeptide released from P(MAA-co-EGMA) hydrogel microparticles in pH 4.0 (□) and 6.0 (◇) buffer solutions.

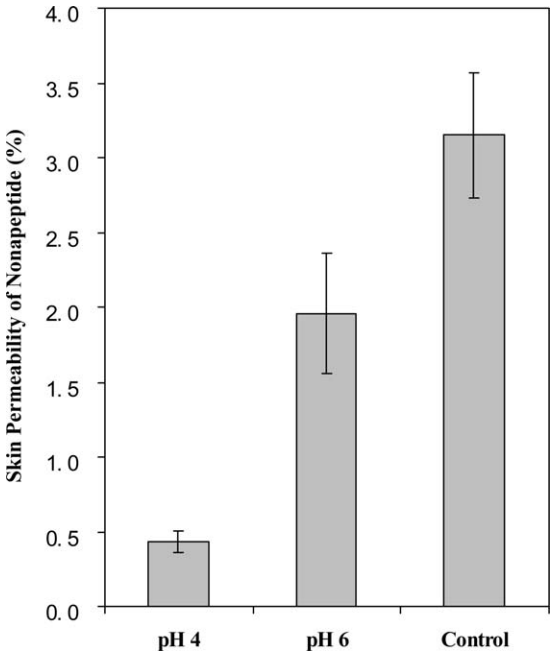

Figure 5. Skin permeability of the nonapeptide from the nonapeptide-loaded P(MAA-co-EGMA) hydrogel microparticles at pH 4.0 and 6.0.

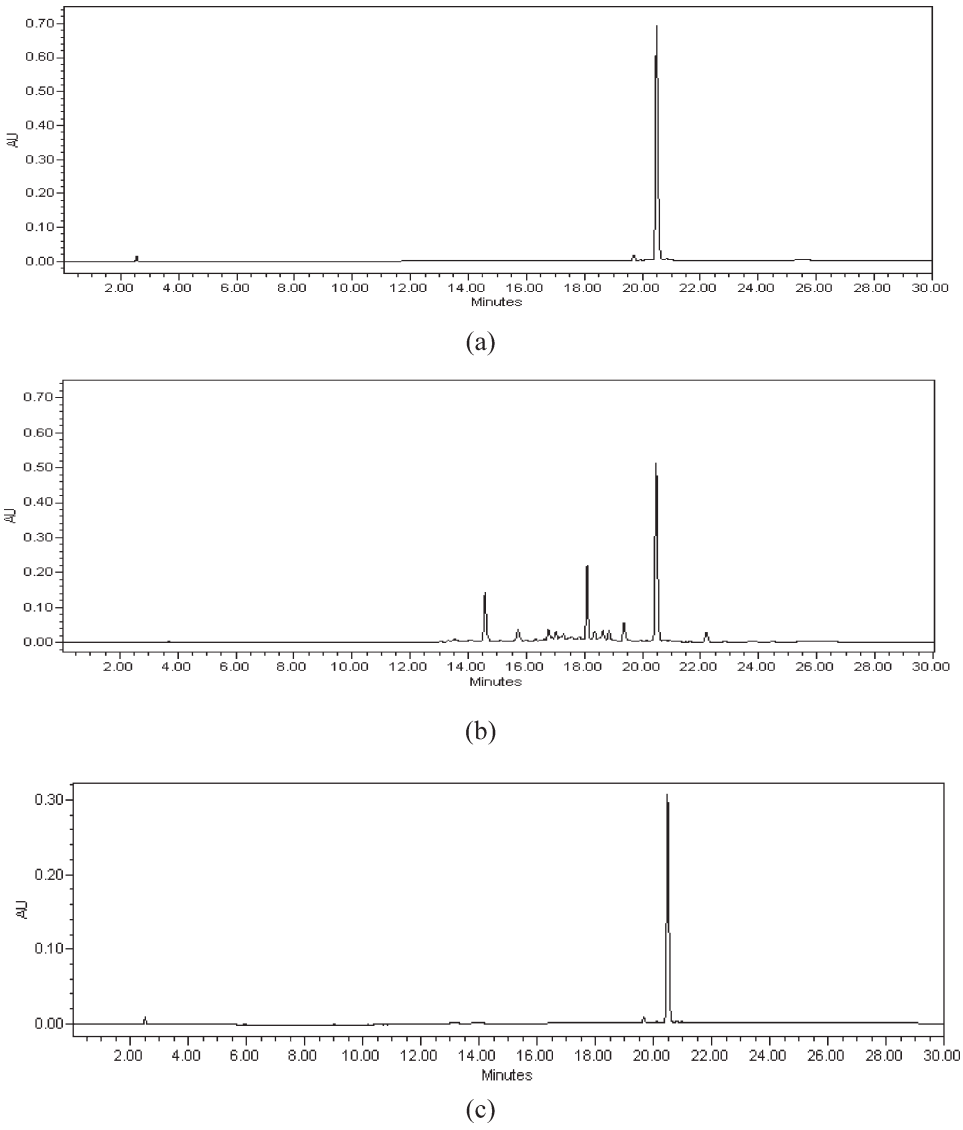

Figure 6. HPLC chromatograms of (a) pure nonapeptide with no pepsin, (b) nonapeptide in pepsin solution, and (c) nonapeptide that was incorporated within hydrogels and then treated with pepsin solution.

Stability of nonapeptide

To investigate the protective ability of the hydrogels for the nonapeptide, the pure nonapeptide and the nonapeptide incorporated microparticles were treated with pepsin at pH2.0 for 24 h and the degradation of nonapeptide was determined using HPLC. Pepsin is one of the proteolytic peptidases that break peptide bonds, resulting in peptide degradation. The resulting HPLC chromatograms are presented in Figure 6. Figure 6(a) shows a chromatogram of the pure nonapeptide, which means that the nonapeptide was not loaded in the hydrogel, dissolved in pH 2.0 buffer solution with no pepsin. There was a peak at 20.5 min, which corresponded to the native nonapeptide. After the nonapeptide was treated with pepsin, the peak area at 20.5 min decreased and a number of new peaks appeared at different retention times (Figure 6b), which represented degraded peptide. However, for the nonapeptide that was incorporated within the microparticles and then placed in the pepsin solution for 24 h, no significant changes in HPLC chromatogram (Figure 6c) were observed compared with Figure 6a. To quantify the effect of the hydrogels on the protection of the nonapeptide, the ratio of the peak area of the native nonapeptide to the area of all the peaks that existed in the HPLC chromatograms was calculated and is listed in Table 5. Without the hydrogel microparticles, about 62% of nonapeptide was degraded by pepsin, but when loaded within the hydrogel particles, $<10\%$ of the peptide was degraded. This result indicates that the hydrogel microparticles prepared in this study can reduce the potential risk that the nonapeptide loses its activity through the interaction with the external environment when it is stored in cosmetic containers and maintain the stability of the nonapeptide.

Conclusions

pH-Responsive P(MAA-co-EGMA) hydrogel microparticles were synthesized by suspension photopolymerization.

Table 5. Ratio of the Peak Area of the Native Nonapeptide to the Area of all the Peaks on the HPLC Chromatograms in Figure 6

Sample	% area of peak at 20.5 min
The pure nonapeptide, which was not loaded into the hydrogel, dissolved in pH 2.0 buffer solution with no pepsin (Figure 6a)	95.2 (±1.2)
The nonapeptide, which was not loaded into the hydrogel, placed in pepsin solution at pH 2.0 (Figure 6b)	33.7 (±2.6)
The nonapeptide, which was incorporated within hydrogels and placed in pepsin solution at pH 2.0, then released at pH 6.0 (Figure 6c)	88.6 (±1.0)

The presence of MAA in the hydrogels resulted in a typical pH-responsive swelling behavior of the anionic hydrogel, that is, low swelling ratios at a pH lower than the pK_a of the hydrogel and high swelling ratios at a pH higher than the pK_a of the hydrogel. Thus, there was a drastic change of the equilibrium weight swelling ratio of P(MAA-co-EGMA) hydrogel microparticles at a pH of around 5, which is the pK_a of PMAA. At a pH below 5, the hydrogels were in a relatively collapsed state but at a pH higher than 5, the hydrogels swelled to a high degree. In addition, as the amount of MAA in the hydrogel increased, the swelling ratio increased at a pH above 5. The incorporation of the nonapeptide into the hydrogel was affected by the electrostatic interaction between the hydrogel and the nonapeptide. The loading efficiency of the nonapeptide at pH 7.0 increased as the amount of MAA in the hydrogel increased. At pH 7.0, where the loading experiments were performed, the hydrogel networks had negative charges, whereas the nonapeptide had positive charges resulting in an electrostatic attraction between charged hydrogel networks and peptides. However, at pH 2.0, where the electrostatic attraction was greatest, the highest loading efficiency was not obtained due to the low swelling ratio of the hydrogel. Therefore, the swelling networks of the hydrogel contributed more to the incorporation of the nonapeptide. The P(MAA-co-EGMA) hydrogel microparticles showed a pH-sensitive release behavior. At low pH (pH 4.0) small amounts of nonapeptide were released from the particles while at high pH (pH 6.0) relatively large amounts of nonapeptide were released from the particles. In addition, at pH 4.0 a very small amount of nonapeptide permeated through the skin while at pH 6.0 relatively high skin permeability was obtained, due to the pH-sensitive release behavior of the hydrogel. For the treatment of the nonapeptide with pepsin, the hydrogel microparticles showed a protective ability for the nonapeptide and maintain the stability of the nonapeptide.

Acknowledgment

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