Preparation and Characterization of pH-Sensitive Anionic Hydrogel Microparticles for Oral Protein-Delivery Applications

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Preparation and Characterization of pH-Sensitive Anionic Hydrogel Microparticles for Oral Protein-Delivery Applications

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Abstract
pH-sensitive P(MAA-g-EG) anionic hydrogel microparticles having an average diameter of approx. 4 µm were prepared by suspension photopolymerization. The pH-sensitive swelling and release behaviors of the P(MAA-g-EG) hydrogel microparticles were investigated as a biological on–off switch for the design of an oral protein delivery system triggered by external pH changes in the human GI tract. There was a drastic change of the equilibrium weight swelling ratio of P(MAA-g-EG) particles at a pH of around 5, which is the pKa of PMAA. At pH < 5, the particles were in a relatively collapsed state, while at a pH > 5 the particles swelled to a high degree. When the concentration of the cross-linker of the hydrogel increased, the swelling ratio of the P(MAA-g-EG) hydrogel microparticles decreased at a pH higher than 5 and the pKa of all the microparticles was in the pH range 4.0–6.0. In release experiments using Rhodamine B (Rh-B) as a model solute, the P(MAA-g-EG) hydrogel microparticles showed a pH-responsive release behavior. At low pH (pH 4.0) only a small amount of Rh-B was released while at high pH (pH 6.0) a relatively large amount of Rh-B was released from the hydrogel particles.

Keywords
Hydrogel microparticles, suspension photopolymerization, pH sensitivity, anionic hydrogel, hydrogel pKa, biological on–off switch, oral protein delivery system

1. Introduction
Oral delivery of therapeutic peptides and proteins is one of the great scientific challenges in the pharmaceutical field. In the development of oral delivery systems for peptides and proteins, major barriers are the degradation of proteins by proteolytic enzymes and the acidic environment in the gastrointestinal (GI) tract and the low penetration of proteins across the intestinal lining into the bloodstream [1, 2].

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order to solve these problems, considerable efforts have been made to use pH-sensitive anionic hydrogels as oral delivery carriers for peptides and proteins [3–8]. Anionic hydrogels are three-dimensional polymer networks and exhibit a drastic change in swelling ratio in response to external pH changes. For an oral protein delivery system, a biological on–off switch triggered by the pH difference between the stomach (pH approx. 2) and the intestine (pH approx. 6) is needed, which would result in the release of proteins from the carrier as it reaches the intestine. In addition, micro- and nanoparticle systems are very attractive for biomedical and pharmaceutical applications, especially in the area of drug-delivery systems [9–14]. Smaller drug carriers are expected to enhance the transport of protein drugs across the intestinal mucosa. As the size of the carrier decreases, the absorption of the carrier to the mucosal layer of the intestine would increase. In this way, the residence time of the carrier on the mucosal layer should increase, thus leading to an improved uptake of proteins. Therefore, the pH-sensitive anionic hydrogel microparticles are one of the most suitable candidates in the design of an oral protein delivery system.

Anionic hydrogels containing poly(methacrylic acid) (PMAA) or poly(acrylic acid) (PAA) have been studied extensively for oral protein delivery applications since they can form polyelectrolyte or hydrogen-bonded complexes that are strongly dependent on the environmental pH and ionic strength [15–21]. In this study, we have set up a method to synthesize PMAA-containing anionic hydrogel microparticles via suspension photopolymerization. The feasibility of hydrogel particles as a biological on–off switch triggered by an external pH change for oral protein delivery applications was evaluated. The pH-sensitive swelling behavior, in particular the effect of the p\(K_a\) of the hydrogel microparticles was investigated. Using Rh-B as a model solute the pH-responsive release behavior of the hydrogel microparticles was determined.

2. Materials and Methods

2.1. Materials

Methacrylic acid (MAA), poly(ethylene glycol) methacrylate (PEGMA, molecular weight 526), poly(ethylene glycol) dimethacrylate (PEGDMA, molecular weight 550), Tween® 20, Span® 20, and silicon oil were purchased from Sigma-Aldrich (USA). 1-Hydroxycyclohexyl phenyl ketone (otherwise known as Igacure® 184) was obtained from Ciba Specialty Chemicals (USA). Rhodamine B (Rh-B) was purchased from Junsei (Japan). MAA was distilled under vacuum prior to use in order to remove an inhibitor. All other chemicals were used as received.

2.2. Hydrogel Microparticle Preparation

Poly(ethylene glycol) (PEG) and poly(methacrylic acid-g-ethylene glycol) (henceforth designated as P(MAA-g-EG)) hydrogel microparticles were prepared by suspension photopolymerization. Monomers with feed compositions (EG/MAA molar ratio) of 1:0 for PEG microparticles and of 1:1 for P(MAA-g-EG) microparticles
were mixed. In each set of the monomer mixtures, PEGDMA was added as a cross-linker at an amount of 1.0, 2.0 or 3.0 mol% of total monomers. Igacure® 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 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 100 ml silicon oil to which Tween® 20 or Span® 20 was added at an amount of 5, 10 or 20 wt% of total monomers. Tween® 20 or Span® 20 was used for the stabilization of the solution containing synthesized hydrogel particles. The mixture of oil and monomers was stirred at 10,000 rpm for 2 min at room temperature to form the suspension. The suspension solution was irradiated with UV light (intensity 1000 mW/cm²) for 300 s for the polymerization. Synthesized particles were separated from oil by several repeated cycles of washing with deionized water, centrifugation and sonications.

2.3. Microparticle Characterization

The size and shape of hydrogel microparticles were observed by scanning electron microscopy (SEM, Jeol 6300).

2.4. Swelling Studies

To determine the pH-sensitive swelling behavior of the hydrogel microparticles, the solution containing hydrogel particles was centrifuged and the supernatant was discarded. The microparticles were then weighed and 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 microparticles before swelling. The equilibrium weight swelling ratio was obtained when the weight of swollen microparticles reached a constant value (±1%).

2.5. Rh-B Incorporation and Release Studies

As a model solute for pH-responsive release studies, Rh-B was used. A stock solution of 0.01 mg/ml Rh-B was prepared and the absorbance of the solution was measured using a UV-visible spectrophotometer (Agilent 8453) at a wavelength of 554 nm. Incorporation of Rh-B was carried out by soaking the hydrogel microparticles in 50 ml of the Rh-B stock solution for 24 h. At specific time points, the solution was centrifuged, 3 ml of the supernatant was withdrawn and the absorbance was recorded in order to calculate the Rh-B loading efficiency, defined as the ratio of the amount of Rh-B incorporated into the hydrogel microparticles to the amount of Rh-B in the stock solution. After 24 h, the Rh-B-loaded hydrogel microparticles were separated from the solution by centrifugation.
To release Rh-B from the particles, Rh-B-loaded hydrogel microparticles were placed in 50 ml of buffer solutions with pH values of 4.0 and 6.0 at 37°C. At specific time-points, 3 ml of the supernatant was withdrawn from the centrifuged solution and the absorbance was measured. The calibration curve of Rh-B concentration versus absorbance was used to obtain quantitative information on incorporated and released Rh-B.

3. Results and Discussion

3.1. Hydrogel Microparticle Synthesis

We first prepared PEG hydrogel microparticles using only PEGMA as a monomer to investigate the effect of Tween® 20 or Span® 20 stabilizers on the preparation of microparticles. Without a stabilizer, there was an agglomeration of hydrogel particles. However, when a stabilizer was used, a well-dispersed hydrogel particle suspension was obtained. Average diameters of PEG microparticles prepared with Tween® 20 and Span® 20 as stabilizer are listed in Table 1. When Tween® 20 was used as a stabilizer, we observed that the microparticles tended to be slightly aggregated and the average size of the particles was larger than that of particles prepared with Span® 20. Thus, Span® 20 was finally chosen as a stabilizer. There was, however, no significant effect of the concentration of the stabilizer on the particle size. Based on the results of the PEG microparticle synthesis, pH-sensitive P(MAA-g-EG) anionic hydrogel microparticles having an average diameter of approx. 4 µm were prepared by suspension photopolymerization. SEM images of PEG and P(MAA-g-EG) hydrogel microparticles are shown in Fig. 1, which demonstrate that the particles are spherical with a uniform shape.

3.2. pH-Sensitive Swelling Behavior

The pH-sensitive swelling behavior of anionic hydrogels results from the ionization or deionization of functional groups in response to external pH changes. Anionic hydrogels contain ionizable groups which become ionized as the pH of the external swelling medium increases above the pK_a of the hydrogel. The pH-sensitive swelling behavior of P(MAA-g-EG) anionic hydrogel microparticles is shown in Fig. 1, which demonstrate that the particles are spherical with a uniform shape.

<table>
<thead>
<tr>
<th>Stabilizer type</th>
<th>Concentration of stabilizer (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Span® 20</td>
<td>4.39 ± 2.0</td>
</tr>
<tr>
<td>Tween® 20</td>
<td>5.75 ± 2.5</td>
</tr>
</tbody>
</table>
is the pH at which the ionizable groups of the hydrogel donate a proton. Figure 2 shows the equilibrium weight swelling ratio of P(MAA-g-EG) hydrogel microparticles as a function of pH in the range from 2.0 to 8.0. The concentration of the cross-linker was 2.0 mol% of the monomers. The presence of MAA in the P(MAA-g-EG) hydrogels resulted in a typical pH-sensitive swelling behavior of the anionic hydrogel, i.e., low swelling ratios at low pH and high swelling ratios at high pH. There was a drastic change in the equilibrium weight swelling ratio of P(MAA-g-EG) hydrogels at a pH of around 5, which is the pK_a of PMAA. At pH < 5, the hydrogels were in a relatively collapsed state, but at pH > 5, the hydrogels swelled to a high degree. The reason for this was that at a pH higher than the pK_a of the hydrogel, the carboxylic acid groups of MAA become ionized and there is electrostatic repulsion between the charged groups, leading to the high swelling ratio (Fig. 3). This sharp transition between the swollen and collapsed states at a specific pH indicates that these hydrogel microparticles can be used as an on–off switch to control the release of the drug from the hydrogel by an external pH change.

Figure 4 presents the effect of the concentration of the hydrogel cross-linker on the equilibrium swelling ratio of the hydrogel microparticles. P(MAA-g-EG) microparticles having 1.0, 2.0 or 3.0 mol% of PEGDMA cross-linker were used. As the concentration of the cross-linker increased, the swelling ratio of the hydrogel particles decreased at a pH higher than 5. As expected, when the concentration of the cross-linker increased the hydrogel networks became denser, making it difficult to expand.

In general, at a pH above the pK_a of the hydrogel, the anionic hydrogel networks swell abruptly. Thus, if a solute is incorporated into the anionic hydrogel, at a pH
Figure 2. Equilibrium weight swelling ratio of P(MAA-g-EG) hydrogel microparticles as a function of pH (average ± SD, n = 3–5).

Figure 3. Molecular structures of carboxylic acid groups of MAA in response to the surrounding pH change; (a) when the external pH is lower than the pKₐ of the hydrogels, (b) when the external pH is higher than the pKₐ of the hydrogels.

above the pKₐ, the solute is released. The swelling behavior, and especially the pKₐ of any polymer hydrogel, depends on the nature and composition of the polymer. It is, therefore, essential to know the correlation between the pKₐ of the hydrogel and the nature and composition of the hydrogel in order to control the release of the drug from the hydrogel by the pKₐ of the hydrogel. To investigate the pKₐ change in relation to the concentration of the cross-linker, the ratios of the change of equilibrium weight swelling ratio (Δq) to the change of pH (ΔpH) in specific pH ranges were calculated. The highest ratio falls in the pH range corresponding to the
pK_{a} of the hydrogel. The pK_{a} change of the hydrogel according to concentration of the cross-linker is shown in Fig. 5. The pK_{a} of all the microparticles was in the pH range 4.0–6.0 and there was no change in pK_{a} in the tested cross-linker concentration range.

### 3.3. pH-Sensitive Release Behavior of P(MAA-g-EG) Hydrogels

Rh-B was incorporated into the hydrogel microparticles by soaking the particles in a Rh-B stock solution. As the hydrogel particles absorbed water, the Rh-B was transported with the water due to the concentration difference of Rh-B between the outside and the inside of the hydrogel. To investigate the pH-sensitive release behavior of the hydrogel microparticles, the Rh-B-loaded hydrogel particles were placed in pH 4.0 and pH 6.0 buffer solutions. The cumulative amount of Rh-B released per hydrogel as a function of time is shown in Fig. 6. The P(MAA-g-EG) hydrogel microparticles showed a pH-sensitive release behavior. At low pH (pH 4.0), small amounts of Rh-B were released from the particles while at high pH (pH 6.0), relatively large amounts of Rh-B were released from the particles. The average cumulative amounts of Rh-B released from the microparticles after 120 h are listed in Table 2. The ratios of the average amount of released Rh-B at pH 6.0 to the
average amount of released Rh-B at pH 4.0 after 120 h were 36.66, 25.18 and 27.19 for P(MAA-g-EG) microparticles having a cross-linker concentration of 1.0, 2.0 and 3.0 mol%, respectively. This pH-sensitive release behavior of P(MAA-g-EG) microparticles indicates that the P(MAA-g-EG) hydrogel microparticles can be used as a biological on–off switch for an oral protein delivery system triggered by an external pH change in the body. In addition, the P(MAA-g-EG) hydrogel microparticles did not release Rh-B for about 5 days at pH 4.0, which means that the P(MAA-g-EG) hydrogel microparticles can potentially keep solutes such as drugs and biologically active materials inside the particles for a long period of time and release the solutes from the particles in response to an increase in the external pH to above the pK\textsubscript{a} of the hydrogel.

4. Conclusions

pH-sensitive P(MAA-g-EG) anionic hydrogel microparticles were prepared by suspension photopolymerization. There was a drastic change in the equilibrium weight swelling ratio of P(MAA-g-EG) hydrogel microparticles at a pH of around 5, which is the pK\textsubscript{a} of PMAA. The microparticles were in a relatively collapsed state at pH < 5, while at a pH > 5 the hydrogels swelled to a high degree. When the concen-
Figure 6. Cumulative amount of Rh-B released from P(MAA-g-EG) hydrogel microparticles in pH 4.0 and pH 6.0 buffer solutions. (♦) 1.0 mol% PEGDMA at pH 6.0, (♦) 1.0 mol% PEGDMA at pH 4.0, (□) 2.0 mol% PEGDMA at pH 6.0, (■) 2.0 mol% PEGDMA at pH 4.0, (△) 3.0 mol% PEGDMA at pH 6.0 and (▲) 3.0 mol% PEGDMA at pH 4.0 (average ± SD, n = 3).

Table 2.
Average cumulative amounts of Rh-B released at pH 4.0 ($M_{R4.0}$) and pH 6.0 ($M_{R6.0}$) after 120 h (average ± SD, n = 3)

<table>
<thead>
<tr>
<th>Concentration of cross-linker (mol%)</th>
<th>$M_{R4.0} \times 10^3$ (mg/g)</th>
<th>$M_{R6.0} \times 10^3$ (mg/g)</th>
<th>$M_{R6.0}/M_{R4.0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.12 ± 0.50</td>
<td>41.06 ± 0.25</td>
<td>36.66</td>
</tr>
<tr>
<td>2.0</td>
<td>1.55 ± 0.26</td>
<td>39.03 ± 0.27</td>
<td>25.18</td>
</tr>
<tr>
<td>3.0</td>
<td>1.34 ± 0.35</td>
<td>36.44 ± 2.55</td>
<td>27.19</td>
</tr>
</tbody>
</table>

The concentration of the cross-linker of PEGDMA increased, the swelling ratio of the hydrogel decreased at a pH higher than 5. The $pK_a$ of all the microparticles was in the range 4.0–6.0 and there was no change of the $pK_a$ with variation of cross-linker concentration. The P(MAA-g-EG) hydrogel microparticles showed a pH-responsive release behavior. At low pH (pH 4.0) small amounts of Rh-B were released, while at high pH (pH 6.0) relatively large amounts of Rh-B were released from the particles. The
results indicate that the P(MAA-g-EG) hydrogel microparticles have the potential to be used as a biological on–off switch to control the release of protein drugs from hydrogel particles by external pH changes and the $pK_a$ of the hydrogels.

Acknowledgement

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References
