Natural polyelectrolyte complex-based pH-dependent delivery carriers using alginate and chitosan

Jun Woo Park, Kyu Ha Park, Sungbaek Seo

Department of Biomaterials Science, Life and Industry Convergence Institute, Pusan National University, Miryang 50463, Republic of Korea

Correspondence to: S. Seo (E-mail: sbseo81@pusan.ac.kr)

ABSTRACT: The pH-dependent complexation behaviors of natural and counterionic polyelectrolyte complexes (PECs) without crosslinkers have been rarely studied. In this work, alginate (0.5–2.5 wt %) and chitosan (0.5–2 wt %) were combined to formulate turbid gel-like assemblies. The PEC was consisted of ~100-μm-sized porous structure observed by scanning electron microscope images and electrostatic interactions inside the complex were newly formed characterized by Fourier transform infrared spectra. Based on visual monitoring and ultraviolet–visible absorption measurement, the complex maintained the gel-like structure under acidic, while the complex was more dissolved under becoming basic. As a demonstration of potential pH-dependent delivery, distinct release profiles of the complexes encapsulating rhodamine 6G (R6G) or doxorubicin, were observed when they were immersed in acidic, neutral, and basic medium. Within 2 h, 7.4, 35.4, and 73.7% of R6G were released under acidic, neutral, and basic condition, respectively, with reasons of degree of protonation or deprotonation of each natural polyelectrolyte at certain pH. © 2019 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 2019, 136, 48143.

KEYWORDS: natural polymers; pH-dependent drug delivery; polyelectrolyte complex

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INTRODUCTION

Polyelectrolyte complexes (PECs) are structured via interactions between polymers possessing oppositely charged groups and are an attractive delivery platform to load water-soluble or charged molecules such as drugs, proteins, and genes. The complexation behaviors of PECs consisting of counterionic synthetic polymers have been primarily investigated. Phase behaviors of PECs were observed as precipitates, polyelectrolyte solutions, or complex coacervates depending on their molecular weight and ionic strength as salt concentration. Among the three phases, complex coacervates have been an attractive option as delivery carriers in the pharmaceutical, food, and cosmetics industries because of their efficient encapsulation of water-soluble components, adhesion to desired wet surfaces, and water immiscibility. Since complex coacervates can be found within adhesions of aquatic organisms from counterionic proteins or single protein, researchers have been inspired to construct complex coacervates using synthetic single polyelectrolytes or surfactants. The synthetic single polyelectrolytes showed pH-dependent complexation behaviors, for example, phase behaviors of precipitates, coacervates, or polyelectrolyte solutions.

The development of natural polyelectrolyte-based delivery carriers, for example, complex coacervates, has been in high demand because of their biocompatibility, biodegradability, nontoxicity, and pH-dependent delivery. However, it is challenging to obtain high loading capability and controlled/desired release of PECs using natural polyelectrolytes. To date, using natural polyelectrolytes, for example, alginate, and chitosan, several approaches to incorporate drug have been developed by layer-by-layer coating and using crosslinkers. Herein, we are interested in preparing the complex between counterionic polyelectrolytes without additional crosslinkers and studying fundamental complexation behaviors of PECs and thereof loading release profiles which have been rarely carried out.

In this study, we envisioned to construct complex coacervates using natural PEC as pH-dependent drug delivery carriers. We rather formulated PECs by mixing two natural polyelectrolytes, alginate, and chitosan, at various concentrations of each polymer in aqueous solution. The complexation behaviors of the chosen PEC at acidic, neutral, and basic pH were compared. To demonstrate the delivery potential of the PEC-based carriers, rhodamine 6G (R6G) or doxorubicin (DOX) was incorporated into the PEC,
and the pH-dependent release profiles of the loaded agents were observed (Figure 1).

**EXPERIMENTAL**

**Materials and Methods**

Chitosan (MW = 50,000–190,000 Da, degree of deacetylation = 75–85%) was purchased from Sigma-Aldrich (St. Louis, MO). Sodium alginate and acetic acid were purchased from DAEJUNG (Seoul, Korea). R6G was purchased from Tokyo Chemical Industry (Tokyo, Japan). DOX hydrochloride was purchased from Alfa Aesar (Ward Hill, MA).

**Preparation of PECs**

Aqueous chitosan solution (0.5, 1, 1.5, and 2 wt %) was prepared by dissolving chitosan in deionized (DI) water containing 0.5 wt % acetic acid. The solution was stored in a water bath at 65 °C for 3–24 h, and then completely dissolved by sonication. Aqueous alginate solution (0.5, 1, 1.5, 2, and 2.5 wt %) was prepared by dissolving sodium alginate in DI water at 22 °C. The two aqueous solutions were mixed and left standing for 30 min after vortexing. The stability and size of the formulated PECs in DI water and ultrasonic cleaner (NXP-1002; KODO Technical Research Co., Ltd., Hwaseong, Korea) were used to dissolve the chemicals in aqueous solution.

**Visual Observation of pH-Dependent Polyelectrolyte Complexation**

The pH solutions were prepared in DI water at pH 2.3, 6.7, and 9.9 by titrating the aqueous solutions with HCl or sodium hydroxide. The formulated PECs (mixture of 2.5 wt % alginate and 2 wt % chitosan) were immersed in each solution. Visual images were taken after 0 min, 30 min, 1 h, and overnight of the incubation. The experiments were performed in triplicate. A pH/mV bench meter (F20-Standard; Mettler Toledo, Columbus, OH) was used to adjust the pH.

**Characterization of Alginate, Chitosan Solution, and PECs**

The morphology of alginate, chitosan, and PEC was observed through scanning electron microscope (SEM). The alginate solution, chitosan solution, and PEC suspension for SEM images was freeze-dried and torn in the middle of the samples, then observed the torn zones. Viscosity of alginate, chitosan, and PEC was measured by rotary viscometer. The range of shear rate/speed is 0–100 rpm. Fourier transform infrared (FTIR) spectra of alginate, chitosan, and PEC were monitored in transmittance mode; the range of wave numbers was scanned from 500 to 4000 cm⁻¹.

**Visual Observation and Ultraviolet–Visible Spectrophotometric Measurement of pH-Dependent Release of R6G in PECs**

R6G solution (0.0125 mM) was mixed with 2 wt % chitosan solution and left standing for 20 min after vortexing. To the mixture solution, 2.5 wt % alginate solution was introduced and left standing for 30 min after vortexing. From the formulated complexes, the amount of released R6G was determined by the absorption intensity (λabs, max = 528 nm) in solutions with corresponding pH. The data are shown as an average and standard deviation of three experiments. A Biochrom Libra S50 ultraviolet/visible (UV/Vis) spectrophotometer (Biochrom, Cambridge, United Kingdom) was used to measure the absorption intensity of the sample solutions.

**Visual Observation and UV–Vis Spectrophotometric Measurement of pH-Dependent Release Profile of DOX in PECs**

DOX (0.2 mg mL⁻¹) was mixed with 2 wt % chitosan solution and left standing for 20 min after vortexing. To the mixture solution, 2.5 wt % alginate solution was introduced and left standing for 30 min after vortexing. From the formulated complexes, the amount of released DOX was determined by the absorption intensity (λabs, max = 480 nm) using a UV/vis spectrophotometer in solutions with corresponding pH. The data are shown as an average and standard deviation of three experiments.

**Encapsulation Efficiency of R6G/DOX in PECs**

The encapsulation efficiency of R6G or DOX in PECs was calculated using the following equation:

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\text{Encapsulation efficiency} = \left( \frac{W_f - W_s}{W_f} \right) \times 100\% 
\]

where \(W_f\) and \(W_s\) are the feeding and supernatant amount of R6G or DOX in the PECs, respectively. The amount of R6G or DOX was measured using UV–vis spectrophotometry and calculated according to a standard calibration curve in concentration ranges of 0.001–0.075 mg mL⁻¹ and 0.0004–0.2 mg mL⁻¹, respectively.
In Vitro Biodegradability Test
The kinetics of weight loss of the PEC in phosphate-buffered saline (PBS) and Dulbecco Modified Eagle Medium (DMEM) was examined over 5 days. The initial mass of PEC was weighted (W_i) right after complexing between alginate and chitosan and removing water at surface of PEC using paper towel. The PEC was immersed into 10 mL of degradation media and placed into an incubator at 37 °C. The mass of PEC was weighted (W_f) after taking out the PEC at each day. The fraction of PEC mass remaining was defined as W_f/W_i × 100%. The experiments with three replicates were examined.

RESULTS AND DISCUSSION
Negatively charged alginate and positively charged chitosan were chosen as counterionic polyelectrolytes in order to investigate phase behaviors at specified concentrations. Since natural polyelectrolytes have broad polydispersity, it is restricted to predict the stoichiometric ratio of acid or base units in natural polyelectrolytes rather than to do this in synthetic polyelectrolytes. It is also challenging for natural polyelectrolytes to interpret the critical parameters, such as polyelectrolyte concentration and ionic strength, for controlling the formation of PECs.25–27 We prepared PECs by mixing two polyelectrolytes and incubating the mixture for a certain amount of time to allow self-assembled complexation. A wide range of concentrations (0.5–2.5 wt % for alginate and 0.5–2 wt % for chitosan) was considered as the mixing conditions. In most conditions, a gel-like, opaque, and amorphous configuration appeared (Figure 2) after 60 min of incubation that enough time to produce the PEC formulations. A gradual process of becoming the opaque formation up to 60 min of incubation was shown in Figures S1–S3.

Among those mixing conditions, a prepared formulation by mixing 2.5 wt % alginate and 2 wt % chitosan was characterized by SEM microscopic morphology evaluation [Figure 3(a–c)]. About 100-μm-sized porous structures could be found within stacks. Before the complexing, the alginate showed film-like structure with holes and the chitosan exhibited sharp stack-like structure. In order to observe mechanical property of the PEC, we measured the viscosity of PEC compared with that of chitosan and alginate aqueous solution before complexing [Figure 3(d)]. The viscosity of the PEC was gradually increased upon shear rate (rpm) while maintaining the middle values between viscosity of alginate (2.5 wt %) solution and viscosity of chitosan (2 wt %) solution. At above 50 rpm of shear rate, the viscosity of PEC was reduced compared with that of the chitosan solution, the rate where the PEC started broken by the shear stress. We also measured FTIR transmittance spectra of PEC, pure alginate and pure chitosan [Figure 3(e)]. We detected disappearance of characteristic amide peak of 1645 cm⁻¹ of chitosan and peak shift of 1591 and 1410 cm⁻¹ from peaks of alginate. That implies formation of electrostatic interaction between alginate and chitosan in the PEC.

We further focused to investigate the pH-dependent complexation behaviors of the PECs. The prepared gel-like formulations were removed from the mixture solutions and immersed in acidic, neutral, and basic aqueous solutions [Figure 4(a), see the photos of preparation steps of PEC in Figure S4]. After immersion for 1 h or overnight, the formulations became more opaque in acidic condition, but were less opaque or disappeared in neutral or basic condition. We additionally conducted UV–vis absorption evaluation of the immersion time-dependent dissolution of PEC in the various media [Figure 4(b–d)]. The absorption intensity of PEC was gradually increased in all the media, indicating counterionic polyelectrolytes became dissociated or dissolved. The rate of the increasing absorbance is more rapid as becoming basic, corresponding to the visual images of dissolving of PEC. This is because the complexation became insoluble at acidic pH, whereas it dissociated at neutral or basic pH. In the acidic condition (pH ~2), the carboxylates of alginate (pK_a 3–4)28,29 become protonated and formed insoluble alginate acid, enabling strong...
Figure 3. SEM images of (a) alginate (2.5 wt %), (b) chitosan (2 wt %), and (c) alginate (2.5 wt %)/chitosan (2 wt %) complex. (d) Plot of viscosity of alginate (2 wt %), chitosan (2 wt %), and alginate/chitosan PEC upon rotation speed. (e) FTIR transmittance spectra of chitosan, alginate, and alginate/chitosan PEC. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 4. (a) Photos of PECs (prepared by mixing 2.5 wt % alginate and 2 wt % chitosan) immersed in pH 2.3, 6.7, and 9.9 for 0 h, 1 h, and overnight. *pHmedium: pH of the medium in which the PECs were immersed. (b) Immersion time-dependent UV–vis absorption spectra of PEC in pH 2.3, 6.7, and 9.9. [Color figure can be viewed at wileyonlinelibrary.com]
interactions with chitosan \(pK_a 6.2-7\)\(^{30,31}\) that are protonated. Also the nonswelling property of the alginate contributed to reduce permeability of the PEC. However, at neutral or basic condition \((pH > 7)\), deprotonated chitosan weakens the interaction between chitosan and alginate, then let the electrostatic repulse between the negatively charged alginates.\(^{32,33}\)

The dramatic pH dependency on complexation behaviors of the PECs generally shows the potential of pH-sensitive drug or gene delivery.\(^{34,35}\) For instance, with strategies of encapsulation with another polymer or crosslinker, the PEC delivery carriers could hold drugs or genes in the stomach \((pH \sim 2)\) and release or deliver them to post-stomach internal organs, for example, the intestine \((pH \sim 7)\).

Since we observed the pH dependency of complexation behavior (from insoluble complexation to dissolution as becoming basic) of PEC, we were interested in the kinetic of load \((R6G, DOX)\) release under acidic, neutral, and basic condition, respectively.

We first prepared PEC-based carriers by loading a charged dye \((R6G)\) or anticancer drug \((DOX)\). The encapsulation efficiency of R6G and DOX was 50.6 \pm 5.2 wt % and 65.7 \pm 15.3 wt %, respectively. To demonstrate the potential of the PEC-based delivery carrier, the pH dependency of the released R6G or DOX was studied. The released amount of R6G in the PEC carrier at corresponding pH values was recorded in Figure 5(a).

Even the initial released amount of R6G was different at the corresponding pH medium where the PEC was immersed; the release profile of R6G at each pH had distinct trend. The release trend of R6G in the PEC carrier was similar to trends of association or dissociation of PEC at each pH [Figure 5(b)]. While the dye was kept in the PEC carrier because of the strong association behavior at acidic conditions, the dye was released more readily from the PEC carrier at neutral and basic conditions because of the dissociation behavior. Even the appearances of the initial complexes immersed at different pH values were different and consistently became separation-like formulations after 120 min [Figure 5(c)].

In order to demonstrate the potential of the PECs as drug carriers, we observed the release profiles of the anticancer drug DOX in the PECs. Depending on the pH of the medium, DOX

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**Figure 5.** (a) UV–vis absorbance of released R6G from PEC carriers. (b) pH-dependent release profiles of R6G from PEC carriers. Each value represents the mean \((n = 3)\). (c) Corresponding photos of PECs at each time point \((0, 30, \text{and } 120 \text{ min})\). *The accumulated release of R6G is expressed as a percentage of the initial amount of encapsulated R6G in the PEC carrier. [Color figure can be viewed at wileyonlinelibrary.com]
showed unique release profiles [Figure 6(a)]. At acidic condition, the accumulated release of DOX reached ~40% with initial burst-like release, which is higher than that of R6G in the same PEC carrier [compare Figure 6(b) with Figure 5(b)]. We believe that this phenomenon is attributed to the weaker interactions at acidic between DOX and polyelectrolyte due to increased solubility and hydrophilicity of DOX at acidic condition.\textsuperscript{36,37} The greater hydrophilicity and solubility of DOX may be partially from more charged status of DOX (pK\textsubscript{a} ~7–9)\textsuperscript{38,39} than that of R6G (pK\textsubscript{a} ~6.5),\textsuperscript{40} resulting in a higher release of DOX at the same acidic pH and duration. Compared to the acidic condition, the amount of DOX released became in overall similar to that of R6G released in at neutral and basic conditions. It is believed to that favored dissociation of the PEC at neutral or basic dominantly induce to the release of DOX, resulting more release amount of DOX as times goes on [Figure 6(c)].

We additionally conducted in vitro biodegradability test of the PEC in PBS (physiological pH of 7.4) DMEM (commonly used medium of mammalian cell culture) medium, respectively. After overnight of incubation at 37 °C, 84.1 and 83.3 wt % of PEC were degraded in PBS and DMEM, respectively (Figure S5). In both media, the kinetic trend of degrading of the PEC after overnight was similar to the kinetic trend of dissolving of PEC at near neutral pH of 6.7.


**CONCLUSIONS**

PECs in opaque gel-like forms were prepared by mixing alginate and chitosan at various concentrations. The complexation behaviors were depended on the pH of the medium, and the pH-dependent release profiles of an optically visualized dye or anticancer drug were obtained. At acidic condition, the complex was insoluble and released less readily while at neutral and basic conditions, the complex was dissociated and much higher amounts of the encapsulated drugs were released. We believe that these findings provide simple but clear insights for the design of drug or cosmetic delivery within PECs consisting of oppositely charged natural polyelectrolytes.

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**Figure 6.** (a) UV–vis absorbance of released DOX from PEC carriers. (b) pH-dependent release profiles of DOX from PEC carriers. Each value represents the mean (n = 3). (c) Corresponding photos of PECs at each time point (0, 30, and 120 min). *The accumulated release of DOX is expressed as a percentage of the initial amount of encapsulated DOX in the PEC carrier. [Color figure can be viewed at wileyonlinelibrary.com]
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CONFLICT OF INTEREST
The authors declare no competing conflict of interest.

AUTHOR CONTRIBUTIONS
The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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