SYNTHESIS AND CHARACTERIZATION OF SELF-ASSEMBLING BLOCK COPOLYMERS CONTAINING ADHESIVE MOIETIES

Kai Huang, Bruce Lee and Phillip B. Messersmith

Biomedical Engineering Department
Northwestern University
2145 Sheridan Road
Evanston, IL 60208

Introduction

Pluronic, a family of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers, are of considerable interest in the biotechnological and pharmaceutical industry for their unique surfactant abilities, low toxicity, and minimal immune response. Aqueous solutions of Pluronic copolymers exhibit interesting temperature-induced aggregation phenomena as a result of the hydrophobic nature of the PPO block. In some cases, gelation of concentrated Pluronic solutions occurs upon heating to temperatures at or just above ambient, a property which is potentially useful for medical drug delivery applications. For example, in-situ gelling materials are potentially useful as carriers for drug delivery to mucosal surfaces, i.e. the oral cavity and the respiratory, gastrointestinal, and reproductive tracts. The overall goal of this work is to synthesize new block copolymers that have the ability to form bioadhesive hydrogels in situ.

One strategy for enhancing the bioadhesive characteristics of polymers is to introduce biological moieties that are known to possess adhesive properties in nature. For example, it is known that marine mussels secrete unique adhesive proteins (mussel adhesive proteins, MAPs) that form strong moisture-resistant bonds to a variety of underwater surfaces. One interesting feature of MAPs is the presence of 3-(4-dihydroxyphenyl)-L-alanine (DOPA), an amino acid which is believed to be responsible for both adhesion and cross-linking of MAPs. The catechol form of DOPA is thought to be responsible for adhesion to surfaces, while the oxidized o-quinone form is responsible for cross-linking of the MAPs. Recently, DOPA-containing synthetic polypeptides have been chemically synthesized by copolymerization of N-carboxyanhydride monomers of lysine and DOPA. The water soluble polypeptides were found to form gels in the presence of oxidizing agents, and adhesion to various substrates was observed. In this paper we describe the preparation of DOPA-modified Pluronic materials which have the ability to form polymer hydrogels by a thermally triggered self-assembly process.

Experimental Materials. PEO100PPO65PEO100 (Pluronic F127) and PEO127PPO5PEO80 (Pluronic F68) were purchased from Sigma (St. Louis, MO). DTPA, thionyl chloride, N,N’-dicyclosuccinimimidyl carbonate as well as 4-(dimethylamino)pyridine (DMAP) were purchased from Aldrich (Milwaukee, WI). All other chemical reagents were used as received. D-OPA methyl ester hydrochloride (DME-HCl) was prepared according to the literature.

Synthesis of Succinimidyl Carbonate Pluronic F127 (SC-PF127). Pluronic F127 (0.60 mmols) was dissolved in 30 mL of dry dioxane. Pluronic F127 (0.60 mmols) was dissolved in 30 mL of dry dioxane. After stirring for 3 hours, the solvent was evaporated off. DME-PF127 was synthesized using the method described above for the synthesis of SC-PF127. A solution of DOPA methyl ester hydrochloride (1.25 mmols) and triethylamine (2.5 mmols) were mixed with SC-PF127 (0.16 mmols) in 10 mL of chloroform. After stirring for 3 hours, the solvent was evaporated off. DME-PF127 was purified by precipitation from cold methanol three times. The removal of the starting materials in DME-PF127 was followed by TLC in chloroform-methanol-acetic acid (5:3:1) system. DME-PF127 gave a positive Arnow test indicating that both catechol hydroxyl groups were free.

75% of product yield was obtained. \( ^1 \text{H NMR (500 MHz, CDCl3): } \delta \text{ ppm 0.98-1.71 (br, 195 H), 2.83-3.04 (m, 4H), 3.15-4.10 (br, 994 H), 4.05-4.30 (d, 4H), 4.55 (m, 2H), 5.30 (d, 2H), 6.45 (m, 2H), 6.61 (s, 2H), 6.78 (d, 2H).} \)

Synthesis of DOPA-Pluronic F127 (DOPA-PF127). L-DOPA (1.56 mmols) was added to 30 mL of 0.1 M Na2B4O7 (pH = 9.32) aqueous solution under the Ar atmosphere, followed by stirring at room temperature for 30 minutes. 5 mL aceton containing SC-PF127 (0.156 mmols) was added to the resulting mixture and stirred overnight at room temperature. The solution pH was maintained with sodium carbonate during the reaction. The solution was acidified with concentrated hydrochloric acid to pH 2 and then was extracted three times with dichloromethane. The combined dichloromethane extracts were dried with anhydrous sodium sulfate and filtered. Dichloromethane was evaporated off. The product was purified by precipitation from methanol twice. The removal of the starting materials in DOPA-PF127 was followed by TLC in chloroform-methanol-acetic acid (5:3:1) system. DOPA-PF127 gave a positive Arnow test indicating that both catechol hydroxyl groups were free. The product yield was 52%. \( ^1 \text{H NMR (500 MHz, CDCl3): } \delta \text{ ppm 0.92-1.81 (br, 195 H), 2.91-3.15 (m, 4H), 3.20-4.11 (br, 991 H), 4.1-4.35 (d, 4H), 4.56 (m, 2H), 5.41 (d, 2H), 6.60 (d, 2H), 6.7 (s, 2H), 6.81 (d, 2H).} \)

Synthesis of Succinimidyl Carbonate Pluronic F68 (SC-PF68). The same procedure as above for the synthesis of SC-PF127 was used to prepare SC-PF68. The product yield was 68%. \( ^1 \text{H NMR (CDCl3, 500 MHz): } \delta \text{ ppm 0.95-1.58 (br, 90 H), 2.80 (s, 8 H), 3.10-4.01 (br, 714 H), 4.40 (s, 4 H).} \)

Synthesis of DOPA methyl ester-Pluronic F68 (DME-PF68). The same procedure as above for the synthesis of DME-PF127 conjugate was used to make DME-PF68. The product yield was 76%. \( ^1 \text{H NMR (500 MHz, CDCl3): } \delta \text{ ppm 0.92-1.81 (br, 195 H), 2.91-3.13 (m, 4H), 3.20-3.95 (br, 710 H), 4.06-4.30 (d, 4H), 4.54 (m, 2H), 5.35 (d, 2H), 6.50 (d, 2H), 6.68 (s, 2H), 6.78 (d, 2H).} \)

Synthesis of DOPA-Pluronic F68 (DOPA-PF68). The same procedure as above for the synthesis of DOPA-PF127 conjugate was used to prepare DOPA-PF68. The product yield was 56%. \( ^1 \text{H NMR (500 MHz, CDCl3): } \delta \text{ ppm 0.92-1.81 (br, 195 H), 2.91-3.13 (m, 4H), 3.20-3.95 (br, 710 H), 4.06-4.30 (d, 4H), 4.54 (m, 2H), 5.35 (d, 2H), 6.50 (d, 2H), 6.68 (s, 2H), 6.80 (d, 2H).} \)

Colorimetric Assay. Coupling yields of DOPA methyl ester and DOPA to Pluronics F127 and F68 were determined by the colorimetric method. DOPA was chosen as the standard for both DOPA methyl ester-Pluronics and DOPA-Pluronics.

Rheology. Rheological measurements were performed using a Bohlin VOR Rheometer. A cone and plate geometry cell (diameter 30 mm, 2.5°) was used for all measurements. The temperature was controlled by a circulating water bath. Storage and loss moduli, \( G' \) and \( G'' \), were measured at a frequency of 0.1 Hz. The heating rate was 0.5°C/min except in the vicinity of the gelation temperature, when it was reduced to 0.1°C/min.

Differential Scanning Calorimetry (DSC). DSC measurements were performed on a TA Instruments DSC-2920 (TA Instruments, New Castle, DE) calorimeter. Spectra were obtained for three samples of each concentration on heating and cooling cycle. Sample volumes of 20 μl in aluminum pans were used and scans were recorded at a heating and cooling rate of 3°C/min with an empty pan as reference.
Results and Discussion

Despite the numerous applications of Pluronics, relatively few attempts have been made to subject them to chemical derivatization.\(^9\) Pluronic F127 and Pluronic F68 were first activated by using N,N'-disuccinimidyl carbonate in the presence of DMAP. The conjugation of succinimidyl carbonate groups to the PEO ends of these copolymers was found to provide an efficient coupling chemistry and regardless of polymer molecular weight. Activated Pluronics SC-PF127 and SC-PF68 can be stably stored in a desiccator at \(-20^\circ C\) and have been found not to lose their activity after months of storage.

The succinimidyl carbonate conjugated derivative was determined to be a useful intermediate for the introduction of DOPA into the Pluronics. The coupling can be performed in both organic and aqueous environments. Based on the assumption of two available succinimidyl carbonate groups in SC-PF127 and SC-PF68, coupling yields of DOPA methyl ester and DOPA to these two Pluronics were found to be in the range from 76% to 82% as obtained from colorimetric analysis. The reported coupling yield is the average value of at least three repeated experiments performed under the same conditions and was not found to increase significantly when a larger excess of DOPA was used. These white DOPA-containing Pluronics could be stored in \(-20^\circ C\) freezer indefinitely with no discoloration or change in properties.

All DOPA-modified Pluronics were freely soluble in cold \(H_2O\). Gelation of concentrated solutions was initially assessed using the qualitative tube inversion method. In this method, the temperature at which the solution no longer flows is taken as the gelation temperature. 22 wt% solutions of DOPA-PF127 and DME-PF127 were found to form a transparent gel at approximately 23 ± 1°C, lowering the polymer concentration to 18 % resulted in a gelation temperature of approximately 31 ± 1°C. These gels were found to be resistant to flow over long periods of time. However, no gelation was observed for solutions with a concentration less than 17 wt %, even at high temperatures.

The temperature-induced gelation of DOPA-modified Pluronics solutions was further studied by oscillatory rheometry. Figure 1 shows the elastic storage and loss moduli, \(G'\) and \(G''\), of a 22 wt % of DME-PF127 aqueous solution as a function of temperature. At subambient temperatures, both storage modulus \(G'\) and loss modulus \(G''\) were negligible, however \(G'\) increased rapidly at the gel temperature \(T_{gel}\). The \(T_{gel}\) value was defined as the onset of the increase of the \(G'\) vs. Temperature plot. DME-PF127 and DOPA-PF127 (not shown) show similar rheological profiles. The \(T_{gel}\) of 22 wt % DME-PF127 solution and 22 wt % DOPA-PF127 solution were found to be about the same, at 20.6 ± 0.5°C. \(T_{gel}\) decreases with increasing concentration of the block-copolymer. The rheological profile of equivalent concentration solution of unmodified Pluronic F127 resulted in a lower \(T_{gel}\) (15.6 ± 0.4°C). \(G'\) of DME-PF127 approaches a plateau value as high as 13 kPa, which is slightly lower than that (16 kPa) of an equivalent concentration solution of Pluronic F127.

Studies\(^\)\(^9\) have shown that aequorin solutions of some Pluronics contain dissolved unimers at low temperatures but form micelles at higher temperatures. It is generally believed that these micelles consist of a core of PPO containing little or no water and a hydrated mantle of PEO and the gel is formed from an interconnected network of close-packed micelles. The increase of \(T_{gel}\) compared with that of pure Pluronic may be due to the introduction of DOPA groups to the both ends of the Pluronics, resulting in an increase in length of the hydrophilic EO chains compared to the hydrophobic PO core.

Differential scanning calorimetry measurements were also made on DME-PF127 and DOPA-PF127 at different concentrations. The DSC profiles obtained for DME-PF127 and DOPA-PF127 were similar. Figure 2 shows the DSC curve of a 22 wt % solution of DME-PF127 during heating. We observed a broad endothermic transition at the micellization temperature and a small transition at the gelation temperature, indicating that gelation is an almost athermal gelation process compared to micellization.\(^11\) Gel temperatures of 22 wt% DME-PF127 and 22 wt% DOPA-PF127 solutions determined by DSC measurements generally coincided with those obtained from rheology (Table 1).

**Figure 1.** Storage and loss moduli at a 22 wt % of DME-PF127 solution, \(G'\) and \(G''\), as a function of the temperature at a frequency of 0.1 Hz.

**Figure 2.** Differential scanning calorimetry (DSC) on a 22 wt % DME-PF127 solution at heating rate of 3°C/min on heating cycle. (1) Micellization transition, (2) Gelation transition.

<table>
<thead>
<tr>
<th>Gel Temperature (°C)</th>
<th>DME-PF127</th>
<th>DOPA-PF127</th>
<th>Pluronic F127</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheological</td>
<td>20.5 ± 0.4</td>
<td>20.9 ± 0.1</td>
<td>15.6 ± 0.4</td>
</tr>
<tr>
<td>DSC</td>
<td>20.7 ± 0.5</td>
<td>21.7 ± 0.2</td>
<td>17.5 ± 0.4</td>
</tr>
</tbody>
</table>

Conclusions

SC-PF127 and SC-PF68 were synthesized using succinimidyl carbonate activation chemistry. These carbonate activated Pluronics react easily with DOPA and its methyl ester in both organic and aqueous solutions. Four DOPA-containing Pluronic compounds, DME-PF127, DOPA-F127, DME-PF68 and DOPA-PF68, were thus successfully synthesized. The coupling efficiencies of all four conjugates were consistently about 80 % and were not found to increase significantly when a larger excess of reagents was used. DOPA-modified Pluronics exhibit temperature-induced gelation. Rheological and DSC studies indicate that DOPA-containing Pluronics exhibit a slightly higher gel temperature than that of the pure Pluronics as a result of the introduction of DOPA to the ends of the copolymers.

**References**