Synthesis and Characterization of Self-Assembling Block Copolymers Containing Bioadhesive End Groups

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3,4-Dihydroxyphenyl-L-alanine (DOPA) is an unusual amino acid found in mussel adhesive proteins (MAPs) that is believed to lend adhesive characteristics to these proteins. In this paper, we describe a route for the conjugation of DOPA moieties to poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO−PPO−PEO) block copolymers. Hydroxyl end groups of PEO−PPO−PEO block copolymers were activated by *N,N*′-disuccinimidyl carbonate and then reacted with DOPA or its methyl ester with high coupling efficiencies from both aqueous and organic solvents. DOPA-modified PEO−PPO−PEO block copolymers were freely soluble in cold water, and dye partitioning and differential scanning calorimetry analysis of these solutions revealed that the copolymers aggregated into micelles at a characteristic temperature that was dependent on block copolymer composition and concentration in solution. Oscillatory rheometry demonstrated that above a block copolymer concentration of approximately 20 wt %, solutions of DOPA-modified PEO−PPO−PEO block copolymers exhibited sol−gel transitions upon heating. The gelation temperature could be tailored between ∼23 and 46 °C by changing the composition, concentration, and molecular weight of the block copolymer. Rheological measurement of the bioadhesive interaction between DOPA-modified Pluronic and bovine submaxillary mucin indicated that DOPA-modified Pluronic was significantly more bioadhesive than unmodified Pluronic.

Introduction

Poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO−PPO−PEO) triblock copolymers are widely used in diverse industrial applications.[1] Recently, PEO−PPO−PEO block copolymers have attracted considerable interest in the biotechnological and pharmaceutical industries for their unique surfactant abilities, low toxicity, and minimal immune response.[2−13] Aqueous solutions of PEO−PPO−PEO block copolymers exhibit interesting temperature-induced aggregation phenomena as a result of the hydrophobic nature of the PPO block.[14,15] At low temperature and concentration, PEO−PPO−PEO block copolymers exist in solution as dissolved monomers but self-assemble at higher concentrations and temperatures into block copolymer micelles that form under conditions defined by the critical micelle concentration (cmc, at constant temperature) and the critical micelle temperature (cmt, at constant concentration).

Highly concentrated solutions of certain PEO−PPO−PEO block copolymers, such as Pluronic F127 (PEO$_{100}$PPO$_{65}$-PEO$_{100}$) and F68 (PEO$_{78}$PPO$_{30}$PEO$_{78}$), exhibit sol−gel transitions when heated.[16−19] Polymer solutions possessing thermogelling and bioadhesive capabilities are potentially useful for medical and dental applications,[16−20] for example as tissue adhesives and as injectable carriers for drug delivery to mucosal surfaces (e.g., the oral cavity and the respiratory, gastrointestinal, and reproductive tracts).[20] In an attempt to

improve the bioadhesive properties of PEO−PPO−PEO block copolymers, which are known to be poorly bioadhesive,[21,22] Chen and co-workers[22] as well as others[23,24] have modified PEO−PPO−PEO block copolymers with bioadhesive anionic polymers such as poly(acrylic acid) (PAA).

An alternative approach is to conjugate PEO−PPO−PEO block copolymers with biological moieties that are known to possess desirable adhesive properties in nature, such as 3,4-dihydroxyphenyl-L-alanine (DOPA). DOPA is an amino acid that is believed to be responsible for the adhesive characteristics of mussel adhesive proteins (MAPs). MAPs are remarkable underwater adhesive materials initially secreted as proteinaceous fluids that harden in situ to form an adhesive plaque that anchors marine and freshwater mussels to the substrates upon which they reside.[25,26] In addition to exhibiting good adhesion to metal, metal oxide, and polymer surfaces,[27−29] DOPA-containing molecules have been found to strongly interact with a pig gastric mucin glycoprotein in dilute solution,[30] suggesting that DOPA-containing proteins and biomimetic polymers may have useful mucoadhesive properties which can be exploited for medical applications.

Although the natural curing and adhesion mechanisms of MAPs are not fully understood, unoxidized DOPA is believed to be responsible for strong water resistant adhesion while the oxidized *o*-quinone is responsible for cross-link formation.[31] In an effort to take advantage of the adhesive properties of DOPA, several groups have reported the synthesis and characterization of DOPA-containing polymers and peptides.[32−35] For example, copolymerization of *N*-

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Scheme 1. Reaction Pathways for the Synthesis of DOPA-Modified PEO−PPO−PEO Block Copolymers (a) in Organic Solvent and (b) in Aqueous Solvent
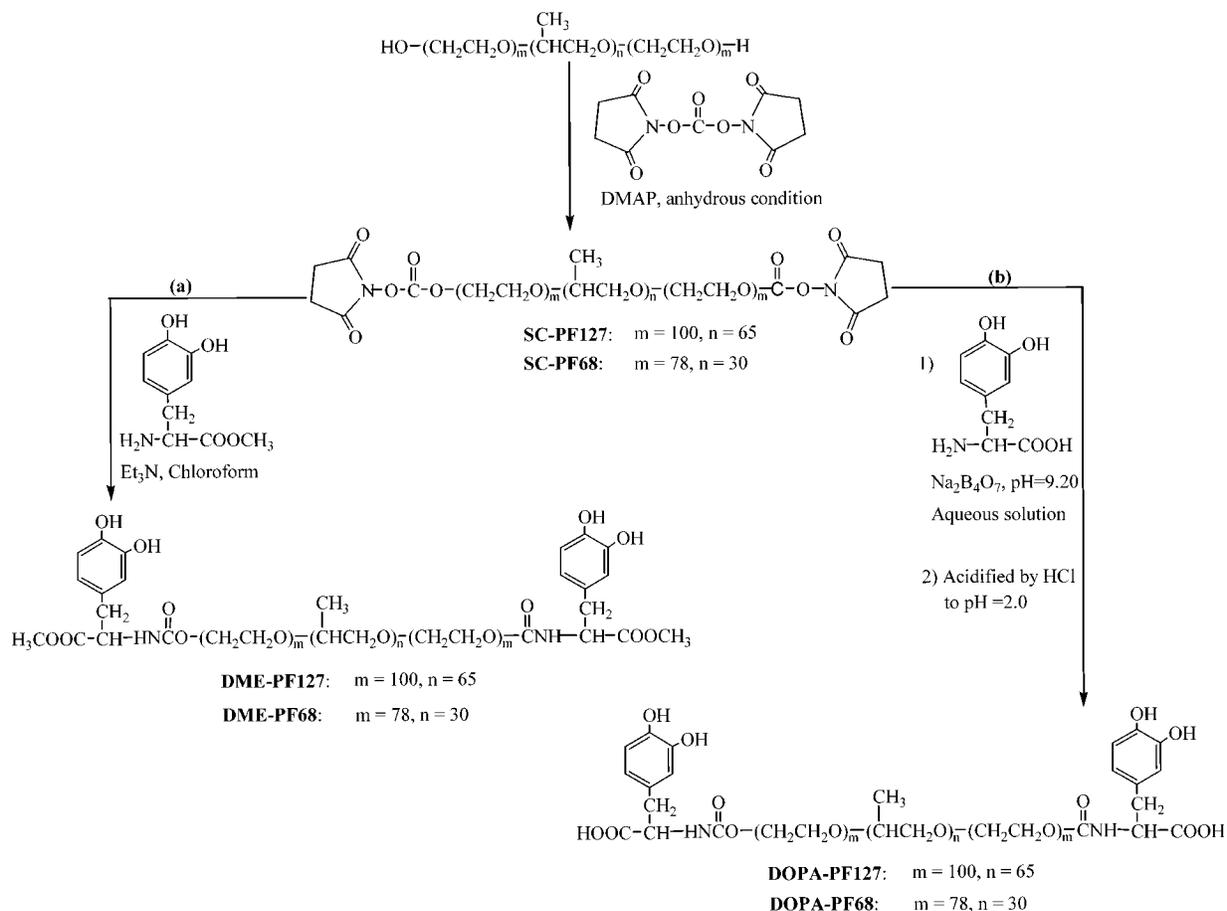
carboxyanhydride monomers of lysine and DOPA yielded DOPA-containing synthetic polymers that exhibited good adhesion to inorganic substrates.[33] However adhesive curing of polymer solutions was accomplished by oxidation of DOPA residues to form DOPA−quinone, which participated in intra- and/or intermolecular reactions with other functional groups to form a cross-linked polymer. The oxidizing reagents necessary for such strategies (NaOH, NaIO₄, H₂O₂, etc.) are expected to be biologically harmful, and the oxidized forms of DOPA that result from such reactions are believed to be less adhesive than unoxidized DOPA.[31] Indeed, DOPA-containing proteins and peptides exhibit better adhesion to both metallic and mucosal surfaces when DOPA residues are not oxidized.[33,36] Thus, there exists a clear need for designing new DOPA-mimetic polymers that employ solidification strategies that are not biologically harmful and which preserve the optimal adhesive form of DOPA; significant benefits may be realized for bioadhesive applications of such polymers.

In this paper, we describe a general synthetic procedure for activation of hydroxyl end groups of PEO−PPO−PEO block copolymers using succinimidyl carbonate chemistry, which was found to be a reliable starting step for the incorporation of DOPA-containing moieties in both organic and aqueous solvents (Scheme 1). Oscillating rheometry, differential scanning calorimetry (DSC), and UV−vis spectroscopy were used to characterize the micellization and gelation behavior of DOPA-modified PEO−PPO−PEO

block copolymers. The DOPA-modified polymers were found to have the ability to form polymer hydrogels by a thermally triggered self-assembly process, and the presence of DOPA end groups significantly improved the mucoadhesive properties of the PEO−PPO−PEO block copolymers.

Experimental Section

Materials. PEO₁₀₀PPO₆₅PEO₁₀₀ (Pluronic F127, $\bar{M}_w$ = 12 600) and PEO₇₈PPO₃₀PEO₇₈ (Pluronic F68, $\bar{M}_w$ = 8400) were purchased from Sigma (St. Louis, MO). L-DOPA, thionyl chloride, *N,N′*-disuccinimidyl carbonate, sodium borate, sodium molybdate dihydrate, sodium nitrite, 1,6-diphenyl-1,3,5-hexatriene (DPH), 4-(dimethylamino)pyridine (DMAP), and purified bovine submaxillary mucin were purchased from Aldrich (Milwaukee, WI). Acetone was dried over 4A molecular sieves and distilled over P₂O₅ prior to use. Triethylamine was freshly distilled prior to use. All other chemical reagents were used as received. L-DOPA methyl ester hydrochloride was prepared according to the literature procedure.[37]

Synthesis of Succinimidyl Carbonate Pluronic F127 (SC-PF127). Pluronic F127 (0.60 mmol) was dissolved in 30 mL of dry dioxane. *N,N′*-Disuccinimidyl carbonate (6.0 mmol) in 10 mL of dry acetone was added. DMAP (6.0 mmol) was dissolved in 10 mL of dry acetone and added slowly under magnetic stirring. Activation proceeded 6 h at room temperature, after which **SC-PF127** was precipitated

into ether. The disappearance of the starting materials during the reaction was followed by TLC in chloroform−methanol (5:1) solvent system. The product was purified by dissolution in acetone and precipitation with ether four times. The product yield was 65%. ^{1}H NMR (500 MHz, CDCl₃): δ, ppm 0.96−1.68 (br, −OCHC*H*₃CH₂O−), 2.80 (s, −COON-(CO)₂(C*H*₂)₂), 3.15−4.01 (br, −OC*H*₂C*H*₂O−; −OC*H*CH₃-C*H*₂O−), 4.40 (s, −OCH₂C*H*₂OCOON(CO)₂CH₂CH₂−).

Synthesis of DOPA Methyl Ester−Pluronic F127 (DME-PF127). A slurry of DOPA methyl ester hydrochloride (1.25 mmol) and triethylamine (2.5 mmol) was mixed with **SC-PF127** (0.16 mmol) in 10 mL chloroform. The disappearance of the starting materials during the reaction was followed by TLC in chloroform−methanol−acetic acid (5:3:1) solvent system. After stirring for 1 h at room temperature, the solvent was evaporated, and **DME-PF127** was purified by precipitation from cold methanol three times. **DME-PF127** gave a positive Arnow test indicating the presence of catechol hydroxyl groups,[38] and a negative Kaiser test indicating the absence of free amino groups. The product yield was 75%. ^{1}H NMR (500 MHz, CDCl₃): δ, ppm 0.98−1.71 (br, −OCHC*H*₃CH₂O−), 2.83−3.06 (m, −NHCHC*H*₂-C₆H₃(OH)₂COOCH₃), 3.15−4.02 (br, −OC*H*₂C*H*₂O−; −OC*H*CH₃C*H*₂O−; −NHC*H*(CH₂C₆H₃(OH)₂COOC*H*₃), 4.05−4.35 (d, −OCH₂C*H*₂OCONHCHCH₂C₆H₃(OH)₂COO-CH₃), 4.55 (br, −NHC*H*CH₂C₆H₃(OH)₂COOCH₃), 5.30 (d, −N*H*CHCH₂C₆H₃(OH)₂COOCH₃), 6.45−6.80 (1s, 2d, −NH-CHCH₂C₆*H*₃(OH)₂COOCH₃).

Synthesis of DOPA-Pluronic F127 (DOPA-PF127). L-DOPA (1.56 mmol) was added to 30 mL 0.1 M Na₂B₄O₇ (pH = 9.32) aqueous solution under Ar atmosphere, followed by stirring at room temperature for 30 min. **SC-PF127** (0.156 mmol) in 5 mL of acetone was added to the resulting mixture and stirred overnight at room temperature. The solution pH was maintained with sodium carbonate during the reaction. The disappearance of the starting materials during the reaction was followed by TLC in chloroform−methanol−acetic acid (5:3:1) solvent system. The solution was acidified to pH 2 with concentrated hydrochloric acid and then extracted three times with dichloromethane. The combined dichloromethane extracts were dried with anhydrous sodium sulfate and filtered, and dichloromethane was evaporated off. The product was further purified by precipitation from cold methanol. **DOPA-PF127** gave a positive Arnow test, indicating the presence of catechol hydroxyl groups,[38] and a negative Kaiser test, indicating the absence of free amino groups. The product yield was 52%. ^{1}H NMR (500 MHz, CDCl₃): δ, ppm 0.92−1.70 (br, −OCHC*H*₃CH₂O−), 2.91−3.15 (m, −NHCHC*H*₂C₆H₃(OH)₂COOCH), 3.20−4.10 (br, −OC*H*₂C*H*₂O−; −OC*H*CH₃C*H*₂O−), 4.1−4.35 (d, −OCH₂-C*H*₂OCONHCHCH₂C₆H₃(OH)₂COOH), 4.56 (m, −NHC*H*-CH₂C₆H₃(OH)₂COOH), 5.41 (d, −N*H*CHCH₂C₆H₅(OH)₂-COOH), 6.60−6.82 (1s, 2d, −NHCHCH₂C₆*H*₃(OH)₂COOH).

Synthesis of Succinimidyl Carbonate Pluronic F68 (SC-PF68). A procedure similar to that described above for the synthesis and purification of **SC-PF127** was used to prepare **SC-PF68**. The product yield was 68%. ^{1}H NMR (500 MHz, CDCl₃): δ, ppm 0.95−1.58 (br, −OCHC*H*₃CH₂O−), 2.80

(s, − COON(CO)₂(C*H*₂)₂), 3.10−4.03 (br, −OC*H*₂C*H*₂O−; −OC*H*CH₃C*H*₂O−), 4.40 (s, −OCH₂C*H*₂OCOON(CO)₂CH₂-CH₂).

Synthesis of DOPA Methyl Ester−Pluronic F68 (DME-PF68). A procedure similar to that described above for the synthesis and purification of **DME-PF127** conjugate was used to make **DME-PF68**. The product yield was 76%. ^{1}H NMR (500 MHz, CDCl₃): δ, ppm 0.98−1.50 (br, −OCHC*H*₃-CH₂O−), 2.85−3.10 (m, −NHCHC*H*₂C₆H₃(OH)₂COOCH₃), 3.15−4.01 (br, −OC*H*₂C*H*₂O−; −OC*H*CH₃C*H*₂O−; −NHC*H*(CH₂C₆H₃(OH)₂COOC*H*₃), 4.03−4.26 (d, −OCH₂C*H*₂OCONHCHCH₂C₆H₃(OH)₂COOCH₃), 4.55 (m, −NHC*H*CH₂C₆H₃(OH)₂COOCH₃), 5.30 (d, −N*H*CHCH₂-C₆H₃(OH)₂COOCH₃), 6.45−6.77 (1s, 2d, −NHCHCH₂-C₆*H*₃(OH)₂COOCH₃).

Synthesis of DOPA−Pluronic F68 (DOPA-PF68). A procedure similar to that described above for the synthesis of **DOPA-PF127** conjugate was used to prepare and purify **DOPA-PF68**. The product yield was 49%. ^{1}H NMR (500 MHz, CDCl₃): δ, ppm 0.92−1.50 (br, −OCHC*H*₃CH₂O−), 2.91−3.10 (m, −NHCHC*H*₂C₆H₃(OH)₂COOH), 3.15−3.95 (br, −OC*H*₂C*H*₂O−; −OC*H*CH₃C*H*₂O−), 4.06−4.30 (d, −OCH₂C*H*₂OCO NHCHCH₂C₆H₃(OH)₂COOH), 4.54 (m, −NHC*H*CH₂C₆H₃(OH)₂COOH), 5.35 (d, −N*H*CHCH₂-C₆H₅(OH)₂COOH), 6.50−6.80 (1s, 2d, −NHCHCH₂C₆*H*₃-(OH)₂COOH).

DOPA Assay. Coupling efficiencies of DOPA methyl ester and DOPA to Pluronics F127 and F68 were determined by the DOPA assay of Waite and Benedict[39] using a Hitachi U-2010 spectrophotometer (Hitachi Instruments, San Jose, CA). Briefly, samples were analyzed in triplicate by diluting aliquots of standards or unknown solutions with 1 N HCl to a final volume of 0.9 mL. Then 0.9 mL of nitrite reagent (1.45 M sodium nitrite and 0.41 M sodium molybdate dihydrate) was added to the DOPA solution, followed immediately by the addition of 1.2 mL of 1 N NaOH. Due to time-dependent changes in absorbance intensity, care was taken to ensure that the time between the addition of NaOH and recording of the absorbance was 3 min for all standards and samples. The absorbance was recorded at 500 nm for all standards and samples. DOPA was used as the standard for both DOPA methyl ester−Pluronics and DOPA−Pluronics.

Rheology. Rheological measurements of the gelation process were performed using a Bohlin VOR Rheometer (Bohlin Rheologi, Cranbury, NJ). A 30 mm diameter stainless steel cone and plate geometry with a cone angle of 2.5° was used for all measurements. The temperature was controlled by a circulating water bath. 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. Samples were cooled in the refrigerator prior to transfer of 0.5 mL of liquid solution to the apparatus. Measurements of storage and loss moduli, G' and G'', were taken in the oscillatory mode at 0.1 Hz and a strain of 0.45%. The strain amplitude dependence of the viscoelastic data was checked for several samples, and measurements were only performed in the linear range where moduli were independent of strain amplitude. Mineral oil

was applied to a ring surrounding the outer surfaces of the sample compartment to prevent dehydration during measurements.

Viscometry of solutions containing block copolymers, bovine submaxillary mucin, and their mixtures was performed as described.[40−43] Mucin was hydrated with distilled water by gentle stirring at room temperature for 1 h to yield a 10.6 wt % solution. Separately, block copolymers were dissolved in distilled water to yield a 10 wt % solution, and the two polymer solutions were mixed to yield a fixed final mucin concentration of 6.55 wt % with variable block copolymer concentration. The mixtures were stirred for 1 h at room temperature, after which viscosity measurements were carried out in the cone and plate geometry at 25 °C and shear rates ranging from 2.02 to 507 s^{-1}. All viscometric experiments were performed in triplicate and results reported in the form of means and standard deviations.

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 hermetically sealed aluminum pans were scanned at a heating and cooling rate of 3 °C/min with an empty pan as reference.

Absorption Spectroscopy. The micellization of DOPA-modified Pluronics in solution was detected using the dye 1,6-diphenyl-1,3,5-hexatriene (DPH), which exhibits a shift in UV absorption when partitioned into the nonpolar environment of the micelle core.[44] DPH-containing polymer solutions were prepared by dissolving solid DOPA-modified Pluronics in Nanopure H$_2$O and diluting to either 2.5% or 5.0% (w/w). A stock solution of DPH in methanol (0.4 mM) was then added to the polymer solutions to achieve a final DPH concentration of 0.004 mM, and the solutions were incubated in the dark at 4 °C overnight. Although the final polymer solutions contained methanol at a level of approximately 1%, probe solvents such as methanol do not measurably affect micellization behavior at this concentration.[45,46] Absorption spectra were measured from 200 to 500 nm using a Hitachi U-2010 spectrophotometer (Hitachi Instruments, San Jose, CA). To eliminate the possible interference of DOPA ($\lambda_{max} = 280$ nm, the tail of which extended into the DPH absorption region) in the detection of DPH absorbance changes associated with micelle formation, the reference cuvette contained a DPH-free copolymer solution during all measurements. Temperatures of the reference and sample solutions were measured with a thermocouple and a Fluke 52 II thermometer. Cuvette temperatures were controlled by a circulating water bath (Neslab RTE−111 circulator, Newington, NH) and measured using a Fluke 52 II thermocouple. Both sample and reference solutions were allowed to equilibrate for at least 45 min at each temperature prior to measurement, and up to 2 h near the cmt.
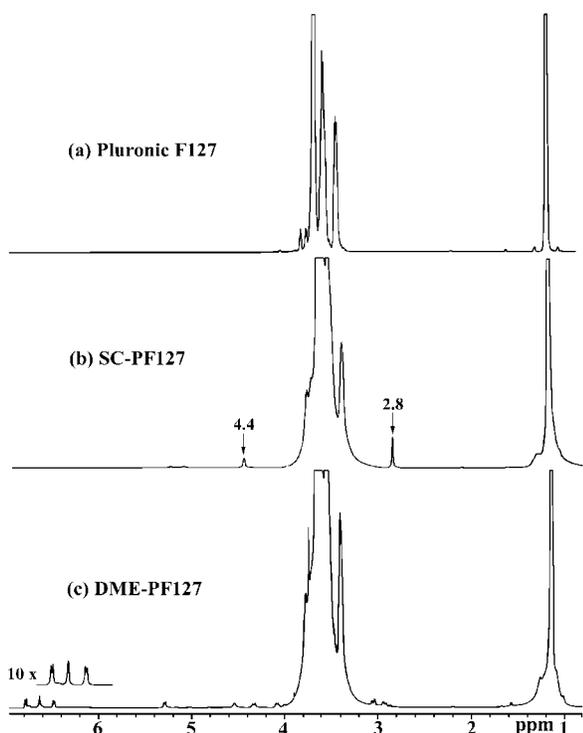
Results and Discussion

Synthesis of DOPA-Modified PEO—PPO—PEO Block Copolymers. Despite the numerous applications of Pluronics,

relatively few attempts have been made to subject them to chemical modification.[22,23,47−49] Succinimidyl carbonate has been recognized as a useful reagent for activating hydroxyl groups of small organic compounds and PEG molecules to form bioconjugates with urethane linkages.[50,51] In this work, succinimidyl carbonate was used to activate the hydroxyl groups of Pluronic F127 and Pluronic F68 in the presence of DMAP. Activated Pluronics **SC-PF127** and **SC-PF68** can be stored as solids in a desiccator at −20 °C for several months without loss of activity.

The succinimidyl carbonate conjugates were determined to be useful intermediates for the coupling of DOPA moieties into Pluronics. Recognizing that the self-assembly and bioadhesive properties of the block copolymers may be affected by end group charge, we synthesized conjugates containing either uncharged (DOPA methyl ester, DME) or charged (DOPA) end groups; the latter is expected to be negatively charged at physiologic pH. Furthermore, it should be noted that the free carboxylic acid in **DOPA-PF127** and **DOPA-PF68** can be funtionalized using standard peptide chemistry to further tailor the properties of the block copolymers; on the other hand it might also be used to study the pH effect on the properties of the block copolymers. Upon the basis of significant solubility differences between free DOPA and DME, we therefore employed two separate routes to couple DOPA and DME to succinimidyl carbonate activated Pluronics. To synthesize uncharged block copolymers, we first prepared DME by the reaction of DOPA with methanol in the presence of thionyl chloride,[37] which was subsequently coupled to **SC-PF127** and **SC-PF68** in organic solvents. The reaction progress was monitored by TLC and NMR, which indicated that the coupling reaction was virtually complete in 1 h (data not shown). After purification from cold methanol, **DME-PF127** and **DME-PF68** were obtained with high product yields.

To make DOPA-modified Pluronic conjugate with free carboxylic acid groups on the DOPA moieties, we performed the coupling of pure DOPA with succinimidyl carbonate activated-Pluronic in alkaline aqueous solution. There have been several reports of the introduction of DOPA into peptides in solid- and liquid-phase chemistry.[33,52−55] It is well-known that one obstacle in working with DOPA is its ease of oxidation (to DOPA−quinone and other products), which readily occurs in alkaline aqueous solutions.[52,53] To prevent unwanted oxidation of DOPA catechol side chains during coupling under alkaline conditions, a borate-protected DOPA was first formed by adding DOPA to aqueous sodium borate (Scheme 1). The resulting complex is remarkably stable in neutral or alkaline solutions,[53] and can be readily deprotected under acidic conditions. Taking advantage of complexation between DOPA and borate, we successfully coupled DOPA to the ends of Pluronic copolymers under alkaline aqueous conditions to yield **DOPA-PF127** and **DOPA-PF68**. Visual inspection of the reaction solution revealed the absence of strongly absorbing DOPA−quinone, an indication that DOPA remains unoxidized during the reaction. At the completion of the reaction, acidification with HCl resulted in deprotection of the DOPA end groups of the block copolymer.

Figure 1. ^{1}H NMR spectra of Pluronic F127, **SC-PF127**, and **DME-PF127** in CDCl₃.

Table 1. Coupling Efficiency and Product Yield of DOPA-Modified Pluronics

	coupling efficiency (%)[a]	product yield (%)
DME-PF127	78.0 ± 4.0	75.0 ± 5.0
DOPA-PF127	80.0 ± 4.0	52.0 ± 3.0
DME-PF68	76.0 ± 2.0	76.0 ± 4.0
DOPA-PF68	81.0 ± 2.0	49.0 ± 2.0

[a] Determined by colorimetric analysis.[30]

Both ^{1}H NMR spectra and DOPA colorimetric assay confirmed the compositions of the succinimidyl activated reaction intermediates and all four DOPA-modified Pluronics. Shown in Figure 1 are ^{1}H NMR spectra of Pluronic F127, succinimidyl carbonate activated Pluronic F127 (**SC-PF127**), and DOPA methyl ester modified Pluronic F127 (**DME-PF127**). In the spectra of **SC-PF127** and **SC-PF68**, the sharp peak at ∼2.8 ppm is due to the −CH_2- protons from the succinimidyl carbonate group, and the peak at ∼4.4 ppm is attributed to the −CH_2−O− protons from the ethylene oxide group adjacent to the succinimidyl carbonate group. These peaks were absent in the ^{1}H NMR spectra of DOPA-containing Pluronics, and a series of new peaks appear due to the introduction of DOPA moieties into the copolymers. A characteristic feature of the ^{1}H NMR spectra of DOPA-containing Pluronics is the appearance of one singlet and two doublets in the range of 6.5−6.9 ppm corresponding to the three protons on the DOPA phenyl ring. The assignments for the catechol hydroxyl protons could not be found, which is consistent with previous studies.[56] Similar features were also observed in the ^{1}H NMR spectrum (not shown) of DOPA−Pluronic conjugates synthesized from aqueous solution. On the basis of the assumption of two available succinimidyl carbonate groups in **SC-PF127** and **SC-PF68**, coupling efficiencies of DME and DOPA to these two Pluronics were quantitatively found to be in the range from 76% to 81% as obtained from colorimetric analysis (Table 1). The reported coupling efficiencies are the average values of at least three repeated syntheses performed under the same conditions and were not found to increase significantly when a larger excess of DOPA was used in the reaction. Similar coupling efficiencies were also found for **DOPA-PF127** and **DOPA-PF68** made from aqueous solutions, suggesting that the presence of Na₂B₄O₇ did not significantly affect the

coupling reaction. On the other hand, the yields of DOPA-modified Pluronics (shown in Table 1) were found to be somewhat lower when synthesized in aqueous solution than in organic solvent. This may be due to the surfactant properties of Pluronics, causing the low efficiency of extraction of DOPA-modified Pluronic with dichloromethane from water. The four DOPA-modified Pluronics could be stored at −20 °C indefinitely with no discoloration or change in properties.

Aggregation Behavior of DOPA-Modified PEO─PPO─PEO Block Copolymers. It is widely acknowledged that PEO−PPO−PEO block copolymers self-assemble in a concentration and temperature-dependent manner into micelles consisting of a hydrophobic PPO core and a water-swollen corona consisting of PEO segments.[14,15,57−60] At high concentration, certain PEO−PPO−PEO block copolymers, such as F127 and F68, transform from a low viscosity solution to a clear thermoreversible gel at elevated temperature. It is generally believed[14] that an increase in the number of polymer chains associating into micelles at elevated temperature leads to the formation of a gel phase, which is stabilized by micelle entanglements. The micellization and gelation processes have been found to depend on factors such as block copolymer molecular weight, relative block sizes, solvent composition, polymer concentration, and temperature.[14,60,61] For example, increasing the length of the hydrophilic PEO blocks relative to the hydrophobic PPO block results in an increase in critical micellization temperature (cmt) and gelation temperature (T_{gel}).[62]

The aggregation of **DME-PF127** and **DOPA-PF127** block copolymers into micelles at the cmt was detected using differential scanning calorimetry (DSC) and a dye partitioning method.[45] DPH, the dye used for the determination of the cmt, is a well-known probe of membrane interiors and molecular aggregation, previously reported for the analysis of unmodified Pluronics.[44,60] In the present study, the intensity of the main absorption peak characteristic of DPH at 356 nm was recorded as a function of temperature through the cmt. Figure 2a shows the absorption intensity of a 0.004 mM DPH/2.5 wt % **DME-PF127** aqueous solution as a function of temperature. Below approximately 20 °C, the DPH absorbance intensity was low, reflecting the existence of a hydrophilic environment consisting of copolymer molecules dissolved in water. At the cmt the absorbance of DPH undergoes a significant increase, indicating the partitioning of DPH into a more hydrophobic environment (micelle core) resulting from the formation of block copolymer micelles. The DPH absorbance continued to increase with temperature until a plateau was reached approximately 5 °C above the cmt, reflecting either the completion of micelle formation or the depletion of DPH from the aqueous
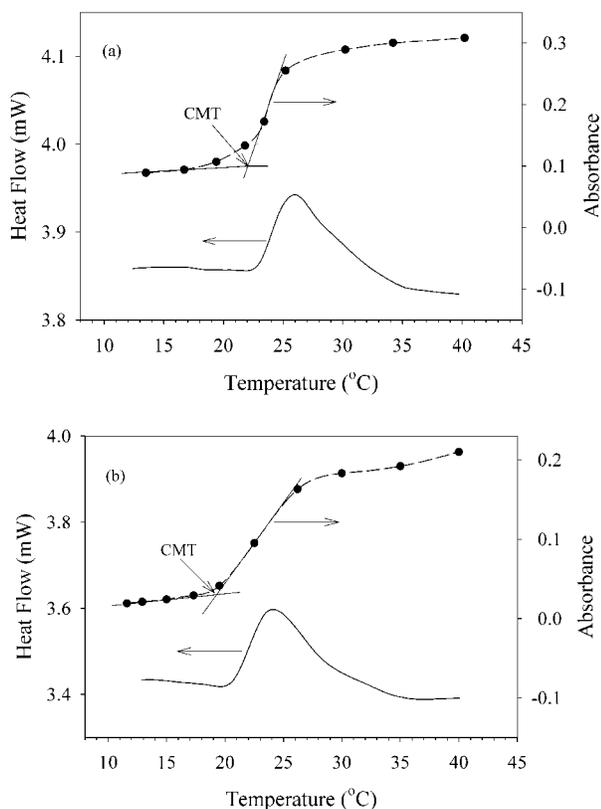
Figure 2. Micellization of (a) 2.5 wt % and (b) 5 wt % solution of **DME-PF127** detected using absorption changes of DPH (356 nm) and differential scanning calorimetry. The estimation of the critical micellization temperature (cmt) from the DPH solubilization method was determined using the intersection of the tangent lines as indicated on the plots.

medium.[44] The cmt was defined as being the onset temperature of the DPH absorption intensity vs temperature sigmoidal curve,[60] and was estimated to be 22.0 ± 1.0 °C for a 2.5 wt % **DME-PF127** solution. Increasing the concentration of **DME-PF127** to 5 wt % resulted in a decrease in the cmt to 19.0 ± 1.0 °C (Figure 2b).

DSC thermograms of DOPA-modified Pluronics at low concentration (<17 wt %) were characterized by a single broad endothermic peak that was attributed to micelle formation (Figure 2).[14] The cmt determined from the DPH partitioning method was found to occur a few degrees below the onset of the micellization endotherm obtained by DSC. The reason for this discrepancy is likely to be in the nonequilibrium nature of the DSC experiment. Whereas in the DPH experiment the copolymer solutions were allowed to reach thermal equilibrium prior to measurement, in the DSC experiment the samples were continually heated at a rate of 3 °C/min through the cmt and therefore were not in thermal equilibrium. Although the formation of micelles is thermodynamically driven, it is also a kinetic process requiring diffusion and assembly of block copolymer chains. Thus, in a nonequilibrium experiment (DSC) it is reasonable to expect that micelle formation will be detected at a higher temperature.

The coupling of DOPA or DME to the hydroxyl end groups of PEO−PPO−PEO block copolymers resulted in changes in the cmt compared to unmodified Pluronics. For example, the cmt and the temperature at maximum heat
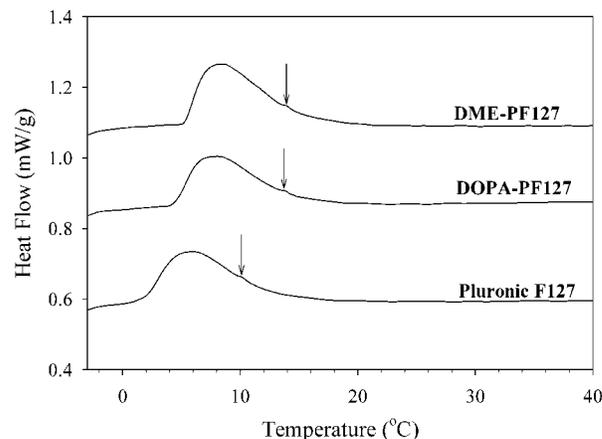
Figure 3. Differential scanning calorimetry thermograms of 30 wt % **DME-PF127**, **DOPA-PF127**, and unmodified Pluronic F127 aqueous solutions. Arrows indicate the location of the gelation endotherm.

capacity were found to be lower for unmodified Pluronic F127 than for **DME-PF127** and **DOPA-PF127**, although the specific enthalpies determined from the areas under the transition were approximately the same (Figure 3 and Table 2). All samples containing greater than 20 wt % copolymer contained a small endothermic peak approximately 10 °C above the cmt, which correlated strongly with gelation as determined by rheometry (described below) and the vial inversion method, and was therefore attributed to the sol−gel transition. This interpretation is in agreement with previous calorimetry studies of unmodified Pluronics,[57] which demonstrated that the broad peak at low temperature is due to micellization while the small peak at higher temperature, only observed in concentrated solutions, corresponds to gelation, a nearly athermal process. Thus, the enthalpies shown in Table 2 include contributions from both micellization and gelation, however due to the small enthalpy of gelation the observed enthalpy changes can be largely attributed to micellization. The micellization peak was seen to extend to temperatures above the onset of gelation, indicating that additional monomers aggregate into micelles at temperatures above the gelation point.[14,59]

Additional DSC studies were performed to more fully explore the concentration dependence of **DOPA-PF127** and **DME-PF127** aggregation. DSC thermograms of **DOPA-PF127** and **DME-PF127** solutions indicate a decrease in the cmt with increasing polymer concentration as shown in Figure 4. Extrapolation of the onset temperatures for micellization and gelation from the DSC data shown in Figure 4 revealed general trends in the concentration dependence of micellization and gelation for the synthesized polymers (Figure 5) and also permitted direct comparison between unmodified Pluronic F127, **DOPA-PF127**, and **DME-PF127**.

Gelation Behavior of DOPA-Modified PEO─PPO─PEO Block Copolymers. The detailed gelation behavior of DOPA-modified PEO−PPO−PEO block copolymers was determined for aqueous solutions containing 10−30% (w/w) of **DOPA-PF127** or **DME-PF127** and 35−54% (w/w) of **DOPA-PF68** or **DME-PF68**. Thermal gelation of concentrated solutions was initially assessed using the simple vial inversion method, in which the gelation temperature (T_{gel}) is defined as the temperature at which the solution no

Table 2. Temperature of Micellization and Gelation of 30 wt % **DME-PF127**, **DOPA-PF127**, and Unmodified Pluronic F127 Solutions[a]
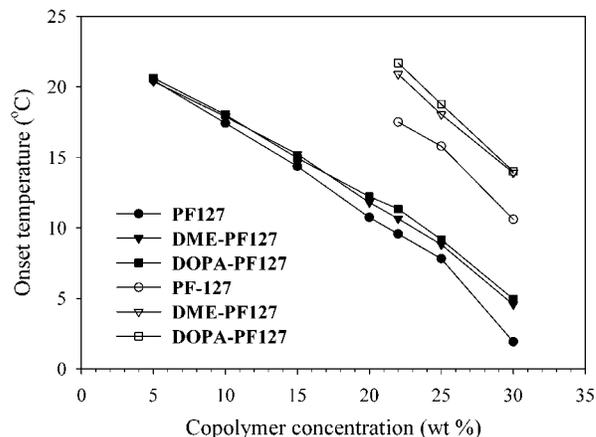
	micellization temp (°C)	temp at max heat capacity (°C)	ΔH (J/g)	gel temp (°C)
DME-PF127 (30 wt %)	5.2 ± 0.2	8.3 ± 0.1	20.3 ± 2.4	14.0 ± 0.4
DOPA-PF127 (30 wt %)	4.6 ± 0.2	8.0 ± 0.6	19.3 ± 1.4	14.0 ± 0.2
Pluronic F127 (30 wt %)	1.9 ± 0.3	6.0 ± 0.4	20.6 ± 1.6	10.6 ± 0.6

[a] Determined by extrapolation of onset temperature of micellization and gelation endotherms.
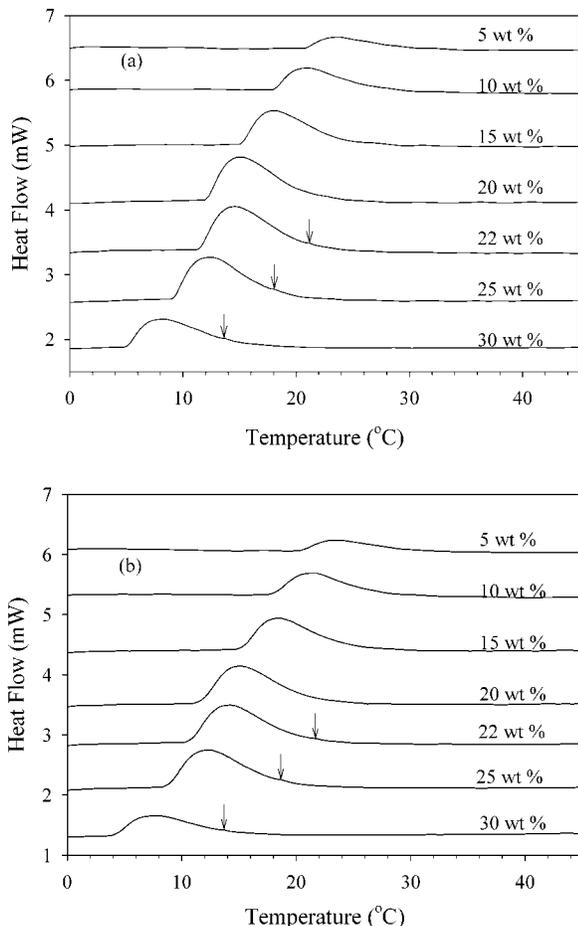
Figure 4. Differential scanning calorimetry thermograms of (a) **DOPA-PF127** and (b) **DME-PF127** at different concentrations. Arrows indicate the location of the gelation endotherm, observed only at polymer concentrations greater than 20 wt %.


Figure 5. Concentration dependence of onset temperature of micellization (filled symbols) and gelation (open symbols) for unmodified Pluronic F127, **DME-PF127**, and **DOPA-PF127**. Onset temperatures were extrapolated from the data shown in Figure 4.

longer flows upon inversion.[15] The gelation temperature of DOPA-modified Pluronics was found to be strongly dependent on copolymer concentration and block copolymer molecular weight (i.e., F127 vs F68), and the gels were found to be resistant to flow over long periods of time. Within the limitations of this simple experimental technique, the gel temperatures were found to be nearly identical for both free acid and methyl ester forms of DOPA-polymer conjugates. For example, 22 wt % solutions of **DOPA-PF127** and **DME-PF127** were found to form a transparent gel at the same temperature, approximately 22.0 ± 1.0 °C, which is approximately 5 °C higher than unmodified Pluronic F127 (17.0 ± 1.0 °C) at the same concentration. Decreasing the concentration of **DOPA-PF127** and **DME-PF127** to 18 wt % resulted in gelation at approximately 31.0 ± 1.0 °C. While solutions of **DOPA-PF127** and **DME-PF127** with concentrations as low as 17 wt % formed gels detectable by the vial inversion method, only solutions with polymer concen-

trations above 20% exhibited an identifiable gelation endotherm in DSC thermograms (indicated by arrows in Figures 3 and 4).

The gelation behavior of **DME-PF68** was qualitatively similar to **DME-PF127**, except that much higher polymer concentrations were required to form **DME-PF68** gels. 54 wt % solutions of **DOPA-PF68** and **DME-PF68** formed gels at 23.0 ± 1.0 °C, which is approximately 16 °C higher than the gel temperature of unmodified Pluronic F68 (7 ± 1.0 °C) at the same concentration. Decreasing the concentration of **DOPA-PF68** and **DME-PF68** to 50 wt % resulted in an increase in gel temperature to 33.0 ± 1.0 °C, and solutions with **DOPA-PF68** and **DME-PF68** concentrations less than 35 wt % did not form gels when heated to 60 °C.

The viscoelastic properties of DOPA-modified PEO−PPO−PEO block copolymer hydrogels were further studied by oscillatory rheometry. Figure 6 shows the elastic storage modulus, G', of 22 wt % solutions of unmodified Pluronic F127 and **DME-PF127** aqueous solutions as a function of temperature. Below the gelation temperature, storage modulus G' was negligible, however G' increased rapidly at the gel temperature (T_{gel}), defined in this case as the onset of the increase of the G' vs. temperature plot.[24] **DOPA-PF127** exhibited a similar rheological profile (not shown). The rheologically determined T_{gel} of 22 wt % solutions of **DME-PF127** and **DOPA-PF127** were found to be identical (20.3 ± 0.6 °C), which is approximately 5° higher than an equivalent concentration of unmodified-Pluronic F127 (15.4 ± 0.4 °C). G' of **DME-PF127** or **DOPA-PF127** approached a plateau value of 13 kPa, which is comparable to that of unmodified Pluronic F127 and in agreement with results reported by other investigators.[63] T_{gel} determined by rheometry was found to coincide with the gel temperature found from DSC and the vial inversion method (Table 3).
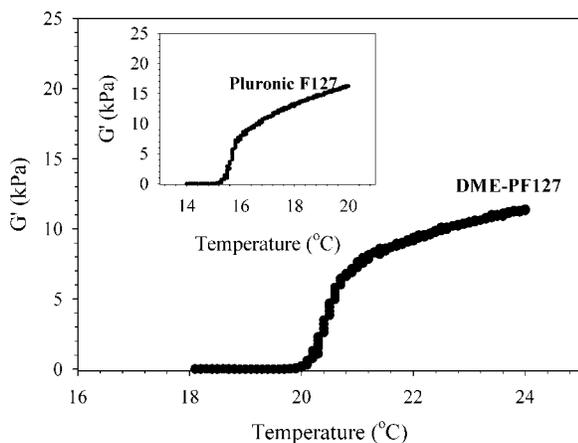
Figure 6. Shear storage modulus, G', of a 22 wt % **DME-PF127** aqueous solution as a function of temperature at 0.1 Hz and a strain of 0.45%. Shown in the inset is the rheological profile of a 22 wt % unmodified Pluronic F127 aqueous solution as a function of temperature.

Table 3. Comparison of Gelation Temperatures Obtained from Vial Inversion Method, Rheology, or Differential Scanning Calorimetry for 22 wt % **DME-PF127**, **DOPA-PF127**, and Pluronic F127 Solutions

	gel temp (°C)		
	vial inversion method	rheological	DSC
DME-PF127 (22 wt %)	22.0 ± 1.0	20.3 ± 0.6	20.9 ± 0.1
DOPA-PF127 (22 wt %)	22.0 ± 1.0	20.4 ± 0.5	21.7 ± 0.2
Pluronic F127 (22 wt %)	17.0 ± 1.0	15.4 ± 0.4	17.5 ± 0.4
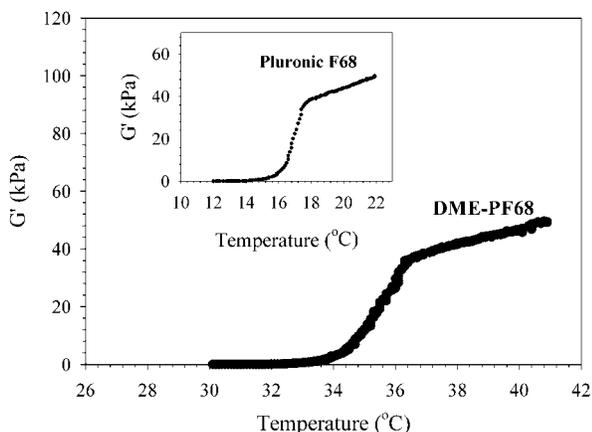
Figure 7. Shear storage modulus, G', of a 50 wt % **DME-PF68** aqueous solution as a function of temperature at 0.1 Hz and a strain of 0.45%. Shown in the inset is the rheological profile of a 50 wt % unmodified Pluronic F68 aqueous solution as a function of temperature.

Shown in Figure 7 are the rheological profiles of 50 wt % solutions of unmodified Pluronic F68 and **DME-PF68** as a function of temperature. The T_{gel} of a 50 wt % **DME-PF68** solution was found to be 34.1 ± 0.6 °C, whereas the T_{gel} of an equivalent concentration of unmodified Pluronic F68 was approximately 18 °C lower (16.2 ± 0.8 °C). The plateau storage moduli of 50 wt % solutions of **DME-PF68** and unmodified Pluronic F68 were not significantly different, approaching a plateau value as high as 50 kPa. Tailoring the gelation temperature of **DME-PF68** was accomplished by altering the polymer concentration as illustrated in Figure 8, which shows the rheological profile of **DME-PF68** at two
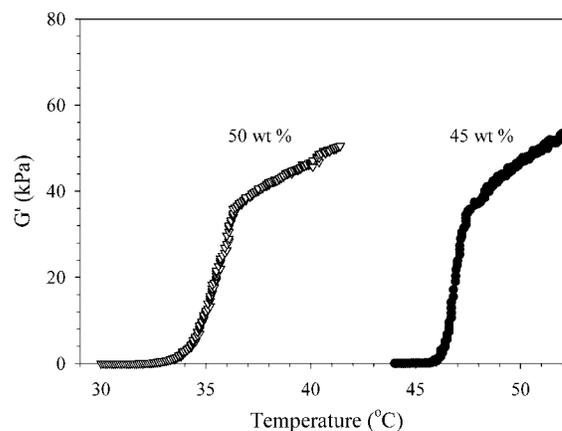

Figure 8. Storage moduli of **DME-PF68** aqueous solutions at 45 and 50 wt %, respectively, as a function of temperature at 0.1 Hz and a strain of 0.45%.

different concentrations as a function of temperature. T_{gel} of 45 wt % solution of **DME-PF68** was observed to be approximately 12 °C higher than that of 50 wt % solution of **DME-PF68**, which is in agreement with the trend of increasing T_{gel} with decreasing concentration as reported in the literature.[63]

DOPA can be considered a hydrophilic moiety due to the presence of the dihydroxyphenyl (catechol) side chain. Thus, coupling of DOPA or DME to the ends of the PEO block can be considered an extension of the hydrophilic PEO segments. The observed increase of T_{gel} in the DOPA-modified Pluronics, compared with that of unmodified Pluronics, is likely due to the increase in molecular weight of the hydrophilic PEO segments resulting from coupling of DOPA to the end groups. It is also clear from the data shown in Figures 6 and 7 that the coupling of DOPA or DME to the Pluronic end groups has a more significant impact on the T_{gel} of F68 compared to F127. This can be rationalized in terms of the overall molecular weights of F68 (approximately 8600) and F127 (approximately 12 600). Addition of DOPA and DOPA methyl ester to both end groups using the chemistry shown in Scheme 1 results in an increase in molecular weight of 446 and 474, respectively. This represents a larger fractional molecular weight increase for F68 compared to F127, due to the lower base molecular weight of F68.

Previous efforts to develop DOPA-mimetic adhesive polymers have provided important practical evidence that these materials may be useful as adhesives.[26,32,33,64] However, the approaches previously used to employ DOPA-mimetic polymers as adhesives typically involve the use of oxidizing agents as the primary means to cross-link the adhesive.[33] Not only is the potential use of strong oxidizing agents in medical applications cause for concern, but current understanding of the adhesive behavior of DOPA suggests that this strategy results in a less adhesive (i.e., oxidized) form of DOPA.[31] By utilizing a thermogelling polymer system, our strategy eliminates the need for use of oxidizing agents to induce polymer gelation, and it preserves the DOPA in its most adhesive form.

To illustrate the bioadhesive potential of DOPA-modified Pluronics, we investigated mucoadhesive interactions be-
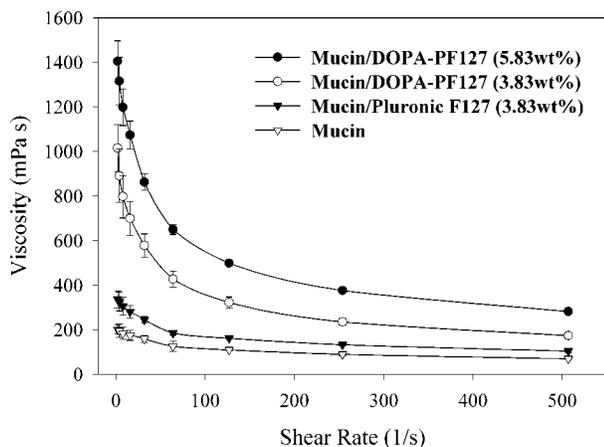
Figure 9. Effect of **DOPA-PF127** and Pluronic F127 on the viscosity of mucin from bovine submaxillary glands as a function of shear rate (1/*s*) at 25 °C. The concentration of bovine submaxillary mucin was constant at 6.55 wt % for all samples. The viscosity of 3.83 wt % solutions of **DOPA-PF127** and Pluronic F127 were less than 1 mPa s at all shear rates tested (data not shown).

tween **DOPA-PF127** and bovine submaxillary mucin, a high molecular weight glycoprotein. Mucin contains O-linked carbohydrate chains of up to 15 or more carbohydrates, and is characterized by enormous variation in monosaccharide composition (i.e., glucose, galactose, galactosamine, glucosamine and sialic acid).[40,65,66] The mucoadhesive properties were evaluated by the Hassan method, in which the forces of interaction between mucin and a bioadhesive polymer are probed by solution viscosity measurements.[41] In this method, the measured viscosity of a solution containing mucin and a bioadhesive polymer is considered to be the result of the contributions of the viscosity of the mucin and the bioadhesive polymer and a contribution due to interaction between the two (i.e., bioadhesion):

$$\gamma_{measured} = \gamma_{mucin} + \gamma_{polymer} + \gamma_{bioadhesion} \qquad (1)$$

Thus, for a poorly bioadhesive polymer, the measured viscosity is little more than the sum of the viscosities of the mucin and polymer, whereas for a bioadhesive polymer the measured viscosity significantly increases, reflecting interactions between the mucin and polymer. This method has been previously used to evaluate mucoadhesive polymers such as poly(vinyl alcohol), hydroxypropylcellulose, and Carbopol.[40,41]

Figure 9 shows the effect of **DOPA-PF127** and Pluronic F127 on the viscosity of bovine submaxillary mucin (6.55 wt %) at 25 °C. It can be seen that the viscosity of the mucin/Pluronic F127 mixture was only slightly higher than that of mucin over a wide range of shear rates. On the contrary, the viscosities of mucin−**DOPA-PF127** mixtures were substantially higher than that of mucin only, of mucin/Pluronic F127 mixtures, and of Pluronic F127 solution (not shown). All mucin and mucin/block copolymer mixtures exhibited shear-thinning behavior, which was particularly evident for mixtures of mucin and **DOPA-PF127**. In the **DOPA-PF127** concentration range tested (3.83−5.83 wt %), the viscosity of the mucin/**DOPA-PF127** mixtures increased with increasing **DOPA-PF127** concentration, an effect that cannot be due to gelation of the block copolymer, since **DOPA-PF127**

solutions do not form gels at concentrations below approximately 20 wt % (Figure 4). For the mixture of mucin (6.55 wt %) and **DOPA-PF127** (3.83 wt %), analysis of the viscosity data resulted in $\gamma_{bioadhesion} = 504.5$ mPa s (at a shear rate of 24s^{-1}). This value is comparable to $\gamma_{bioadhesion}$ obtained for a mixture of mucin (15 wt %) and Pluronic F127−poly-(acrylic acid) (1 wt %) mucoadhesive polymer.[67]

We attribute the enhanced viscosity of the mucin−**DOPA-PF127** mixture to the mucoadhesive properties conferred by the presence of DOPA. The catechol side chains of DOPA residues are capable of undergoing intermolecular OH bonds with the carbohydrate hydroxyl groups and the carbonyl groups on the *N*-acetyl residues of galactosamine, glucosamine and sialic acid in the mucin, thus enhancing the strength of interaction between the block copolymer and the mucin.[36] It is also important to note that the **DOPA-PF127** polymer was in the form of micelles at the concentration and temperature employed in this experiment. Given that the aggregation number of PF127 in water is believed to be approximately 50,[68] each **DOPA-PF127** micelle could contain as many as 100 DOPA residues at the surface, which could further enhance the mucoadhesive interactions.

Conclusions

Succinimidyl carbonate activated PEO−PPO−PEO block copolymers were synthesized and reacted in either organic or aqueous solvent to yield block copolymers containing DOPA and DOPA methyl ester end groups. The efficiency of DOPA coupling to the block copolymer end groups was approximately 80%. The resulting DOPA-modified block copolymers were readily soluble in water and underwent a micellization transition at a temperature higher than the corresponding unmodified Pluronics. Concentrated solutions of DOPA-modified Pluronics exhibit temperature-induced gelation which was found to be dependent on the composition and molecular weight of the copolymer, and the concentration of the copolymer in aqueous solution. Sol−gel transitions of DOPA-modified Pluronic F127 could be tailored between ∼22 and 31 °C by changing the polymer concentration, whereas DOPA-modified Pluronic F68 exhibited sol−gel transitions between ∼22 and 46 °C. Viscometry measurements demonstrated that DOPA-modified Pluronic is significantly more mucoadhesive than unmodified Pluronic.

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