Self-Assembled Poly(2-ethyl-2-oxazoline)/Malonic Acid Hollow Fibers in Aqueous Solutions

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ABSTRACT

Well-defined poly(2-ethyl-2-oxazoline) (PEOX)/Malonic Acid (MA) fibers having hollow tubular morphology were shown to form in aqueous solutions at 25 °C by complexation induced self-assembly between PEOX and MA. The fibers had diameter of ~1-3 μm and a wall thickness of ~40 nm. Different interactions between PEOX and MA were identified for complexation as a function of pH. At pH2, when both ends of MA were protonated, H-bonded complexation was the driving interaction in the fiber formation. IR data showed both PEOX -C=O band and MA -COOH band in dried fibers formed at pH2. The downshift in the -C=O stretching of PEOX by as much as 15 cm^{-1} confirmed the H-bonded complexation. The interaction enthalpy of PEOX and MA was determined by isothermal titration calorimetry (ITC) as -49.38 kJ/mol which is consistent with H-bonding. Thermogravimetric analysis (TGA) of the fibers showed two distinct decomposition temperatures one between 100-150 °C corresponding to MA and the other one at 350-450 °C corresponding to PEOX which also indicated the presence of both components in the fibers. At pH4, when one end of MA was protonated and the other end was ionized, electrostatic complexation between carboxylate ($-\text{COO}^-$) group of MA and the amide group of PEOX was the driving interaction in the fiber formation. At pH7, when both ends of MA were ionized, fiber formation was significantly hindered. The results are important in understanding the role of different interactions in the hollow fiber formation mechanism as a function of pH. pH-responsive hollow fibers have great potential to be used in biomedical applications for drug delivery and release purposes.

1. Introduction

Polymer self-assembly - a spontaneous process driven by non-covalent interactions – is commonly used to obtain ordered structures with different morphologies and properties at nanometer length scales [1-4]. The dynamic nature of non-covalent interactions allow these self-assembled structures to respond chemically or structurally to environmental changes which are quite important in various applications such as drug release, drug delivery, tissue engineering in the biomedical area [4,5], and sensors, multi-functional coatings and catalysis in material technologies [3,6]. Complexation induced self-assembly of block copolymers has also been demonstrated to be effective in tuning the morphology and the properties of various polymeric systems such as nanoporous materials, smart membranes and proton conducting materials [7,8].

Multiple hydrogen bonds (H-bonds) between simple building blocks have been cooperatively used to self-assemble complex, but well-defined reversible structures [9,10]. In polymer blends, H-bonds have been employed to enhance miscibility [11]. Polymers having proton accepting groups also complex with molecules having proton donating groups in solutions through hydrogen bonds [12-16]. H-bonded interpolymer complexes of poly(carboxylic acid)s and non-ionic polymers in aqueous solutions are important in pharmaceutical applications as pH-responsive materials [17]. For H-bonded complexes, poly(acrylic acid) (PAA) and poly(methacrylic acid) were commonly used as hydrogen donors and poly(acrylate)s, poly(alcohol)s, poly(saccharides) and poly(amide)s were used as hydrogen acceptors [17,18]. The onset of poly(N-vinylpyrrolidone) (PVP)/PAA complexation, aggregation, and the structure of the formed particles have been shown to depend on PAA molecular weight and solution composition [19]. H-bonding has been concluded to be the main factor in stabilizing the complexation of polyacrylamide (PAM) or poly(N-isopropylacrylamide) (PNIPAM) with PAA in aqueous solutions [12]. The pH dependent solubility of maleic acid-styrene copolymer and poly(vinylcaprolactam) H-bonded complexes was analyzed as a function of mixing ratio [13].

Mixing the two complexing components in solutions typically forms disordered aggregates. By separating the solutions of the two H-bonding components (H-acceptor and H-donor) and alternately adsorbing one component onto a substrate, it is possible to obtain relatively more ordered H-bonded layer-by-layer (LbL) films [20-24]. LbL films typically do not result in the formation of any ordered self-assembled structures, because the kinetically trapped adsorbed molecules have limited mobility and can not spontaneously relax [23]. The restructuring of free-floating poly(2-ethyl-2-oxazoline) (PEOX)/Tannic Acid (TA) LbL multilayers in an acidic phosphate buffer solution was recently shown to self-assemble to H-bonded, pH-responsive PEOX/TA fibers [25]. Water soluble polymers, such as poly(2-alkyl-2-oxazoline)s (PAOX) and polyvinylpyrrolidone (PVP), were also shown to self-assemble in dilute solutions through weak physical interactions [26-29].

Polyoxazolines (POX) are biocompatible polymers with the -N- end of the amide group on the polymer backbone and the -C=O end on the side chain [30-32]. They can exhibit lower critical solution temperature behavior in aqueous solutions. Physicochemical properties such as solubility in water, crystallinity, temperature-responsiveness, of PAOX polymers depend on the structure and the length of the side chains [33-35]. Driven by their biocompatibility together with exceptional physico-chemical properties, POX have recently been much studied especially regarding the utilization of microparticles, microspheres or microbeads, layer-by-layer capsules or films, micelles, polymersomes or nanogels in biological applications [24, 36-38]. On the self-assembly side, formation of crystalline nanofibers above the cloud point temperature (T_{cl}) in aqueous solutions of poly(2-isopropyl-2-oxazoline) (PIPOX) [26, 27] and PEOX [28] (Chart 1a) was previously reported. PEOX fiber formation was relatively slower than that of PIPOX and enhanced by addition of sodium salts [39].

Dicarboxylic acids are essential component of living systems, important in biological and also industrial processes [40]. Dicarboxylic acids have two pK_a values ($pK_{a1} < pK_{a2}$) which is useful in constructing self-assembled structures exhibiting more than one pH-responsive transitions. Dicarboxylic acids adopt an equilibrated structure in aqueous solutions depending on the pH [41]. Malonic Acid (MA) (Chart 1b) has $pK_{a1} = 2.8$ and $pK_{a2} = 5.7$. Complexation of PEOX-b-PCL (poly(ϵ -caprolactone)) micelles with multifunctional carboxylic acids, including malonic acid have previously been investigated [42]. The observation of intermicellar aggregation was attributed to the H-bond induced complexation between PEOX and carboxylic acids. In another study, the effect of monocarboxylic acids on T_{cl} of PEOX has been studied [43]. The observed shift in T_{cl} as a function of the molar ratio of acid to PEOX repeat units was also attributed to the H-bonding between the carboxylic acid and PEOX together with the hydrophobic interactions.

Chart 1. Chemical structures of (a) poly(2-ethyl-2-oxazoline) (PEOX), (b) Malonic Acid (MA), (c) schematic of bridging hydrogen bonds between PEOX and MA.

In aqueous MA solutions at pH2, ~90% of the MA molecules have both ends protonated and neutral. The protonated ends can make H-bonds with the amide groups of the PEOX chains, most probably bridging two different polymer chains as illustrated in Chart 1c. The ability of the rather short MA molecule to make H-bond on the same PEOX chain is significantly hindered. In this manuscript, we report that complexation between PEOX and MA induce self-assembly into well-defined fibers having hollow tubular morphology. The self-assembly process was investigated as a function of pH and the driving molecular interactions were discussed together with possible mechanisms. pH-responsive hollow fibers have great potential to be used in biomedical applications for drug delivery and release purposes.

2. Experimental

2.1 Materials

PEOX ($M_w \sim 200,000$ g/mol) was purchased from Alfa-Aesar. Malonic acid (MA) (molar mass = 104.06 g/mol) was purchased from Sigma-Aldrich. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck. All chemicals were used without further purification. Deionized water (DI) (18 M Ω -cm resistivity) was purified through a Milli-Q system (Millipore).

2.2 Formation of Fibers through Self-Assembly of PEOX and Malonic Acid

0.8 mg mL⁻¹ PEOX solution and 0.1 M MA solution was separately prepared in DI water. Equal volumes of PEOX and MA solutions were then taken and mixed. pH of the mixed solution was ~2, and the final concentrations of PEOX and MA became 0.4 mg mL⁻¹ and 0.05 M, respectively. pH adjustments were done as needed by addition of HCl or NaOH aqueous solutions. PEOX/MA solutions were placed in an incubator at 25 °C and observed daily. After ~7 days, tiny aggregates (~0.5-1.0 μ m) were visible to the eye which grew into larger aggregates. The aggregates were taken from the solution and characterized after ~15-20 days of incubation. Separate PEOX (0.4 mg mL⁻¹) and MA (0.05 M) aqueous solutions were prepared as reference. No fiber formation was observed in these solutions under the same conditions in 20 days.

To investigate the effect of pH on fiber formation, three different pH values (pH2, pH4 and pH7) were selected according to the pK_a values of MA (pK_{a1} = 2.8 and pK_{a2} = 5.7). At pH2 < pK_{a1} < pK_{a2}, both ends of MA are protonated; at pK_{a1} < pH4 < pK_{a2}, one end of MA is protonated and the other ionized; and at pK_{a1} < pK_{a2} < pH7, both ends of MA are deprotonated. pH of the PEOX/MA mixed solutions were adjusted using aqueous HCl or NaOH solutions. All prepared samples were kept in an incubator at 25 °C.

2.3 Characterization of Fibers

The fibers formed in the solutions were transferred to a 10 mL vial by Pasteur pipet and washed several times with DI water. The morphology of the fibers was characterized using optical microscopy (OM) (Leica LM DM) and field emission scanning electron microscopy (FESEM) (Zeiss Ultra Plus SEM). The fibers were transferred from the silicon wafer to carbon tape and analysis with FESEM by secondary electrons was done without any prior sputtering of conductive layer. The height and horizontal distance of the fibers were determined by atomic force microscopy (AFM) (Bruker Dimension) in tapping mode using silicon cantilevers. FTIR characterization of washed and dried fibers was performed by using a Thermo Scientific iS10 ATR-FTIR. Renishaw Invia Raman Spectrometer was used for the Raman spectroscopy of wet fibers. The fibers were first washed in water to remove free or weakly bound MA molecules and then the wet fibers were placed under the objective. 100 acquisitions were collected with x50 objective using 633 nm laser light. The interaction parameters between PEOX and MA were determined using isothermal titration calorimetry (ITC) (TA Instruments Affinity ITC) at 25°C. 0.05 mM PEOX aqueous solution was placed in sample cell (960 μ L) and 2.4 mM aqueous MA solution was placed in syringe (250 μ L). The titration was achieved by injection of MA solution in the syringe to the PEOX solution in the sample cell using 25 titrations, 10 μ L aliquots in each titration. Between each injection, there was 10 min intervals for equilibration and to ensure uniform mixing. The sample cell was continuously stirred at 125 rpm. Each injection produces a characteristic peak due to the absorbed or released heat. In the analysis of the data, baseline is subtracted from the data, since it corresponds to the signal between consecutive injections when no change in heat flow was detected. The raw heat data was integrated using the instrument software. After integration, to determine the thermodynamic parameters (ΔH , ΔS , ΔG) and the binding constant (K_a), independent binding model was used to fit the integrated data. The first data that derived from the first injection was neglected to ensure proper fitting to the model. Thermal stability of the fibers was characterized by thermogravimetric analyzer (TA Q500, TA instruments).

3. Results and Discussion

3.1 Hydrogen Bonded PEOX/Malonic Acid Fibers at $pH < pK_{a1} < pK_{a2}$

Aqueous PEOX (0.4 mg mL⁻¹) and MA (0.05 M) mixed solutions were prepared at pH2 and incubated at 25 °C. Small aggregates (~0.5-1.0 μ m) visible to the eye appeared in the solutions after ~7 days and grew into entangled micron sized fibrous structures in ~20 days. Fig. 1 shows the optical microscopy (OM) and SEM images of the dried fibrous structures collected from one such solution. The entangled meshes of the fibers having a range of different diameters (~1-2 μ m) are clearly seen in the OM images (Fig. 1a, 1b).

SEM image of the fibers (Fig. 1c) showed that the fibers had hollow tubular morphology which clearly differentiates them from ribbon like crystalline PEOX fibers formed in aqueous solutions above the cloud point temperature [28]. The hollow morphology was clearly observed with the fibers having the smallest diameter in the range of 700-1000 nm. The wall thickness of the fibers was $\sim 40 \pm 6$ nm.

The IR data of the dried fibers in the range of 1800 - 1525 cm^{-1} is seen in Fig. 1d together with those of PEOX and MA. In this range, PEOX ($-\text{C}=\text{O}$ band) was observed at 1629 cm^{-1} and MA ($-\text{COOH}$ band) showed a peak at 1695 cm^{-1} (intermolecular H-bonded MA) with a shoulder at 1721 cm^{-1} (residual water H-bonded to MA) [44]. Two peaks were observed in the spectra of the fibers at 1614 cm^{-1} and 1721 cm^{-1} indicating the presence of both PEOX and MA, respectively, in the fibers. The downshift of the PEOX $-\text{C}=\text{O}$ vibrational band by 15 cm^{-1} to 1614 cm^{-1} clearly indicates the presence of H-bonding interactions between PEOX and MA [45].

Fig. 1. a,b) Optical microscopy images of the PEOX/MA fibers formed in aqueous solutions at pH2 after 21 days: a) 2740 μm x 2055 μm ; b) 548 μm x 411 μm . c) SEM image of the PEOX/MA fibers seen in (a) and (b). Scale bar = 1 μm . d) IR data of PEOX, MA and dried fibers of PEOX/MA formed at pH2.

To understand the shifts in the IR data better, we have investigated the IR data of PEOX/MA blends systematically as a function of MA mole ratio. Fig. 2a shows the IR data of the blends having PEOX/MA

mole ratio in the range of 10 to 1. With increasing amount of MA in the blend, PEOX carbonyl band shifted from 1629 cm^{-1} for pure PEOX to 1594 cm^{-1} at PEOX/MA mole ratio of 1. MA carboxyl band shifted from 1695 cm^{-1} for pure MA to 1720 cm^{-1} at PEOX/MA mole ratio of 10. We attribute these shifts with increasing amount of MA in the blend to the increasing contribution of H-bonds between PEOX and MA. Comparing the shift of the vibrational bands for the PEOX/MA fiber (Fig. 1d) with the shifts in the blends (Fig. 2a), we estimate PEOX/MA mole ratio in the fibers as 8.3. Fig. 2b shows the TGA data of the fibers formed at pH2 together with those of PEOX and MA. MA shows a single thermal decomposition with onset at $\sim 150\text{ }^{\circ}\text{C}$ and PEOX shows a single thermal decomposition with onset at $350\text{ }^{\circ}\text{C}$. The fibers formed at pH2 showed two distinct decomposition temperatures one between $100\text{--}150\text{ }^{\circ}\text{C}$ and the other one at $350\text{--}450\text{ }^{\circ}\text{C}$ clearly indicating the presence of both MA and PEOX, respectively, in the fibers. At $350\text{ }^{\circ}\text{C}$, the total weight loss of the fibers was $\sim 11.2\%$. This weight loss, mainly due to the decomposition of MA with minor contributions from the adsorbed water, is quite consistent with the estimate of PEOX/MA mole ratio of 8.3 based on the IR band shifts.

Fig. 2. a) IR data of PEOX/MA blends. PEOX/MA mole ratio was changed from 1:0.1 to 1:1 (the direction of the curved arrows). **b)** TGA data of PEOX/MA fibers formed in aqueous solution at pH2 together with that of PEOX and MA.

Thus, we conclude that the fibers with a unique hollow tubular morphology form at pH2 by H-bonding between PEOX and MA. Although MA can form intermolecular H-bonds, any self-assembled structures of MA in aqueous solutions were not observed in the pH range of 2-7. Crystalline PEOX fibers were previously observed in aqueous solutions above the cloud point temperature [28]. However, the morphology of crystalline PEOX fibers -ribbon-like flat morphology- was very different from that of PEOX/MA fibers formed at pH2 -hollow tubular morphology. Polyvinylpyrrolidone (PVP) was previously reported to self-assemble into branched hollow fibers in an aqueous solution after aging the PVP solution for about two weeks [29]. The fiber formation mechanism was attributed to hydrophobic interactions originating from the polymer backbone and the methylene groups together with the possibility of bridging of the amide groups by solvent-mediated H-bonding. Although these interactions

also apply to PEOX in aqueous solutions, any self-assembled aggregates were not observed at 25 °C in the absence of MA. The complexation between PEOX and MA was investigated by ITC. According to the independent binding model, the thermodynamic interaction parameters enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) were determined from ITC data as -49.39 kJ/mol, -82.37 J/mol K and -24.83 kJ/mol, respectively (See Fig. S1-S2 in Supplementary Material). These values indicate that the interaction between PEOX and MA is enthalpy driven. We attribute the large exothermic enthalpy to the formation of hydrogen bonds between PEOX and MA. The measured enthalpy value is significantly larger compared to those of H-bonded interpolymer complexes [46] and of tannic acid/poly(alkyl oxazoline) complexes [47]. The rather fast and strong interaction between MA and PEOX as observed by ITC should form the precursor aggregates which are expected to self-assemble in a much slower rate by most probably a dynamic attachment/detachment mechanism.

3.2 Effect of pH on the Formation of Hydrogen Bonded PEOX/Malonic Acid Fibers

At $\text{pH} > \text{pK}_{a2} > \text{pK}_{a1}$, both ends of MA are ionized and deprotonated. Therefore, MA is not expected to make H-bonds with PEOX chains. To check whether the absence of H-bonds prevent the formation of hollow fibers, PEOX/MA solutions were investigated at pH7. At pH7, more than 95% of MA are doubly ionized and have carboxylate ($-\text{COO}^-$) groups at both ends.

Some fibrous aggregates were still observed at pH7 (Fig. 3c, 3f), but the total amount of fibers was significantly less than those obtained at $\text{pH} < \text{pK}_{a1}$. Enough fibers could not be collected at pH7 to pursue further thermal or spectroscopic characterization. In addition, the fibers were larger in diameter and showed different morphologies. We would like to emphasize that T_{cl} of the PEOX-200K solution containing MA was much larger than the incubation temperature of 25 °C. T_{cl} of PEOX (0.4 mg mL^{-1}) was measured as ~ 68 °C and did not show any significant change with pH in the range of pH2-pH7. In the presence of MA (0.05 M), T_{cl} of PEOX solutions (0.4 mg mL^{-1}) decreased to 63 °C at pH2, and to 60 °C at pH7. Therefore, we do not attribute the observation of fibers at pH7 to the slow crystallization process above T_{cl} as reported previously [28]. We hypothesize that electrostatic complexation of carboxylate groups ($-\text{COO}^-$) of MA and the amide groups ($-\text{N}-\text{C}=\text{O}$) of PEOX might have induced the formation of the observed fibers at pH7. The tertiary amide group has an exceptionally high dipole moment (4.6 D for N,N-dimethylacetamide) which makes the stable electrostatic complexation between the amide group and the carboxylate group very probable. The bridging of the PEOX chains might have been limited due to electrostatic repulsion between carboxylate ion complexed PEOX chains, since the other end of MA was also a carboxylate ion at pH7. Therefore, the amount of fibers was significantly less compared to that formed at pH2 and any further thermal or spectroscopic characterization could not be done.

Fig. 3. **a)** SEM image of the PEOX/MA fibers at pH2 (Scale bar = 200 nm), **b)** SEM image of the PEOX/MA fibers at pH4 (Scale bar = 200 nm), **c)** SEM image of the PEOX/MA fibers at pH7 (Scale bar = 1 μ m), **d)** AFM height image of the PEOX/MA fibers at pH2, **e)** AFM height image of the PEOX/MA fibers at pH4, **f)** AFM height image of the PEOX/MA fibers at pH7, **g)** AFM height profile of the fibers at pH2 along the solid line in (d), **h)** AFM height profile of the fibers at pH4 along the solid line in (e), **i)** AFM height profile of the fibers at pH7 along the solid line in (f).

The significant decrease in the amount of the observed fibers at pH7 clearly indicates the dominant role of H-bonded complexation between PEOX and MA at pH2 in the fiber formation mechanism. At pH7, ~95% of MA are deprotonated at both ends and therefore H-bonded complexation is not dominant. To check whether MA having one end protonated and the other deprotonated induced any fiber formation ($pK_{a2} > pH > pK_{a1}$), we have investigated the fiber formation at pH4 where the concentration of $HOOC-CH_2-COO^-$ is close to its maximum value of ~95%.

Fig. 3 compares the SEM (Fig. 3a-3c) and AFM height images (Fig. 3d-3f) of some typical fibers formed at pH2, pH4 and pH7. At pH4, the amount of fibrous structures after ~21 days was similar to that

obtained at pH2. The formed fibers (Fig. 3b) also had hollow tubular morphology similar to those formed at pH2 (Fig. 3a). In the AFM height image of Fig. 3e, a flat ribbon like fiber formed at pH4 is also seen in the lower right quadrant having a width of $\sim 1.5 \mu\text{m}$ and a height of $\sim 70 \text{ nm}$ (Fig. 3g). This might be the precursor of the larger diameter hollow tubular fibers. Such flat fibers were not observed at all at pH2.

The IR and TGA data of PEOX/MA fibers formed at pH2 and pH4 are compared in Fig. 4. Despite the observation that similar amounts of fibers having hollow tubular morphology were formed both at pH2 and pH4, there were clear differences in the IR and TGA data of the two. IR spectrum of fibers formed at pH2 showed two clear peaks at 1614 cm^{-1} corresponding to $-\text{C}=\text{O}$ stretching of PEOX and at 1721 cm^{-1} corresponding to $-\text{COOH}$ stretching band of MA. IR spectrum of fibers formed at pH4 showed PEOX peak at 1624 cm^{-1} and a shoulder at 1580 cm^{-1} . This shoulder corresponds to asymmetric stretching vibration of the carboxylate ($-\text{COO}^-$) group of MA. The lack of any visible shoulder at 1695 cm^{-1} in IR spectra of PEOX/MA fibers is due to the lack of intermolecular H-bonds between MA molecules. The fibers formed at pH2 and pH4 were also characterized by Raman spectroscopy after washing them in water to remove free or weakly bound MA molecules. Raman spectrum of the washed wet PEOX/MA fibers formed at pH4 clearly showed an enhanced asymmetric stretching vibration of the carboxylate ($-\text{COO}^-$) group of MA compared to that at pH2 (Fig. S3 in Supplementary Material).

Fig. 4. a) IR data of dried PEOX/MA fibers formed at pH2 and at pH4 together with that of PEOX and MA, b) TGA data of dried PEOX/MA fibers formed at pH2 and at pH4 together with that of PEOX and MA.

Based on the observation of the carboxylate peak in the IR spectrum of the washed and dried fibers and the Raman spectrum of the washed wet fibers formed in pH4 aqueous solutions, we conclude that the fibers dominantly consisted of MA molecules having carboxylate ($-\text{COO}^-$) end groups. This will be possible if the charged $-\text{COO}^-$ end groups complex with the amide group ($-\text{N}-\text{C}=\text{O}$) of PEOX by electrostatic interactions. In this case, the uncomplexed end at pH4 will be $-\text{COOH}$ which can make H-bond to amide group of another chain. But, any $-\text{COOH}$ stretching vibration band was not observed in

the IR spectrum of Fig. 4a. We interpret this as the shift of -COOH ionization reaction in the forward direction in favor of -COO^- ions, since the complexation of carboxylate ions with the amide group decreases their concentration in the solution. A similar shift due to H-bonded complexation of PAA with poly(acrylamide)s was previously reported in favor of -COOH groups [12]. The shift of -COOH ionization reaction in favor of carboxylate ions will eventually lead to the dominant bridging complexation of carboxylate groups of MA and the amide groups of PEOX.

TGA data of PEOX/MA fibers formed at pH2 and pH4 also show some differences as shown in Fig. 4b. Fibers formed both at pH2 and pH4 showed two major decomposition temperatures corresponding to MA (in the range of 100-200 °C) and PEOX (in the range of 330-450 °C) indicating that the fibers consisted of both MA and PEOX. While the weight loss of the fibers formed at pH2 at 330 °C was ~9.5%, the fibers formed at pH4 showed less weight loss which was only ~5.3 %. We attribute this to less amount of MA complexation with PEOX at pH4 compared to pH2. While PEOX showed a single transition at ~400 °C, both fibers formed at pH2 and pH4 showed double transition at ~400 °C with a clear shoulder in both. This can be due to the existence of two different morphology in the fibers having slightly different thermal stabilities. The first weight loss below 400 °C was ~53.2 % for the fibers formed at pH2 and ~32.3% for the fibers formed at pH4. We attribute the first weight loss around 400 °C to the PEOX complexed with MA. The weight fractions lost for the fibers in the first stage are also consistent with the weight loss of MA below 330 °C. The weight loss for the fibers above 400 °C can be attributed to the portions of the fibers where PEOX chains did not interact and form complex with MA. The residue above 450 °C is the sodium salts in the system due to NaOH addition to adjust pH. Since the TGA measurements were done in air, sodium carbonate forms as the final residue up to 700 °C [48].

We hypothesize that bridging of PEOX chains by MA through H-bonded complexation at pH2 or through electrostatic complexation at pH4 might have formed planar sheets which then curled into hollow fibers, similar to the curling of PEOX/TA LbL films [25]. Because the aggregates formed after ~7 days were fibrous, curling might have happened immediately after the planar precursors are formed due to stress in the sheets which might be due to chemical anisotropy of the sides of the films. Such anisotropy can originate from the orientation of the ethyl side chains of PEOX chains and the hydrophobic interactions in aqueous solution can bring the two opposite sides together to minimize hydrophobic/aqueous interface by bending the sheet. A slower growth of these curled nuclei by a dynamic attachment/detachment mechanism might then follow. Further structural investigations are needed to identify both the mechanism of formation and the structure of the hollow fibers.

4. Conclusions

Well-defined PEOX/MA fibers having hollow tubular morphology were shown to form by complexation induced self-assembly between PEOX and MA. The fibers had diameter of ~1-3 μm and a wall thickness of ~40 nm. At pH2, when both ends of MA were protonated, H-bonded complexation

was the driving interaction in the fiber formation. IR data showed the presence of both PEOX and MA in dried fibers formed at pH2. The downshift in the -C=O stretching of PEOX by as much as 15 cm^{-1} and the presence of -COOH stretching of MA confirmed the H-bonded complexation. The interaction enthalpy of PEOX and MA was determined by ITC as -49.38 kJ/mol which is consistent with H-bonding. Based on the IR band shifts of PEOX/MA blends as a function of PEOX repeat unit/MA mole ratio, PEOX/MA mole ratio in the fibers formed at pH2 was estimated to be 8.3. This ratio was consistent with the weight loss of $\sim 11.2\%$ at $350\text{ }^{\circ}\text{C}$ in TGA data. TGA analysis of the fibers showed two distinct decomposition temperatures one between $100\text{-}150\text{ }^{\circ}\text{C}$ corresponding to MA and the other one at $350\text{-}450\text{ }^{\circ}\text{C}$ corresponding to PEOX which clearly indicated the presence of both components in the fibers, as well. Hollow fibers also formed at pH4 when one end of MA was protonated and the other end was ionized. IR and TGA data also showed that both PEOX and MA were present in the fibers. -COOH peak was not observed, but asymmetric stretching vibration of the carboxylate (-COO^-) group of MA was present. It is concluded that the fibers formed at pH4 dominantly consisted PEOX chains bridged by electrostatic complexation of MA molecules through carboxylate (-COO^-) end groups. At pH7, when both ends of MA were ionized, electrostatic carboxylate/amide complexation between MA and PEOX induced the formation of few fibers, but the amount was not enough for further characterizations. The investigation of complexation induced self-assembly of PEOX/MA hollow fibers as a function of pH showed the role of different driving molecular interactions at different pH values. Further investigations are needed to understand the formation mechanism and the structure of the hollow fibers. pH-responsive hollow fibers have great potential to be used in biomedical applications for drug delivery and release purposes.

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Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

References

- [1] G.M. Whitesides, B. Grzybowski, Self-Assembly at All Scales, *Science* 295 (2002) 2418-2421, <https://doi.org/10.1126/science.1070821>.
- [2] D.S. Lawrence, T. Jiang, M. Levett, Self-Assembling Supramolecular Complexes, *Chemical Reviews* 95 (1995) 2229-2260, <https://doi.org/10.1021/cr00038a018>.

- [3] M. Motornov, Y. Roiter, I. Tokarev, S. Minko, Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems, *Progress in Polymer Science* 35 (2010) 174-211, <https://doi.org/10.1016/j.progpolymsci.2009.10.004>.
- [4] M.C. Branco, J.P. Schneider, Self-assembling materials for therapeutic delivery, *Acta Biomaterialia* 5 (2009) 817-831, <https://doi.org/10.1016/j.actbio.2008.09.018>.
- [5] R. Palao-Suay, L.G. Gómez-Mascaraque, M.R. Aguilar, B. Vázquez-Lasa, J.S. Román, Self-assembling polymer systems for advanced treatment of cancer and inflammation, *Progress in Polymer Science* 53 (2016) 207-248, <https://doi.org/10.1016/j.progpolymsci.2015.07.005>.
- [6] M.A.C. Stuart, W.T.S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G.B. Sukhorukov, I. Szleifer, V.V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, Emerging applications of stimuli-responsive polymer materials, *Nature Materials* 9 (2010) 101, <https://doi.org/10.1038/nmat2614>.
- [7] J. Ruokolainen, R. Mäkinen, M. Torkkeli, T. Mäkelä, R. Serimaa, G.t. Brinke, O. Ikkala, Switching Supramolecular Polymeric Materials with Multiple Length Scales, *Science* 280 (1998) 557-560, <https://doi.org/10.1126/science.280.5363.557>.
- [8] O. Ikkala, G. ten Brinke, Functional Materials Based on Self-Assembly of Polymeric Supramolecules, *Science* 295 (2002) 2407-2409, <https://doi.org/10.1126/science.1067794>.
- [9] R.P. Sijbesma, E.W. Meijer, Self-assembly of well-defined structures by hydrogen bonding, *Current Opinion in Colloid & Interface Science* 4 (1999) 24-32, [https://doi.org/10.1016/S1359-0294\(99\)00011-4](https://doi.org/10.1016/S1359-0294(99)00011-4).
- [10] D.C. Sherrington, K.A. Taskinen, Self-assembly in synthetic macromolecular systems multiple hydrogen bonding interactions, *Chemical Society Reviews* 30 (2001) 83-93, <https://doi.org/10.1039/B008033K>.
- [11] Y. He, B. Zhu, Y. Inoue, Hydrogen bonds in polymer blends, *Progress in Polymer Science* 29 (2004) 1021-1051, <https://doi.org/10.1016/j.progpolymsci.2004.07.002>.
- [12] G. Staikos, K. Karayanni, Y. Mylonas, Complexation of polyacrylamide and poly(N-isopropylacrylamide) with poly(acrylic acid). The temperature effect, *Macromolecular Chemistry and Physics* 198 (1997) 2905-2915, <https://doi.org/10.1002/macp.1997.021980920>.
- [13] C. Vasile, G. Bumbu, Y. Mylonas, I. Cojocaru, G. Staikos, Hydrogen-bonding interaction of an alternating maleic acid-vinyl acetate copolymer with poly(ethylene glycol), polyacrylamide and poly(N-isopropylacrylamide): a comparative study, *Polymer International* 52 (2003) 1887-1891, <https://doi.org/10.1002/pi.1274>.
- [14] T. Aoki, M. Kawashima, H. Katono, K. Sanui, N. Ogata, T. Okano, Y. Sakurai, Temperature-Responsive Interpenetrating Polymer Networks Constructed with Poly(acrylic acid) and Poly(N,N-dimethylacrylamide), *Macromolecules* 27 (1994) 947-952, <https://doi.org/10.1021/ma00082a010>.
- [15] A.R. Nesic, S.S. Trifunovic, A.S. Grujic, S.J. Velickovic, D.G. Antonovic, Complexation of amidated pectin with poly(itaconic acid) as a polycarboxylic polymer model compound, *Carbohydrate Research* 346 (2011) 2463-2468, <https://doi.org/10.1016/j.carres.2011.08.021>.
- [16] M. Kalagasidis Krušić, E. Džunuzović, S. Trifunović, J. Filipović, Polyacrylamide and poly(itaconic acid) complexes, *European Polymer Journal* 40 (2004) 793-798, <https://doi.org/10.1016/j.eurpolymj.2003.11.016>.
- [17] V.V. Khutoryanskiy, Hydrogen-bonded interpolymer complexes as materials for pharmaceutical applications, *International Journal of Pharmaceutics* 334 (2007) 15-26, <https://doi.org/10.1016/j.ijpharm.2007.01.037>.
- [18] Z.S. Nurkeeva, G.A. Mun, V.V. Khutoryanskiy, Interpolymer Complexes of Water-Soluble Nonionic Polysaccharides with Polycarboxylic Acids and Their Applications, *Macromolecular Bioscience* 3 (2003) 283-295, <https://doi.org/10.1002/mabi.200390037>.
- [19] Henke, S. Kadłubowski, M. Wolszczak, P. Ulański, V. Boyko, T. Schmidt, K.F. Arndt, J.M. Rosiak, The Structure and Aggregation of Hydrogen-Bonded Interpolymer Complexes of Poly (acrylic

- acid) With Poly(N-vinylpyrrolidone) in Dilute Aqueous Solution, *Macromolecular Chemistry and Physics* 212 (2011) 2529-2540, <https://doi.org/10.1002/macp.201100409>.
- [20] J.F. Quinn, F. Caruso, Facile Tailoring of Film Morphology and Release Properties Using Layer-by-Layer Assembly of Thermoresponsive Materials, *Langmuir* 20 (2004) 20-22, <https://doi.org/10.1021/la0360310>.
- [21] H. Lee, R. Mensire, R.E. Cohen, M.F. Rubner, Strategies for Hydrogen Bonding Based Layer-by-Layer Assembly of Poly(vinyl alcohol) with Weak Polyacids, *Macromolecules* 45 (2012) 347-355, <https://doi.org/10.1021/ma202092w>.
- [22] Erel, H. Schlaad, A.L. Demirel, Effect of structural isomerism and polymer end group on the pH-stability of hydrogen-bonded multilayers, *Journal of Colloid and Interface Science* 361 (2011) 477-482, <https://doi.org/10.1016/j.jcis.2011.05.033>.
- [23] H.E. Karahan, L. Eyüboğlu, D. Kiyılar, A.L. Demirel, pH-stability and pH-annealing of H-bonded multilayer films prepared by layer-by-layer spin-assembly, *European Polymer Journal* 56 (2014) 159-167, <https://doi.org/10.1016/j.eurpolymj.2014.04.015>.
- [24] S. Hendessi, P. Tatar Güner, A. Miko, A.L. Demirel, Hydrogen bonded multilayers of poly(2-ethyl-2-oxazoline) stabilized silver nanoparticles and tannic acid, *European Polymer Journal* 88 (2017) 666-678, <https://doi.org/10.1016/j.eurpolymj.2016.10.039>.
- [25] E.B. Adatoz, S. Hendessi, C.W. Ow-Yang, A.L. Demirel, Restructuring of poly(2-ethyl-2-oxazoline)/tannic acid multilayers into fibers, *Soft Matter* 14 (2018) 3849-3857, <https://doi.org/10.1039/c8sm00381e>.
- [26] M. Meyer, M. Antonietti, H. Schlaad, Unexpected thermal characteristics of aqueous solutions of poly (2-isopropyl-2-oxazoline), *Soft Matter* 3 (2007) 430-431, <https://doi.org/10.1039/B616678D>.
- [27] A.L. Demirel, M. Meyer, H. Schlaad, Formation of Polyamide Nanofibers by Directional Crystallization in Aqueous Solution, *Angewandte Chemie International Edition* 46 (2007) 8622-8624, <https://doi.org/10.1002/anie.200703486>.
- [28] P.T. Güner, A. Mikó, F.F. Schweinberger, A.L. Demirel, Self-assembled poly(2-ethyl-2-oxazoline) fibers in aqueous solutions, *Polymer Chemistry* 3 (2012) 322-324, <https://doi.org/10.1039/C1PY00463H>.
- [29] P. Qiu, C. Mao, Biomimetic branched hollow fibers templated by self-assembled fibrous polyvinylpyrrolidone structures in aqueous solution, *ACS nano* 4 (2010) 1573-1579, <https://doi.org/10.1021/nn9009196>.
- [30] R. Hoogenboom, Poly (2-oxazoline) s: a polymer class with numerous potential applications, *Angewandte Chemie International Edition* 48(43) (2009) 7978-7994, <https://doi.org/10.1002/anie.200901607>.
- [31] R. Luxenhofer, G. Sahay, A. Schulz, D. Alakhova, T.K. Bronich, R. Jordan, A.V. Kabanov, Structure-property relationship in cytotoxicity and cell uptake of poly (2-oxazoline) amphiphiles, *Journal of Controlled Release* 153 (2011) 73-82, <https://doi.org/10.1016/j.jconrel.2011.04.010>.
- [32] R. Hoogenboom, H. Schlaad, Thermoresponsive poly(2-oxazoline)s, polypeptoids, and polypeptides, *Polymer Chemistry* 8 (2017) 24-40, <https://doi.org/10.1039/C6PY01320A>.
- [33] H. Schlaad, C. Diehl, A. Gress, M. Meyer, A.L. Demirel, Y. Nur, A. Bertin, Poly(2-oxazoline)s as Smart Bioinspired Polymers, *Macromolecular Rapid Communications* 31 (2010) 511-525, <https://doi.org/10.1002/marc.200900683>.
- [34] R. Hoogenboom, H.M.L. Thijs, M.J.H.C. Jochems, B.M. van Lankvelt, M.W.M. Fijten, U.S. Schubert, Tuning the LCST of poly(2-oxazoline)s by varying composition and molecular weight: alternatives to poly(N-isopropylacrylamide)?, *Chemical Communications* 44 (2008) 5758-5760, <https://doi.org/10.1039/B813140F>.
- [35] A.L. Demirel, P. Tatar Güner, B. Verbraeken, H. Schlaad, U.S. Schubert, R. Hoogenboom, Revisiting the crystallization of poly(2-alkyl-2-oxazoline)s, *Journal of Polymer Science Part B: Polymer Physics* 54 (2016) 721-729, <https://doi.org/10.1002/polb.23967>.

- [36] P. Wilson, P.C. Ke, T.P. Davis, K. Kempe, Poly (2-oxazoline)-based micro-and nanoparticles: A review, *European Polymer Journal* 88 (2017) 486-515, <https://doi.org/10.1016/j.eurpolymj.2016.09.011>.
- [37] G. Paramasivam, M. Vergaelen, M.-R. Ganesh, R. Hoogenboom, A. Sundaramurthy, Hydrogen bonded capsules by layer-by-layer assembly of tannic acid and poly(2-n-propyl-2-oxazoline) for encapsulation and release of macromolecules, *Journal of Materials Chemistry B* 5 (2017) 8967-8974, <https://doi.org/10.1039/C7TB02284K>.
- [38] C. Wang, Y. Li, Y. Ma, Y. Gao, D. Dong, J. Fang, N. Zhang, Thermoresponsive polymeric nanoparticles based on poly(2-oxazoline)s and tannic acid, *Journal of Polymer Science Part A: Polymer Chemistry* 56 (2018) 1520-1527, <https://doi.org/10.1002/pola.29033>.
- [39] P. Tatar Güner, A.L. Demirel, Effect of anions on the cloud point temperature of aqueous poly (2-ethyl-2-oxazoline) solutions, *The Journal of Physical Chemistry B* 116 (2012) 14510-14514, <https://doi.org/10.1021/jp310648d>.
- [40] D. Curiel, M. Más-Montoya, G. Sánchez, Complexation and sensing of dicarboxylate anions and dicarboxylic acids, *Coordination Chemistry Reviews* 284 (2015) 19-66, <https://doi.org/10.1016/j.ccr.2014.09.010>.
- [41] L. Alderighi, P. Gans, A. Ienco, D. Peters, A. Sabatini, A. Vacca, Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species, *Coordination chemistry reviews* 184 (1999) 311-318, [https://doi.org/10.1016/S0010-8545\(98\)00260-4](https://doi.org/10.1016/S0010-8545(98)00260-4).
- [42] C. Kim, S.C. Lee, I.C. Kwon, H. Chung, S.Y. Jeong, Complexation of Poly(2-ethyl-2-oxazoline)-block-poly(ϵ -caprolactone) Micelles with Multifunctional Carboxylic Acids, *Macromolecules* 35 (2002) 193-200, <https://doi.org/10.1021/ma010511l>.
- [43] J. Ambreen, J. Yang, X. Ye, M. Siddiq, Effect of carbon chain length of monocarboxylic acids on cloud point temperature of poly(2-ethyl-2-oxazoline), *Colloid and Polymer Science* 291 (2013) 919-925, <https://doi.org/10.1007/s00396-012-2810-z>.
- [44] X. Shao, Y. Zhang, S.-F. Pang, Y.-H. Zhang, Vacuum FTIR observation on hygroscopic properties and phase transition of malonic acid aerosols, *Chemical Physics* 483-484 (2017) 7-11, <http://dx.doi.org/10.1016/j.chemphys.2016.11.001>.
- [45] N. S. Myshakina, Z. Ahmed, S. A. Asher, Dependence of amide vibrations on hydrogen bonding, *J. Phys. Chem B Lett.* 112 (2008) 11873-11877, <https://doi.org/10.1021/jp8057355>.
- [46] S. C. Bizley, A. C. Williams, V. V. Khutoryanskiy, Thermodynamic and kinetic properties of interpolymer complexes assessed by isothermal titration calorimetry and surface plasmon resonance, *Soft Matter* 10 (2014) 8254-8260, <https://doi.org/10.1039/c4sm01138d>.
- [47] A. Sundaramurthy, M. Vergaelen, S. Maji, R. Auzély-Velty, Z. Zhang, B. G. De Geest, R. Hoogenboom, Hydrogen Bonded Multilayer Films Based on Poly(2-oxazoline)s and Tannic Acid, *Adv. Healthcare Mater.* 3 (2014) 2040-2047, <https://doi.org/10.1002/adhm.201400377>.
- [48] F.J. Caires, L.S. Lima, C.T. Carvalho, R.J. Giagio, M. Ionashiro, Thermal behaviour of malonic acid, sodium malonate and its compounds with some bivalent transition metal ions, *Thermochimica Acta* 497 (2010) 35-40, <https://doi.org/10.1016/j.tca.2009.08.013>.