Electronic Supplementary Material (ESI) for RSC Applied Polymers.

Melt Stability of Carbonic Anhydrase in Polyethylene oxide for Extrusion of Protein-Polymer Composite Materials

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Supplemental Information:

Neglecting pressure gradients, the shear rate can be expressed via the following equation:

$$\dot{\gamma}_{yz} = \frac{V\,cos\theta}{h} \quad \text{(Eq. 1)}$$

where V is the following:

$$V = \frac{2\pi NR}{60} \quad \text{(Eq. 2)}$$

N is the rpm of the screws, R is the screw radius, h is the channel height, and θ is defined as the angle of the screw with respect to the axis perpendicular to the flow direction. As the screw is conical, the maximum shear rate experienced by the melt is when the screw is largest in diameter. The variables estimated to produce a maximum shear rate of approximately 10 s^{-1} are shown below, based on measurements of the screws:

Table S1. Screw measurements of the HAAKE™ MiniCTW Micro-conical Twin Screw Compounder at the largest screw diameter and accompanying screw speed.

N (rpm)	R (mm)	h (mm)	θ (°)	$\dot{\gamma}$ (s^{-1})
20	7.25	1	45	10.7

Table S2. Densities of 0.04 M phosphate buffer pH 7.4 and buffer-PEO solutions

	(%)	(g/mL)
Buffer	-	1.006 ± 0.001
PEO 3350 solution	14%	1.032 ± 0.001
PEO 20k solution	14%	1.031 ± 0.001
PEO 100k solution	11%	1.023 ± 0.001

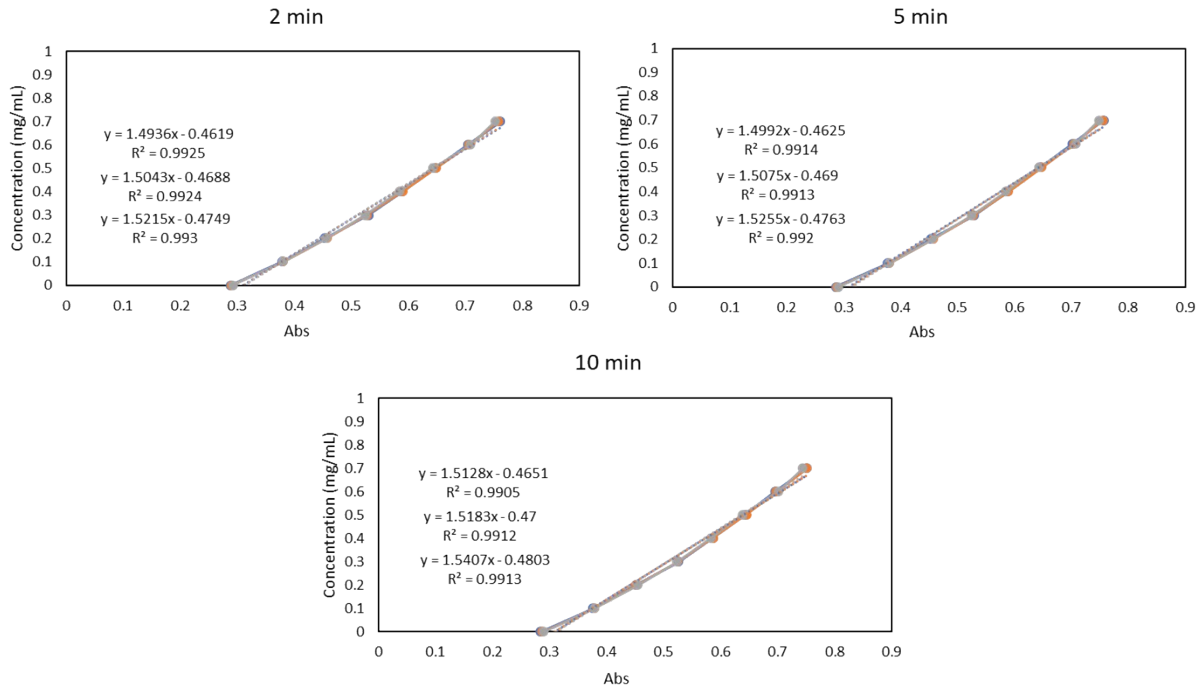

Figure S1. Bovine carbonic anhydrase Bradford assay calibration containing PEO 3350 in the solution. Absorbance measurements at 595 nm were taken at 2 minutes, 5 minutes, and 10 minutes. Lines of best fit were found and plotted using standard linear regression, and are shown in each figure.

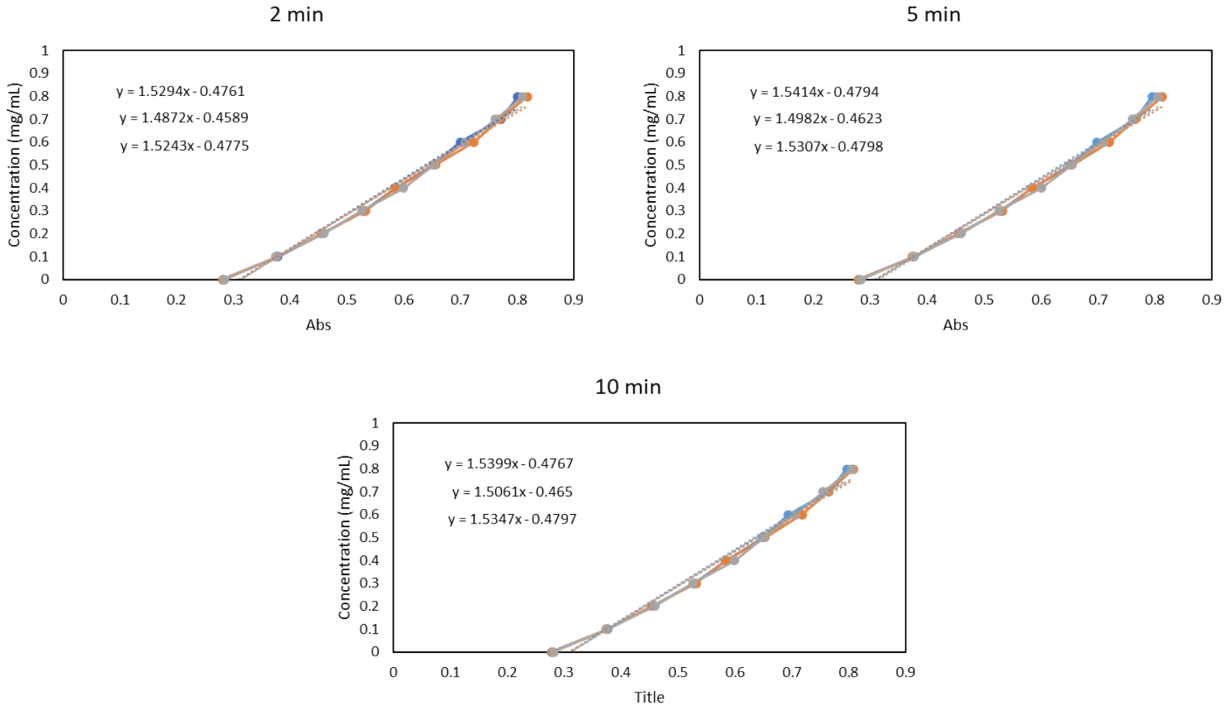

Figure S2. Bovine carbonic anhydrase Bradford assay calibration containing PEO 20k in the solution. Absorbance measurements at 595 nm were taken at 2 minutes, 5 minutes, and 10 minutes. Lines of best fit were found and plotted using standard linear regression, and are shown in each figure.

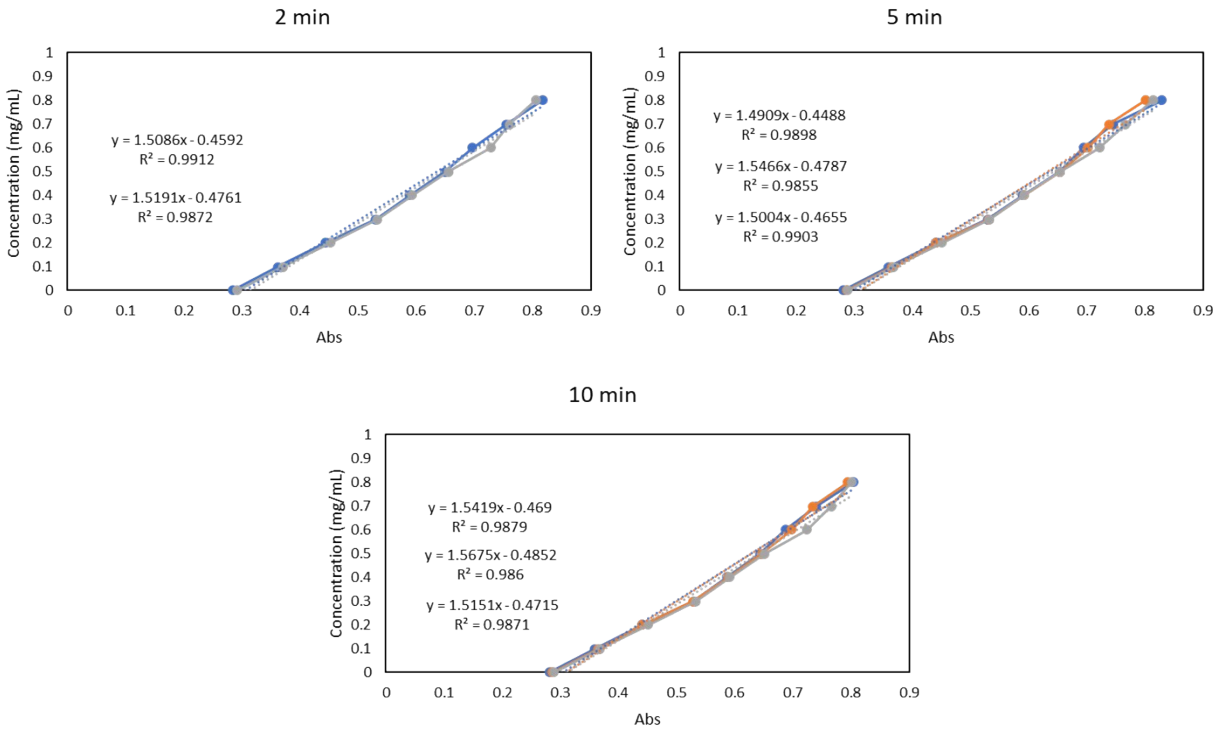

Figure S3. Bovine carbonic anhydrase Bradford assay calibration containing PEO 100k in the solution. Absorbance measurements at 595 nm were taken at 2 minutes, 5 minutes, and 10 minutes. Lines of best fit were found and plotted using standard linear regression, and are shown in each figure.

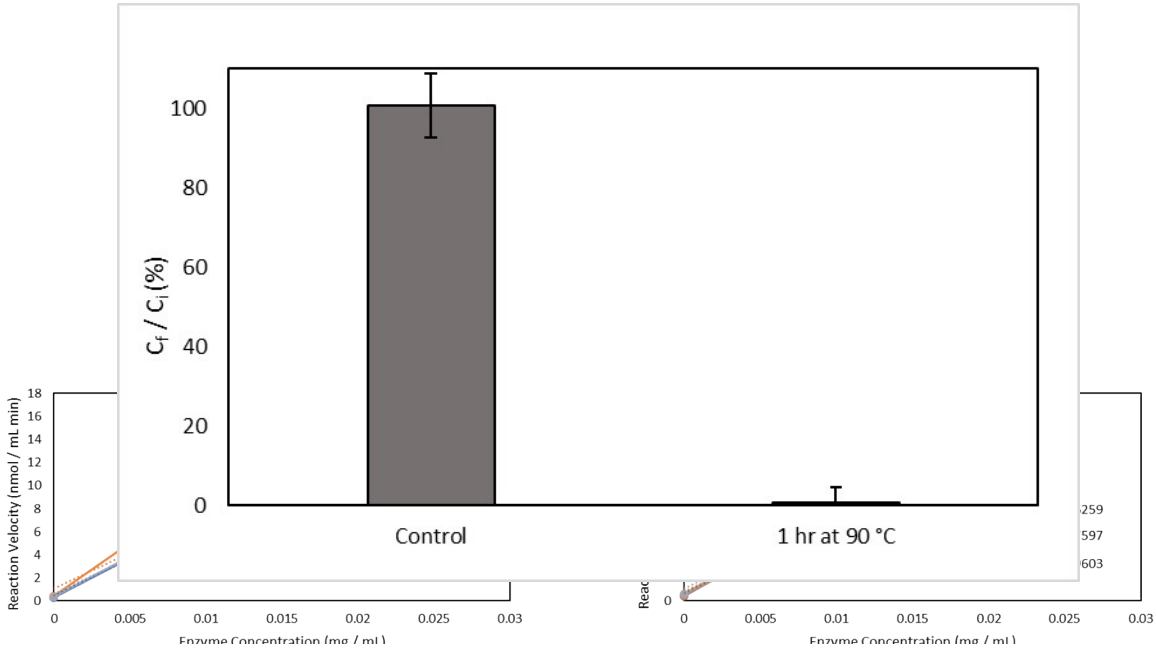

Figure S4. Bradford assay for carbonic anhydrase heated at 90 °C for 1 hour in a phosphate buffer solution, compared to a native control sample. Error bars represent 1 standard deviation from the mean. C_i was calculated by taking the mass of protein added to a known volume of buffer.

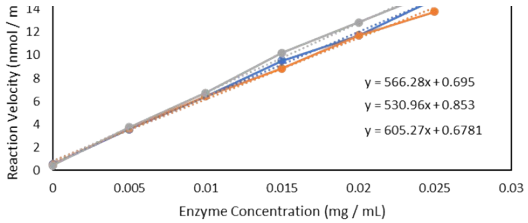

Figure S5. PNPA hydrolysis activity assay results for control bovine carbonic anhydrase in varying PEO MW and phosphate buffer pH 7.4 solutions. Ratio of carbonic anhydrase to PEO molecules matched those of samples sheared on the rheometer, equating to roughly 1.5wt%. Linear regression was performed to find the lines of best fit, shown in each plot.

Table S3. Bradford assay results for 1" pieces from the extrudate using PEO 100k with 1.5 wt% carbonic anhydrase, processed at 100 °C and 20 rpm for 15 minutes

Sample	Concentration (mg/mL)
1	**0.98 ± 0.05**
2	**0.95 ± 0.04**
3	**0.99 ± 0.04**
4	**1.00 ± 0.05**

Molecular Weight	Viscosity (Pa s)

Table S4. Melt viscosities obtained for PEO using a 20mm parallel plate with an 800 µm sample gap, shearing at 10 s^{-1}.

(g/mol)	100 °C	130 °C	160 °C	190 °C
3350	0.35	0.15	0.10	0.06
20000	23.9	13.5	7.75	4.40
100000	3920	1200	781	346

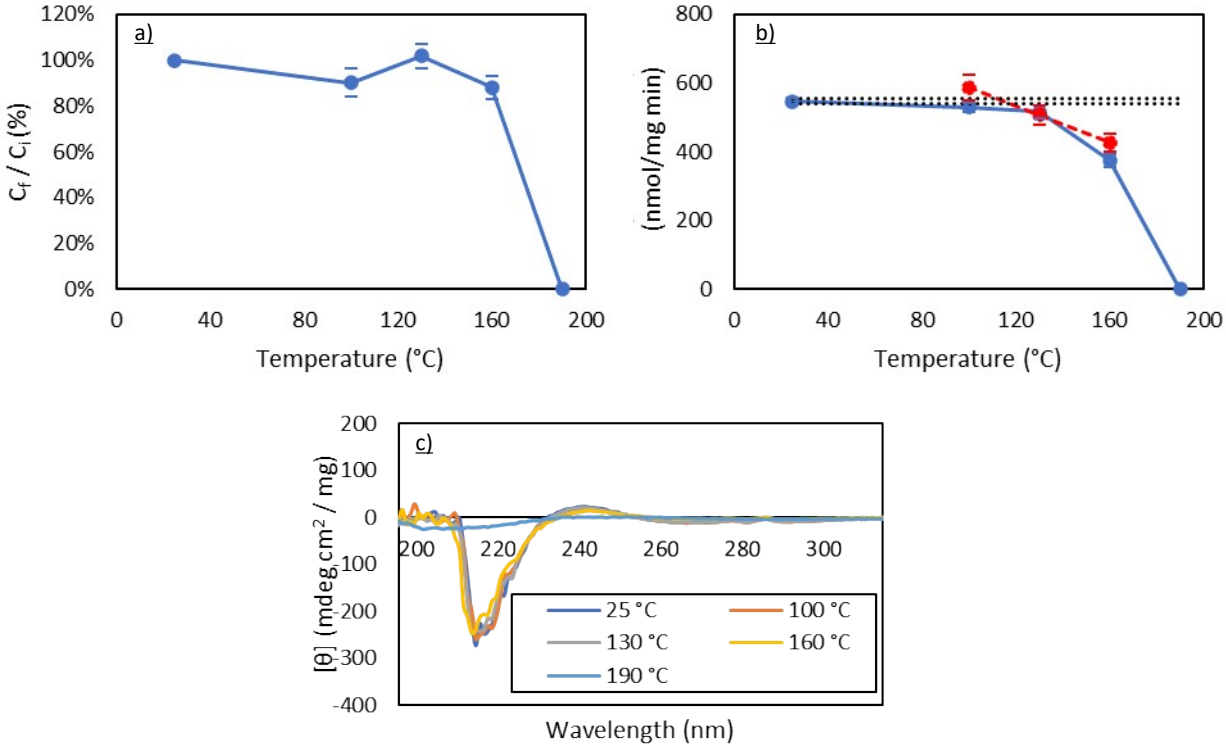

Figure S6. Thermal stability of only lyophilized carbonic anhydrase (no PEO). Samples were exposed to either 100, 130, 160, or 190 C for 15 minutes while in aluminum foil, then cooled to room temperature and dissolved in 0.04 M phosphate buffer pH 7.4. Once dissolved, a) Bradford assays, b) activity assays, and c) circular dichroism spectra were obtained, all at room temperature.

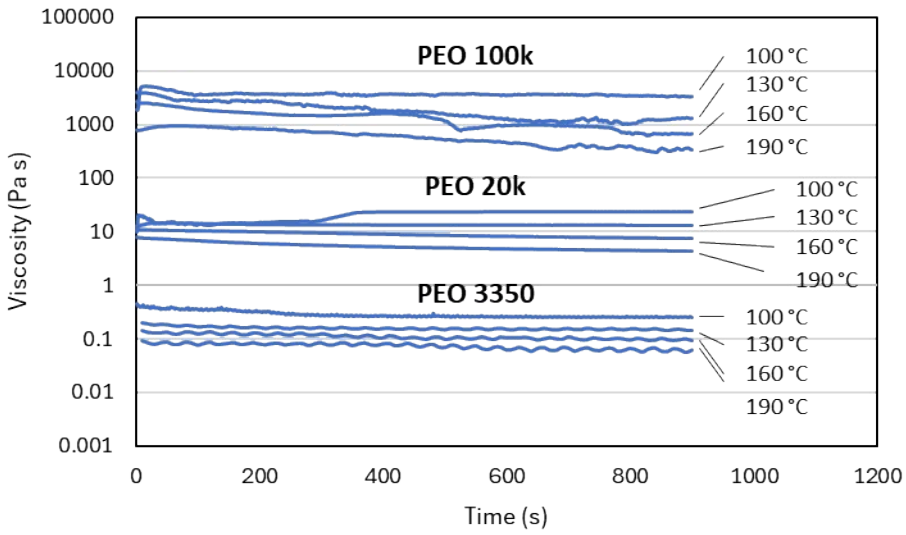

Figure S7. Viscosity curves obtained for PEO using a 20 mm parallel plate with an 800 µm sample gap, shearing at 10 s^{-1}.

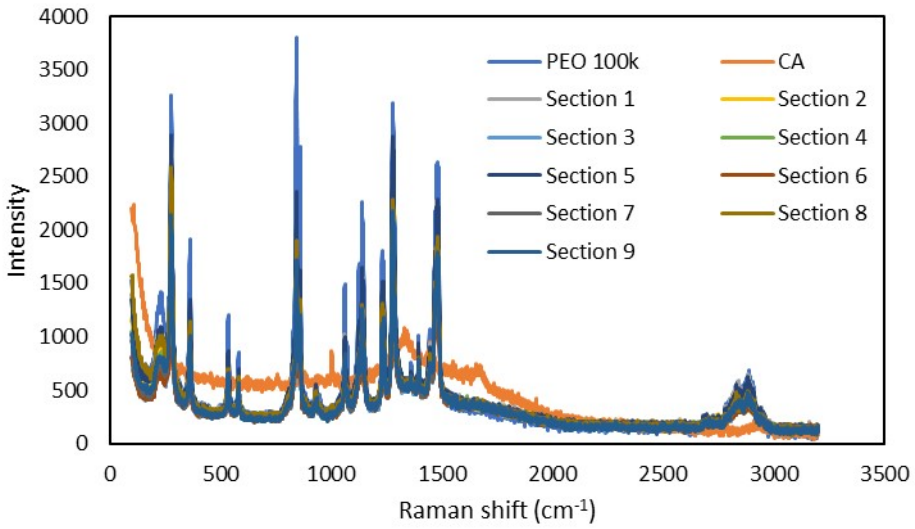

Figure S8. Raman spectra for PEO 100000, lyophilized bovine carbonic anhydrase, and protein-polymer composites extruded at 100 °C and 20 rpm. Sections 1-9 are from the same extrusion-processed sample, spaced apart by 20 µm. Spectra were obtained using a 785nm laser.