Electronic Supplementary Information For:

Influence of Surfactant on Glass Transition Temperature of Poly(lactic-co-glycolic acid) Nanoparticles

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Intrinsic viscosity measurement of PLGA samples

The intrinsic viscosity was applied to evaluate whether significant degradation was occurring during the DSC measurements and the effect of this potential molecular weight change on the glass transition temperature before and after DSC measurements using the Mark-Houwink equation.[1] Intrinsic viscosity measurements allow us to determine if the potential degradation of PLGA in wet conditions and high temperature has a significant influence on T_g. To eliminate the influence of the second component (surfactant) on intrinsic viscosity, non-surfactant PLGA particles was prepared and dissolved in chloroform. The experiment was done in ice water which provided consistent temperature and avoided excessive evaporation of chloroform. The principle of intrinsic viscosity is based on the Mark-Houwink relation according to equation (1):

$$[\eta] = K \cdot M_w^{\alpha} \tag{1}$$

Where α = 0.73,[2] K is calculated from the known molecular weight of non-surfactant PLGA particles and the accordingly measured intrinsic viscosity, which resulted in a value of 2.275 X 10^{-4}.

Preparation of non-surfactant PLGA nanoparticles

20 mg of PLGA was weighed and dissolved in 2 ml of acetone solution to create the organic phase and injected into 100 of ultra-pure water. The mixture was stirred overnight to evaporate the organic solvent and the sample was collected with 40 μm cell strainers to remove the large chunks of aggreged polymer. The collected samples were frozen at -80°C and freeze dried to get nanoparticles.

Intrinsic viscosity measurement

Different amounts of non-surfactant PLGA particles (5 mg, 10 mg, 15 mg, and 20 mg) were dissolved in chloroform to create varying concentrations of PLGA-chloroform solution. The intrinsic viscosity measurement was performed with an Ubbelohde viscometer (Fisher Scientific catalog# 13614F). According to Figure S1, intrinsic viscosity of PLGA particles at 0°C before T_g measurement was 0.6127 dL/g, which was set as reference to calculate the K value using the know molecular weight of the PLGA as 50,000 g/mol. The intrinsic viscosities of DSC treated PLGA particles (dry condition and wet condition) were 0.4532 dL/g and 0.4287 dL/g, respectively, which gave molecular weights of 33,100 and 30,100 g/mol.

It has been reported that when the molecular weight of PLGA is higher than 10,000 g/mol, the T_g remains stable despite changes in the molecular weight.[3] The decrease in molecular weight in samples after DSC measurement indicate that the DSC treated PLGA particles experienced some chain-scission during T_g measurement, however the degree of degradation of PLGA chains is minor and not significant enough to shift the T_g. Also, the Gordon-Taylor equation suggested that the effect of molecular weight on $T_{g,max}$ is negligible when it's large than 20 kDa.[4] As a conclusion, we can confirm that the T_g change before and after DSC scans was not caused by the degradation of PLGA chains.
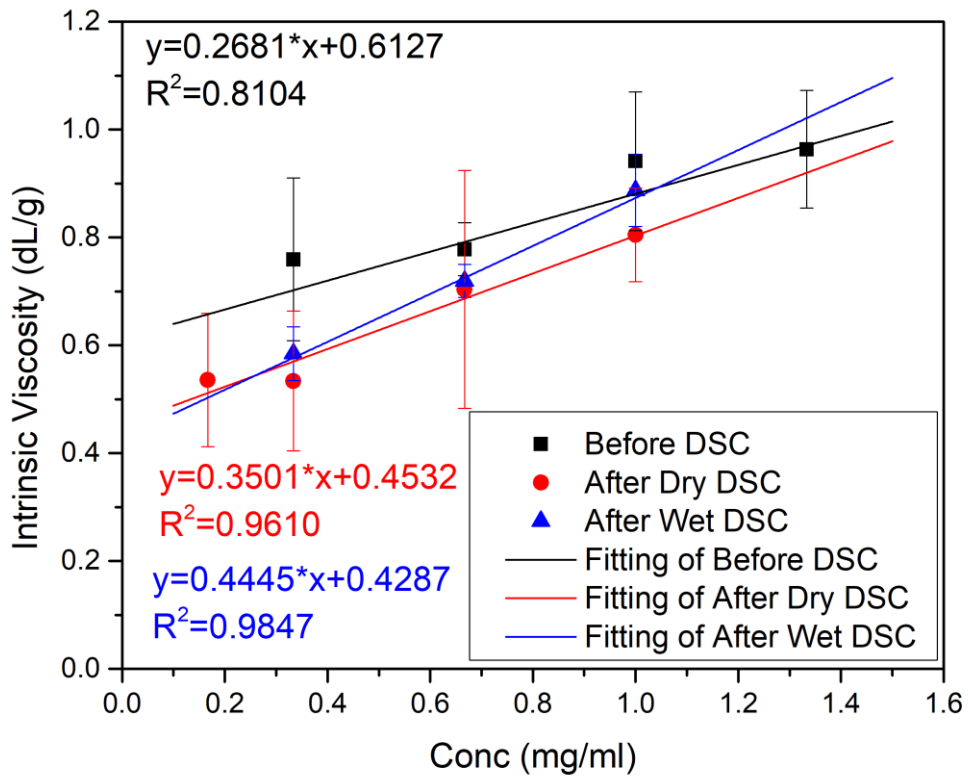
Figure S1 Intrinsic viscosity of non-surfactant PLGA nanoparticles before DSC scan (black square), after DSC scan in dry condition (red circle), and after DSC scan in wet condition (blue triangle), and their fitting lines as measured at 0°C.

One-way ANOVA: PVA-PLGA NP diameter vs. initial concentration
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	0.50%, 1%, 1.50%, 2%, 2.50%

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	8178	2044.45	119.26	0.000
Error	70	1200	17.14		
Total	74	9378			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.14042	87.20%	86.47%	85.31%

Means

Factor	N	Mean	StDev	95% CI
0.50%	15	146.37	4.50	(144.24, 148.51)
1%	15	136.02	4.11	(133.89, 138.15)
1.50%	15	125.093	2.998	(122.961, 127.225)
2%	15	120.75	5.19	(118.62, 122.89)
2.50%	15	118.447	3.560	(116.315, 120.579)

Pooled StDev = 4.14042

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping			
0.50%	15	146.37	A			
1%	15	136.02		B		
1.50%	15	125.093			C	
2%	15	120.75				D
2.50%	15	118.447				D

Means that do not share a letter are significantly different.

Figure S2 Single Factor ANOVA analysis of PVA-PLGA particle diameter and initial

PVA concentration. Equal variances were assumed for the analysis according to

the results of a multiple comparisons test for equal variance.

One-way ANOVA: DMAB-PLGA NP diameter vs. initial concentration

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	0.25%, 0.50%, 1.00%, 1.50%, 2.00%

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	29710	7427.6	13.25	0.000
Error	70	39231	560.4		
Total	74	68942			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
23.6738	43.09%	39.84%	34.68%

Means

Factor	N	Mean	StDev	95% CI
0.25%	15	140.07	25.86	(127.88, 152.26)
0.50%	15	157.06	23.79	(144.87, 169.25)
1.00%	15	166.05	22.51	(153.86, 178.24)
1.50%	15	107.77	19.15	(95.58, 119.96)
2.00%	15	140.92	26.35	(128.73, 153.11)

Pooled StDev = 23.6738

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
1.00%	15	166.05	A		
0.50%	15	157.06	A	B	
2.00%	15	140.92		B	
0.25%	15	140.07		B	
1.50%	15	107.77			C

Means that do not share a letter are significantly different.

Figure S3 Single Factor ANOVA analysis of DMAB-PLGA particle diameter and

initial DMAB concentration. Equal variances were assumed for the analysis according to the results of a multiple comparisons test for equal variance.

One-way ANOVA: T_g of PVA-PLGA Dry-NPs vs. residual PVA

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	0.0542, 0.0645, 0.0697, 0.0826, 0.0872

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	2.164	0.5410	3.98	0.035
Error	10	1.360	0.1360		
Total	14	3.524			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.368755	61.41%	45.97%	13.17%

Means

Factor	N	Mean	StDev	95% CI
0.0542	3	49.1800	0.0400	(48.7056, 49.6544)
0.0645	3	49.7000	0.1058	(49.2256, 50.1744)
0.0697	3	49.993	0.367	(49.519, 50.468)
0.0826	3	50.327	0.454	(49.852, 50.801)
0.0872	3	49.677	0.571	(49.202, 50.151)

Pooled StDev = 0.368755

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
0.0826	3	50.327	A	
0.0697	3	49.993	A	B
0.0645	3	49.7000	A	B
0.0872	3	49.677	A	B
0.0542	3	49.1800		B

Means that do not share a letter are significantly different.

Figure S4 Single Factor ANOVA analysis of T_g of PVA-PLGA dry particles and residual PVA. Equal variances were assumed for the analysis according to the results of a multiple comparisons test for equal variance.

One-way ANOVA: T_g of DMAB-PLGA Dry-NPs vs. residual DMAB

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	5	0.0462, 0.0447, 0.0508, 0.0592, 0.0866

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	4	0.1551	0.03878	0.19	0.936
Error	10	2.0067	0.20067		
Total	14	2.1618			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.447966	7.17%	0.00%	0.00%

Means

Factor	N	Mean	StDev	95% CI
0.0462	3	49.9233	0.1150	(49.3471, 50.4996)
0.0447	3	49.997	0.550	(49.420, 50.573)
0.0508	3	49.867	0.808	(49.290, 50.443)
0.0592	3	49.847	0.187	(49.270, 50.423)
0.0866	3	50.1267	0.0208	(49.5504, 50.7029)

Pooled StDev = 0.447966

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
0.0866	3	50.1267	A
0.0447	3	49.997	A
0.0462	3	49.9233	A
0.0508	3	49.867	A
0.0592	3	49.847	A

Means that do not share a letter are significantly different.

Figure S5 Single Factor ANOVA analysis of T_g of DMAB-PLGA dry particles and residual DMAB. Equal variances were assumed for the analysis according to the

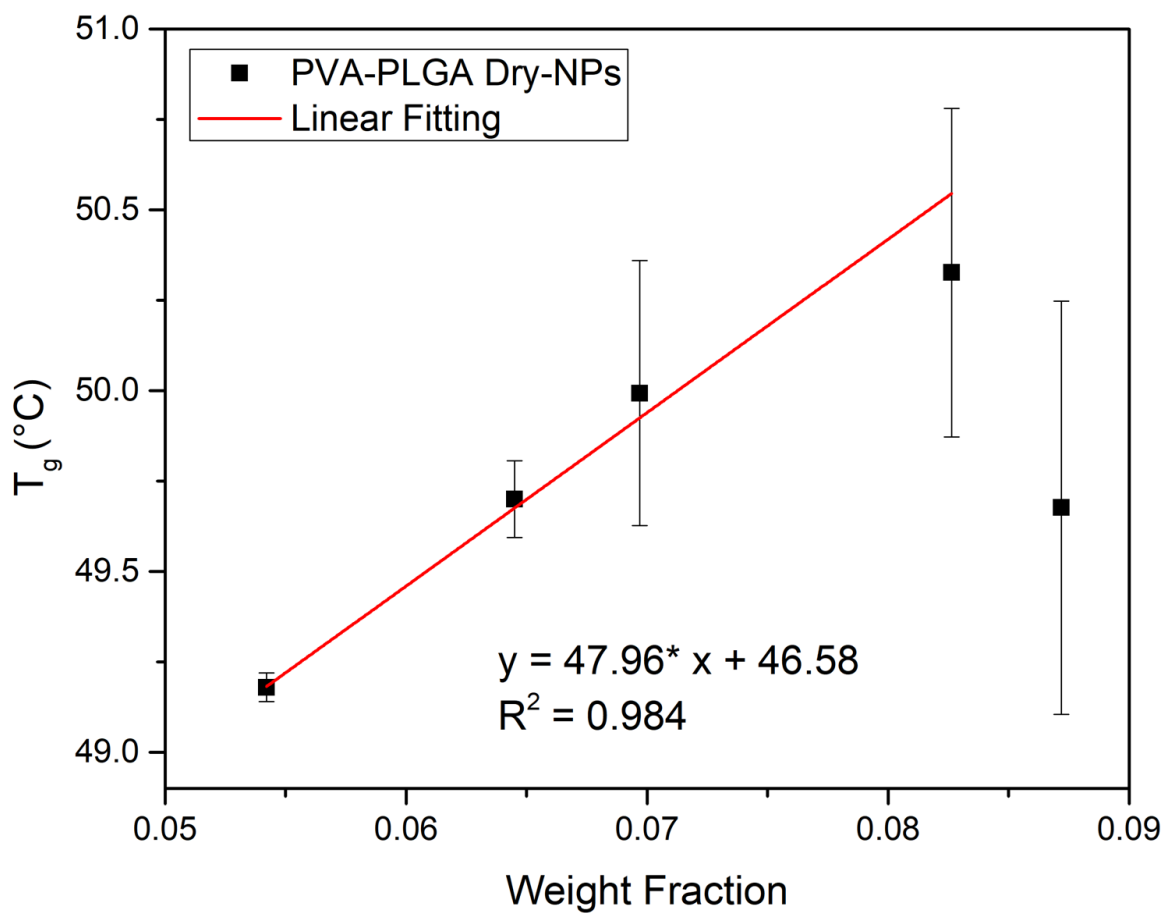results of a multiple comparisons test for equal variance.

Figure S6 T_g of PVA-PLGA dry particles as a function of residual PVA. The red fitting line of the first four data points shows the increase trend.