Supporting Information

Note: Supplemental Tables and Figures are intermingled and appear in the order referenced in the main article.

Contents

Screening designs for H_2SO_4, SO_3:DMF and SO_3:pyridine

Table S1. Range of different factors in PB design based on literature survey

Reagent	Temperature (°C)	Time (min)	Polymer concentration (gL^{-1})	Molar Ratio (Reagent/OH)
H_2SO_4 (98%)				
Lo (-1)	10	350	30	2
Hi (+1)	45	1200	120	8
SO_3:DMF				
Lo (-1)	5	60	10	1
Hi (+1)	25	180	35	4
SO_3:pyridine				
Lo (-1)	35	385	40	1
Hi (+1)	105	1200	80	4

Table S2. PB design for H_2SO_4 as sulfating agent and experimentally determined degree-of-substitution values (%)

Run Order	Temperature (°C) (V_1)	Time (min) (V_2)	Polymer concentration (gL^{-1}) (V_3)	Molar Ratio (Reagent/OH) (V_4)	%DS
1	-1	+1	-1	-1	1
2	+1	+1	-1	+1	1
3	0	0	0	0	1
4	+1	-1	+1	-1	1
5	-1	+1	+1	-1	1
6	0	0	0	0	0
7	-1	+1	+1	+1	9
8	-1	-1	-1	+1	0
9	-1	-1	-1	-1	0
10	-1	-1	+1	+1	8
11	+1	+1	-1	+1	1
12	+1	+1	+1	-1	2
13	+1	-1	-1	-1	0
14	0	0	0	0	1
15	+1	-1	+1	+1	13

Table S3. Analysis of variance of PB design for H_2SO_4 as sulfating agent.

Source	d.f.	Mean square	Sum of squares	F-value	P-value*
Model	5	33.2963	166.482	4.47	0.025 (S)
Temperature (V_1)	1	0.0177	0.018	0.00	0.962 (NS)
Time (V_2)	1	5.8493	5.849	0.79	0.398 (NS)
Polymer Con (V_3)	1	76.4838	76.484	10.28	0.011 (S)
MRatio (V_4)	1	68.1500	68.150	9.16	0.014 (S)
Curvature	1	15.9808	15.981	2.15	0.177 (NS)

* S and NS stand for significant and non-significant, respectively.

Table S4. PB design for SO_3:DMF as sulfating agent and experimentally determined degree-of-substitution values (%)

Run Order	Temperature (˚C) (W_1)	Time (min) (W_2)	Polymer Conc. (gL^{-1}) (W_3)	Molar Ratio (Reagent/OH) (W_4)	DS (%)
1	0	0	0	0	84
2	0	0	0	0	84
3	-1	-1	+1	+1	87
4	+1	-1	+1	+1	82
5	-1	+1	-1	-1	54
6	-1	-1	-1	+1	85
7	0	0	0	0	84
8	+1	+1	-1	+1	77
9	-1	+1	+1	-1	69
10	+1	-1	-1	-1	50
11	+1	+1	-1	+1	44
12	+1	-1	+1	-1	60
13	+1	+1	+1	-1	58
14	-1	-1	-1	-1	59
15	-1	+1	+1	+1	84

Table S5. Analysis of variance of PB design for SO_3:DMF as sulfating agent.

Source	d.f.	Mean square	Sum of squares	F-value	P-value*
Model	5	509.89	2549.46	7.30	0.005 (S)
Temperature (W_1)	1	366.10	366.10	5.24	0.048 (S)
Time (W_2)	1	114.95	114.95	1.64	0.232 (NS)
Polymer Con (W_3)	1	424.86	424.86	6.08	0.036 (S)
MRatio (W_4)	1	992.28	992.28	14.20	0.004 (S)
Curvature	1	651.28	651.28	9.32	0.014 (S)

* S and NS stand for significant and non-significant, respectively

Table S6. PB design for SO_3:pyridine as sulfating agent and experimentally determined degree-of-substitution values (%)

Run Order	Temperature (˚C) (Z_1)	Time (min) (Z_2)	Polymer Conc. (gL^{-1}) (Z_3)	Molar Ratio (Reagent/OH) (Z_4)	DS (%)
1	+1	-1	+1	+1	49
2	-1	-1	-1	+1	55
3	-1	-1	-1	-1	43
4	-1	+1	+1	+1	53
5	-1	+1	-1	-1	43
6	+1	-1	-1	-1	21
7	+1	-1	+1	-1	17
8	-1	-1	+1	+1	80
9	0	0	0	0	59
10	0	0	0	0	65
11	-1	+1	+1	-1	21
12	+1	+1	-1	+1	38
13	+1	+1	+1	-1	4
14	+1	+1	-1	+1	17
15	0	0	0	0	68

Table S7. Analysis of variance of PB design for SO_3:pyridine as sulfating agent.

Source	d.f.	Mean square	Sum of squares	F-value	P-value*
Model	5	1202.53	6012.64	12.55	0.001 (S)
Temperature (Z_1)	1	18.26.71	18.26.71	19.09	0.002 (S)
Time (Z_2)	1	680.30	680.30	7.10	0.026 (S)
Polymer Con (Z_3)	1	4.65	4.65	0.05	0.831 (NS)
MRatio (Z_4)	1	1748.03	1748.03	18.24	0.002 (S)
Curvature	1	1752.95	1752.95	18.29	0.002 (S)

* S and NS stand for significant and non-significant, respectively

Table S8. Range of different factors in BBD-RSM design based on literature survey, results of PB design and preliminary runs

Range	Temperature (°C) (X_1)	Time (min) (X_2)	Molar Ratio (Reagent/OH) (X_3)
Lo (-1)	35	385	1
Central point (CP (0))	70	792.5	2.5
Hi (+1)	105	1200	4

Model fitting, transformation and reduction

Not-reduced, not-transformed model equation:

$$DS = -34.4 + 1.968X_1 + 0.0548X_2 + 36.35X_3 - 0.01446X_1X_1 - 0.000008X_2X_2 - 5.36X_3X_3 - 0.0003717X_1X_2 - 0.0812X_1X_3 + 0.00201X_2X_3$$

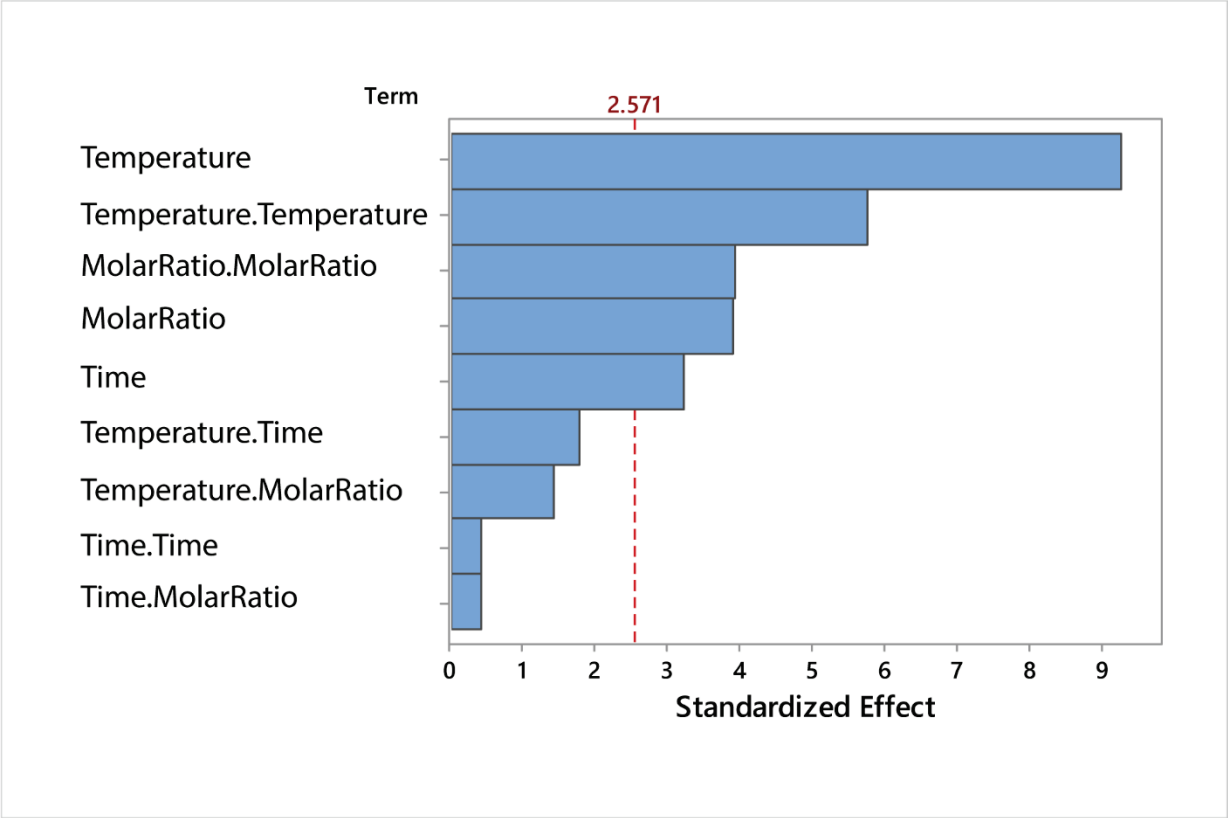


Figure S1. Standardized effect of all terms in the original model (not transformed-not reduced) on the %DS (α=0.05).

A quadratic model was used to explain the mathematical relationship between the independent variables and dependent response.

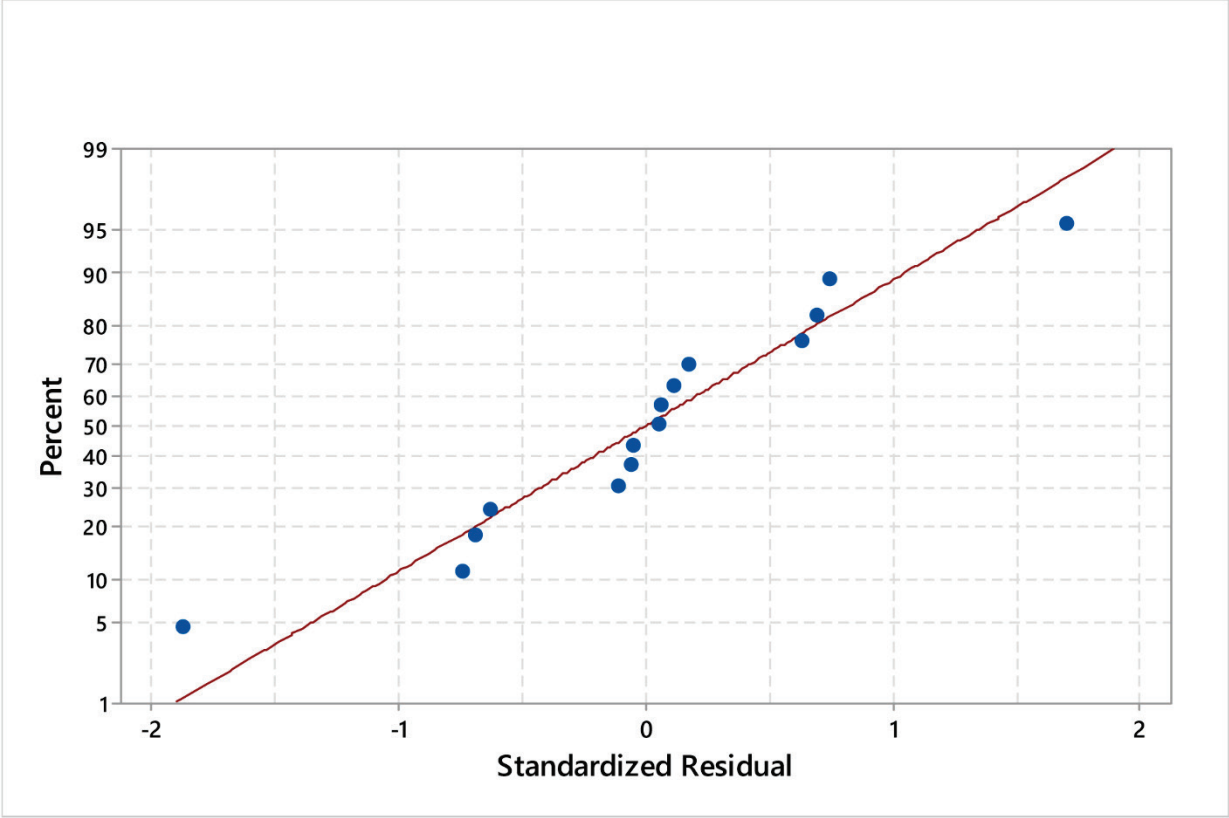


Figure S2. Normal probability of residuals for not-reduced, not-transformed model.

The points of normal probability plot followed a line suggesting the data set is approximately normal.

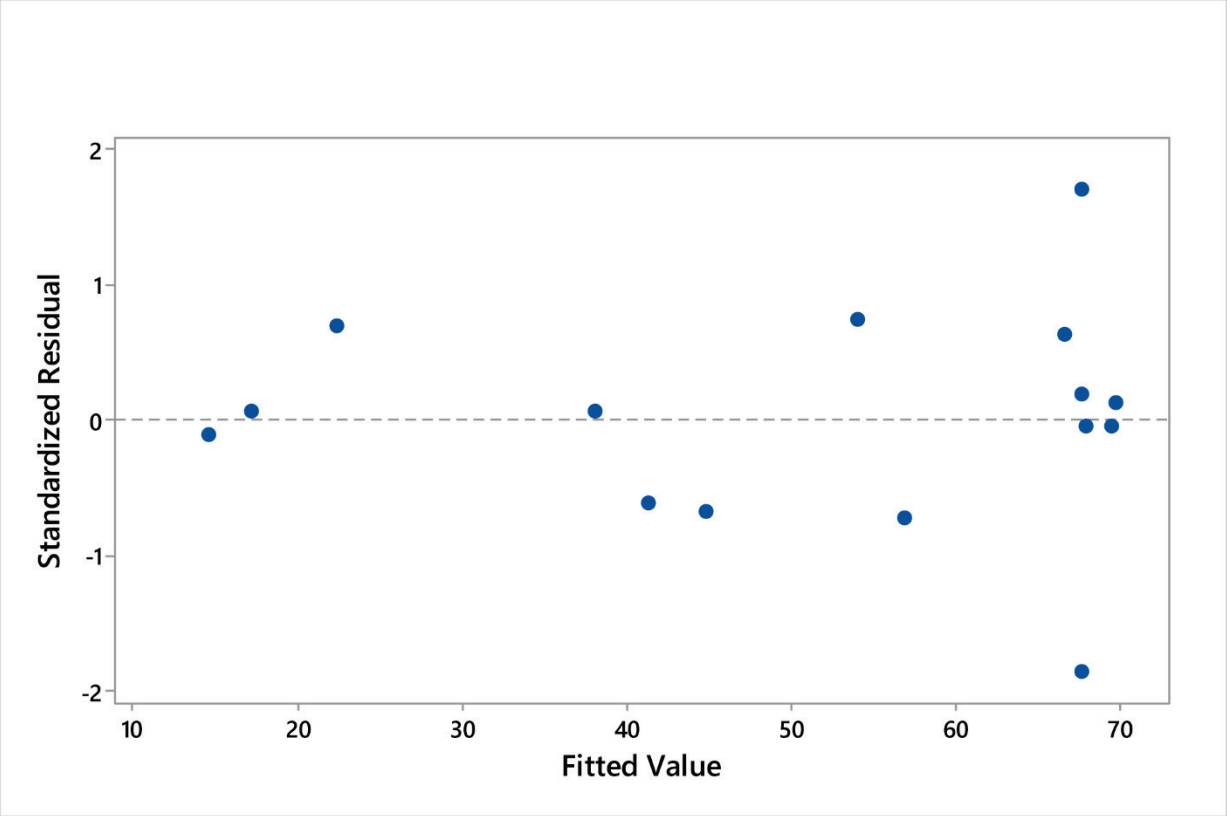


Figure S3. Standardized residuals vs fitted values plot for not-reduced, not-transformed model.
The residuals fall randomly around 0, suggesting the assumption of the linearity is reasonable. No residual stands out from the basic pattern of residuals, suggesting there are no outliers. The residuals show a fanning effect from left to right, suggesting the variances of the error terms are not equal. To improve the homoscedasticity of the model, Box-Cox transformation can be performed on the data.

Table S9. Analysis of variance for transformed data.*

Source	d.f.	Mean Square	Sum of Squares	F-value	P-value
Model	9	629.86	5668.78	17.95	0.003 (S)
Temperature (X_1) (Linear)	1	2997.51	2997.51	85.431.00	0.000 (S)
Time (X_2) (Linear)	1	361.15	361.15	10.291.00	0.024 (S)
MRatio (X_3) (Linear)	1	533.20	533.20	15.201.00	0.011 (S)
Temperature*Temperature (X_1X_1) (Square)	1	1158.05	1158.05	33.011.01	0.002 (S)
Time*Time (X_2X_2) (Square)	1	6.34	6.34	0.181.01	0.688 (NS)
MRatio*MRatio (X_3X_3) (Square)	1	537.40	537.40	15.321.01	0.011 (S)
Temperature*Time (X_1X_2) (2-way interaction)	1	112.18	112.18	3.201.00	0.134 (NS)
Temperature*MRatio (X_1X_3) (2-way interaction)	1	72.77	72.77	2.071.00	0.209 (NS)
Time*MRatio (X_2X_3) (2-way interaction)	1	6.02	6.02	0.171.00	0.696 (NS)
Lack of fit	3	8.36	25.09	-0.11	0.946 (NS)

*Lack of fit is greater than 0.05, which means the model fits the data well. Some terms have a P-value of higher than 0.05 and suggest the model might be reducible. S and NS stand for significant and non-significant, respectively

Table S10. Model summary for not-reduced, not-transformed data.*

R^2	R^2 (adj)	R^2 (pred)
97.00	91.60	87.34

* Adjusted R^2 compares the explanatory power of regression models that contain different numbers of predictors, while predicted R^2 indicates how well a regression model predicts responses for new observations. The difference between predicted R^2 and adjusted R^2 may suggest that there are too many terms in the model.

Not-reduced, Box-Cox transformed model equation:

$$DS^{0.5} = 0.92 + 0.1568X_1 + 0.00086X_2 + 2.408X_3 - 0.001174X_1X_1 - 0.000000X_2X_2 - 0.3861X_3X_3 - 0.000036X_1X_2 - 0.00315X_1X_3 + 0.000195X_2X_3$$

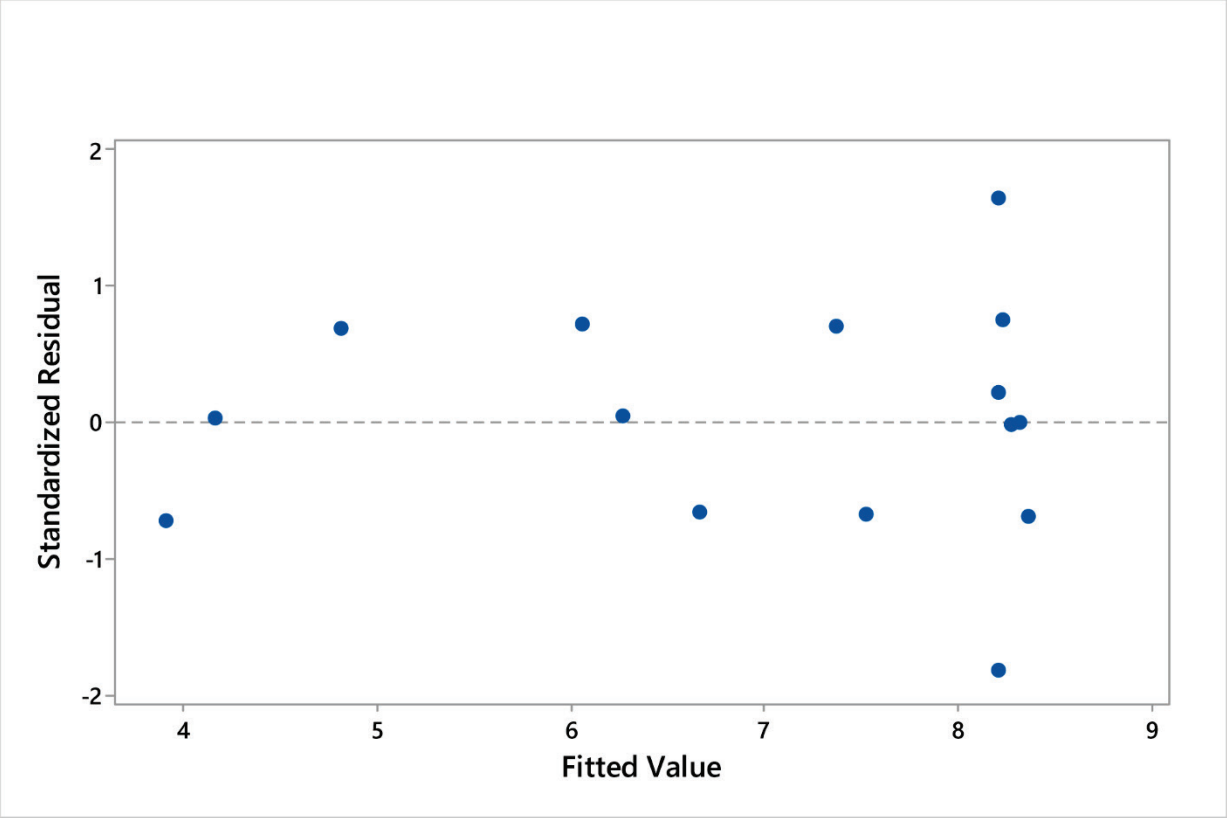


Figure S4. Standardized residuals vs fitted values plot for not-reduced, Box-Cox-transformed data.

The residuals fall randomly around 0, suggesting the assumption of the linearity is reasonable. No residual stands out from the basic pattern of residuals, suggesting there are no outliers. The residuals show a better behaving residual versus fits plot. Homoscedasticity of the model improved by Box-Cox transformation.

Table S11. Analysis of variance for not-reduced, transformed model.*

Source	d.f.	Mean Square	Sum of Squares	F-value	P-value
Model	9	3.9386	35.4477	28.04	0.001 (S)
Temperature (X_1) (Linear)	1	19.0088	19.0088	135.33	0.000 (S)
Time (X_2) (Linear)	1	2.3101	2.3101	16.45	0.010 (S)
MRatio (X_3) (Linear)	1	3.0429	3.0429	21.66	0.006 (S)
Temperature*Temperature (X_1X_1) (Square)	1	7.6417	7.6417	54.40	0.001 (S)
Time*Time (X_2X_2) (Square)	1	0.0008	0.0008	0.01	0.944 (NS)
MRatio*MRatio (X_3X_3) (Square)	1	2.7870	2.7870	19.84	0.007 (S)
Temperature*Time (X_1X_2) (2-way interaction)	1	1.0589	1.0589	7.54	0.041 (S)
Temperature*MRatio (X_1X_3) (2-way interaction)	1	0.1092	0.1092	0.78	0.418 (NS)
Time*MRatio (X_2X_3) (2-way interaction)	1	0.0568	0.0568	0.40	0.553 (NS)
Lack of fit	3	0.0462	0.0462	0.16	0.912 (NS)

*Term X_2X_2 shows a very high P-value in the model, suggesting it does not have a significant effect on the model and can be omitted and the model can be reduced. The stepwise model reduction approach was adopted. The term with the highest P-value (X_2X_2) will be omitted and the analysis of variance will be performed again. This procedure will be continued until a satisfactory model is achieved. S and NS stand for significant and non-significant, respectively

Table S12. Model summary for not-reduced, transformed data.*

R^2	R^2 (adj)	R^2 (pred)
98.06	94.56	90.36

* Transformation improved both adjusted R^2 and predicted R^2 but does not decrease the difference between them.

Reduced (step 1), Box-Cox transformed model equation:

$$DS^{0.5} = 0.98 + 0.1567X_1 + 0.00072X_2 + 2.405X_3 - 0.001173X_1X_1 - 0.3856X_3X_3 - 0.000036X_1X_2 - 0.00315X_1X_3 + 0.000195X_2X_3$$

Table S13. Analysis of variance for reduced (step 1), transformed model (Term X_2X_2 is omitted).*

Source	d.f.	Mean Square	Sum of Squares	F-value	P-value
Model	8	4.4309	35.4469	37.81	0.000 (S)
Temperature (X_1) (Linear)	1	19.0088	19.0088	162.22	0.000 (S)
Time (X_2) (Linear)	1	2.3101	2.3101	19.71	0.004 (S)
MRatio (X_3) (Linear)	1	3.0429	3.0429	25.97	0.002 (S)
Temperature*Temperature (X_1X_1) (Square)	1	7.6753	7.6753	65.50	0.000 (S)
MRatio*MRatio (X_3X_3) (Square)	1	2.7964	2.7964	23.86	0.003 (S)
Temperature*Time (X_1X_2) (2-way interaction)	1	1.0589	1.0589	9.04	0.024 (S)
Temperature*MRatio (X_1X_3) (2-way interaction)	1	0.1092	0.1092	0.93	0.372 (NS)
Time*MRatio (X_2X_3) (2-way interaction)	1	0.0568	0.0568	0.48	0.512 (NS)
Lack of fit	4	0.0348	0.1393	0.12	0.961 (NS)

*Term X_2X_3 shows a very high P-value in the model, suggesting it does not have a significant effect on the model and can be omitted and the model can be reduced. S and NS stand for significant and non-significant, respectively

Table S14. Model summary for reduced (step 1), transformed data.*

R^2	R^2 (adj)	R^2 (pred)
98.06	95.46	93.30

* Omitting term X_2X_2 does not have a significant effect on adjusted R^2 but increases predicted R^2 and makes the two values closer to each other.

Reduced (step 2), Box-Cox transformed model equation:

$$DS^{0.5} = 0.59 + 0.1567X_1 + 0.001207X_2 + 2.560X_3 - 0.001173X_1X_1 - 0.3856X_3X_3 - 0.000036X_1X_2 - 0.00315X_1X_3$$

Table S15. Analysis of variance for reduced (step 2), transformed model (Terms X_2X_2 and X_2X_3 are omitted).*

Source	d.f.	Mean Square	Sum of Squares	F-value	P-value
Model	7	5.0557	35.3901	46.57	0.000 (S)
Temperature (X_1) (Linear)	1	19.0088	19.0088	175.10	0.000 (S)
Time (X_2) (Linear)	1	2.3101	2.3101	21.28	0.002 (S)
MRatio (X_3) (Linear)	1	3.0429	3.0429	28.03	0.001 (S)
Temperature*Temperature (X_1X_1) (Square)	1	7.6753	7.6753	70.70	0.000 (S)
MRatio*MRatio (X_3X_3) (Square)	1	2.7964	2.7964	25.76	0.001 (S)
Temperature*Time (X_1X_2) (2-way interaction)	1	1.0589	1.0589	9.75	0.017 (S)
Temperature*MRatio (X_1X_3) (2-way interaction)	1	0.1092	0.1092	1.01	0.349 (NS)
Lack of fit	5	0.0392	0.1962	0.14	0.966 (NS)

*Term X_1X_3 shows a high P-value in the model, suggesting it does not have a significant effect on the model and can be omitted and the model can be reduced under the conditions that the assumptions of linear regression are met. If normality (no drastic deviation from normal probability plot), homoscedasticity (no drastic deviation from the random distribution in residual versus fitted values plot) and multicollinearity (no VIF value significantly greater than 1) of the model are satisfactory, this term can be omitted. S and NS stand for significant and non-significant, respectively

Table S16. Model summary for reduced (step 2), transformed data.*

R^2	R^2 (adj)	R^2 (pred)
97.90	95.80	94.20

*Omitting term X_2X_3 does not have a significant effect on adjusted R^2 but increases predicted R^2 and makes the two values closer to each other.

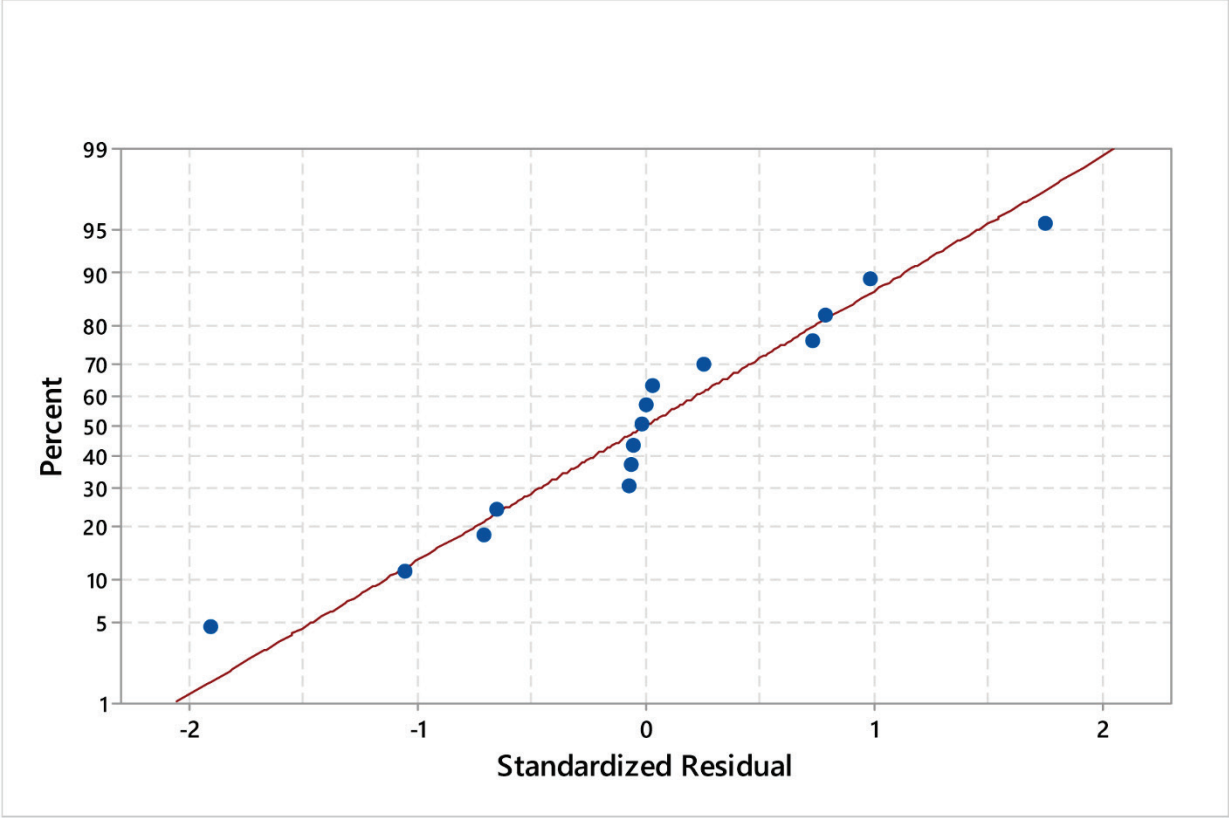


Figure S5. Normal probability of residuals for reduced (step 2), transformed model.

The points of normal probability plot approximately followed a line suggesting the data set is approximately normal. Normal probability assumption is achieved better in this model than the further reduced model (step 3, Box-Cox transformation; see Figure S7).

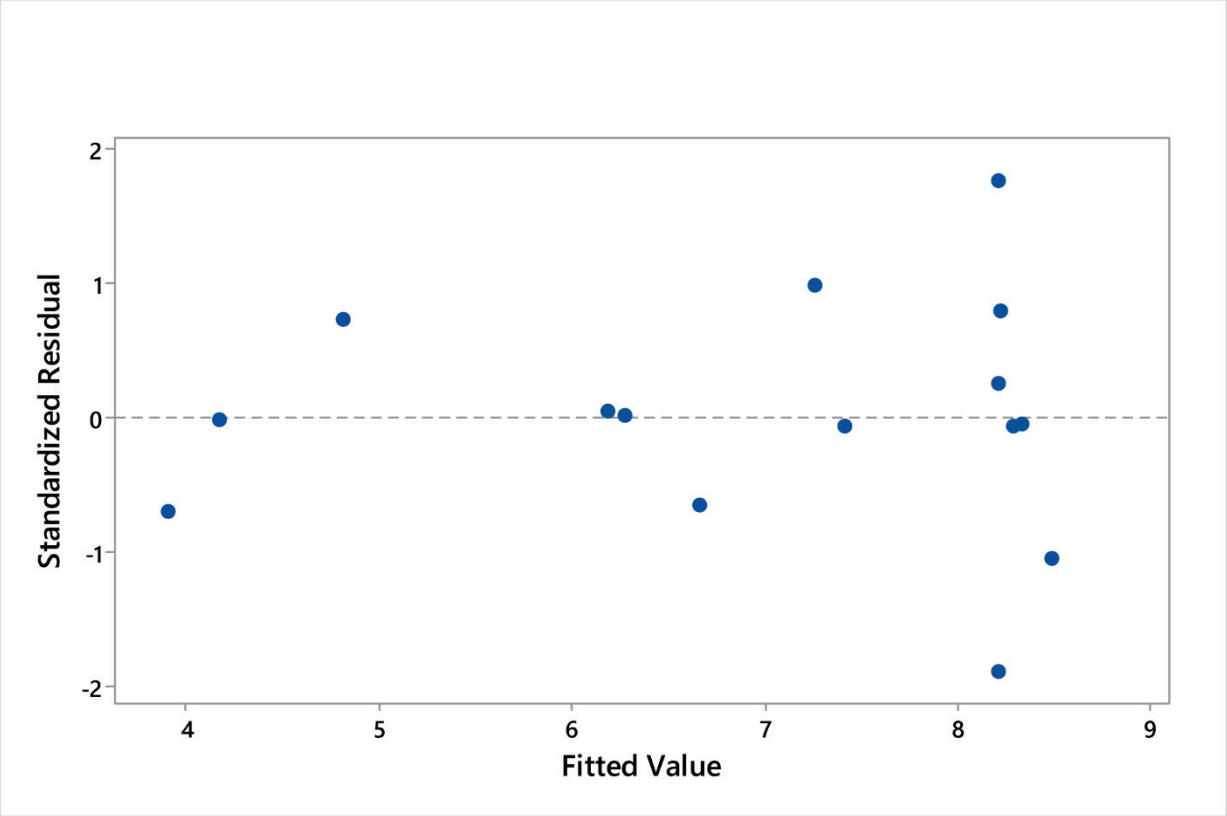


Figure S6. Standardized residuals vs fitted values plot for reduced (step 2), Box-Cox-transformed model.

The residuals fall randomly around 0 line, suggesting the assumption of the linearity is reasonable. No residual stands out from the basic pattern of residuals, suggesting there are no outliers. The residuals show a relatively better behaving residual versus fits plot in comparison to the further reduced (step 3), Box-Cox transformed model. Homoscedasticity of this model is better than the further reduced model (step 3, Box-Cox transformed; see Figure S8).

Reduced (step 3), Box-Cox transformed model equation:

$$DS^{0.5} = 1.14 + 0.1488X_1 + 0.001207X_2 + 2.339X_3 - 0.001173X_1X_1 - 0.3856X_3X_3 - 0.000036X_1X_2$$

Table S17. Analysis of variance for reduced (step 3), transformed model (Terms X_2X_2, X_2X_3 and X_1X_3 are omitted).

Source	d.f.	Mean Square	Sum of Squares	F-value	P-value*
Model	6	5.8801	35.2809	54.12	0.000 (S)
Temperature (X_1) (Linear)	1	19.0088	19.0088	174.97	0.000 (S)
Time (X_2) (Linear)	1	2.3101	2.3101	21.26	0.002 (S)
MRatio (X_3) (Linear)	1	3.0429	3.0429	28.01	0.001 (S)
Temperature*Temperature (X_1X_1) (Square)	1	7.6753	7.6753	70.65	0.000 (S)
MRatio*MRatio (X_3X_3) (Square)	1	2.7964	2.7964	25.74	0.001 (S)
Temperature*Time (X_1X_2) (2-way interaction)	1	1.0589	1.0589	9.75	0.014 (S)
Lack of fit	6	0.0509	0.3054	0.18	0.957 (S)

* S and NS stand for significant and non-significant, respectively

Table S18. Model summary for reduced (step 3), transformed data.*

R^2	R^2 (adj)	R^2 (pred)
97.60	95.79	94.64

* Omitting term X_1X_3 does not have a significant effect on adjusted R^2 or predicted R^2.

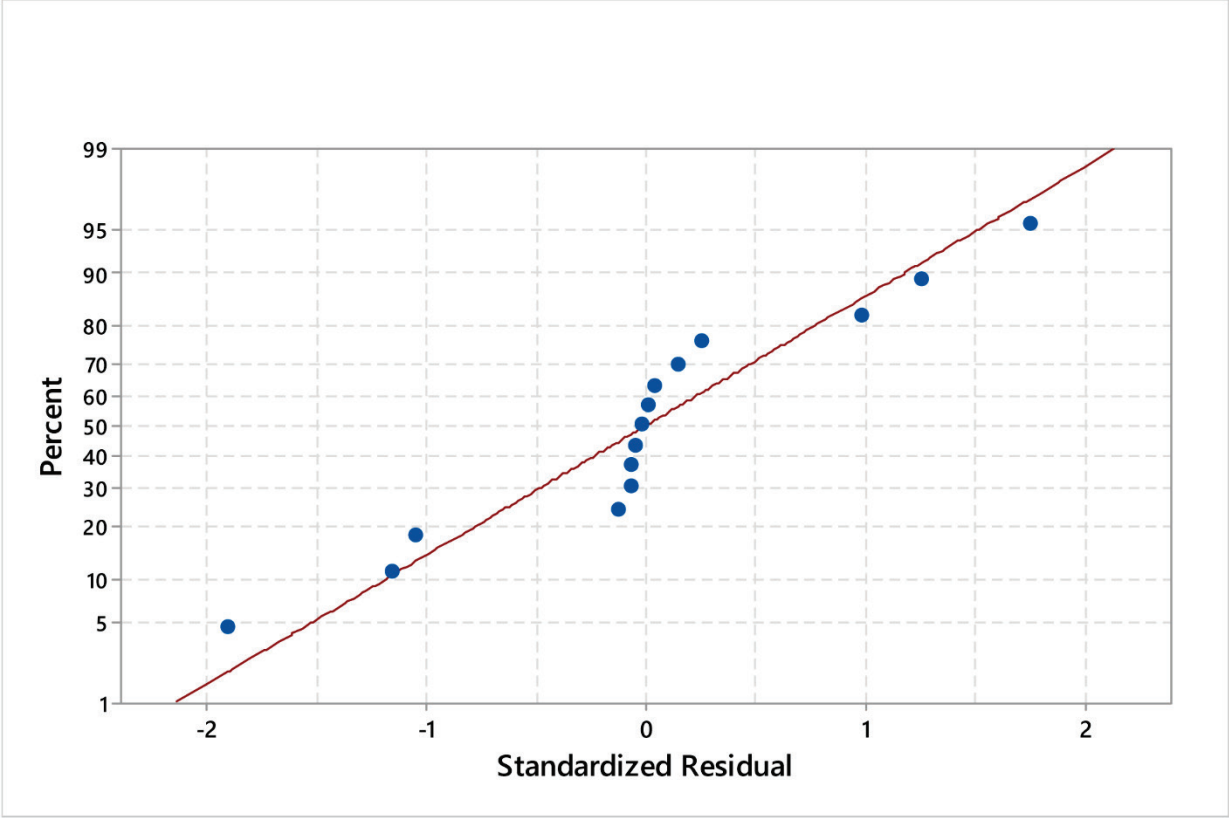


Figure S7. Normal probability of residuals for reduced (step 3), transformed model.

The points of normal probability plot approximately followed a line but the normal probability assumption in this model is not better than the model in step 2 (step 2, Box-Cox transformation; see Figure S5).

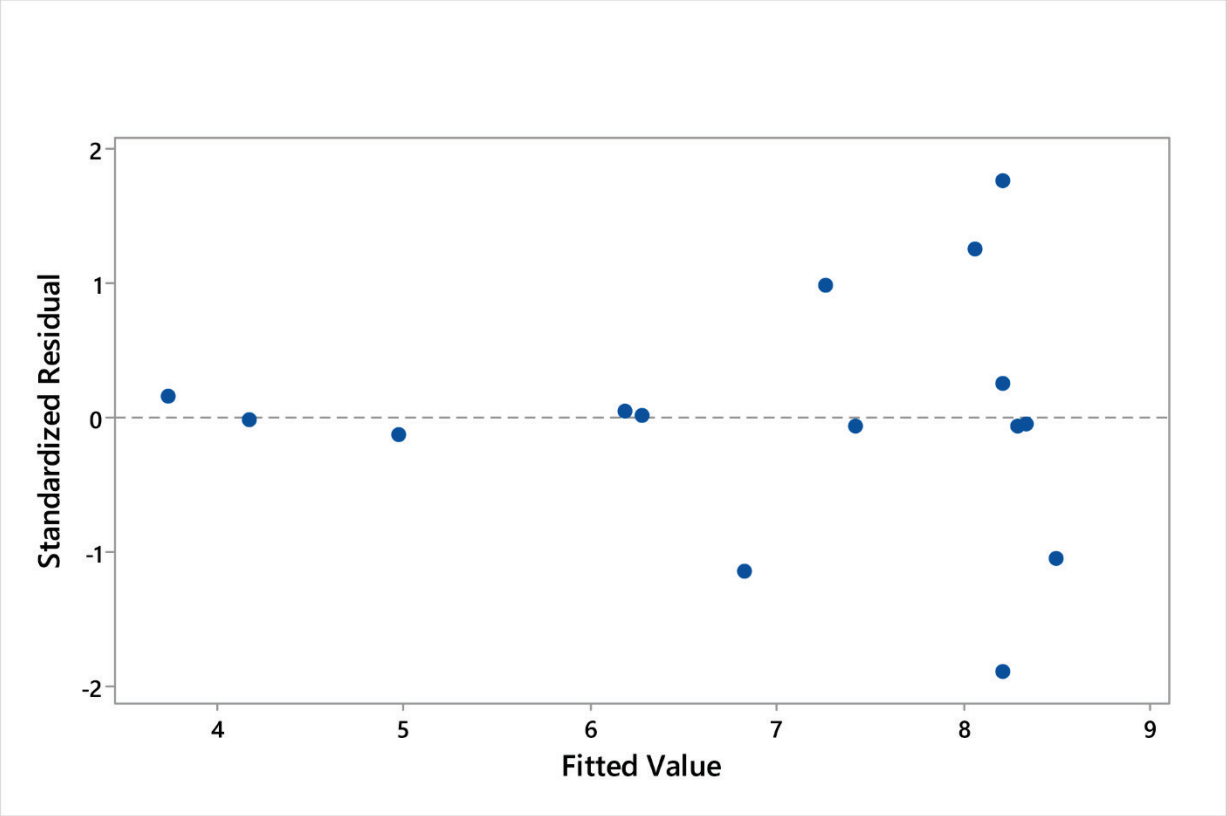


Figure S8. Standardized residuals vs fitted values plot for reduced (step 3), Box-Cox-transformed data.

The residuals fall randomly around 0 line, suggesting the assumption of the linearity is reasonable. No residual stands out from the basic pattern of residuals, suggesting there are no outliers. The residuals show a bad behaving residual versus fits plot in comparison to the reduced model in step 2 (step 2, Box-Cox transformed model). Homoscedasticity of this model is worse than the step 2 reduced model (step 2, Box-Cox transformed; see Figure S6). Based on the analysis of variance, omitting X_1X_3 does not improve the model significantly (not a significant increase in adjusted or predicted R^2 or in P-value of the model). However, it is detrimental to normal probability plot and homoscedasticity. Therefore, in the reduced (step 2), Box-Cox transformed model was selected as the optimal model in this study. Reduced (step 2) model shows no evidence of lack of fit and shows high values of adjusted R^2 and predicted R^2, while the normality, homoscedasticity and multicollinearity assumptions are satisfactory. Therefore, this model was chosen to be used in this study.

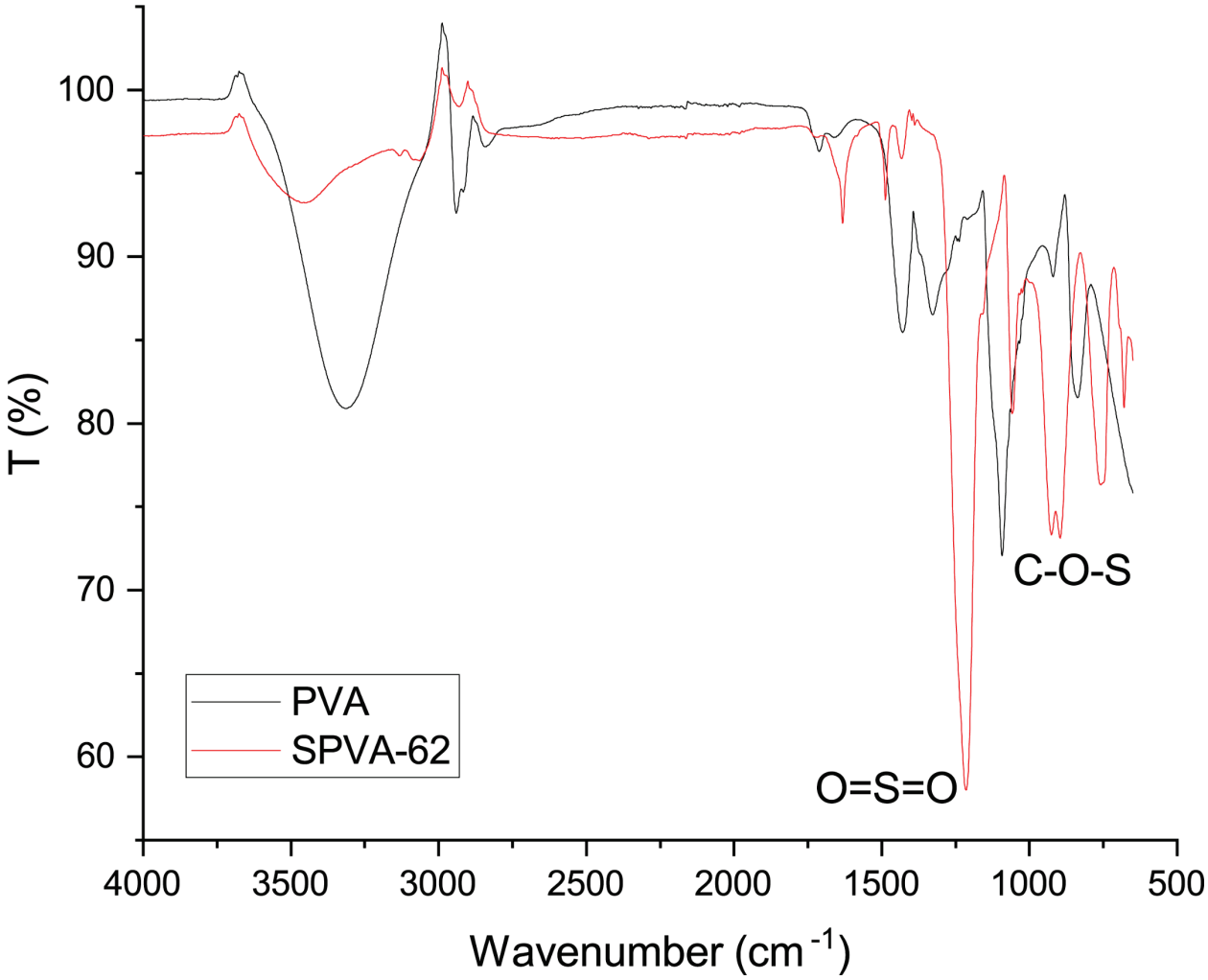
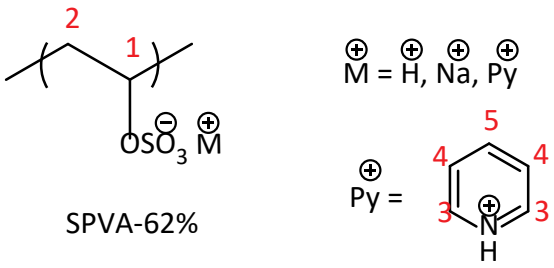


Figure S9. FT-IR spectra of PVA and SPVA-62.

The peaks at 901 cm^{-1} and 1213 cm^{-1} correspond to C-O-S and O=S=O, respectively. The peak at 3337 cm^{-1} in both spectra corresponds to OH, and the peak at 2941 cm^{-1} corresponds to C-H.



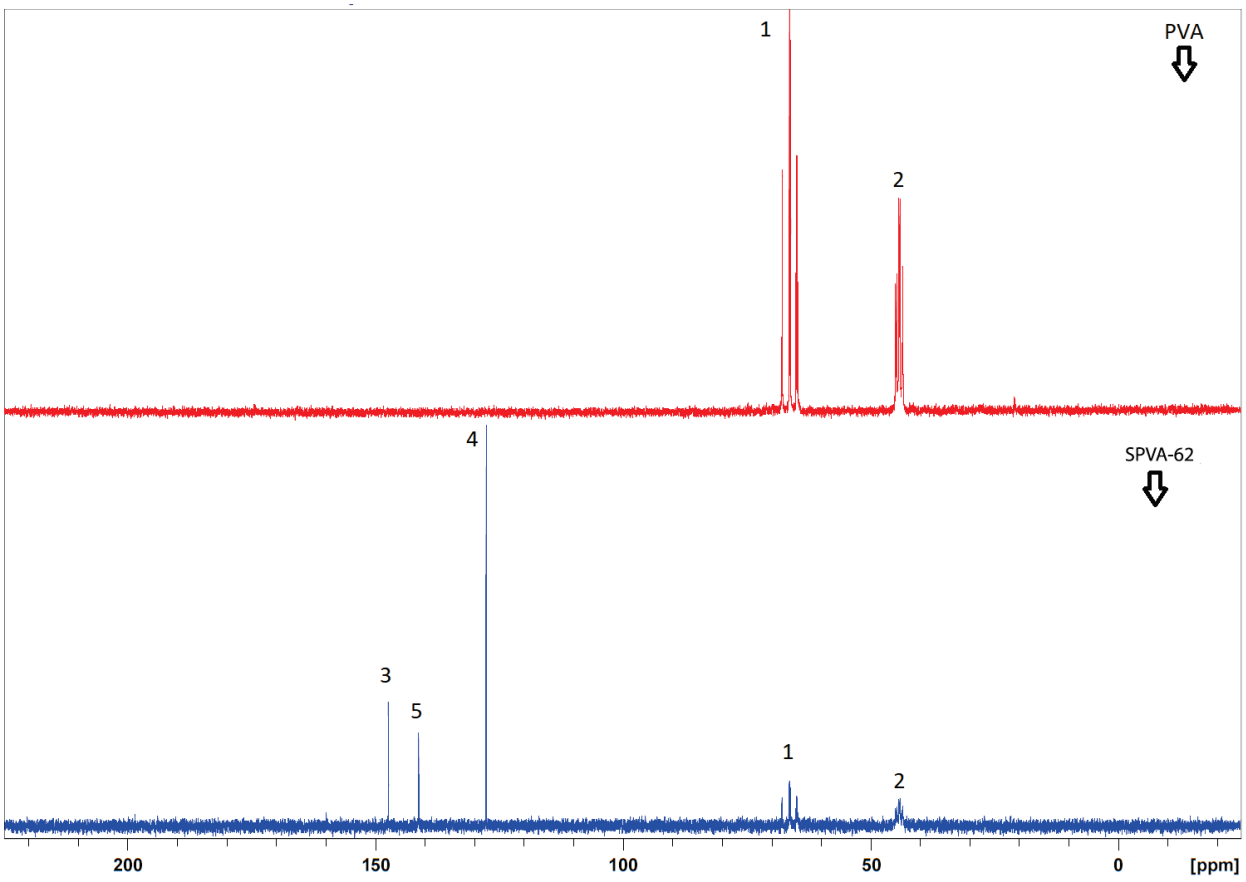


Figure S10. ^{13}C-NMR and molecular structure of PVA and SPVA-62.

The peaks and the corresponding carbon atoms are numbered in the chemical structure and the spectrum. Peaks in the range of 64.9-67.9 ppm for CH and 43.6-45 ppm for CH_2. The peaks at 147.4, 127.6, and 141.3 ppm were assigned to C_3, C_4 and C_5 of pyridinium ion, respectively, which remained despite extensive dialysis.

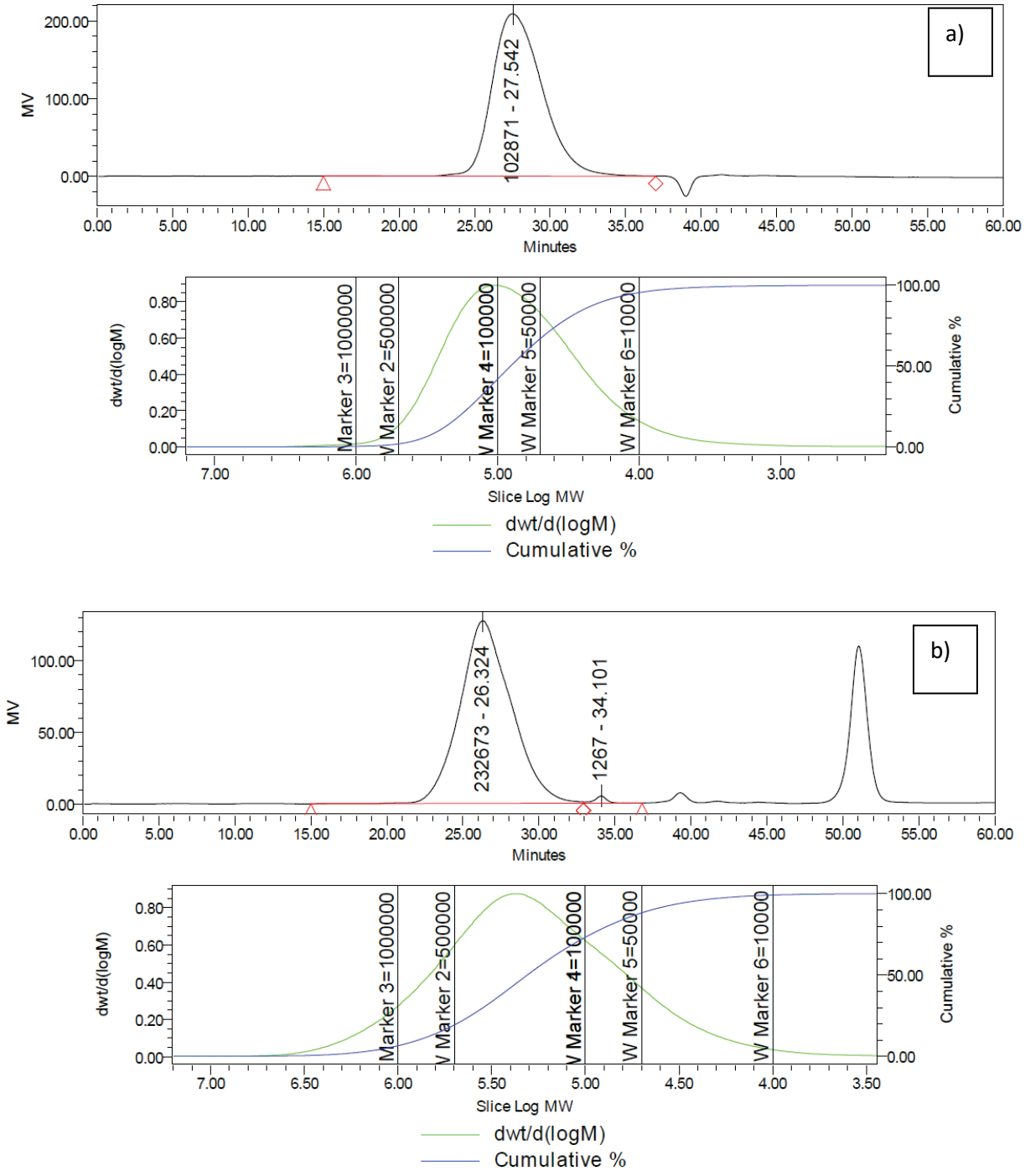


Figure S11. GPC of PVA (a) and SPVA-62 (b).

The M_p of PVA was 103 KDa (PDI 4.08) versus a molecular weight of 125 kDa reported by the manufacturer. The M_p of SPVA-62 was 233 KDa (PDI of 3.70), which suggests no major degradation of the polymer under the sulfation conditions. The apparently higher molecular weight of SPVA-62 may arise from a combination of the additional molar mass due to sulfation and increased radius of gyration due to the anionic nature of the polymer.

Table S19. Tukey pairwise comparisons for the APTT test.

Group	N	Mean*	Grouping
Pure Plasma	3	224.10	A
Pure water	3	164.467	B
PVA- 25 µg.ml^{-1}	3	140.100	C
PVA- 50 µg.ml^{-1}	3	79.633	D
PVA-100 µg.ml^{-1}	3	79.533	D
PVA- 200 µg.ml^{-1}	3	71.333	E
PVA-1000 µg.ml^{-1}	3	61.067	F
SPVA-38- 25 µg.ml^{-1}	3	48.900	G
SPVA-38- 50 µg.ml^{-1}	3	47.333	G
SPVA-38- 100 µg.ml^{-1}	3	44.600	H
SPVA-38- 200 µg.ml^{-1}	3	43.433	H
SPVA-62- 25 µg.ml^{-1}	3	38.4333	I
SPVA-62- 50 µg.ml^{-1}	3	36.667	I, J
SPVA-62- 100 µg.ml^{-1}	3	35.233	J
SPVA-62- 200 µg.ml^{-1}	3	32.500	K
SPVA- 84- 25 µg.ml^{-1}	3	31.833	K
SPVA- 84- 50 µg.ml^{-1}	3	30.367	K, L
SPVA- 84- 100 µg.ml^{-1}	3	30.333	K, L
SPVA- 84- 200 µg.ml^{-1}	3	29.367	L, M
Heparin- 1 µg.ml^{-1}	3	27.700	M
Heparin- 5 µg.ml^{-1}	3	27.233	M
Heparin- 10 µg.ml^{-1}	3	27.233	M
Heparin- 25 µg.ml^{-1}	3	27.167	M
Heparin- 50 µg.ml^{-1}	3	27.067	M

* Means that do not share a letter are significantly different.

Table S20. Analysis of variance for one-way ANOVA vs group.*

Source	d.f.	Adjusted sum of squares	Adjusted mean square	F-Value	p-Value*
Group	23	171370	7450.85	12487.46	<0.001
Error	48	29	0.6		
Total	71	171398			

* Null hypothesis: All means are equal.

Table S21. Tukey pairwise comparisons for the P-selectin test.

Group	N	Mean*	Grouping
Blank	3	1.000	A
P-selectin	3	0.5969	B
PVA	3	0.4953	B, C
SPVA-38	3	0.4864	B, C
SPVA-62	3	0.4623	C
SPVA-84	3	0.2455	D
Fucoidan	3	0.0000	E

* Means that do not share a letter are significantly different.

Table S22. Analysis of variance for one-way ANOVA vs group.*

Source	d.f.	Adjusted sum of squares	Adjusted mean square	F-Value	P-Value
Group	6	1.70774	0.284623	126.21	0.000
Error	14	0.03157	0.002255		
Total	20	1.73931			

* Null hypothesis: All means are equal.