Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information for

Functional cationic derivatives of starch as antimicrobial agents

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Notes on determination of degree of substitution (DS) by ^{1}H NMR

DS was calculated by using the following formula:

DS = Non exchangeable (NE) proton per anhydroglucose units (AGU)/(3*[$I_{2.6 - 8.0} - I_s$])

NE proton per AGU = 7; 3 is used to account for the possibility that there are 3 reactive sites per AGU. In our calculation, contribution from degree of branching – i.e., the change in the possible reactive sites from 3 to 2 at the branching site, has been ignored; $I_{2.6 - 8.0}$ = Integral value corresponding to all the protons within 2.6 – 8.0 ppm range; I_s = Integral value corresponding to the NE protons from the substitution reaction within 2.6 – 8.0 ppm range.

Example calculation using a representative example - *Starch-Allyl-Bn(20-80)*:

$$DS = 7/(3*[29.99 - 1*(5+6+7) + 0.13(5+6+5)]) = 0.24$$

The phenyl region $I_{7.0 - 8.0} = 5.0$, corresponding to 5H of benzene ring within 7.0 – 8.0 ppm range, was used to normalize the *N,N*-dimethylbenzylamine substitution reaction and $I_{5.95 - 6.20} = 0.13$, corresponds to 1H proton from the allylic-alkene proton within 5.95 – 6.20 ppm range, was used to estimate the *N,N*-dimethylallylamine substitution reaction (NE protons from glyceryl-subunit = 5; NE protons from dimethyl groups= 6; NE protons from benzyl-subunit = 7; NE protons from allyl-subunit = 5).

Ratio of substitution of two different amines were calculated as follows by using the integration values per 1H obtained from $I_{7.0 - 8.0}$ and $I_{5.95 - 6.20}$: $R^1 : R^2 = 0.13/(1+0.13) : 1/(1+0.13) = 12 : 88$

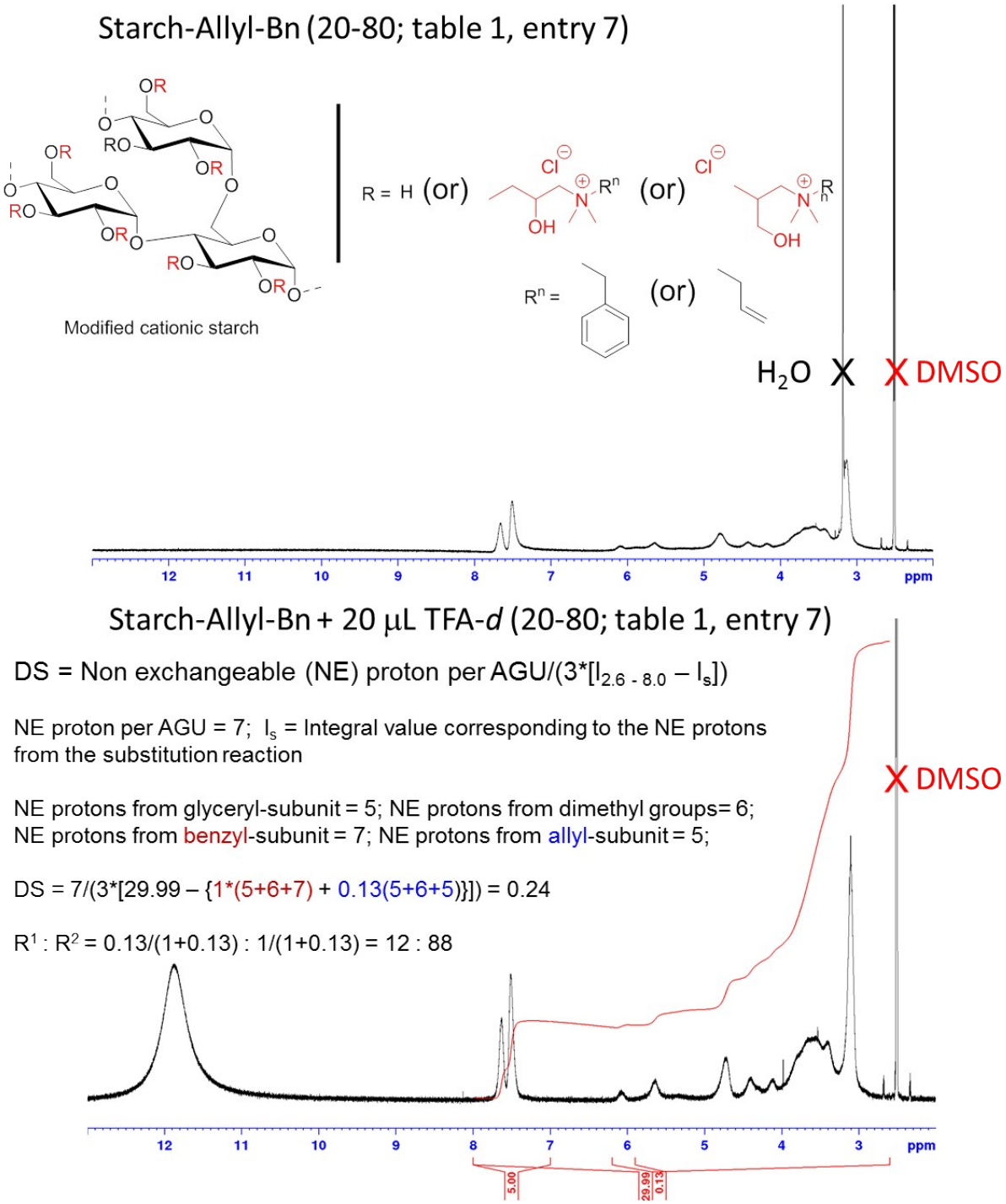
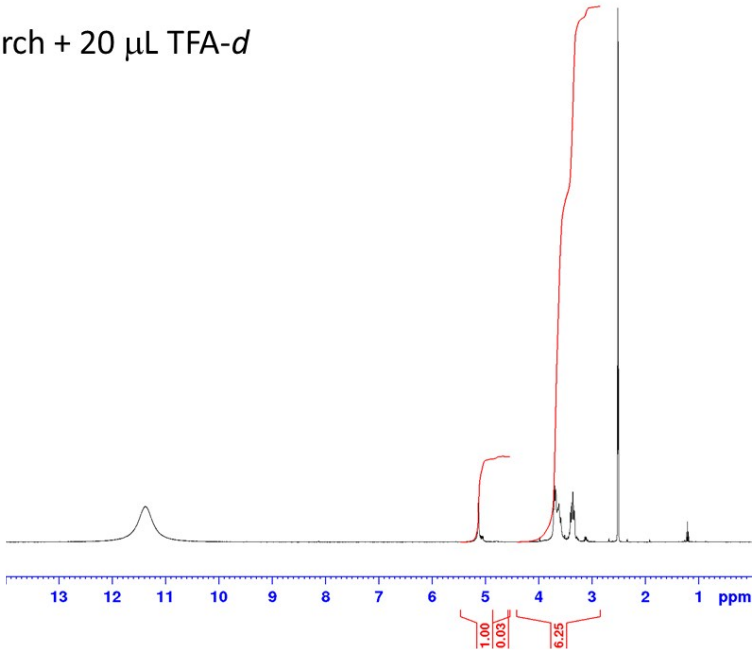


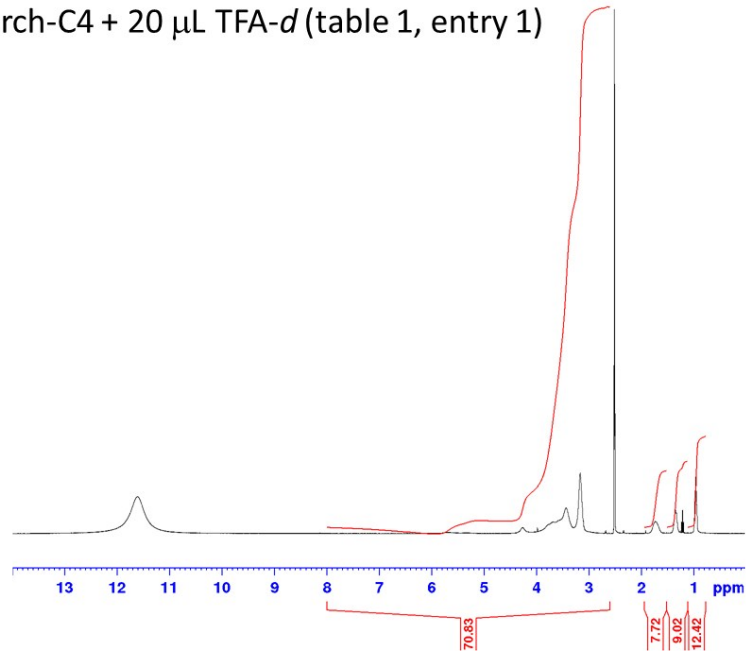
Figure S1. Determination of degree of substitution by ^{1}H NMR.

Compilation of ^{1}H NMR spectra of starch derivatives

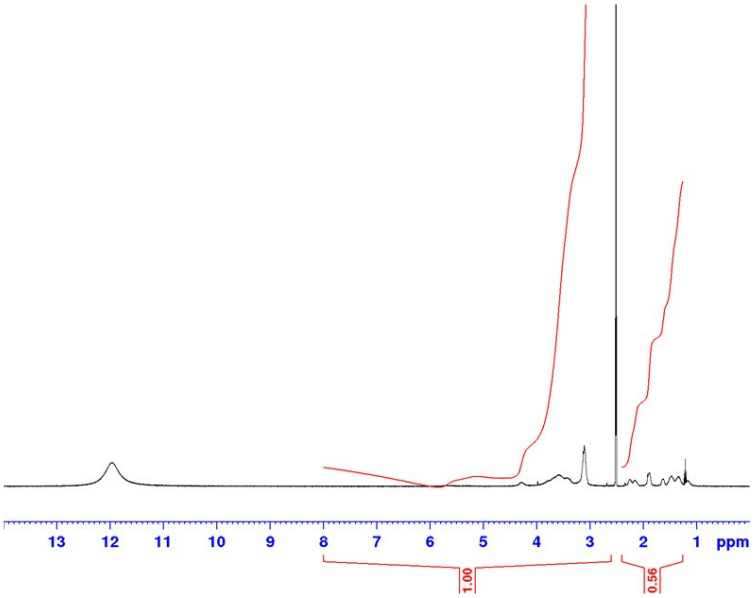
Starch + 20 μL TFA-*d*



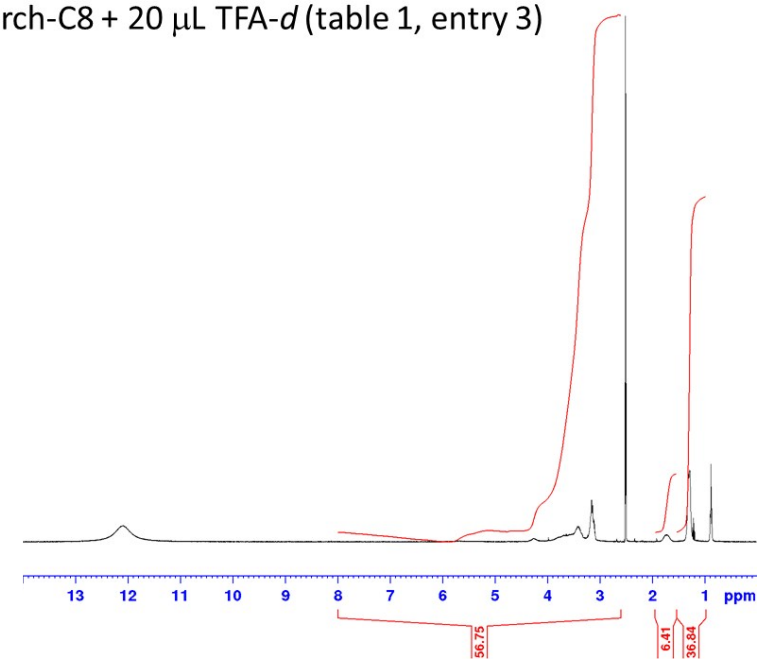
Starch-C4 + 20 μL TFA-*d* (table 1, entry 1)



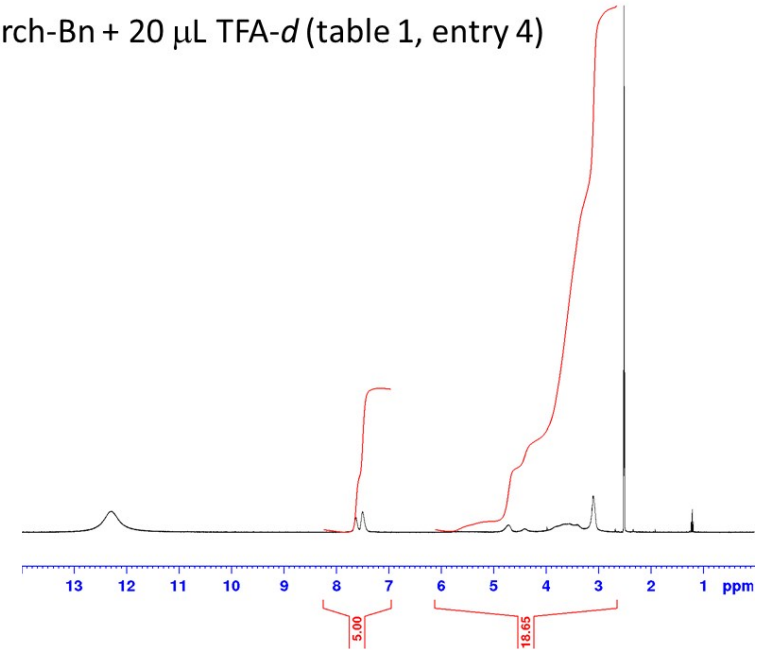
Starch-Cyclohexyl + 20 μL TFA-*d* (table 1, entry 2)



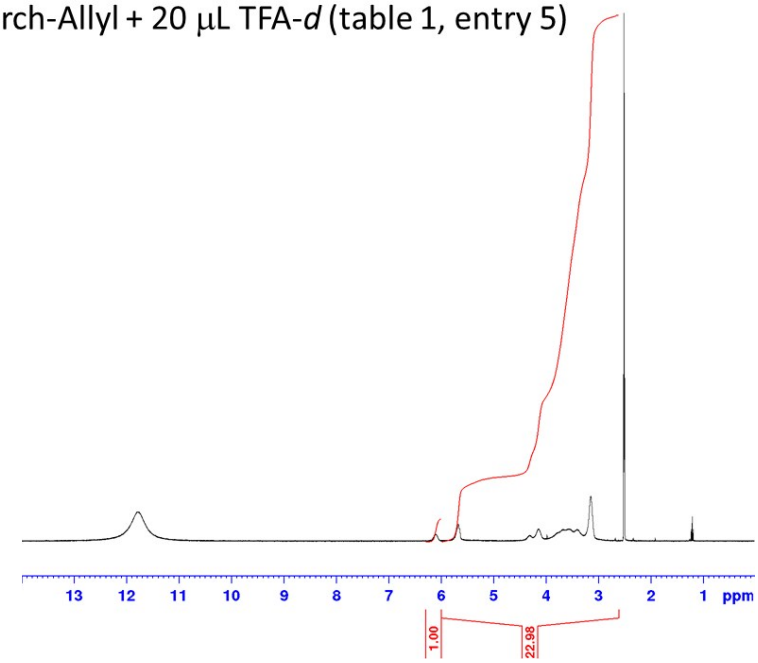
Starch-C8 + 20 μL TFA-*d* (table 1, entry 3)



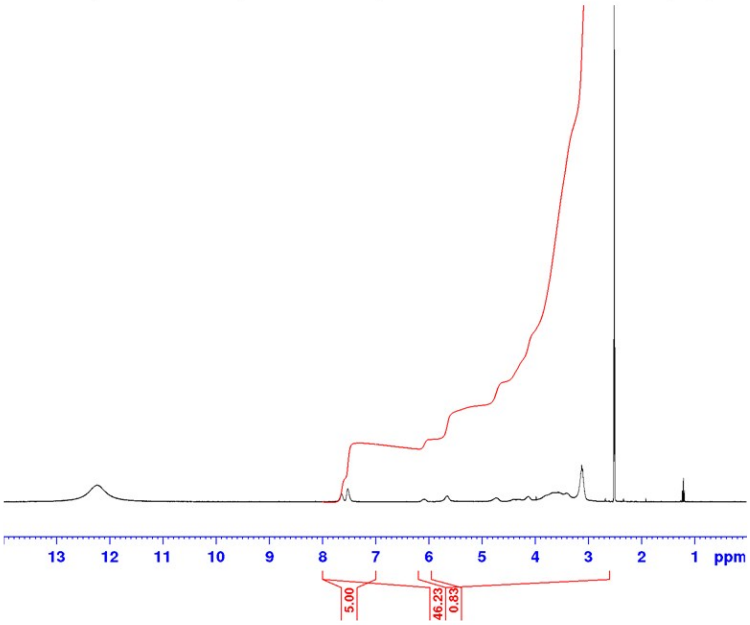
Starch-Bn + 20 μL TFA-*d* (table 1, entry 4)



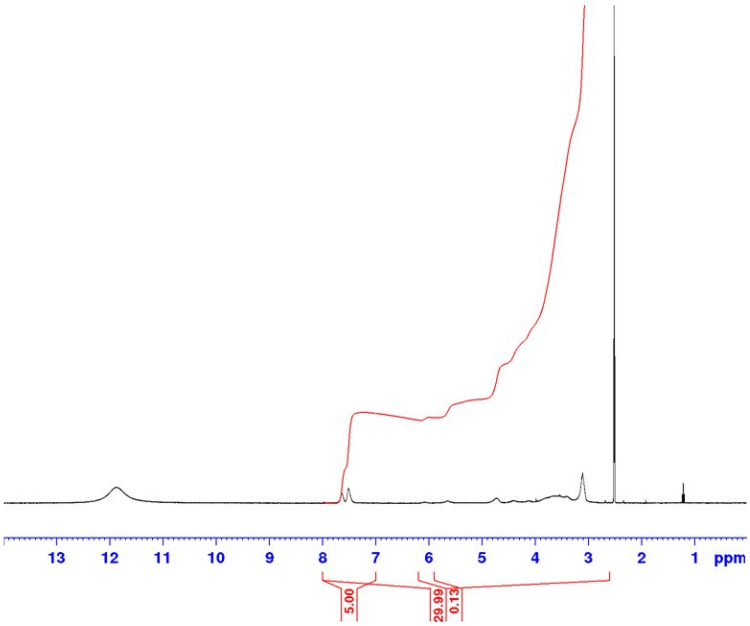
Starch-Allyl + 20 μL TFA-*d* (table 1, entry 5)



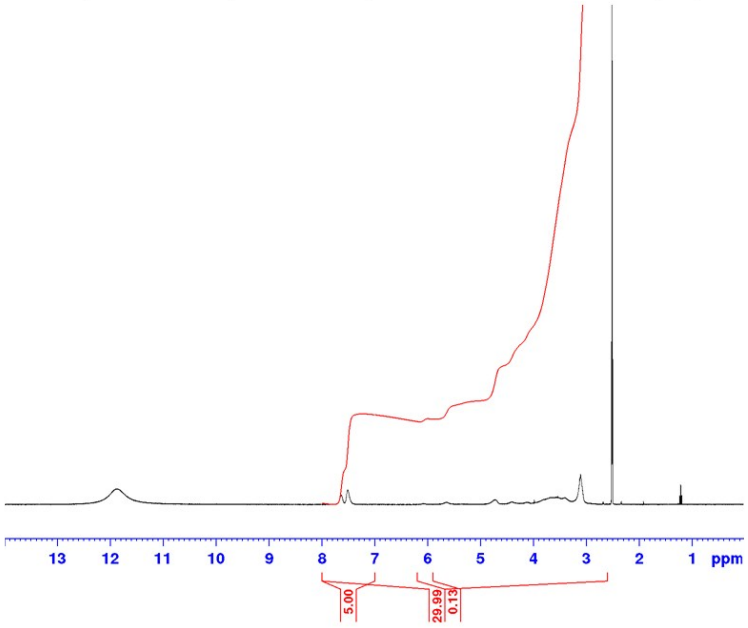
Starch-Allyl-Bn + 20 μL TFA-*d* (50-50; table 1, entry 6)



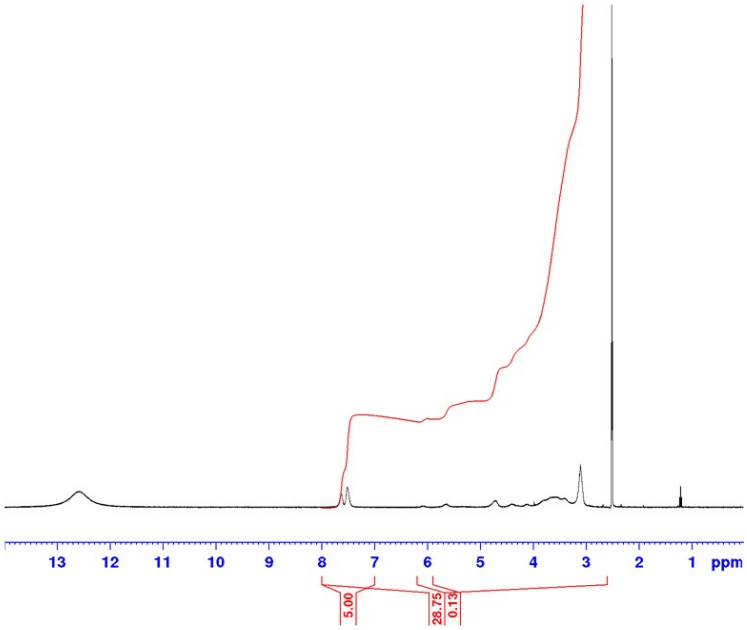
Starch-Allyl-Bn + 20 μL TFA-*d* (20-80; table 1, entry 7)



Starch-Allyl-Bn + 20 μL TFA-*d* (20-80; table 1, entry 8)



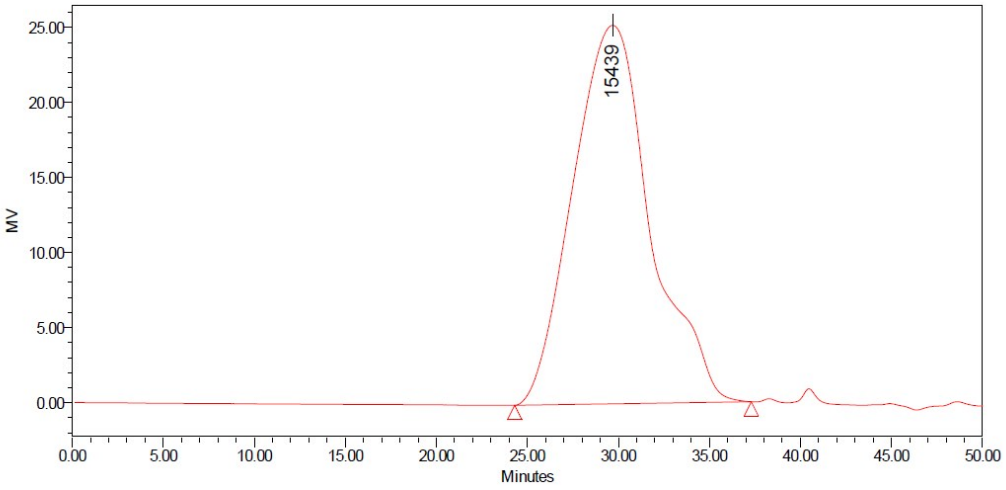
Starch-Allyl-Bn + 20 μL TFA-*d* (20-80; table 1, entry 9)



Compilation of SEC chromatographs of starch derivatives

Starch (unmodified, commercial sample - Sigma Aldrich # 33615, lot # SZBE0520V)

Eluent: 0.1(v/v) % TFA in HPLC H_2O

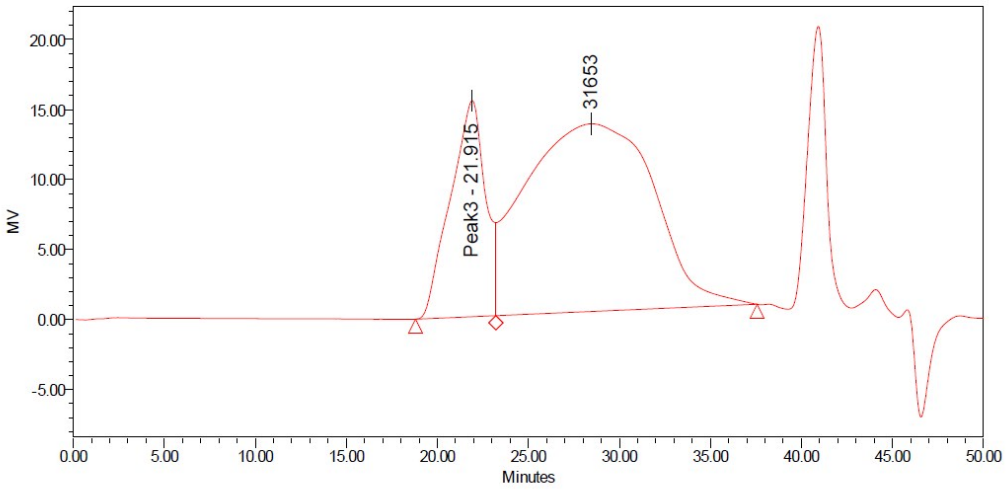


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		8446	25736	15439	60677	103999		3.047208		
2										
3										

Starch-C4 (*entry 1, Table 1*)

Eluent: 0.1(v/v) % TFA in HPLC H_2O

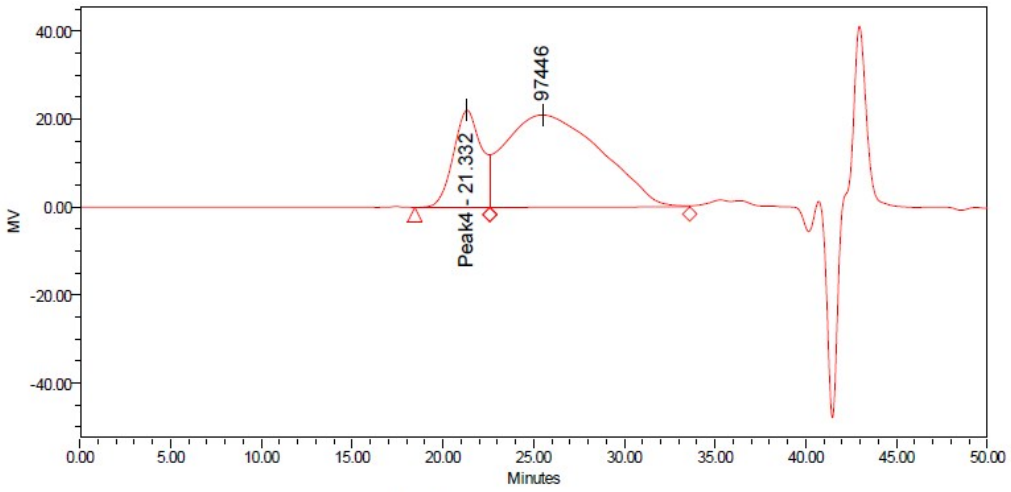


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		9798	52506	31653	123176	165966		5.358629		
3										
4										

Starch-C4 (*entry 1, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

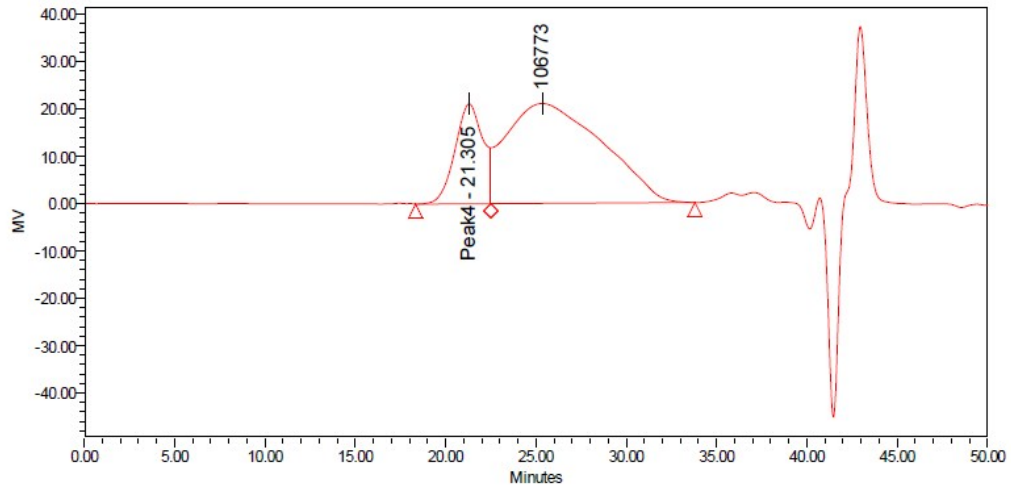


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		19777	71341	97446	135384	174675		3.607236		
3										

Starch-Cyclohexyl (*entry 2, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

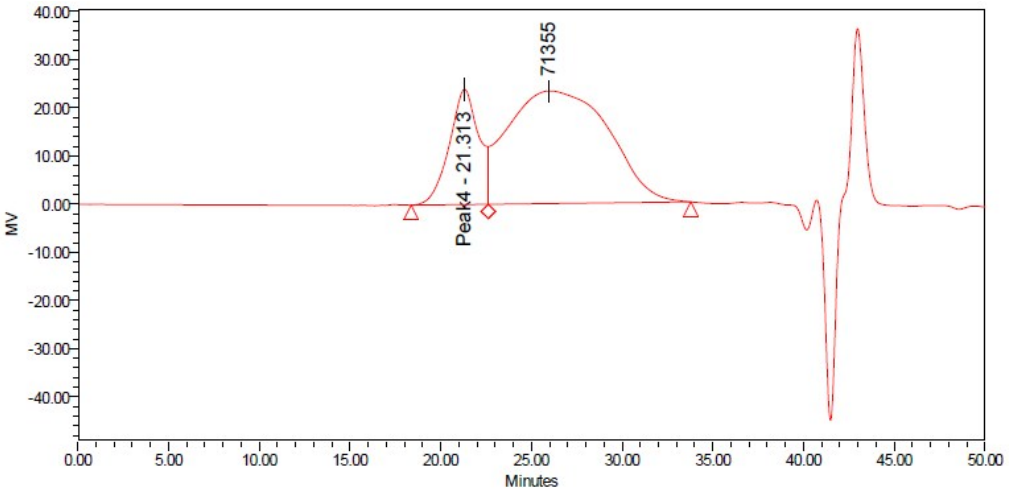


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		19988	72143	106773	136520	175308		3.609351		
3										

Starch-Bn (*entry 4, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

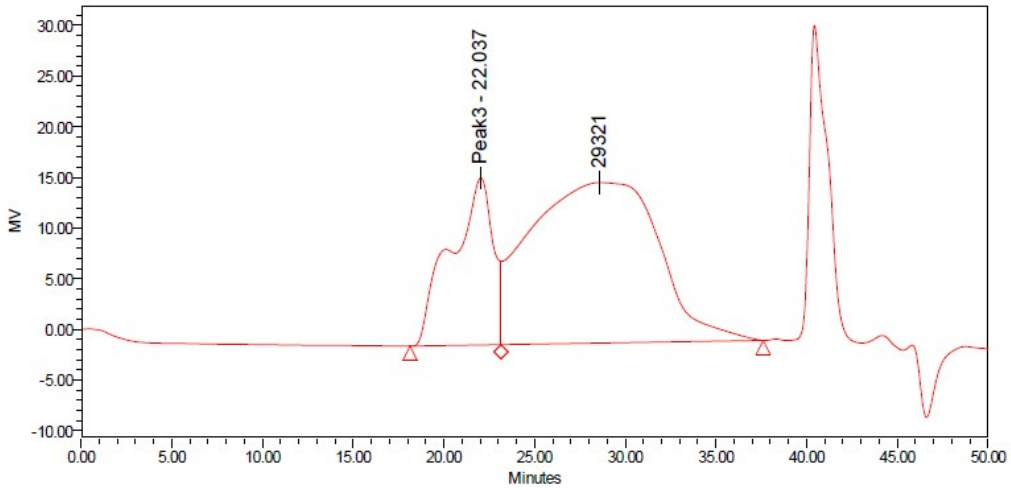


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		18698	65179	71355	129903	171811		3.485773		
3										

Starch-Allyl (*entry 5, Table 1*)

Eluent: 0.1(v/v) % TFA in HPLC H_2O

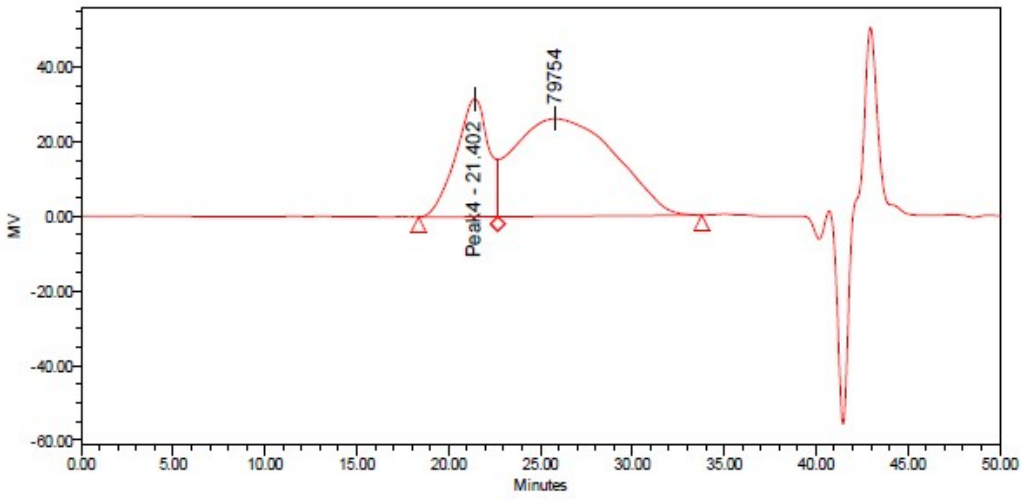


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		10065	53290	29321	124059	166891		5.294709		
3										
4										

Starch-Allyl (*entry 5, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

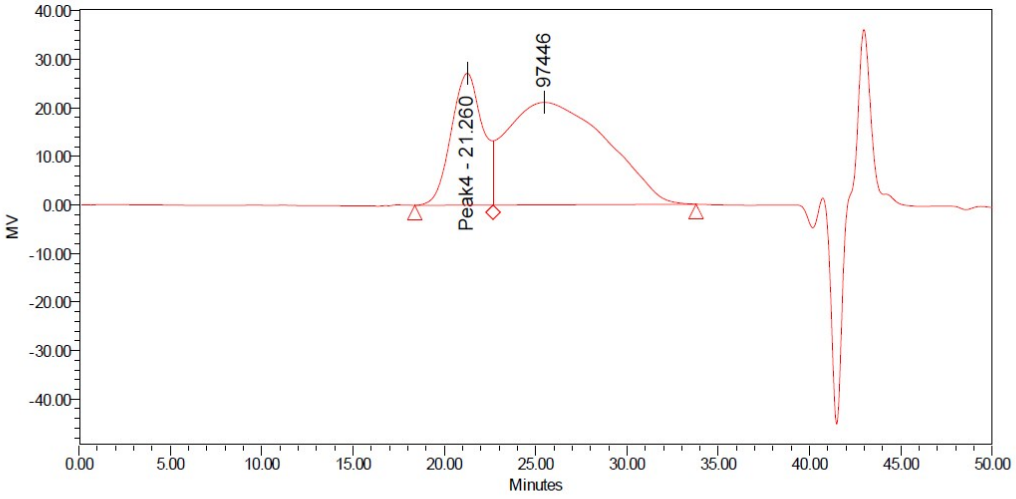


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		19053	67535	79754	132493	173410		3.544540		
3										

Starch-Allyl –Bn (50-50) (*entry 6, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

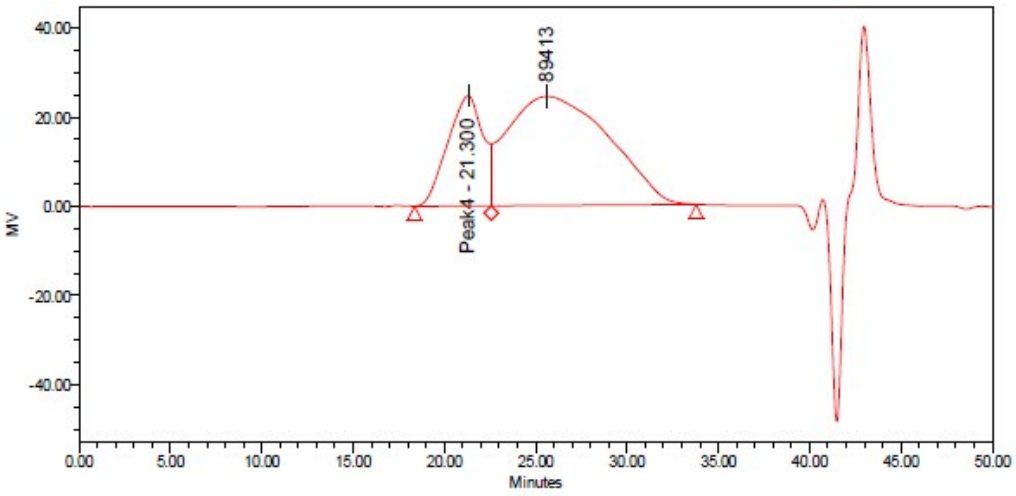


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		19124	69759	97446	135179	175048		3.647812		
3										

Starch-Allyl–Bn (20-80) (*entry 7, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

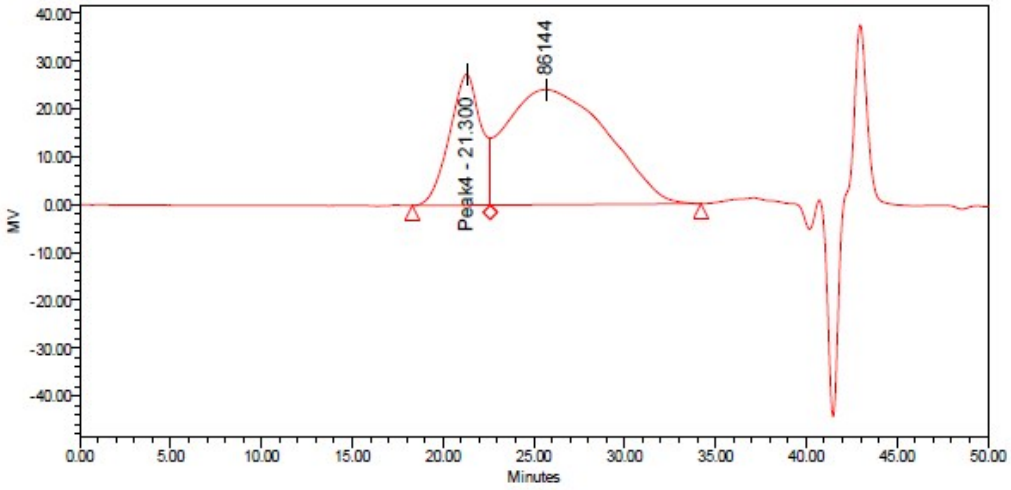


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		18385	68220	89413	133750	174078		3.710671		
3										

Starch-Allyl–Bn (20-80) (*entry 8, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)

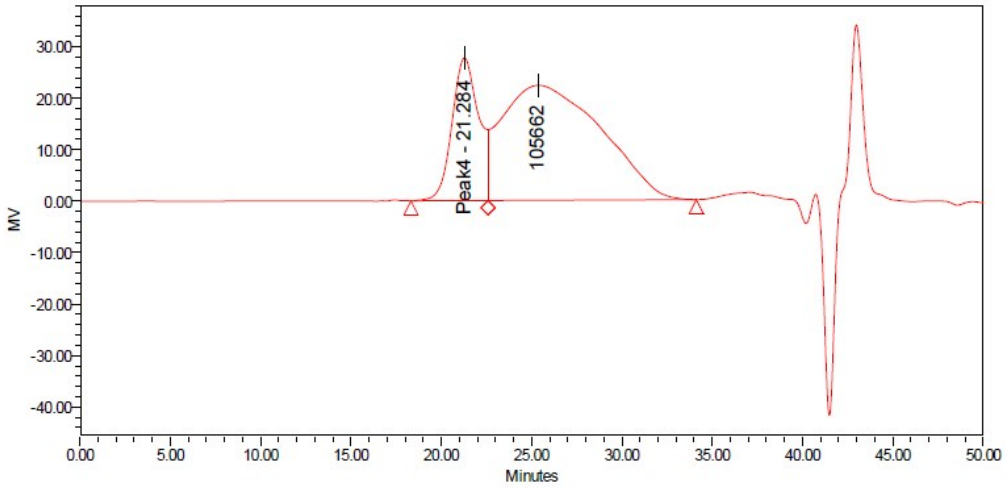


GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		17937	67806	86144	133508	173983		3.780158		
3										

Starch-Allyl–Bn (20-80) (*entry 9, Table 1*)

Eluent: HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture)



GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1										
2		18584	70333	105662	136006	175480		3.784541		
3										

Table S1. Summary of SEC data

Sample	Entry in table 1	Eluent[a]	Major fraction			Minor fraction[c]	
			M_n (kDa)	$Đ$	% area under the curve	Peak elution time (min)	% area under the curve
Starch (unmodified)	NA	**1**	8.4	3.05	100	NA	NA
Starch-C4	1	**1**	9.8	5.36	76.2	21.92	23.8
Starch-C4	1	**2**	20.0	3.61	77.3	21.31	22.7
Starch-Cyclohexyl	2	**2**	19.8	3.61	76.6	21.33	23.4
Starch-Bn	4	**2**	18.7	3.49	77.0	21.31	23.0
Starch-Allyl	5	**1**	10.1	5.29	74.7	20.1, 22.04[b]	25.3
Starch-Allyl	5	**2**	19.1	3.54	71.9	21.40	28.1
Starch-Allyl-Bn (50-50)	6	**2**	19.1	3.65	71.2	21.26	28.8
Starch-Allyl-Bn (20-80) - 1	7	**2**	18.3	3.71	74.0	21.30	26.0
Starch-Allyl-Bn (20-80) - 2	8	**2**	17.9	3.78	73.7	21.30	26.3
Starch-Allyl-Bn (20-80) - 3	9	**2**	18.6	3.78	75.0	21.28	25.0

NA = not applicable; a = **1,** 0.1 (v/v) % TFA in HPLC H_2O or **2**, corresponding to HPLC H_2O : methanol : acetic acid = 54 : 23 : 23 with 0.5 M sodium acetate (salt concentration with respect to entire solvent mixture); b = minor fraction was multimodal with two peaks; c = minor fraction was beyond the exclusion limit of our column.

Table S2. Zeta potential of selected samples at 1.0 mg/mL concentration at 25 °C

Sample		Zeta potential (mV)	Standard deviation
Starch (unmodified)	-	- 36.0	± 3
Starch-Bn	*entry 4, table 1*	+ 62.3	± 2
Starch-Allyl	*entry 5, table 1*	+ 60.4	± 2
Starch-Allyl-Bn (20-80) - 2	*entry 8, table 1*	+ 61.5	± 1

Table S3. Minimum inhibitory concentrations (MIC) of different batches of Starch-Allyl-Bn(20-80)

Sample	SA (ppm)	EC (ppm)	PA (ppm)
Batch 1 (*entry 7, table 1*)	15.6	31.3	31.3
Batch 2 (*entry 8, table 1*)	15.6	31.3	31.3
Batch 3 (*entry 9, table 1*)	15.6	31.3	31.3

***In vitro* cytotoxcity**

Cytoxicity of selected polymers was investigated against primary human keratinocytes (NK 103) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after incubation for 18 h. The cells and media were kindly donated by CellResearch Corp. The detailed protocol is provided in our previous publication.[1]

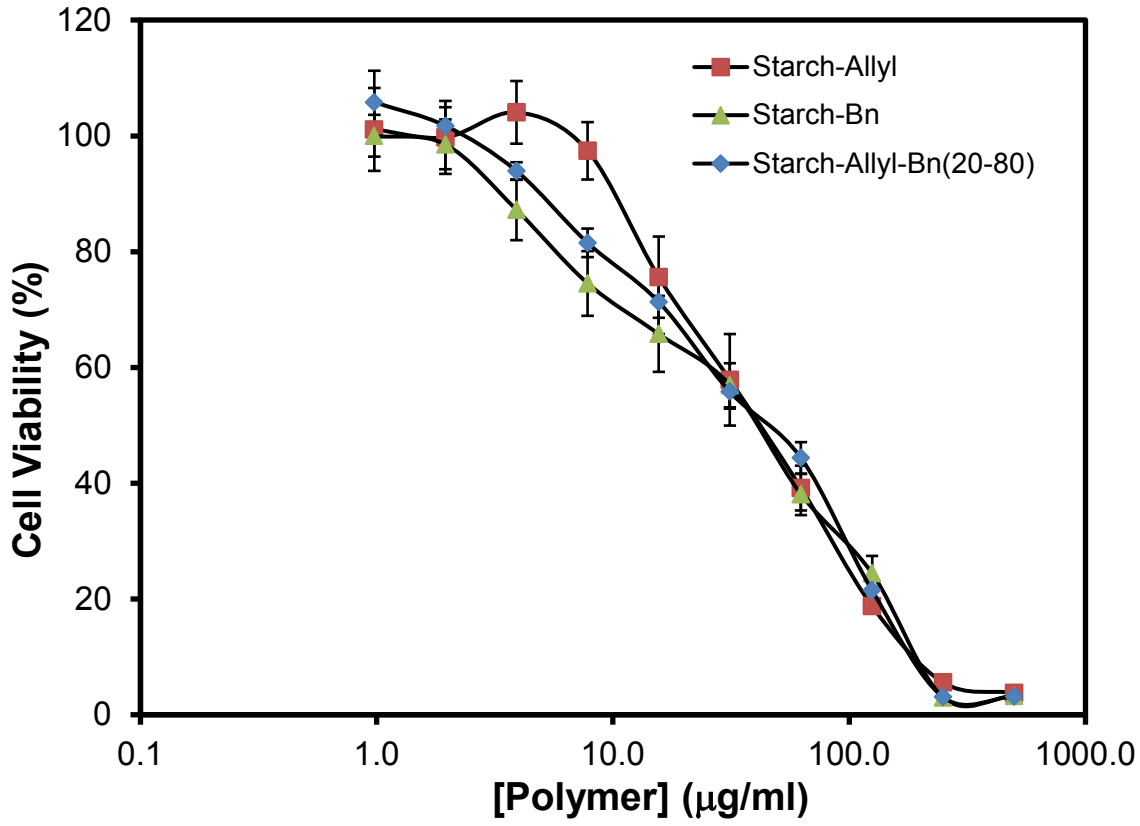


Figure S2. Viability of keratinocytes when tested against Starch-Allyl, Starch-Bn and Starch-Allyl-Bn(20-80) after 18 h incubation.

Reference:

1. Y. Li, K. Fukushima, D. J. Coady, A. C. Engler, S. Liu, Y. Huang, J. S. Cho, Y. Guo, L. S. Miller, J. P. K. Tan, P. L. R. Ee, W. Fan, Y. Y. Yang, J. L. Hedrick, *Angew. Chem., Int. Ed.* **2013**, *52*, 674.