Electronic Supplementary Material (ESI) for RSC Advances.

ESI - Electronic Supplementary Information

Solid liquid liquid extraction of porcine gastric mucins from homogenized animal material

Veronika J. Schoemig[a], Eda Isik[a], Lea Martin[a], Sonja Berensmeier[a*]

a Bioseparation Engineering Group, Department of Mechanical Engineering, Technical University of Munich, Boltzmannstr. 15, D-85748 Garching, Germany

* Corresponding author: Prof. Dr. Sonja Berensmeier, s.berensmeier@tum.de

Compatibility of mucin with solvents

Purified mucin was incubated in each solvent for 24 h, and completely evaporated before functionality was tested in terms of viscoelasticity at pH 2 in 10 mM sodium phosphate buffer, pH 2.

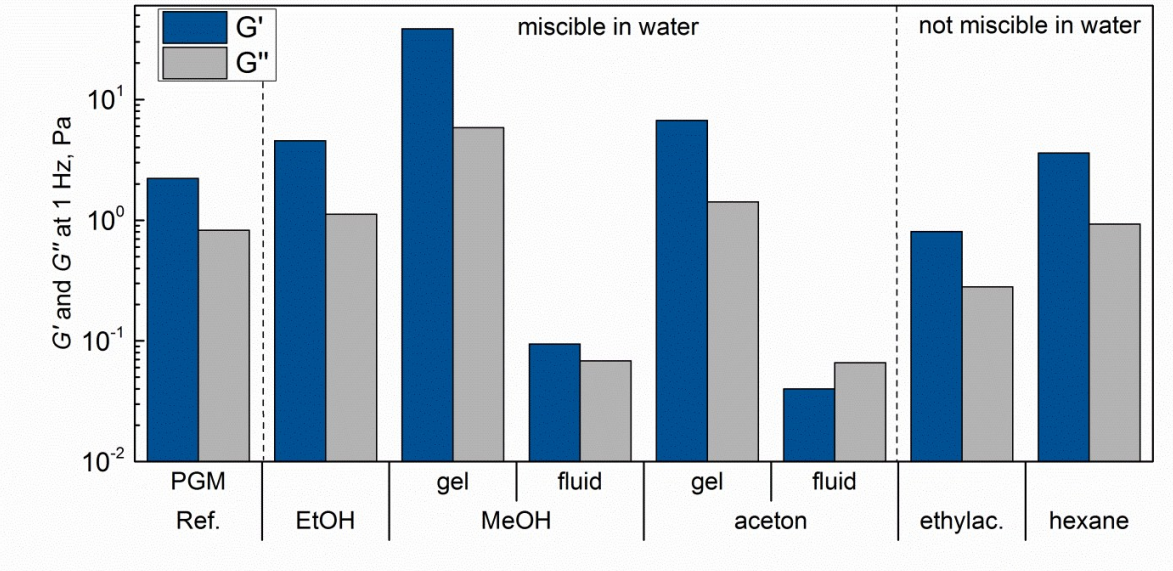

Figure S 1. Comparison of mucin viscoelasticity after 24 h incubation in ethanol (EtOH), methanol (MeOH), aceton, ethylacetate (ethylac.) and hexane. Storage modulus *G'* and loss modulus *G''* were measured at 1 Hz in 10 mM sodium phosphate buffer pH 2. Solvents were completely evaporated before hydrating in buffer and measuring viscoelasticity. *G'* and *G''* were compared to purified PGM from the reference process (PGM_Ref) based on [1]. MeOH and acetone formed two aqueous phases after incubation at pH 2 (gel and fluid).

Design of Experiments (DoE)

Table S 1. Summary of factors and response variables with minimum and maximum values

factor	name	unit	min.	max.	center point
A	time	h	-1 = 0.25	+1 = 6	3.13
B	hexane/water (v/v)	-	-1 = 3/2	+1 = 1/15	1/2
response Y_1	glycoprotein	mg	4.22	7.08	
response Y_2	Muc5AC	mg	0.09	0.17	

Table S 2. Set up of experimental space with the factors A: time and B: ratio hexane/water. The mass of glycoproteins (Y_1) and Muc5AC (Y_2) were set as response variables

experiment	*A*: time, h	*B*: ratio hexane/water	Volume water based on 8 mL	Y_1: glycoprotein, mg	Y_2: Muc5AC, mg
4	6.00	3/2	3.15	6.12	0.10
21	3.13	1/2	5.33	6.03	0.15
3	6.00	3/2	3.15	5.90	0.12
17	3.13	1/2	5.33	6.05	0.09
18	3.13	1/2	5.33	4.83	0.09
12	6.00	1/2	5.33	7.08	0.09
20	3.13	1/2	5.33	5.92	0.15
11	6.00	1/2	5.33	5.38	0.15
6	0.25	1/15	7.50	4.38	0.13
2	0.25	3/2	3.15	4.27	0.09
19	3.13	1/2	5.33	5.98	0.13
1	0.25	3/2	3.15	4.48	0.09
14	3.13	3/2	3.15	5.43	0.09
7	6.00	1/15	7.50	6.18	0.17
8	6.00	1/15	7.50	6.27	0.15
5	0.25	1/15	7.50	4.51	0.13
10	0.25	1/2	5.33	4.22	0.11
9	0.25	1/2	5.33	4.56	0.10
15	3.13	1/15	7.50	6.34	0.15
13	3.13	3/2	3.15	6.07	0.09
16	3.13	1/15	7.50	6.35	0.14

Table S 3. Analysis of variance table (ANOVA) for response variable Y_1 mass of glycoproteins

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	11.3455698	3	3.78185661	20.013771	< 0.0001	significant
A-Time	9.1871413	1	9.1871413	48.6188032	< 0.0001	
B-Ratio	0.25794288	1	0.25794288	1.36504638	0.2588	
A^2	1.90048564	1	1.90048564	10.0574634	0.0056	
Residual	3.21236624	17	0.18896272			
Lack of Fit	0.35336449	5	0.0706729	0.29663317	0.9056	not significant
Pure Error	2.85900176	12	0.23825015			

Cor Total	14.5579361	20

Table S 4. Analysis of variance table (ANOVA) for response variable Y_2 mass of Muc5AC

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.00841741	4	0.00210435	5.4952514	0.0056	significant
A-Time	0.00132347	1	0.00132347	3.45606599	0.0815	
B-Ratio	0.00704843	1	0.00704843	18.4060765	0.0006	
AB	5.4741E-07	1	5.4741E-07	0.0014295	0.9703	
B^2	4.497E-05	1	4.497E-05	0.11743362	0.7363	
Residual	0.00612704	16	0.00038294			
Lack of Fit	0.00039795	4	9.9488E-05	0.20838415	0.9288	not significant
Pure Error	0,00572909	12	0.00047742			
Cor Total	0.01454446	20				

Table S 5. Model validation of DoE. Overview over predicted and measured response values Y_1 and Y_2

	experiment	**1**	**2**	**3**
A	time, h	5.5	3.5	1.5
B	hexane/water	9:7	7:9	1:15
	predicted	6.07 ± 0.43	5.94 ± 0.43	5.37 ± 0.43
	measured	7.26 ± 0.13	7.49 ± 0.07	7.69 ± 0.02
Y_1	difference	1.19	1.55	2.32
	95 % (CI) low	5.43	5.32	4.67
	95% (CI) high	6.72	6.56	5.99
	predicted	0.11 ± 0.02	0.11 ± 0.02	0.14 ± 0.02
	measured	0.04 ± 0.02	0.11 ± 0.01	0.11 ± 0.01
Y_2	difference	0.07	0.0	0.03
	95 % (CI) low	0.08	0.09	0.11
	95% (CI) high	0.14	0.14	0.17

Purification of Muc5AC with SEC

- **Time of SLLE incubation: 15 min**

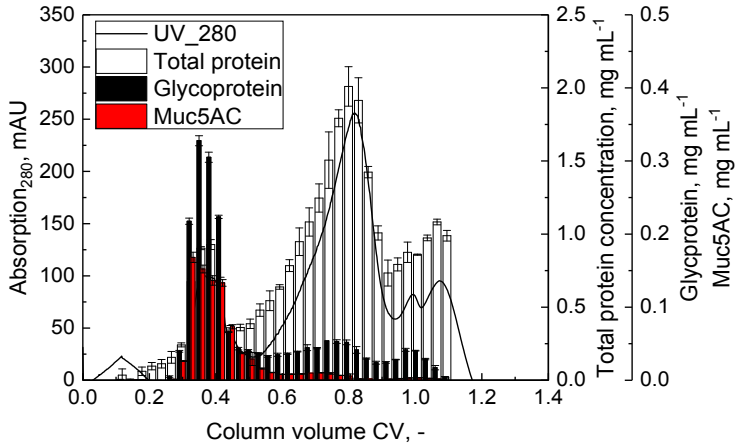

Figure S 2. SEC chromatogram of the solvent phase after application of SLLE (5.6 g homogenate 75 % v/v, hexane/water ratio 1/15, incubation time 15 min. Material: Sepharose 6 *Fast Flow*, Molecular weight cut off: approx. 4 MDa, injection volume: 20 mL, flow rate: 30 cm h^{-1}, column volume (CV) 167 mL, column diameter: 16 mm, running buffer: 10 mM sodium phosphate buffer with 170 mM NaCl, pH 7.0. All fractions of 5 mL each were analyzed in terms of total protein (280 nm, white), glycoprotein (PAS Assay, black) and Muc5AC (ELISA, red).

- **Time of SLLE incubation: overnight**

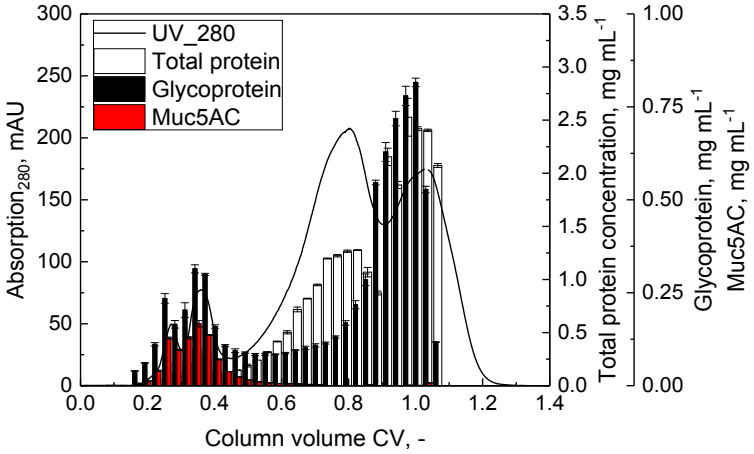

Figure S 3. SEC chromatogram of the solvent phase after application of SLLE (5.6 g homogenate 75 % v/v, hexane/water ratio 1/15, incubation time overnight. Material: Sepharose 6 *Fast Flow,* Molecular weight cut off: approx. 4 MDa, injection volume: 20 mL, flow rate: 30 cm h^{-1}, column volume (CV) 167 mL, column diameter: 16 mm, running buffer: 10 mM sodium phosphate buffer with 170 mM NaCl, pH 7.0. All fractions of 5 mL each

were analyzed in terms of total protein (280 nm, white), glycoprotein (PAS Assay, black) and Muc5AC (ELISA, red).

Viscoelasticity of purified mucin (SLLE) w/ and w/o supplemented DNA

Purified mucin that has been extracted overnight was supplemented with DNA (150 µg mL^{-1} fish sperm DNA) in order to investigate if DNA influences viscoelasticity.

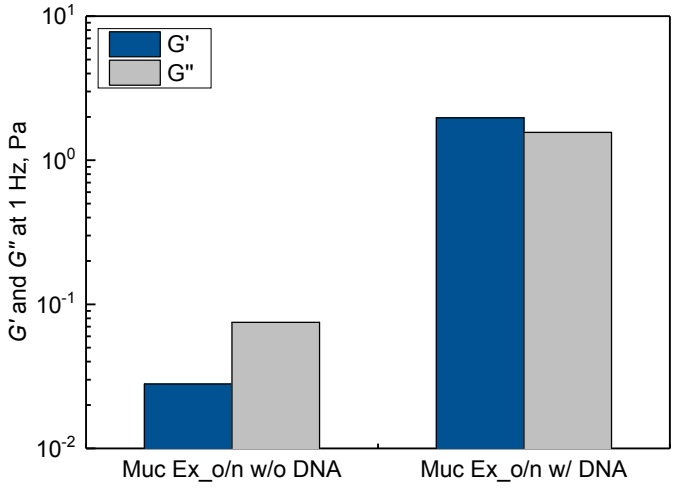

Figure S 4. Comparison of storage modulus *G'* and loss modulus *G''* for 1 % (w/v) PGM at a frequency of 1 Hz. PGM has been purified with the extraction process (extraction overnight, Ex_o/n) with and without additional dsDNA (150 µg mL^{-1} fish sperm DNA, 15 µg respectively).

Lubricity of purified mucin (extraction protocol) w/ and w/o supplemented DNA

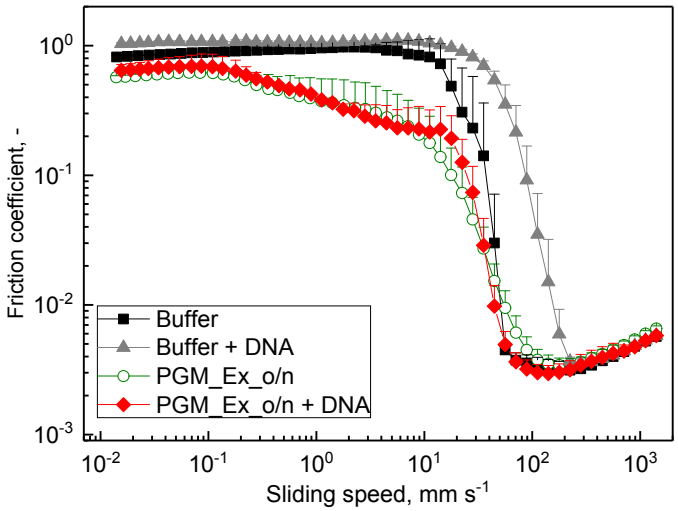

Figure S 5. Lubricity of 0.1 % (w/v) mucin purified with the extraction process (SLLE) (extraction overnight, PGM_Ex_o/n) hydrated in 20 mM HEPES buffer pH 7.4 with and without additional dsDNA (0.15 mg mL^{-1} fish sperm DNA). Pure HEPES buffer with and without additional DNA (0.5 mg mL^{-1}) are shown for comparison. Friction coefficients were measured for sliding speeds between 10^{-2} - 10^{3} mm s^{-1}. Error bars represent the standard deviation of analytical triplicates.

DNA content in crude and purified mucin

Table S 6. Summary of dsDNA concentration and the ratio A260/280 for mucin samples, determined with the BioSpectrometer basic. 1 g mucus and homogenate were hydrated in 5 mL 10 mM sodium phosphate buffer pH 7.0 or ddH_2O overnight, respectively. The samples were centrifuged for 30 min at 17000 x g and the supernatant was analyzed for dsDNA. Lyophilized mucins were hydrated in ddH_2O and also analyzed for dsDNA content.

	dsDNA, µg mL^{-1}	**A260/280**
Mucus (hydrated)	734	1.38
PGM_Ref, 1 mg mL^{-1}	110	1.49
Homogenate	431	1.24
PGM_Ex_3h, 1 mg mL^{-1}	68	1.13