Modeling of the Effects of Metal Complexation on the Morphology and Rheology of Xanthan Gum Polysaccharide Solutions

Kolattukudy P. Santo¹, Kristina I. Fabijanic², Chi-Yuan Cheng,² Andrei Potanin², and Alexander V.

Neimark^{1,*}

¹ Department of Chemical and Biochemical Engineering, Rutgers University, Piscataway, NJ, 08854,

United States. ²Colgate-Palmolive, Piscataway, NJ, 08854, United States.

*Corresponding author: aneimark@rutgers.edu

Table of Contents

1. NMR Measurements of Pyruvate/Acetate Contents of XG Chains	2
2. Details of the Atomistic XG-Zn Systems	3
3. Snapshots from Atomistic Simulations	4
4. DPD Parameterization	5
4.1 XG-Water Parameters	5
4.2 Bond and Angle Parameters	8
4.3 Zinc-Ligand Coordination Parameters	10
5. XG DPD Systems; Radius of Gyration and Cluster Distributions in DPD Simulations	11
References	12

1. NMR Measurements of Pyruvate/Acetate Contents of XG Chains

Experimental Setup. Xanthan Gum is first added in D₂O with 5g/L in presence of 2.4 mM of sodium citrate (Sigma-Aldrich, St Louis, MO). The sample is mixed at 40°C until completely dissolved. ¹H NMR spectra of xanthan samples are acquired at 75°C. NMR measurements are performed on a Bruker Avance NEO spectrometer (Bruker Biospin, Billerica, MA) with a 5 mm CryoProbe operating at 500.13 MHz for ¹H. The typical NMR parameters is as follows: pulse length: 5 ms, recycle delay: 20 sec, number of scans: 16, sweep width: 5000 Hz.

Determination of Acetate and Pyruvate Contents of XGs. The acetate and pyruvate contents are determined by ¹H NMR spectroscopy by reference to an internal standard, sodium citrate. Three peaks are observed in NMR spectrum: 1.47 ppm and 2.16 ppm are attributed to pyruvate and acetate groups bound to Xanthan, and peak at 3.1ppm assigned to citrate. From the peak integral corresponding to both substituents by reference to the standard protons, one can calculate the number of substituent (N_A , N_P) of XG

$$N_A = \frac{I_A}{I_R} \frac{c_R}{c_0} \tag{S1}$$

$$N_P = \frac{I_P c_R}{I_R c_0} \tag{S2}$$

Where I_A , I_P , and I_R corresponds to the peak integral of acetate, pyruvate group and reference peak, respectively. c_R is the concentration of sodium citrate, and c_0 is the concentration of XG.

2. Details of the Atomistic XG-Zn Systems

Atomistic simulations are performed with XG-A and XG-B single polymers that are 30

n _z	Run	No of water/Na+	XG wt%	Zn wt%	$W_{z/x}$
0	R1	56654/50	2.546	0	0
0	R2	56654/50	2.546	0	0
0	R3	56654/50	2.546	0	0
5	R1	53738/40	2.68	0.033	0.0255
5	R2	54620/40	2.64	0.032	0.0255
5	R3	54899/40	2.63	0.032	0.0255
10	R1	54620/30	2.64	0.065	0.0511
10	R2	54183/30	2.66	0.065	0.0511
10	R3	54898/30	2.63	0.064	0.0511
20	R1	54532/10	2.64	0.129	0.1021
20	R2	54992/10	2.62	0.128	0.1021
20	R2	55008/10	2.62	0.128	0.1021

monomers long in water, at different Zn concentrations with number of Zn per chain $n_z = 0, 5$,

Table S1. Details of the XG-A-Zn-Water atomistic simulations.

n _z	Run	No of water/Na+	XG wt%	Zn wt%	$W_{z/x}$
0	R1	56597/44	2.526	0	0
0	R2	56597/44	2.526	0	0
0	R3	56597/44	2.526	0	0
5	R1	55617/34	2.569	0.032	0.0258
5	R2	54269/34	2.631	0.033	0.0258
5	R3	55391/34	2.580	0.032	0.0258
10	R1	56421/24	2.533	0.063	0.0515
10	R2	56397/24	2.535	0.063	0.0515
10	R3	56208/24	2.543	0.063	0.0515
20	R1	56349/4	2.536	0.125	0.0103
20	R2	56092/4	2.547	0.126	0.0103
20	R2	56103/4	2.547	0.126	0.0103

Table S2. Details of the XG-B-Zn-Water system.

10 and 20. We set up 3 independent runs named R1, R2 and R3 for each n_z , this means a total of 24 simulations. The details of the simulations are provided in Tables S1 and S2. Molecular mass of XG-A 30 monomer xanthan chain is 26697.8 Da, while that of XG-B is 26451.7 Da. The average wt% of Xanthan in the simulations is ~ 2.6 %.



Figure S1. Snapshots of the XG chains at the end of 200 ns of various atomistic systems with (a) XG-A and (b) XG-B polymers. The cellulosic backbone is colored orange. Side chains that are linked with Zn are colored by the cluster ID. The boxes marks the bend in the chains due to hydrogen bonding (green) and Zn complexation (red). Zn ions can be visible in cyan (dummy atoms) and purple (Zn atom).

3. Snapshots from Atomistic Simulations

Figure S1a-b shows snapshots of XG chains in various systems with different Zn concentrations. The snapshots show that structural characteristics such as bend and hairpin can be induced on the chain by Zn ions as well as hydrogen bonding interactions between the side chains. Conformational changes due to H-bonded interactions are indicated by green boxes while, that induced by Zn ions are indicated with red boxes. Comparing of XG-A and XG-B snapshots, one can see that XG-B conformations consist of several *abruptly* bent backbones. This may be an indication that XG-B back bone is more flexible and undergo larger conformational fluctuations as presented in the R_G values in Figure 7.

4. DPD Parameterization

4.1 XG-Water Parameters

We follow the parameterization scheme of Anderson et al,¹ which allow variable bead sizes and densities. Water is coarse-grained into a CG bead W representing 6 water molecules and the number density is chosen to be $\rho_w=3$. Other beads are defined to be each saccharide beads (G, C), the acetate group (A) and the pyruvate group (P) as described in the Methods section. The interaction lengths R_{ij} are defined for each pair interaction, with the intra bead interaction ranges found from the molar volume, as $R_{ii}^3 \sim V_{molar}$. Water is taken to be the reference system, the diameter of which $R_{WW} = (\rho_w N_m v_w)^{1/3} = 8.14$ Å is taken as the unit of length in the DPD simulations with $R_c = R_w = 1$. The other bead sizes and interaction lengths are given in units of R_c . The G bead diameter is obtained as 0.9923 R_c , assuming a density 1.7 g/cm³ for β -D glucopyranose (by Advanced Chemistry Development Inc. (ACD/Labs)), the corresponding DPD number density is 3.07 R_c^{-3} . For the C beads, the diameter is found to be 0.9637 R_c , with a density 2.0 g/cm³ and the corresponding DPD density is 3.35 R_c^{-3} . The acetate bead is considerably small and diameter of the A bead is estimated to be 0.8079 R_c using the experimental density of 1.05 g/cm³ of acetic acid, which gives a high DPD density 5.69 R_c^{-3} , as R_c being much larger than the A bead size. The pyruvate bead P has a diameter of 0.8548 R_c and a DPD density 4.80 taking the Pyruvic acid density to be 1.3 g/cm³. A mixing rule R_{ii} = $\frac{1}{2}(R_{ii}+R_{jj})$ is used then to find the interaction ranges between different bead types (Table 1 of the paper).

The intracomponent interaction parameter of water beads a_{WW} is taken as 25.0 k_BT . Initially a_{II} of other beads are estimated from matching the DPD densities of the corresponding compounds, using short simulations of single bead type consisting of 1500 beads. Then intercomponent parameters a_{IJ} are calculated by matching water-octanol partition coefficients. For this, the octanol bead is parametrized with diameter 1.1311 R_c , a_{00} =31.0 k_BT and DPD density 2.08 R_c^3 , matching with the experimental density of 0.83 g/cm³. The partition coefficient is defined as

$$\log P = \log_{10}([S]_0 / [S]_W)$$
(S3)

which is the logarithm of the ratio of the solute concentration in octanol phase to the solute concentration in water phase. The immiscible water-octanol systems are constructed as sufficiently large boxes, in order to have traces of the solute in the phases that are measurable, when the overall solute concentration is 5%. A positive and large value of log *P* indicates highly hydrophobic solute, while a negative log *P* implies hydrophilicity of the substance. Generally, larger boxes are required as the $|\log P|$ becomes larger, as in these cases, the solute concentration in one of the phases becomes considerably smaller.¹ The simulations boxes are constructed with sizes, $60 \times 20 \times 20 R_c^3$, $60 \times 40 \times 40 R_c^3$ and $60 \times 60 \times 60 R_c^3$, depending on the value of log *P*. Initially, equilibrated water and octanol slabs of 30 R_c height in the Xdirection is constructed and fused together. In the case of 1-bead solutes, they are equally and randomly distributed in each phase, by replacing W/O beads. In the case of oligomeric solutes, they are distributed randomly in the system using the *packmol*² program. Snapshot of a typical water-octanol-solute system given in Figure S2a.

log *P*s are calculated for the compounds represented by single beads, as well as for oligomers of several bead types. Since the CG beads in our simulations except water, represent large



Figure S2. The water (pink), octanol (red) and C bead (cyan) system to measure log P od glucuronic acid. The solute (C) density is calculated away from the interfacial region.

molecular groups such as saccharide units, reference compounds that consist of different types of such beads are rare and experimental log *P*s are mostly unavailable. For example, to parametrize a_{AG} one needs a compound A-G or glucose acetate, for which no experimental data available. Same is the case with G-P (glucose pyruvate) or CGP (glucuronic glucose pyruvate) and so on.

COMPOUND	DPD LOGP	ACD OR EXPT LOGP
WATER	-1.88	-1.38 (Expt) ³
OCTANOL	2.1	$3.0 (Expt)^{3-4}$
BETA D GLUCOSE	-1.12	$-1.7 (Expt)^3$
BETA D GLUCURONIC ACID	-1.97	-2.3 to -2.9 (predicted by various methods ALOGPS, ChemAxon, ACD/Labs) ^{3, 5}
ACETIC ACID	0.31	$-0.17 (Expt)^4$
PYRUVIC ACID	-0.38	-0.43 (Expt) ³
AG (GLUCOSE ACETATE)	-0.79	0.36+/-0.64 (ACD/Labs) ⁶
GP (GLUCOSE PYRUVATE)	-1.27	0.84+/-0.65 (ACD/Labs) ⁶
AGGP (ACETATE GLUCOSE GLUCOSE PYRUVATE)	-1.5	0.90+/-0.94 (ACD/Labs) ⁶
CGP (GLUCURONIC GLUCOSE PYRUVATE)	-0.9	-0.89+/-0.92 (ACD/Labs) ⁶
AGCG	-2.21	-2.15+-0.98 (ACD/Labs) 6

Table S3. The computed logP values of the reference compounds and experimental/ACD log Ps.

To resolve this, we constructed this compounds using the *Chemsketch* software of the ACD/Labs⁶ and computed log *P* using their computational algorithm for log P calculation from chemical structures, that is based on a fragmental method in which log P has contributions from atomic fragments of the structure.⁷ The DPD log *P* is computed from the average bulk density of the solute in both phases (see Figure S2b), measured away from the water-octanol interface, as prescribed by Anderson et al.¹ a_{IJ} are iteratively refined so that the expected log *P* values are fairly reproduced.

Table S3 provide the matching of log *P*s of different reference compounds. The agreement with experiment is somewhat fair while computed values themselves have wide range of uncertainties. However, this level of agreement is very fair on CG level of N_m =6. Note that at a smaller CG level, N_m =2, Anderson et al¹ reported deviations of about 10 % . Furthermore, all our CG beads are hydrophilic and the relative hydrophilicity of the beads is fairly reproduced. Values in Table S2 led to the a_{IJ} values given in the main text plus the interaction parameters a_{OJ} with octanol beads given below.

$a_{0J}\left(k_{B}T\right)$	W	0	G	С	Α	Р
0	41.0	31.0	37.0	42.0	29.0	32.0

4.2 Bond and Angle Parameters

To find bond lengths of A-G, G-G, C-G and G-P, the respective fragments in water are simulated with atomistic and DPD simulations. For the calculation of the angle parameters, fragments of G-G-G, G-G-M (with mannose in the side chain position, in DPD G-G-(G)) G-M-C (G-G-C), G-M-A (G-G-A), A-M-C (A-G-C), M-C-M (G-C-G) and C-G-P (Figure S3a) are simulated. The bond lengths and bond angles in atomistic simulations are calculated over 10 ns after a 4 ns



Figure S3. (a) The coarse-graining scheme and the bead definitions. (b) Bond length distributions in MD and DPD. (c) Angle distributions in MD and their DPD counter parts.

equilibration, between the center of mass of the respective CG groups, while DPD distributions are calculated over 400000 steps of 500000 steps long trajectory. The respective distributions are given in Figure S3b-c. The atomistic bond distributions of G-G, C-G and G-P are fitted with DPD very well (Figure S3b), while A-G bond is found to have two peaks, arising possibly from two stable orientations of the acetate group with respect to the sugar ring. As such multiple orientations cannot be taken into account in the current CG model of just two beads A and G, we fit the corresponding DPD distribution with an average A-G bond length assuming that both bond lengths are somewhat equally probable (which is also obvious from the distributions). Atomistic angle distributions of A-G-C, G-G-G, G-G-G, G-G-C, G-C-G, are fitted very well with DPD distributions (Figure S3c). However, A-G-G and C-G-P angles are found to have multiple peaks. As in the case of A-G bond length, C-G-P angle distribution is fitted with average equilibrium angle value, while the A-G-G angle, being distributed over a wide range, is neglected and unrestrained in the C-G simulations. All parameters that are obtained by the fitting procedure is reported in the Tables 3&4 in the main text.

4.3 Zinc-Ligand Coordination Parameters

The Morse parameters of Zn-ligand coordination interaction are determined so that reasonable



Figure S4. (a) The Z-ligand radial distribution functions in DPD simulations. (b) The running coordination numbers of various ligands around Zn in DPD simulations.

coordination numbers are reproduced. The Z-W coordination is modelled so that water bead W is coordinated at the center of the central Z bead at $r_{ZL} = 0$ (L represents ligand bead). Both Z-C and Z-P coordination distances are taken to be $r_{ZL} = 0.5$. DPD NPT simulations of small systems of 5 Zn ions, 30 P/C beads and 500 water beads (P and C beads are not simultaneously present) are performed for 50000 steps at pressure 23.7. The obtained radial distribution functions (RDFs) are presented in Figure S4a, which shows the coordination distances and first coordination shells are finely reproduced. Figure S4b presents running coordination numbers; while Z-W coordination number is about half (means 3 real water) the Z-C/P coordination numbers are 2.5-3. Also, Z-C and Z-P RDFs and RCNs are identical. Note that these Z-C/P coordination numbers are obtained when the C/P concentration is small and the beads are free in water. With polymers making crosslinks, this coordination number is found to increase with polymer concentration. Thus, 4-links are observed in both atomistic and DPD simulations.



Figure S5. (a) Radius of gyration at different Zn concentration of 30 monomer DPD chains of XG-A and XG-A. The errorbar represent fluctuations over time. The plot shows quantitative and qualitative agreement with MD results in Figure 7 in the text. (b) XG-B clusters at different polymer and Zn concentrations. Polymers are colored by their cluster ID, while Zn ion is represented with dummy atoms in cyan and the central Zn atom in purple.

5. XG DPD Systems; Radius of Gyration and Cluster **Distributions in DPD** Simulations Figure S5a shows the average radius of gyration of the 30monomer chains in DPD simulations that are done to compare with atomistic results. For this, single chain simulations with sizes $40 \times 20 \times 20 R_c^3$ with a typical number of 47700 water beads are performed. Simulations are performed for 1.5 million steps and the R_g values are calculated over 100 frames in the last 500000

steps. The plot shows excellent agreement with the MD results.

Figure S5b shows clustering in DPD XG-B systems of different polymer and Zn concentrations. At low polymer concentrations and high Zn concentrations (Figure S5b, left) isolated clusters are formed. At a higher polymer concentration, clusters are formed even at low Zn concentrations (see text section 3.2 for more description). Figure S6 depicts cluster size distributions in XG-A and XG-B systems of 30 chains and 60 chains at different Zn concentrations and shear rates. N_{clust} is the number of clusters of size n_c , the maximum value of which increases as the Zn



Figure S6. The XG cluster size distributions at different shear rates and Zn concentrations in (a) XG-A and (b) XG-B systems

concentration increases. However, clusters decompose to smaller ones with application of a shear

0.05 τ^{-1} . Upon application of a shear, XG-A clusters decompose larger aggregates, compared to

XG-B, which indicates that XG-A systems form more strongly bound clusters compared to XG-

B, due to its higher pyruvate content that helps forming more Zn-induced interchain crosslinks.

References

- 1. Anderson, R. L.; Bray, D. J.; Ferrante, A. S.; Noro, M. G.; Stott, I. P.; Warren, P. B., Dissipative particle dynamics: Systematic parametrization using water-octanol partition coefficients. *The Journal of Chemical Physics* **2017**, *147* (9), 094503.
- 2. Martínez, L.; Andrade, R.; Birgin, E. G.; Martínez, J. M., PACKMOL: a package for building initial configurations for molecular dynamics simulations. *Journal of computational chemistry* **2009**, *30* (13), 2157-64.

3. <u>www.chemspider.com</u>.

4. Schnackenberg, L. K.; Beger, R. D., Whole-Molecule Calculation of Log P Based on Molar Volume, Hydrogen Bonds, and Simulated 13C NMR Spectra. *J. Chem. Inf. Model.* **2005**, *45* (2), 360-365.

5.

https://foodb.ca/compounds/FDB021897. ACD/ChemSketch, v., Advanced Chemistry Development, Inc., Toronto, ON, Canada, 6. www.acdlabs.com, 2018. 7. Petrauskas, A. A.:

Petrauskas, A. A.; Kolovanov, E. A., ACD/Log P method description. Perspect. Drug Discov. Des. 2000, 19 (1), 99-116.