

Electronic Supplementary Information 3

Alginate hydrogel swelling

Context

The aim of this supplementary information section is to provide data and theory for the swelling of the alginate hydrogel at different ionic force. This can be used to calculate the effective alginate concentration as a function of the ionic force, and thus to obtain a more precise estimate of the pore radii at different ionic force.

Theory

Swelling of covalently crosslinked alginate hydrogels has recently been investigated in great depth for an aldehyde-based crosslinking chemistry¹, in the general framework of the Flory-Huggins theory. With minor adaptations, these results can be used for the adipic dihydrazide crosslinked alginate used here.

The basic idea of the Flory-Huggins theory is that a hydrogel is at swelling equilibrium when the expansive forces exerted by osmotic pressure and solvent/polymer mixing energy are balanced by the retractive nature of the polymer network.

The retractive energy contribution is calculated in reference¹ for a random coil configuration (Gaussian network), with corrections for limited molecular weight of the monomers and chain extension effects (eq. 17d in ¹). As the correction terms have almost no effect in our conditions (less than 1%), we model the retractive energy contribution taking only the main Gaussian terms in ¹ into account:

$$\Delta G_{\text{elastic}} = \frac{RT \cdot V_{\text{H}_2\text{O}}}{\bar{v} \cdot M_c} \cdot \frac{2\nu_0}{f} \cdot [\varphi^{-1/3} - \varphi^{-1}] \quad (\text{S19})$$

where $R=8.3144\text{J/mol}\cdot\text{K}$ is the universal gas constant, $T=293\text{K}$ the absolute temperature, $V_{\text{H}_2\text{O}}=1.8\cdot 10^{-5}\text{m}^3/\text{mol}$ the molar volume of the solvent (e.g. water), and $\bar{v}=0.8\cdot 10^{-3}\text{m}^3/\text{kg}$ the specific volume of the polymer chains¹, M_c the mean molar mass between crosslink points (to be fitted) and ν_0 the polymer volume fraction at synthesis. φ is the swelling ratio as compared to the gel volume at synthesis (to be compared to experimental values), and f is the valency of the elementary crosslinking points ($f=3$ in our case since we attach 1 adipic dihydrazide molecule to a continued alginate strand). The coefficients in S19 are adjusted such $\Delta G_{\text{elastic}} = 0$ for $\varphi=1$, reflecting the assumption that the gel is considered at equilibrium after synthesis. $\Delta G_{\text{elastic}}$ thus rises from 0 to a

maximum positive value for $\varphi = 3\sqrt{3}$ and then slowly decays with further increase in φ ; for practical purposes, since we never observe a swelling ratio beyond 5, this means that $\Delta G_{\text{elastic}}$ is a monotonously rising function in our experimental conditions, and translates the idea that at least for realistic experimental conditions, the gel retraction force increases with increasing swelling.

The solvent/polymer mixing energy is given by eq. 10b in ref ¹:

$$\Delta G_{\text{mixing}} = RT \cdot \left[\ln(1 - \nu) + \nu + \chi_1 \nu^2 \right] \approx RT \cdot \left[\chi_1 - \frac{1}{2} \right] \cdot \nu^2 \quad (\text{S20})$$

where ν is the final polymer volume fraction, and χ_1 the Flory-Huggins interaction parameter; the approximation by Taylor development is possible due to the low polymer volume fractions used. The Flory-Huggins interaction parameter reflects the affinity of the alginate chains for water, and we use the ionic force dependence reported in ¹ (Fig. 2):

$$\chi_1 = 0.0751 \cdot \ln(c_{\text{bulk}}) + 0.3737 \quad (\text{S21})$$

where c_{bulk} is the concentration in the surrounding monovalent electrolyte (KCl in our case). Since lower values of χ_1 imply more negative mixing energies (cf. eq. S20), the ionic force dependency expressed in S21 implicitly translates increasing interchain repulsion with decreasing KCl concentration.

We model the osmotic pressure as being the Gibbs-Donnan value, corrected for counter-ion condensation:

$$\Delta G_{\text{osm}} = -a \cdot V_{\text{H}_2\text{O}} \cdot RT \cdot [c^+ + c^- - 2c_{\text{bulk}}] \quad (\text{S22})$$

where a is an activity coefficient accounting for counter-ion condensation, R the ideal gas constant, T the absolute temperature, c^+ and c^- the Donnan concentration for a 1:1 strong electrolyte in the presence of the fixed charge density associated with the alginate chains. We use $a=0.2$, in accordance with the typical values of effective alginate charge of 0.1 to 0.3 found in numerical simulation of the interchain repulsion (electronic supplementary information 2). The Donnan expressions for the concentrations of the monovalent negative and positive electrolyte ions are:

$$c^- = -\frac{c_{\text{fixed}}}{2} + \sqrt{c_{\text{bulk}}^2 + c_{\text{fixed}}^2/4}, \quad c^+ = \frac{c_{\text{fixed}}}{2} + \sqrt{c_{\text{bulk}}^2 + c_{\text{fixed}}^2/4} \quad (\text{S23})$$

where c_{bulk} is the molarity of the externally imposed KCl electrolyte, and where the fixed charge concentration is here taken in absolute values, that is

$$c_{\text{fixed}} = \frac{c_{\text{alg}}}{M_{\text{residue}}} \quad (\text{S24})$$

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where c_{alg} is the alginate concentration (w/v) at the swelling state under concentration, and M_{residue} is the molar mass per residue (198g/mol for sodium alginate). Overall, the total energy of additional swelling is therefore expressed in terms of the swelling ratio ϕ compared to synthesis:

$$\Delta G_{\text{total}}(\phi) = \Delta G_{\text{elastic}} + \Delta G_{\text{osm}} + \Delta G_{\text{mixing}} =$$

$$RT \cdot \left\{ \frac{V_{\text{H}_2\text{O}}}{M_c} \cdot \frac{2c_{\text{alg},0}}{f} \cdot [\phi^{-1/3} - \phi^{-1}] - a \cdot V_{\text{H}_2\text{O}} \cdot \left[\sqrt{\left(\frac{c_{\text{alg},0}}{\phi \cdot M_{\text{residue}}} \right)^2 + 4c_{\text{bulk}}^2} - 2c_{\text{bulk}} \right] + \right.$$

$$\left. \left(\chi_1(c_{\text{bulk}}) - \frac{1}{2} \right) \cdot \left(\frac{\bar{v} \cdot c_{\text{alg},0}}{\phi} \right)^2 \right\} \quad (\text{S25})$$

where $c_{\text{alg},0}$ denotes the alginate concentration (in units of weight per volume) at synthesis. Equilibrium swelling is predicted at $\phi_{\text{equilibrium}}$ such that:

$$\Delta G_{\text{total}}(\phi_{\text{equilibrium}}) \equiv 0 \quad (\text{S26})$$

$\phi_{\text{equilibrium}}$ generally needs to be found numerically, due to the presence of the different powers of ϕ in eq. S25; the numerical solution of eq. S25 and S26 is however generally relatively simple, since negative ΔG_{total} values call for an increase in ϕ , while positive ΔG_{total} values indicate a tendency of the gel to loose water and shrink.

Fundamental and empirical parameters

The swelling ratio $\phi_{\text{equilibrium}}$ can be calculated numerically from eq. S25 and eq. S26, for the desired initial gel concentration and bulk free salt concentrations, provided all the parameters in eq. S25 are known. We carry out the parameter estimation based on a priori assumptions, except for M_c . Indeed, we have to obtain M_c by fitting of the observed swelling ratios for each gel synthesis recipe, since it reflects the underlying network structure and therefore resistance against swelling. For a given gel synthesis recipe, M_c is independent of the ionic force. We find the following best fit values for M_c :

$c(\text{alg},0)$	25mg/mL	50mg/mL	65mg/mL
M_c	961g/mol	398g/mol	331g/mol

Table S27: M_c values for the three gel recipes.

The values obtained for M_c are realistic, since the crosslinks are of two types: the adipic dihydrazide elements have a molecular weight of 174g/mol, giving a

lower bound, whereas along the alginate chains, we provide enough adipic dihydrazide to crosslink at best every tenth residue, such that we expect M_c to lie in the range between 174g/mol and 2000g/mol, which it does. We also expect the M_c values to decrease with increasing alginate concentration, since an increased alginate concentration is expected to favor inter-chain crosslinking over ineffective intra-chain bridges. We did evaluate the M_c values when taking all the corrections proposed in ¹ into account, the largest deviation was found for the 65mg/mL gel ($M_c=321$ g/mol instead of 331g/mol, representing a little over 3% deviation).

The fundamental parameters are not fitted, but known in advance. They are summed up in Table S28.

Symbol	Physical quantity	Value
V_{H_2O}	Molar volume of water	$1.8 \cdot 10^{-5} \text{m}^3/\text{mol}$
f	Network topology	3
a	Counter-ion activity	0.2
\bar{v}	Specific volume of alginate	$0.8 \cdot 10^{-3} \text{m}^3/\text{kg}^1$
M_{residue}	Molecular weight per residue	198g/mol
χ_1	Flory-Huggins interaction	Needs to be calculated by eq. S21

Table S28: Common Flory-Huggins parameters.

Comparison to experimental data

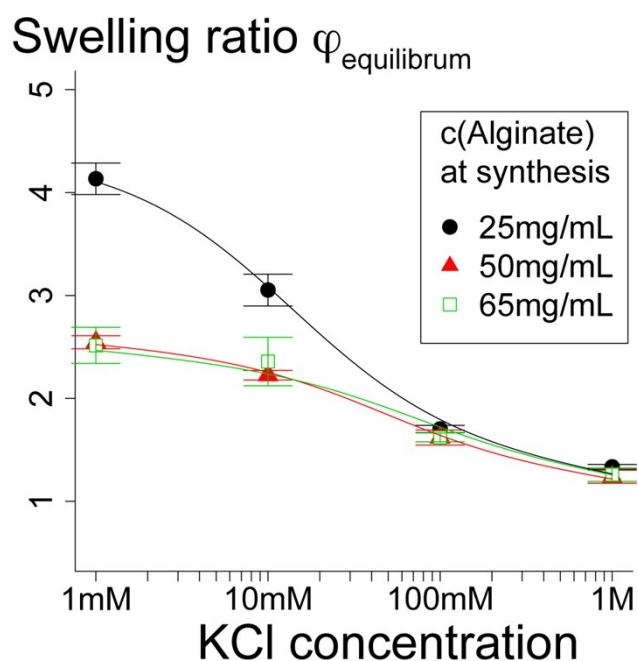


Figure S29: Equilibrium hydrogel swelling as function of monovalent electrolyte concentration. Hydrogel disks were placed in KCl solutions of known concentrations and let to equilibrate for at least 24h with a large excess of electrolyte. Gel volume was assessed at fabrication and after swelling by weighing, the swelling ratio is the ratio of the weight after swelling compared to the fabrication weight. The theoretical lines are the solutions to eq. S25 and S26, with the parameter values as given by Table 27 and Table 28, and eq. S21 for χ_1 .

Relatively accurate fits are obtained between predicted and observed swelling ratios when using the parameters given in Table S27 and S28, as shown in Figure S29. As a control, virtually identical curves are obtained when using the entire series of corrections proposed in ¹. We use the theoretical swelling values to calculate the effective final alginate concentration at the different ionic force conditions, and therefore the pore size via the best fit formula indicated in Figure 3C in the main text.

Bibliography

1. A. W. Chan and R. J. Neufeld, *Biomaterials*, 2009, **30**, 6119-6129.