

SUPPORTING INFORMATION

Polyelectrolyte *vs.* Polyampholyte Behavior of Composite Chitosan/Gelatin Films

Daniel Pinto Ramos,[†] Sharon Sarjinsky,[†] Moien Alizadehgiashi,[†] Juri Möbus,[◇] Eugenia Kumacheva^{†‡§}*

[†] Department of Chemistry, University of Toronto, Toronto, Ontario, M5S 3H6, Canada

[◇] Suncayr, Ltd., 2660 Speakman Drive, Mississauga, Ontario, L5K 2L1, Canada

[‡] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, M5S 3G9, Canada

[§] Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, M5S 3E5, Canada

S1. Assay calibration curves

S1.1. Lowry assay. Lowry assay was used to determine the dissolution of gelatin (GEL) films.¹ Five standard GEL solutions were prepared with concentrations of 20, 40, 60, 80, and 100 $\mu\text{g/mL}$. Each solution (300 μL) was mixed with 1.5 mL of Lowry reagent solution, incubated for 10 min at 25 $^{\circ}\text{C}$, mixed with 150 μL of diluted Folin's phenol reagent, and incubated for 30 min at 25 $^{\circ}\text{C}$. Absorbance of the solution was measured at 750 nm using a Varian Cary 50 UV-Vis spectrometer. Lowry reagent solution and diluted Folin's phenol reagent were prepared immediately prior to their use by mixing 50 mL 2% Na_2CO_3 in 0.1 M NaOH with 1 mL 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1% Na·K tartrate, and 1 mL Folin's phenol reagent with 1 mL of deionized water.

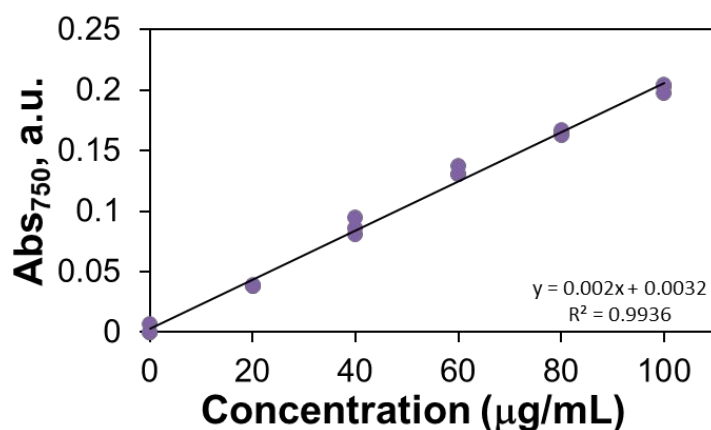


Figure S1. Calibration graph for Lowry assay, plotted for the absorbance of gelatin standard solutions at $\lambda = 750 \text{ nm}$ vs. the concentration of gelatin. All samples were done in triplicate.

S1.2. O-phthalaldehyde assay. O-phthalaldehyde assay was used to determine the dissolution of chitosan (Ch) and Ch/GEL films.² Standard Ch solutions were prepared in 0.25% (v/v) acetic acid solutions. The concentrations of NaCl concentrations were 0, 0.2, 0.4, 0.6, and

0.8 M. The concentrations of Ch were 0, 20, 40, 60, 80, and 100 $\mu\text{g}/\text{mL}$. Standard solutions of GEL in deionized water were prepared with concentrations of 20, 40, 60, 80, 100 $\mu\text{g}/\text{mL}$. Each standard solution (1 mL) was mixed with 1 mL of the reagent solution of o-phthalaldehyde and incubated for 1 h at 25 $^{\circ}\text{C}$. Absorbance of this solution was measured at 340 nm. O-phthalaldehyde reagent solution was prepared immediately prior to its use by adding 200 μL of 0.11 M o-phthalaldehyde and 0.071 M N-Acetyl-L-cysteine solutions in ethanol to 5 mL of 0.2 M borate buffer at pH 8.9. Two separate absorbance intensity-concentration calibration curves for GEL and Ch were using the standard solutions (Figure S2).

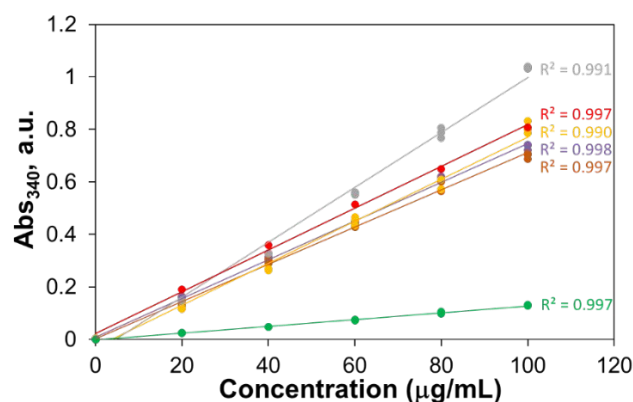


Figure S2. Calibration graph for o-phthalaldehyde assay, plotted for the absorbance of Ch standard solutions in (●) 0 M NaCl, (●), 0.2 M NaCl, (●), 0.4 M NaCl, (●) 0.6 M NaCl, (●) 0.8 M NaCl solutions and GEL standard solutions in (●) deionized water at $\lambda = 340$ nm vs. the concentration of chitosan or gelatin. All measurements were conducted three times.

S2. Effect of Film Dissolution and Swelling on Ionic Strength of Salt Solutions

Figure S3 shows the variation in the fraction of the Ch film dissolved, M_D , and the degree of film swelling, Q , upon its incubation in NaCl, Na_2SO_4 , and CaCl_2 solutions, both plotted as a function of the solution ionic strength. In Figure S3A, for all solutions, the extent of Ch film dissolution decreased with an increasing ionic strength, with a M_D reducing from 45% (deionized water) to almost 0 for NaCl, Na_2SO_4 , and CaCl_2 solutions at ionic strengths of 0.8, 2.4, and 2.4 M, respectively. The effect of a specific ion can be compared for salt solutions at an ionic strength of 0.6 M: the values of M_D in the NaCl and CaCl_2 solutions were similar, while film dissolution in

the Na₂SO₄ solution was suppressed. Furthermore, for CaCl₂ and Na₂SO₄ solutions with a similar ionic strength of 1.2 M, weaker film dissolution was observed in the Na₂SO₄ solution.

Figure S3b shows the variation in Q after Ch film incubation in NaCl, CaCl₂ and Na₂SO₄ solutions at the ionic strength in the range of 0 to 2.4 M. The trend in the variation in Q correlated with the change in film dissolution: in all salt solutions, the swelling of the Ch films decreased with an increasing ionic strength. At an ionic strength of 0.6 M film exposure to the solutions containing the Cl⁻ anions resulted in similar Q values (333 vs. 386% for NaCl and CaCl₂ solutions, respectively), while exposure to the Na₂SO₄ solution showed a significantly lower Q value of 164%.

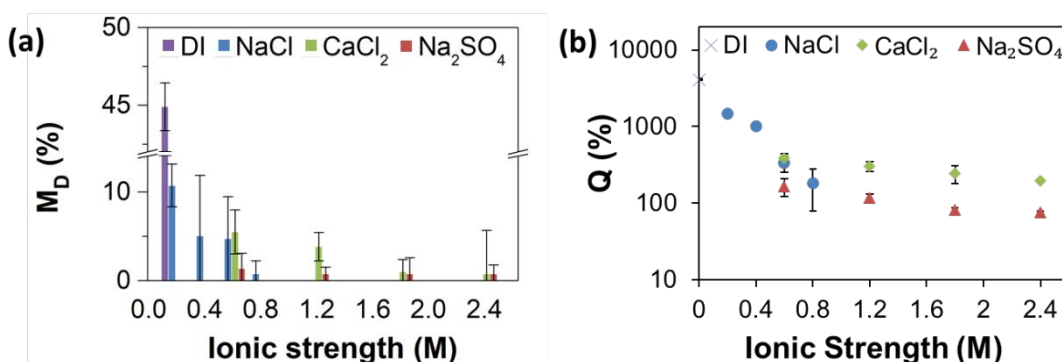


Figure S3. Stability of Ch films in salt solutions. (a) Variation in the fraction of the film dissolved and (b) the degree of swelling (Q) after 1 h film incubation in water (DI) and NaCl, CaCl₂ and Na₂SO₄ solutions, plotted as a function of their ionic strength. The error bars show the standard deviation obtained in three independent experiments.

Figure S4a shows the variation in M_D and Q for the GEL films upon incubation in NaCl, CaCl₂ and Na₂SO₄ solutions, plotted vs. ionic strengths of NaCl, Na₂SO₄, and CaCl₂ solutions. For films incubated in the NaCl solutions, the changes in M_D , in comparison with films incubated in deionized water were not statistically significant ($p > 0.05$). The dissolution of GEL films in solutions containing a divalent anion SO₄²⁻, reduced with increasing ionic strength, in contrast with that in the NaCl solutions. Film dissolution in solutions containing a divalent cation Ca²⁺ drastically increased with increasing ionic strength. For example, GEL films incubated in CaCl₂ solution at an ionic strength of 2.4 M showed almost complete dissolution, with an M_D value of 79%.

Figure S4b shows the variation in Q after GEL film incubation in salt solutions at ionic strengths in the range of 0 to 2.4 M. Similar to the trends observed for the variation in M_D , the variation in

Q showed a strong dependence on the type of solution it was exposed to. For films incubated in NaCl and CaCl₂ solutions, the value of Q increased with ionic strength, that is, from 630 to 985 to 1220%, corresponding to the deionized water, NaCl at an ionic strength of 0.8 M and CaCl₂ solution at an ionic strength of 1.8 M, respectively (in CaCl₂ solutions at ionic strength of 2.4 M the GEL films completely dissolved). When comparing film swelling in the salt solutions at the ionic strength of 0.6 M, the Q values for the GEL films exposed to NaCl and CaCl₂ solutions were close, i.e., 940 and 890%, respectively. In solutions containing the divalent anion SO₄²⁻, the swelling of the GEL films, first, increased from 630 (deionized water) to 750% in the Na₂SO₄ solution at an ionic strength of 0.2 M, and then decreased with increasing ionic strength to 307% at 2.4 M.

The enhanced swelling of the GEL films with increasing ionic strength of NaCl solutions was caused by the polyampholyte nature of GEL, which has both amine and carboxyl groups in approximately 36 mmol⁶ and 100 to 115 mmol per 100 g, respectively.⁷ In our work, the NaCl solutions had 5.2 < pH < 5.5, that is, close to the isoelectric point of GEL of 4.7 to 5.2.⁷ Under these conditions, both positive NH₃⁺ and negative COO⁻ groups co-existed on the GEL chains (Figure 3.12). In ionic solutions, attraction between these groups was screened by the counterions, e.g. Na⁺ and Cl⁻ ions, thus resulting in weaker attraction within the polymer and an increased water penetration between polymer chains and enhanced swelling.⁸

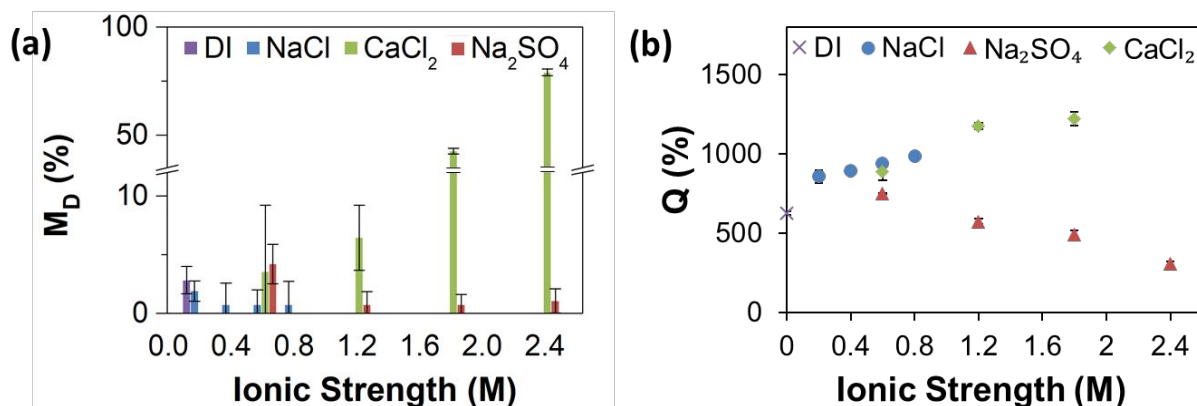


Figure S4. Stability of GEL films in ionic solutions. (a) Variation in the fraction of the film dissolved and (b) the degree of swelling (Q) after 1 h film incubation in water (DI), and in NaCl, CaCl₂, and Na₂SO₄ solutions, plotted as a function of their ionic strength. Due to the complete dissolution of GEL films in the CaCl₂ solution at an ionic strength of 2.4 M, no swelling data is shown for CaCl₂ at that ionic strength. The error bars show the standard deviation obtained in three independent experiments.

Figure S5 shows the swelling and dissolution behavior of the composite films prepared at the Ch/GEL mass ratio of 1:12 and exposed to solutions of NaCl, CaCl₂ and Na₂SO₄ with various ionic strengths. The variation in M_D (Figure S5a) showed a similar trend to that of the pure GEL films. For films incubated in NaCl solutions, the change in M_D was not statistically significant ($p > 0.05$), in comparison with M_D in deionized water. The value of M_D of films incubated in solutions containing the divalent cation Ca²⁺ increased with an increasing ionic strength. For films incubated in solutions containing the divalent anion SO₄²⁻ the value of M_D decreased with increasing ionic strength, in contrast with that of films incubated in CaCl₂ solutions.

Figure S5b shows the variation in Q of the Ch/-GEL films incubated in NaCl, Na₂SO₄, and CaCl₂ solutions at varying ionic strengths. In solutions containing the monovalent anion Cl⁻, the swelling of Ch/GEL films increased with increasing ionic strength. For example, swelling of the C/GEL films increased from 525 to 723 and 992% for deionized water, NaCl solution at an ionic strength of 0.8 M and CaCl₂ solution at an ionic strength of 2.4 M, respectively. When comparing film swelling in these solutions at an ionic strength of 0.6 M, the Q values for the Ch-GEL films exposed to NaCl and CaCl₂ solutions were similar, i.e., 682 and 668%, respectively. In solutions containing a divalent anion SO₄²⁻, the swelling of the Ch-GEL films decreased with increasing ionic strength.

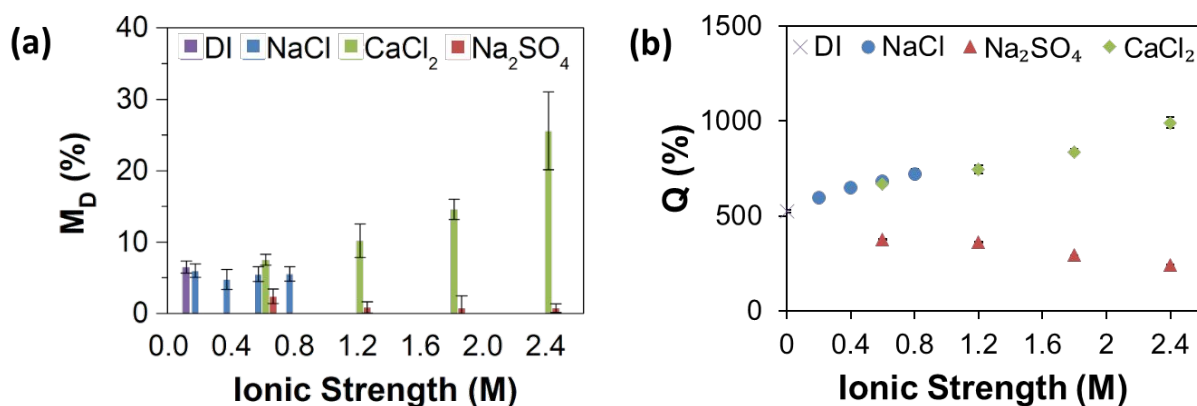


Figure S5. Stability of Ch/GEL films in ionic solutions. (a) Variation in the fraction of the Ch-GEL film dissolved and (b) the degree of swelling (Q) after 1 h film incubation in water (DI) and in NaCl, CaCl₂ and Na₂SO₄ solutions, plotted as a function of their ionic strength. The error bars show the standard deviation obtained in three independent experiments.

S3. Film Morphology

Figures S6-8 show the morphologies of original Ch, GEL, and Ch/GEL films prior to swelling experiments. Solutions of Ch, GEL, and Ch/GEL films were prepared with a solid content of 2.5% (w/v), with Ch/GEL weight ratios of 1:0, 1:12, and 0:1. Acetic acid was added in a ratio of 60% (w/w Ch) to the Ch/GEL solution and of 20% (w/w Ch) for Ch solutions. Films were prepared by casting 650 μL of a Ch, GEL, or a Ch/GEL solution onto 18 mm diameter glass cover slips and drying for 24 h. The films were then frozen in liquid propane and lyophilized for 2 days. The films were fractured, gold coated using a SC7640 high-resolution sputter coater (QuorumTechnologies) for 30 s at 2.0 kV, and their cross-sections were imaged using an FEI Quanta FEG 250 SEM.

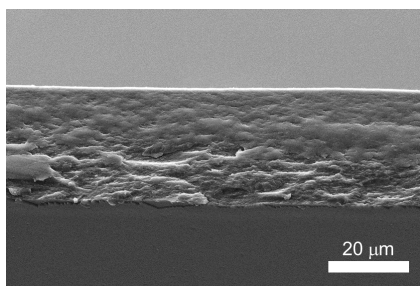


Figure S6. SEM image of the Ch film prior to swelling experiments.

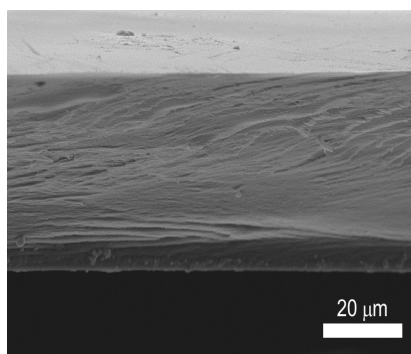


Figure S7. SEM image of the GEL film prior to swelling experiments.

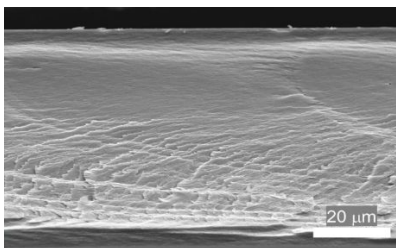


Figure S8. SEM image of the Ch/GEL film prior to swelling experiments.

S4. Dissolution of Ch films under agitation

Figure S9 shows the variation in the fraction of the film dissolved, M_d , for the Ch films incubated under agitation in 0 to 0.8 M NaCl solutions. Films were prepared by casting 12 mL of the Ch, Ch/GEL, or GEL solution (S2) into a PTFE mold (2 mm x 6 cm x 6 cm) and drying the solution at room temperature for 24 h. Dried films were cut into 1 x 1 cm squares (14 mg), and placed in 20 mL of 0 to 0.8 M NaCl solution and stirred at 100 rpm. At different time intervals, a sample of solution was removed with a syringe, filtered through a filter with a pore size 0.45 μm, and the amount of dissolved GEL and/or Ch was determined using the colorimetric Lowry (S1.1) and o-phthalaldehyde assay (S1.2), in which supernatant samples were used, instead of standard solutions. The same trends were observed for film dissolution without agitation, yet dissolution with agitation reached an equilibrium in 10 min for all NaCl solutions. The Ch films dissolved completely in 0 to 0.4 M NaCl solutions, while M_d decreased with increasing NaCl concentration from 0.4 to 0.8 M.

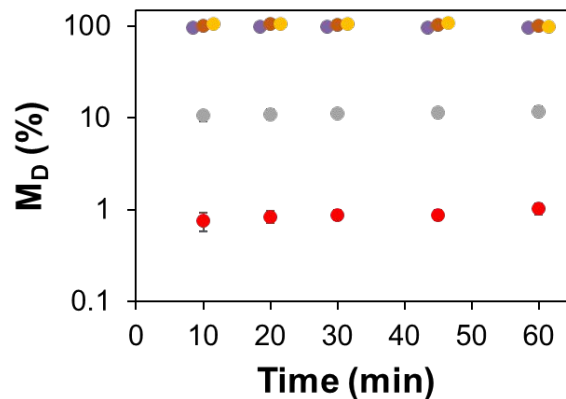


Figure S9. Temporal variation in the fraction of Ch dissolved in solutions with varying NaCl concentration: (●) 0 M, (●) 0.2 M, (●) 0.4 M, (●) 0.6 M, and (●) 0.8 M. The error bars show the standard deviation obtained in three independent experiments.

References

- (1) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
- (2) Larionova, N. I.; Zubaerova, D. K.; Guranda, D. T.; Pechyonkin, M. A.; Balabushevich, N. G. Colorimetric Assay of Chitosan in Presence of Proteins and Polyelectrolytes by Using O-Phthalaldehyde. *Carbohydr. Polym.* **2009**, *75*, 724–727.