



UNIVERSITÀ DEGLI STUDI DI SALERNO

Department of Industrial Engineering
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Analysis of the swelling behavior of low methoxy calcium pectin hydrogels

Thesis in
Transport Phenomena in Food Processes

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Abstract

Nowadays, food and pharmaceutical sector require the employment of materials that possess essential qualities, including biocompatibility, stability, non-toxicity, and, where applicable, controlled release capabilities. Hydrogels are peculiar macromolecules, characterized by a polymeric matrix, an interstitial fluid and, eventually, some ionic species. There is an obvious interest in natural polymer-based hydrogels. Polysaccharide are gaining attention as hydrogel forming biopolymers, as they are synonyms of biocompatibility, and water absorption capacity.

Pectin is a natural water-soluble polysaccharide that can be found in plant cells. It consists of linear chains of 1-4-D-galacturonic acid residues that contain carboxyl groups. Low methoxy pectin (degree of esterification <50%) is capable of forming insoluble hydrogels, such as calcium pectinate, when the acid chains are crosslinked with a divalent cation, calcium, which can be used, for instance, for nutraceutical molecules delivery purpose. Calcium pectinate(CaP) hydrogels are known to be nontoxic and can be degraded by colonic bacteria, being able to remain into the upper gastrointestinal tract. It has been investigated as a carrier for controlled drug release and the protection of drugs against gastric environment. Specific properties of these gels based on new models and their applications in functional food structure design deserve a further study.

Calcium Pectin Hydrogels(Ca-PEC) can be characterized as physically cross-linked network, whose swelling behavior is not fully understood, despite its significance in many applications. In simulating the swelling response of such systems, the experiments are of high importance because of two reasons: finding the necessary parameters for simulation; verification of the theory and numerical implementation. One of the two external stimuli that can alter the swelling response of Ca-PEC hydrogels, are the pH and ionic strength of the medium these gels interact with. pH is a very important factor in studying pectin gels because these are always used for food and pharmaceutical products

with different pH values. pH can alter the dissociation of carboxylic groups in pectin thus its binding with calcium ions. On the other hand, many studies have also investigated the effects of pectin on the in vitro absorption of minerals, such as calcium, zinc, magnesium and iron. Thus, it is essential to discuss the influence of the ionic strength on the general behavior in solution as ions could potentially replace the bound calcium, leading to structure weakening and loss of stability.

Given that swelling properties of hydrogels are important factors for any application, aim of this thesis project is to study the swelling behavior of calcium pectin gel, obtained through ionotropic gelation, in aqueous solutions. Dynamic swelling experiment were carried out with a gravimetric method. Swelling kinetics were well fitted by a saturation growth model. Equilibrium swelling response was analysed as function of the external medium pH and ionic strength. As an additional tool to have more insights about the pH role on the gel diffusive properties in solution, EDTA complexometric titration was performed to monitor calcium ions release in solution. Unconfined compression tests were performed to characterize the main mechanical properties of the prepared hydrogels. In the end, the prepared pectin hydrogels did not show pH responsive properties, in classical sense and this is more typical of chemically crosslinked systems with superabsorbent properties. Ionic strength contributed to swelling through a general polyelectrolyte behavior. The findings can be further exploited for modelling purpose and formulating delivery systems with desired properties.

Chapter One

Introduction

In this chapter, a general description of polysaccharide-based hydrogels is presented. Pectin Hydrogels properties are highlighted. At the end of the chapter, after a review of the state of the art, the aims of this work are illustrated.

Bibliography

1. Chai, Q., Y. Jiao, and X. Yu, *Hydrogels for biomedical applications: their characteristics and the mechanisms behind them*. Gels, 2017. **3**(1): p. 6.
2. Aswathy, S., U. Narendrakumar, and I. Manjubala, *Commercial hydrogels for biomedical applications*. Heliyon, 2020. **6**(4).
3. Kirchhof, S., et al., *Diels–Alder hydrogels for controlled antibody release: correlation between mesh size and release rate*. Molecular Pharmaceutics, 2015. **12**(9): p. 3358-3368.
4. Peppas, N.A., et al., *Hydrogels in biology and medicine: from molecular principles to bionanotechnology*. Advanced materials, 2006. **18**(11): p. 1345-1360.
5. Ricciardi, R., et al., *X-ray diffraction analysis of poly (vinyl alcohol) hydrogels, obtained by freezing and thawing techniques*. Macromolecules, 2004. **37**(5): p. 1921-1927.
6. Fernandes, C.S., A.S. Pina, and A.C.A. Roque, *Affinity-triggered hydrogels: Developments and prospects in biomaterials science*. Biomaterials, 2021. **268**: p. 120563.
7. Varaprasad, K., et al., *A mini review on hydrogels classification and recent developments in miscellaneous applications*. Materials Science and Engineering: C, 2017. **79**: p. 958-971.
8. Ullah, F., et al., *Classification, processing and application of hydrogels: A review*. Materials Science and Engineering: C, 2015. **57**: p. 414-433.
9. Dechiraju, H., et al., *Ion-Conducting Hydrogels and Their Applications in Bioelectronics*. Advanced Sustainable Systems, 2022. **6**(2): p. 2100173.
10. Thoniyot, P., et al., *Nanoparticle–hydrogel composites: Concept, design, and applications of these promising, multi-functional materials*. Advanced Science, 2015. **2**(1-2): p. 1400010.
11. Coviello, T., et al., *Polysaccharide hydrogels for modified release formulations*. Journal of controlled release, 2007. **119**(1): p. 5-24.
12. Li, Z. and Z. Lin, *Recent advances in polysaccharide-based hydrogels for synthesis and applications*. Aggregate, 2021. **2**(2): p. e21.
13. Rimdusit, S., et al., *Biodegradability and property characterizations of methyl cellulose: effect of nanocompositing and chemical crosslinking*. Carbohydrate polymers, 2008. **72**(3): p. 444-455.
14. Matricardi, P., F. Alhaique, and T. Coviello, *Polysaccharide hydrogels: Characterization and biomedical applications*. 2016: CRC Press.

15. Gowder, S. and H. Devaraj, *A review of the nephrotoxicity of the food flavor cinnamaldehyde*. Current Bioactive Compounds, 2010. **6**(2): p. 106-117.
16. Cook, M.T., et al., *Production and evaluation of dry alginate-chitosan microcapsules as an enteric delivery vehicle for probiotic bacteria*. Biomacromolecules, 2011. **12**(7): p. 2834-2840.
17. Hu, X., et al., *Formation of self-assembled polyelectrolyte complex hydrogel derived from salectan and chitosan for sustained release of Vitamin C*. Carbohydrate polymers, 2020. **234**: p. 115920.
18. Yang, Q., et al., *Polysaccharide hydrogels: Functionalization, construction and served as scaffold for tissue engineering*. Carbohydrate Polymers, 2022. **278**: p. 118952.
19. Attaran, A., K. Keller, and T. Wallmersperger, *Modeling and simulation of hydrogels for the application as finger grippers*. Journal of Intelligent Material Systems and Structures, 2018. **29**(3): p. 371-387.
20. Waddell, L.S., *Colloid osmotic pressure and osmolality*, in *Small animal critical care medicine*. 2009, Elsevier. p. 868-871.
21. Caccavo, D., et al., *Modeling the mechanics and the transport phenomena in hydrogels*, in *Computer Aided Chemical Engineering*. 2018, Elsevier. p. 357-383.
22. Flory, P.J., *Principles of polymer chemistry*. 1953: Cornell university press.
23. Quesada-Pérez, M., et al., *Gel swelling theories: the classical formalism and recent approaches*. Soft Matter, 2011. **7**(22): p. 10536-10547.
24. Bouklas, N. and R. Huang, *Swelling kinetics of polymer gels: comparison of linear and nonlinear theories*. Soft Matter, 2012. **8**(31): p. 8194-8203.
25. Jia, D. and M. Muthukumar, *Theory of charged gels: swelling, elasticity, and dynamics*. Gels, 2021. **7**(2): p. 49.
26. Ganji, F., F.S. Vasheghani, and F.E. Vasheghani, *Theoretical description of hydrogel swelling: a review*. 2010.
27. Rubinstein, M. and R. Colby, *Polymer Physics Oxford University Press*. New York, 2003.
28. Horkay, F., *Polyelectrolyte gels: a unique class of soft materials*. Gels, 2021. **7**(3): p. 102.
29. De Piano, R., et al., *Hydrogel: Ph Role on Polyelectrolyte Behaviour in Aqueous Media*. Chemical Engineering Transactions, 2023. **100**: p. 397-402.
30. Narayan, S. and L. Anand, *A coupled electro-chemo-mechanical theory for polyelectrolyte gels with application to modeling their chemical stimuli-driven swelling response*. Journal of the Mechanics and Physics of Solids, 2022. **159**: p. 104734.
31. Drozdov, A. and J.d. Christiansen, *Modeling the effects of pH and ionic strength on swelling of anionic polyelectrolyte gels*. Modelling and Simulation in Materials Science and Engineering, 2015. **23**(5): p. 055005.
32. Bajpai, A.K., et al., *Responsive polymers in controlled drug delivery*. Progress in Polymer Science, 2008. **33**(11): p. 1088-1118.
33. Jarvis, M.C., *Structure and properties of pectin gels in plant cell walls*. Plant, Cell & Environment, 1984. **7**(3): p. 153-164.
34. Mbewana, S., *Functional analysis of a lignin biosynthetic gene in transgenic tobacco*. 2010, Stellenbosch: University of Stellenbosch.
35. Bahú, J.O., et al., *Plant polysaccharides in engineered pharmaceutical gels*. Bioengineering, 2022. **9**(8): p. 376.

36. Medina, L.A. and J. Dzalto, *1.11 Natural Fibers*. 2018.
 37. Assenza, S. and R. Mezzenga, *Soft condensed matter physics of foods and macronutrients*. Nature Reviews Physics, 2019. **1**(9): p. 551-566.
 38. Zdunek, A., P.M. Pieczywek, and J. Cybulska, *The primary, secondary, and structures of higher levels of pectin polysaccharides*. Comprehensive Reviews in Food Science and Food Safety, 2021. **20**(1): p. 1101-1117.
 39. Diener, M., et al., *Primary, secondary, tertiary and quaternary structure levels in linear polysaccharides: From random coil, to single helix to supramolecular assembly*. Biomacromolecules, 2019. **20**(4): p. 1731-1739.
 40. Sundar Raj, A., et al., *A Review on Pectin: Chemistry due to General Properties of Pectin and its Pharmaceutical Uses. 1: 550 doi: 10.4172/scientificreports. 550 Page 2 of 4 Volume 1• Issue 12• 2012 in a chain-like configuration; this corresponds to average molecular weights from about 50,000 to 150,000 daltons*. Large differences may exist between samples and between molecules within a sample and estimates may differ between methods of measurement, 2012.
 41. Grant, G.T., et al., *Biological interactions between polysaccharides and divalent cations: the egg-box model*. FEBS letters, 1973. **32**(1): p. 195-198.
 42. Cao, L., et al., *Egg-box model-based gelation of alginate and pectin: A review*. Carbohydrate polymers, 2020. **242**: p. 116389.
 43. Braccini, I. and S. Pérez, *Molecular basis of Ca²⁺-induced gelation in alginates and pectins: the egg-box model revisited*. Biomacromolecules, 2001. **2**(4): p. 1089-1096.
 44. Axelos, M. and J. Thibault, *The chemistry of low-methoxyl pectin gelation. The chemistry and technology of pectin*, 1991. **6**: p. 109-108.
 45. Said, N.S., I.F. Olawuyi, and W.Y. Lee, *Pectin hydrogels: Gel-forming behaviors, mechanisms, and food applications*. Gels, 2023. **9**(9): p. 732.
 46. Liu, H., X. Xu, and S.D. Guo, *Rheological, texture and sensory properties of low-fat mayonnaise with different fat mimetics*. LWT-Food Science and Technology, 2007. **40**(6): p. 946-954.
 47. Francis, F.P. and R. Chidambaram, *Hybrid hydrogel dispersed low fat and heat resistant chocolate*. Journal of Food Engineering, 2019. **256**: p. 9-17.
 48. Tarifa, M.C., et al., *Microencapsulation of Lactobacillus casei and Lactobacillus rhamnosus in pectin and pectin-inulin microgel particles: Effect on bacterial survival under storage conditions*. International Journal of Biological Macromolecules, 2021. **179**: p. 457-465.
 49. Ishwarya S, P. and P. Nisha, *Advances and prospects in the food applications of pectin hydrogels*. Critical reviews in food science and nutrition, 2022. **62**(16): p. 4393-4417.
 50. Vancauwenberghe, V., et al., *Development of a coaxial extrusion deposition for 3D printing of customizable pectin-based food simulant*. Journal of Food Engineering, 2018. **225**: p. 42-52.
 51. Sarioglu, E., et al., *Theophylline-loaded pectin-based hydrogels. II. Effect of concentration of initial pectin solution, crosslinker type and cation concentration of external solution on drug release profile*. Journal of Applied Polymer Science, 2019. **136**(43): p. 48155.
 52. Popov, S., et al., *Swelling, Protein Adsorption, and Biocompatibility In Vitro of Gel Beads Prepared from Pectin of Hogweed Heracleum sosnowskyi*
-

- Manden in Comparison with Gel Beads from Apple Pectin*. International Journal of Molecular Sciences, 2022. **23**(6): p. 3388.
53. Kazemzadeh, B., H. Hosseinzadeh, and M. Babazadeh, *Synthesis of a novel pectin-based superabsorbent hydrogel with salt and pH-responsiveness properties*. Biomedical and Pharmacology Journal, 2015. **6**(1): p. 41-48.
54. Davidovich-Pinhas, M. and H. Bianco-Peled, *A quantitative analysis of alginate swelling*. Carbohydrate Polymers, 2010. **79**(4): p. 1020-1027.
55. Ventura, I., J. Jammal, and H. Bianco-Peled, *Insights into the nanostructure of low-methoxyl pectin-calcium gels*. Carbohydrate polymers, 2013. **97**(2): p. 650-658.
56. Popov, S., et al., *Effect of Cross-Linking Cations on In Vitro Biocompatibility of Apple Pectin Gel Beads*. International Journal of Molecular Sciences, 2022. **23**(23): p. 14789.
57. Fraeye, I., et al., *Influence of pectin structure on texture of pectin-calcium gels*. Innovative food science & emerging technologies, 2010. **11**(2): p. 401-409.
58. Brazel, C.S. and N.A. Peppas, *Mechanisms of solute and drug transport in relaxing, swellable, hydrophilic glassy polymers*. Polymer, 1999. **40**(12): p. 3383-3398.
59. Kim, B., K. La Flamme, and N.A. Peppas, *Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery*. Journal of Applied Polymer Science, 2003. **89**(6): p. 1606-1613.
60. Chelpanova, T. and E. Efimtseva, *Alkaline phosphatase immobilization on spherical pectin gel particles*. Applied biochemistry and microbiology, 2016. **52**: p. 36-42.
61. Varnier, K., et al., *Polysaccharide-based hydrogels for the immobilization and controlled release of bovine serum albumin*. International journal of biological macromolecules, 2018. **120**: p. 522-528.
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