

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Ionically Cross-Linked Alginate Hydrogels as
Drug Delivery Systems for Analgesics in Broiler
Chickens**

Thesis presented in partial fulfilment of
the requirement for the degree of

Masters of Science
In
Chemistry

At Massey University, Palmerston North, Manawatu,
New Zealand

Samuel James Booty

2017

Thesis Abstract

Treating birds with analgesic drugs requires continuous injections of near lethal concentrations to maintain the therapeutic dose in the blood plasma. This is due to birds having higher metabolic rates than mammals. Therefore, there is a need to develop drug delivery systems that can control and slow down the release of analgesics in birds. This study was designed to analyse the sustained release of the model analgesics, sodium salicylate and sodium aspirin, from ionically cross-linked alginate hydrogels, in *in vitro* and *in vivo* experiments using broiler chickens as the model bird. Analgesic loaded hydrogels separated into two layers, unlike the homogeneous blank hydrogels. This was labelled as the separation effect. Swelling studies indicated the absence of the insoluble cross-linked alginate material in the hydrogels where the separation effect occurred, with most of the hydrogels dissolving back into the medium. The highest equilibrium swelling percentage achieved in the loaded hydrogels was 68 %. In comparison, the highest equilibrium swelling percentage in the blank hydrogels was 622 %. *In vitro* drug release profiles showed that the hydrogels released up to 100 % of the sodium salicylate within 3.33 hours. In contrast, the hydrogels containing sodium aspirin released only 35 % of the encapsulated drug. Hydrogels containing a drug concentration of 150 mg/mL were injected into the model birds at a dose rate of 150 mg/Kg. No chicken reacted negatively to the hydrogel injection. *In vivo* results indicate sustained release of the model analgesic from the hydrogels compared to the release from the aqueous solutions of the drug. The effective concentration for an analgesic effect of sodium salicylate was maintained by the group injected with an aqueous solution of sodium salicylate 18 hours after the injection. The groups injected with the hydrogel with the maximum calcium chloride content saw the largest sustained release, with the plasma concentration of sodium salicylate remaining over the effective concentration for up to 36 hours after the injection.

Keywords: Sodium salicylate, sodium aspirin, hydrogel, analgesia, sustained release, broiler chicken.

Acknowledgements

I want to first acknowledge my three gracious supervisors, Professor David Harding, Dr Catherine Whitby, and Dr Preet Singh. Without their help, suggestions, critical analysis skills and wonderful guidance, this project and thesis would not be possible. I also want to give huge thanks for their help at the start of the project, during my health problems. Thank you for all the support and patience you gave to me during a hard part of the project. It is a beautiful kindness I will always remember. It has been an honour to work with some of the best scientists in the world and will be something I will remember with pride for the rest of my life. Words cannot thank you all enough.

I want to do a special thanks to Professor David Harding, for always being there no matter the situation and always making me strive to be the best I can be not only as a person, but also as a chemist. Thank you for always making me laugh with your jokes, even the ones I did not understand - they always brightened even the darkest days. Again, words cannot thank you enough for all the work you put into the thesis and the project, and I wish you all the luck in the world for the future.

To my loving family, Carl, Debra, Tyler, and Jordan, thank you for all the support you have given me throughout my journey. It has been very hard and highly straining but my days became better after you gave me your love and support. I love you to the ends of the earth and cannot wait to see where the future takes all of us. Special mention to my father, Carl for his Photoshop skills - you saved my terrible attempts at taking photos!!

I want to thank all the staff of the IFS Institute at Massey for making this journey possible, and giving me the chance to learn from the best. A special thanks to the beautiful office ladies for helping with all the paper work needed to complete the project as well as our long conversations about life. You all lighten up the Institute.

A huge thank you to Rafea Naffa for guiding me through the HPLC analysis of the chicken plasma samples and Shashwati Mathurkar for helping me to effectively process the samples for the analysis.

To all the staff at Massey Accommodation Services, thank you for the last year and for giving me a chance at working as a residential assistant in Palmerston North. Your support during this period has been unforgettable and I cannot wait to see where the future takes you all. A very special thanks to Sarah Sandilands, you made my year with Accommodation Services even better with your beautiful smile, unrelenting support and much needed advice.

To Yvette Jones, thank you for the support you have given me throughout the project. You have been in my life from the start of my journey here at Massey, and through our ups and downs, we always come out on top better than before. You are like a sister to me and even as I am writing this up, I cannot stop smiling and thinking of all the good times we have had. Thank you for all the support you have given me in life and this project and I cannot wait what the future outside of Massey holds for the both of us.

To Massey University, thank you for giving me the opportunity to do my chemistry course and this project. Your help even before starting at university has truly made me feel like part of the Massey family. As my journey ends at Massey and the new journey begins, I will always look back on my journey with a smile on my face and know that what I achieved was due to my time at Massey.

Finally, for anyone I missed, thank you all for your support. As you may not be here, please know that I have not forgotten about you or your support and thank you from the bottom of my heart for everything.

Table of Contents

Abstract	ii
Acknowledgments	iii
Table of Contents	v
List of Tables	ix
List of Figures	x
Chapter 1	1
1. Introduction	1
1.1. Definitions and Categorisations of a Gel	2
1.1.1. Definition of a Gel According to IUPAC	2
1.1.2. Definition of a Gel According to the Academic World	2
1.1.3. Classification of Gels	3
1.2. Hydrogels	4
1.3. Classification of Hydrogels	4
1.3.1. Six-step Classification of Hydrogels	5
1.3.2. Stimuli-Sensitive Hydrogels	5
1.4. Characterisation of Hydrogels	6
1.4.1. Swelling Capabilities	6
1.4.2. Toxicity and Biodegradability	7
1.4.3. Flexibility	7
1.4.4. Biocompatibility	8
1.4.5. Drug Entrapment and Release	8
1.5. Preparation of Hydrogels	9
1.5.1. Chemical Cross-Linking	9
1.5.2. Physical Cross-Linking	12
1.6. Alginate and Alginate Based Hydrogels	13
1.6.1. Composition of Alginate	13
1.6.2. Production of Alginate	14
1.6.3. Properties of Alginate	14

1.6.4. Alginate Based Hydrogels	15
1.7. The Use of Analgesics in Poultry	16
1.7.1. Salicylic Acid	17
1.7.1.1. Salicylic Acid Mechanism of Action	18
1.7.2. Present Drug Delivery Techniques	19
1.7.3. Present Sustained Release Drug Delivery Techniques	20
1.7.4. Composition of Blood	21
1.7.4.1. Salicylic Acid Affinity for Plasma Proteins	21
1.7.5. Previous Studies of Analgesic Effects of Salicylic Acid in Birds	22
Thesis Outline	23
Chapter 2	24
2. Material and Methods	24
2.1. Materials	24
2.2. Instrumentation and Equipment	24
2.3. Methods for Preparation of Hydrogel Films	24
2.3.1. Synthesis of Sodium Aspirin	25
2.3.2. Stability of the Model Analgesics	25
2.3.3. Synthesis of Calcium Salicylate	25
2.3.4. Preparation of Calcium Hydrogel Films	25
2.3.5. Preparation of Films with Alternative Cross-Linkers	26
2.3.6. Entrapment of the Model Analgesic	26
2.4. Methods for <i>In Vitro</i> Characterisation of Hydrogel Films	26
2.4.1. Fourier Transform Infrared Spectroscopy (FTIR)	26
2.4.2. Equilibrium Swelling Studies	26
2.4.3. <i>In Vitro</i> Cumulative Release Studies	27
2.4.3.1. SIF Buffer	27
2.4.3.2. Water	27
2.5. Methods for <i>In Vivo</i> Release in Poultry	28
2.5.1. Study Design	28
2.5.2. Hydrogel Preparation	28

2.5.3. Drug Administration	28
2.5.4. Sample Collection	28
2.5.5. Sample Preparation	29
2.5.6. Validation Protocol	29
2.5.6.1. Lower Limit of Quantification (LLQ) and Detection (LLD)	29
2.5.6.2. Linearity	29
2.5.6.3. Recovery	29
2.5.6.4. Specificity	30
2.5.7. Sample Analysis	30
Chapter 3	32
3. Results and Discussion	32
3.1. Synthesis of Calcium Salicylate	32
3.2. Synthesis of Sodium Aspirin	32
3.3. Model Analgesic Calibration Curves	32
3.4. Stability of the Model Analgesics	35
3.5. Hydrogel preparation	36
3.5.1. Blank Hydrogel	36
3.5.2. Loaded Hydrogel	38
3.5.3. Calcium Salicylate Cross-Linked Hydrogel	42
3.5.4. Drying Method	43
3.6. FTIR	44
3.7. Swelling Studies for Select Hydrogels	45
3.7.1. Blank Hydrogels	45
3.7.2. Hydrogels A, B, and C	49
3.7.3. Hydrogels D, E, and F	52
3.8. Drug Release Profiles	54
3.8.1. Hydrogels A, B, and C	54
3.8.2. Hydrogels D, E, and F	55
3.8.3. Hydrogels G and H	56
3.8.4. Hydrogels I and J	59
3.8.5. Hydrogel K	62

3.9. <i>In Vivo</i> Experiments on Poultry Using the Hydrogels	63
3.9.1. HPLC Analysis of Processed Plasma Samples	64
3.9.1.1. Preparation of Samples for Analysis	64
3.9.1.2. HPLC Problems	66
3.9.2. HPLC Validation Results	66
3.9.3. Analysis of Plasma Samples	67
Chapter 4	71
4. Conclusions	71
4.1. Recommendations for Future	72
Bibliography	79
Appendix	87
Appendix A. IR spectrum of alginate.....	87
Appendix B. IR spectrum of sodium salicylate.....	88
Appendix C. IR spectrum of hydrogel B1.....	89
Appendix D. IR spectrum of hydrogel B3.....	90
Appendix E. IR spectrum of hydrogel A.....	91
Appendix F. IR spectrum of hydrogel D.....	92

List of Tables

Chapter 1

1. Classification of gels 3

Chapter 2

2. Composition of physically cross-linked alginate hydrogels 31

Chapter 3

3. Stability results of sodium salicylate measured in SIF buffer at 4, 21, and 37.5 °C using UV-Vis spectroscopy at λ_{\max} of 300 35
4. Stability results of sodium aspirin measured in SIF buffer at 4, 21, and 37.5 °C using UV-Vis spectroscopy at both λ_{\max} of 300 and 260 35
5. Equilibrium swelling values for hydrogels B1, B2, B3, and B4 left overnight in water 46
6. Equilibrium swelling values for hydrogels B1, B2, B3, and B4 left overnight in SIF 47
7. Comparison of maximum swelling values for hydrogels B1, B2, B3, B4, and B5 in water and SIF 48
8. Equilibrium swelling values of hydrogels A, B, and C left overnight in water . 50
9. Equilibrium swelling values of hydrogels D, E, and F left overnight in water .. 53
10. Table Illustrating the 5 groups of chickens injected with the altering substances through subcutaneous injection 67

List of Figures

Chapter 1

1. Simple Schematic of a hydrogel showing the hydrophilic polymer chains and the chemical or physical cross-links (or bonds) 4
2. Hydration of a hydrogel. (a) Dried, water free hydrogel, (b) Hydrogel introduced to water resulting in the formation of primary water (blue), (c) Interaction between primary water and polar hydrophilic group of polymer chain, (d) Formation of secondary water (green), and (e) Formation of free water (purple). 7
3. Example mechanism of Schiff's base reaction between: (a) chitosan and (b) glutaraldehyde 11
4. Mechanism of cross-linking between chitosan and genipin 12
5. Chemical composition of alginate. A) Composition of β -D-mannuronic acid and α -L-guluronic acid residues. B) composition of alginate with G-blocks, M-blocks, and MG-blocks 13
6. Binding of Ca^{2+} by alginate. A) Binding of Ca^{2+} to G-block. B) inter chain formation 16
7. Chemical structures of salicyl alcohol glucoside (a), salicylic acid (b), and sodium salicylate (c) 17
8. Proposed mechanism of inhibition of COX by aspirin 18
9. Selected chemical structures of prostaglandins released when cells are damaged 19
10. Illustration of the component of blood samples 21

Chapter 3

11. UV-Vis absorption spectrums of sodium salicylate (blue) and sodium aspirin (red) in water over wavelength range of 250-300 nm 33
12. Calibration curve of sodium salicylate in SIF buffer using λ_{max} at 300 nm 34
13. Calibration curve of sodium aspirin in SIF buffer using λ_{max} at 260 nm 34

14. Images of blank hydrogels B4 (left) and B1 (right). Hydrogel composition: both contain 1.0 g alginate and 20 ml water, B4 contains 0.2 g calcium chloride and B5 contains 1.0 g calcium chloride	37
15. Image of hydrogel B4 and its ability to coat the back of a spoon. Hydrogel composition: 1.0 g alginate, 20 mL water, and 0.2 g calcium chloride	37
16. Image of a loaded hydrogel illustrating the separation effect. Hydrogel composition: 9 g sodium salicylate, 1.0 g alginate, 1.0 g calcium chloride, and 60 mL water	39
17. Image of loaded hydrogels illustrating the differences between the colloidal suspensions at different calcium contents, 0.3 g calcium chloride (upper image) and 1.0 g calcium chloride (lower image). Hydrogel composition of left image: 1.0 g alginate and 0.3 g calcium chloride for all, 3 g sodium salicylate and 20 mL water (A), 6 g sodium salicylate and 40 mL water (B), and 9 g sodium salicylate and 60 mL water (C). Hydrogel composition of right image: 1.0 g alginate and 1.0 g calcium chloride for all, 3 g sodium salicylate and 20 mL water (D), 6 g sodium salicylate and 40 mL water (E), and 9 g sodium salicylate and 60 mL water (F)	40
18. Image illustrating the formation of copper salicylate during the preparation of a hydrogel. Hydrogel composition: 9 g sodium salicylate, 1.0g alginate, 1.0 g copper sulfate, and 60 mL water	41
19. Images illustrating the hydrogel cross-linked with calcium salicylate, before oven drying (left) and after oven drying (right). Hydrogel composition: 1.0 g alginate, 0.3 g calcium salicylate, and 20 mL water	43
20. Image illustrating the comparison between the drying techniques used in this project, oven drying (upper image) and lyophilisation (lower image). Hydrogel composition: All hydrogels have 1.0 g alginate and 0.3 g calcium chloride, 3 g sodium salicylate and 20 mL water (A1 and A2), 6 g sodium salicylate and 40 mL water (B1 and B2), and 9 g sodium salicylate and 60 mL water (C1 and C2)	44
21. Swelling profiles of hydrogels B1 (blue), B2 (red), B3 (green) and B4 (purple) cross-linked with decreasing calcium chloride content. Swelling profile completed in water as the medium. Hydrogel composition: All hydrogels have 1.0 g alginate and 20 mL water, 1.0 g calcium chloride (B1), 0.5 g calcium chloride (B2), 0.3 g calcium chloride (B3), and 0.2 g calcium chloride (B4)	46

22. Swelling profiles of hydrogels B1 (blue), B2 (red), B3 (green) and B4 (purple) cross-linked with decreasing calcium chloride content. Swelling profile completed in SIF as the medium. Hydrogel composition: All hydrogels have 1.0 g alginate and 20 mL water, 1.0 g calcium chloride (B1), 0.5 g calcium chloride (B2), 0.3 g calcium chloride (B3), and 0.2 g calcium chloride (B4)	47
23. Swelling profiles of hydrogels, A (blue), B (red), and C (green) cross-linked with 0.3g calcium chloride. Swelling profile completed in water as the medium. Hydrogel composition: The hydrogels have 1.0 g alginate and 0.3 g calcium chloride, 3 g sodium salicylate and 20 mL water (A), 6 g sodium salicylate and 40 mL water (B), and 9 g sodium salicylate and 60 mL water (C)	50
24. Swelling profiles of hydrogels D (blue), E (red), and F (green) cross-linked with 1.0 g calcium chloride. Swelling profile completed in water as the medium. Hydrogel composition: All hydrogels have 1.0 g alginate and 1.0 g calcium chloride, 3 g sodium salicylate and 20 mL water (D), 6 g sodium salicylate and 40 mL water (E), and 9 g sodium salicylate and 60 mL water (F)	53
25. Drug release profiles of hydrogels A (blue), B (red), and C (green) in water. Hydrogel compositions: All hydrogels have 1.0 g alginate and 0.3 g calcium chloride, 3 g sodium salicylate and 20 mL water (A), 6 g sodium salicylate and 40 mL water (B), and 9g sodium salicylate and 60 mL water (C)	55
26. Drug release profiles of hydrogels D (blue), E (red), and F (green) in water. Hydrogel compositions: All hydrogels have 1.0 g alginate and 1.0 g calcium chloride, 3 g sodium salicylate and 20 mL water (D), 6 g sodium salicylate and 40 mL water (E), and 9 g sodium salicylate and 60 mL water (F)	56
27. Drug release profile of hydrogel H in SIF buffer. Hydrogel composition: 3.0 g alginate, 1.0 g calcium chloride, 12.0 g sodium salicylate, and 80 mL water ...	57
28. Comparison of drug release profiles for hydrogels G (blue) and H (red) in SIF medium. Hydrogel composition: both have the 3.0 g alginate and 1.0 g calcium chloride, 3.0 g sodium salicylate and 20 mL water (G) and 12.0 g sodium salicylate and 80 mL water (H)	59
29. Drug release profiles for hydrogels I (blue) and J (red) in SIF medium. Hydrogel composition: Both have the 1.0 g alginate, 3.0 g sodium aspirin, 0.2 g calcium chloride and 20 mL water (I) and 3.0 g sodium aspirin, 1.0 g calcium chloride and 20 mL water (J)	61

30. Drug release profile one of hydrogel K in water as the medium. Hydrogel composition: 1.0 g alginate, 0.3 g calcium salicylate, and 20 mL water	62
31. Drug release profile one of hydrogel K in water as the medium. Hydrogel composition: 1.0 g alginate, 0.3 g calcium salicylate, and 20 mL water	63
32. Concentration time curve for sodium salicylate after subcutaneous injection of group 1 (orange), group 2 (red), group 3 (blue), group 4 (purple), and group 5 (green) in broiler chickens. The data points represent the mean of the 6 chickens, with a total of 24 chickens in the whole study. The red dotted line indicates the minimum effective plasma concentration of sodium salicylate required to maintain analgesia in chickens	68

Chapter 4

33. Molecular structures of meloxicam (left) and butorphanol tartrate (right)	74
34. Chemical structures of cyclodextrin monomer (upper left image) and cyclodextrin α -(1,4) linkage (upper right image). Structural schematics of β -cyclodextrin (bottom image).....	76