

NOVEL POLYMERIC MICROCAPSULES FOR TARGETED DRUG DELIVERY

**A thesis submitted in partial fulfillment
of the requirements for the degree
of
Doctor of Philosophy
in
Materials and Process Engineering**

YUEWEN LIN



**THE UNIVERSITY OF
WAIKATO**
Te Whare Wananga o Waikato

2009

Abstract

Microencapsulation is a technology in which small particles or droplets are entrapped by a coating to give the particles with many useful properties. Microcapsules have been used to protect the encapsulated material from the environmental conditions or to release the active agent in a sustained and controlled manner into the surrounding medium. This thesis investigated how to develop a post-ruminal delivery system based on polymeric microencapsulation. A major criterion was to develop microcapsules with reversible switching in response to changes of pH in the *in vivo* environment so contents could be released. The microcapsules must have a thin shell strong enough to withstand harsh physical conditions and a large hollow centre, which will maximise drug-carrying capacity.

Two processes were developed to manufacture pH-responsive microcapsules - conventional interfacial polymerisation with plasma-induced grafting and a novel phase-inversion technique with chemical grafting. This research demonstrated that microcapsules with a porous shell could be manufactured by interfacial polymerisation. Carboxyl groups introduced onto the pores in the microcapsule by plasma-induced graft polymerisation of acrylic acid allowed pore openings to be controlled by the pH change of the environment.

Polyamide microcapsules made by interfacial polymerisation had a hollow core and a porous shell with smooth external and rough internal surfaces. Their average diameter of 28 μm decreased with increased stirring rate during polymerisation. Shell porosity could be changed by adjusting the ratio of amine monomers used to form the microcapsule shell. An argon plasma treatment was developed to graft acrylic acid onto the microcapsule surface. Plasma treatment with 90 seconds produced 0.56 mmol/g of grafting extent. The effect of pH on releasing contents from poly(acrylic acid)-grafted microcapsules was investigated using two different sized molecules (vitamin B₁₂ and cytochrome *c*) to simulate model drugs. Release rate was not significantly affected by molecular size; contents were retained when pH was between 7 and 5.5 and released when pH was between 5 and 3.5. Full release occurred at pH below 3.

Further studies showed that micron-sized microcapsules with a hollow core and a thick matrix wall could be made from polycaprolactone, polysulfone and polystyrene. A modified solvent evaporation process reduced shell thickness but the microcapsules still had a non-porous skin. Process parameters such as polymer concentration, temperature, surfactant, solvent composition, stirring speed, processing pressure, and co-polymer additives influenced structure and internal morphology of the microcapsules. Morphological characteristics of the microcapsules strongly depended on the way the coating polymer is precipitated, particularly the non solvent–polymer–solvent interactions. Chemical grafting was not successful for grafting acrylic acid onto the polycaprolactone and polysulfone microcapsules.

Polystyrene microcapsules with a hollow core and porous micro-channel shell structure were successfully produced using a novel formulation in a phase-inversion process. The outer skin could be removed by re-dissolving the formed microcapsules in a suitable solvent. Open pores with inter-connected micro-channels on the microcapsule surface can be produced by carefully controlling the time the microcapsules are in the solvent. The microcapsule was functionalised using free radical polymerisation to graft acrylic acid onto its surface. However, these microcapsules did not completely retain their contents at pH 7 and had a slow release profile when pH was decreased. Recommendations are given on how to improve and optimise the process.

Polystyrene microcapsules made with a simple and inexpensive phase-inversion technique have potential as a targeted drug delivery system. They could be reusable, compared to most other systems that degrade or disintegrate, and may have other applications as a carrier to immobilise desired molecules onto the microcapsules. Further investigations to optimise manufacturing polystyrene microcapsules should include: larger scale trials; further investigations, using orthogonal tests with multi-variation analysis to optimise factors affecting microcapsule porosity and extent of grafting; identifying an alternative, faster way to analyse pore size; and developing a mathematical model for the release rate.

Acknowledgements

No thesis can be completed without the dedicated help of many people. First of all, I wish to sincerely and gratefully acknowledge my chief supervisor Professor Janis Swan and co-supervisor Professor Conan Fee for their contributions to the studies. Their excellent supervision, indispensable advice, patient guidance and constant encouragement have guided me throughout the research to bring this thesis to life. I would also like to thank my co-supervisor Dr Susanne Meier and the NERF team Dr Michael Rathbone and Dr Keith Ellis for their valuable advice and encouragement.

I am especially grateful to the Department's technicians Lisa Li, Indar Singh and Paul Ewart for their expertise, crucial assistance and providing all possible facilities for my practical work. Special thanks go to Associate Professor Lyndsey Main from the Department of Chemistry for his valuable suggestions on the chemistry of grafting reactions. Thanks also to Pat Greed from the Department of Chemistry, who found time from her busy schedule to carry out the FT-IR spectrometry analysis. Thanks to Stuart Pilling for modifying a plasma reactor in this study. Thanks to all my lab colleagues and other individuals for their inspiration and help.

The New Enterprise Research Fund (NERF) Scholarship provided by Foundation for Research Science and Technology (FRST) is gratefully acknowledged.

Finally, and most importantly, I acknowledge the love, support and endless encouragement from my family.

Preface

This thesis is a part of the NERF (New Enterprise Research Fund) project UOWX030 *Post-Ruminal controlled Release Drug Delivery Technology for Animal Health*.

Publications arising from this study:

1. Lin, Y., Swan, J.E., Fee, C.J. (2006) *Polymer microcapsules for pH-responsive controlled release*. Proceedings of 33rd Annual Meeting & Exposition of the Controlled Release Society, Vienna, Austria.
2. Lin, Y., Swan, J.E., Fee, C.J. (2006) *Preparing porous polyamide microcapsules with a pH-sensitive chemical valve*. Proceedings of CHEMECA, Auckland, New Zealand.
3. Lin, Y., Swan, J.E., Fee, C.J. (2006) *Micron-scale gates: a new approach for controlled release drug delivery*. NZBio Conference, Auckland, New Zealand.
4. Lin, Y., Swan, J.E., Fee, C.J. (2005) *Hollow microcapsules with a porous shell for drug delivery*. Proceedings of 7th Conference on Formulation and Delivery of Bioactives, Dunedin, New Zealand.
5. Lin, Y., Swan, J.E., Fee, C.J. (2004) *Biomaterials for controlled drug delivery*. Proceedings of a joint Conference of Society of Chemical Engineers of New Zealand (SCENZ)/Society of Materials New Zealand Institute (SMNZI)/Food Engineering Association of New Zealand (FEANZ), Hamilton, New Zealand. pp 162.

Awards:

- NERF Scholarship from Foundation for Research Science and Technology.
- Claude McCarthy Fellowship from New Zealand Vice-Chancellors' Committee.
- Winner of *Highlights of Student Posters*, 33rd Annual Meeting & Exposition of the Controlled Release Society.

Table of Contents

Abstract	i
Acknowledgements	iii
Preface.....	iv
Table of Contents	v
List of Figures	viii
List of Tables	xi
Abbreviations	xii
1 Overview	1
1.1 Introduction	1
1.2 Thesis Outline	4
2 Critical Review of the Literature	5
2.1 Controlled release technology	5
2.2 Controlled delivery in animals	7
2.2.1 Ruminant physiology	8
2.2.2 Post-ruminal delivery systems	10
2.3 Overview of biomaterials	11
2.3.1 Stimuli-sensitive biomaterials	11
2.3.2 Hydrogels	12
2.3.3 Hydrophobic biomaterials	12
2.3.4 Hydrophilic biomaterials	13
2.3.5 Biodegradable polymers	14
2.4 Polymeric biomaterials and their applications	15
2.4.1 Polymers used for drug delivery systems	17
2.4.2 Poly(amides)	18
2.4.3 Poly(esters)	18
2.4.4 Poly(ortho esters)	19
2.4.5 Poly(anhydrides)	19
2.4.6 Polysulfone	20
2.4.7 Polystyrene	21
2.5 Surface modification of polymer materials	23

2.5.1	Polymer brushes	23
2.5.2	Plasma-induced grafting.....	28
2.5.3	Radiation grafting.....	30
2.5.4	Chemical methods	30
2.5.5	Photochemical grafting	32
2.5.6	Crosslinking	32
2.6	Microencapsulation techniques	33
2.6.1	Manufacturing techniques.....	34
2.6.2	Preparative routes.....	38
2.7	Research plan.....	40
2.7.1	Objectives.....	40
2.7.2	Criteria and considerations.....	42
3	Materials and Methods.....	43
3.1	Reagents and materials	43
3.2	Equipments	44
3.3	Preparing microcapsules.....	45
3.3.1	Interfacial polymerisation	45
3.3.2	Solvent evaporation.....	46
3.3.3	Phase inversion.....	46
3.4	Morphological analyses	46
3.4.1	Particle size analysis	46
3.4.2	Porosity	46
3.4.3	Optical microscope.....	47
3.4.4	Scanning electronic microscope (SEM).....	47
3.4.5	Cross-sectioning technique	47
3.5	Surface modification.....	48
3.5.1	Plasma-induced grafting.....	48
3.5.2	Determining grafting yield.....	48
3.5.3	Microcapsule functional processing.....	49
3.5.4	Freeze-pump-thaw method for degassing liquids	49
3.5.5	Free radical grafting polymerisation	50
3.5.6	Dialysing grafted microcapsules	51
3.6	Model drug release study.....	52
3.7	Statistical significance	52
4	Plasma Grafted Microcapsules.....	53
4.1	Introduction	53
4.2	Fabricating microcapsule and morphological analyses	54

4.2.1	Morphology and size	54
4.2.2	Specific surface area and porosity	56
4.2.3	Stability and rigidity	57
4.2.4	Influence of process parameters	58
4.3	Functionalised microcapsules	62
4.3.1	Plasma-induced grafting	62
4.3.2	Determining carboxyl groups	63
4.4	Release studies	66
4.4.1	Loading capacity.....	66
4.4.2	Model drug molecules release studies	66
4.4.3	Effect of pH on release	69
4.4.4	Factors affecting release rate	71
4.5	Conclusions	75
5	Chemically Grafted Microcapsules	77
5.1	Introduction	77
5.2	Exploratory study	78
5.2.1	Morphology	78
5.2.2	Effects of process parameters	81
5.2.3	Chemical grafting trials	86
5.2.4	Summary	88
5.3	Polystyrene microcapsules	89
5.3.1	Influence of process parameters on morphology.....	89
5.3.2	Phase-inversion technique – a novel approach.....	97
5.3.3	Release study	103
5.3.4	Summary	107
5.4	Conclusions	107
6	Conclusions and Recommendations	109
6.1	Conclusions	109
6.2	Recommendations	111
	References	113
	Appendix	127

List of Figures

Figure 2.1 Comparison of conventional (- - -) and ideal controlled release delivery (—) systems	6
Figure 2.2 Ruminant stomach (from Meier, personal communication)	9
Figure 2.3 Schematic of the gastro-intestinal tract of the cattle	10
Figure 2.4 Method for preparing hydrogel-based drug delivery systems.....	13
Figure 2.5 Action of an ‘encrypted’ polymeric drug carrier	15
Figure 2.6 Chemical structure of polysulfone	20
Figure 2.7 Chemical structure of polystyrene	21
Figure 2.8 Polymer systems with polymer brushes	25
Figure 2.9 Preparing polymer brushes by physisorption or grafting.....	27
Figure 2.10 Criteria and considerations for the research.....	42
Figure 3.1 Plasma reactor	45
Figure 3.2 Schematic of microcapsule functional processing	49
Figure 3.3 Free radical grafting polymerisation	50
Figure 3.4 Scheme of the chemical grafting process.....	51
Figure 3.5 Dialysing grafted microcapsules	52
Figure 4.1 SEM images of polyamide microcapsules: (a) microcapsule appearance, (b) single capsule, (c) external surface, (d) internal surface	55
Figure 4.2 Particle size distribution of polyamide microcapsules (n=3).....	55
Figure 4.3 Effect of reaction times on thickness of polyamide microcapsule: (a) 30, (b) 60, (c) 120, (d) 180 minutes	56
Figure 4.4 Pore size distribution of polyamide microcapsule	57
Figure 4.5 Particle size distribution of microcapsules in various pH solutions stored for one month (n=3).....	58
Figure 4.6 Effect of magnetic stirring rates on microcapsule stability (n=3).....	58
Figure 4.7 Effect of stirring speed during emulsification on microcapsule diameter (n=3).....	59
Figure 4.8 Effect of varying ratio of EDA:DETA on microcapsules appearance: column a - 2:1; column b - 1:2 ration.	60
Figure 4.9 Effect of monomer on cross-linking during the polymerisation reaction	61
Figure 4.10 Fragility of polyamide microcapsule	62
Figure 4.11 FT-IR of polyacrylic acid (top), grafted (middle) and ungrafted (bottom) polyamide microcapsules.....	64
Figure 4.12 Effect of plasma treatment time on extent of grafting (n=3)	65

Figure 4.13 Effect of pH on release of vitamin B ₁₂ from PAA-grafted polyamide microcapsules (n=3).....	67
Figure 4.14 Effect of changing pH on release of vitamin B ₁₂ from PAA-grafted polyamide microcapsules (n=3)	68
Figure 4.15 Effect of changing pH on release of cytochrome <i>c</i> from PAA-grafted polyamide microcapsules (n=3)	68
Figure 4.16 Effect of changing pH either from 2 to 7 (top) or from 7 to 2 (bottom) on contents release from PAA-grafted microcapsules.....	69
Figure 4.17 Hysteresis for release of vitamin B ₁₂ from PAA-grafted microcapsules when the pH is switched between 2 and 7	70
Figure 4.18 Effect of capsule size on vitamin B ₁₂ release from PAA-grafted microcapsules (n=3); (a), (b) and (c) average diameter of microcapsules	72
Figure 4.19 Effect of microcapsule pore size on vitamin B ₁₂ release from PAA-grafted microcapsules (n=3).....	73
Figure 4.20 Effect of graft extent on vitamin B ₁₂ release from PAA-grafted microcapsules (n=3)	74
Figure 4.21 Effect of types of model drugs on release from PAA-grafted microcapsules (n=3)	75
Figure 5.1 SEM images of polycaprolactone microcapsules morphology: (a) and (b) microcapsules, (c) single capsule, (d) cross-section.....	78
Figure 5.2 SEM images of polysulfone microcapsules morphology: (a) and (b) microcapsules, (c) single capsule, (d) cross-section	79
Figure 5.3 SEM images of polystyrene microcapsules morphology: (a) and (b) microcapsules, (c) and (d) cross-section	79
Figure 5.4 Particle size distribution of microcapsules prepared from polycaprolactone (top), polysulfone (middle), and polystyrene (bottom)	80
Figure 5.5 SEM images of polycaprolactone microcapsules prepared in different concentration: (a) 2%, (b) 5%, (c) 10%	82
Figure 5.6 SEM images of polystyrene microcapsules prepared in different concentration: (a) 2%, (b) 5%, (c) 10%	82
Figure 5.7 Effect of processing temperature on particle size distribution of polystyrene microcapsules.....	83
Figure 5.8 SEM images of polysulfone microcapsules prepared at room temperature (a, b) and 37°C (c, d)	83
Figure 5.9 SEM images of polystyrene microcapsules prepared at room temperature (a, b) and 37°C (c, d)	84
Figure 5.10 Effect of PVA concentrations on particle size distribution of prepared polystyrene microcapsules.....	85
Figure 5.11 Effect of stirring rate on particle sizes of microcapsules prepared from polycaprolactone, polysulfone, and polystyrene.....	86

Figure 5.12 Possible chemical grafting reaction	88
Figure 5.13 Morphology of polystyrene microcapsules prepared by reducing ambient pressure during solvent evaporation.....	89
Figure 5.14 Effect of ethyl acetate/dichloromethane ratio on polystyrene microcapsules structure: 2:1(a); 1:1(b); 1:2(c) and (d)	90
Figure 5.15 Morphology of polystyrene microcapsules prepared when ammonium bicarbonate was added to the internal aqueous phase.....	91
Figure 5.16 Effect of 2% CaCl ₂ in the internal aqueous phase on polystyrene microcapsules structure	92
Figure 5.17 SEM images of polystyrene microcapsules prepared by blending with polymers: PEG (a, b); PEO (c, d); PVP (e, f); PEG-PPG-PEG (g, h).....	93
Figure 5.18 Morphology of PSt microcapsules prepared with different PSt/PVP ratios: (a) 5%:2%, (b) 5%:5%, (c) 5%:10%; (d) 2%:2% (e) 2%:5%, (f) 2%:10%	94
Figure 5.19 SEM images of polystyrene microcapsules prepared with 5% 1-dodecanol in polymer mixture of (a) 5% PSt + 10% PVP; (b) 5% PSt + 5% PVP	95
Figure 5.20 Polystyrene microcapsules prepared by phase inversion: (a) in the coagulating solution, (b) dried	97
Figure 5.21 Morphology of polystyrene microcapsules prepared by phase inversion (a) microcapsule, (b) cross-section, (c, d, e, f) enlarged cross-section.....	98
Figure 5.22 Morphology of polystyrene microcapsules prepared by phase inversion with (a, b) and without (c, d) THF used as co-solvent.....	99
Figure 5.23 Morphology of polystyrene microcapsules prepared by phase inversion with PVP	100
Figure 5.24 Morphology of mechanically remove surface skins of polystyrene microcapsule prepared by phase inversion.....	101
Figure 5.25 Effect of re-dissolving times in DMAa on skin layer morphology of prepared polystyrene microcapsule.....	102
Figure 5.26 Effect of acrylic acid concentration extent of grafting on polystyrene microcapsules	103
Figure 5.27 Effect of pH on release profile of vitamin B ₁₂ from polystyrene microcapsule prepared with skin re-dissolving times of 30 sec (top), 1 min (middle), and 2 min (bottom).....	104
Figure 5.28 Effect of changing pH on vitamin B ₁₂ released from polystyrene microcapsule.....	105
Figure 5.29 Effect of changing pH on BSA released from polystyrene microcapsule	106
Figure 5.30 SEM images of surface splits on polystyrene microcapsules prepared by phase inversion.....	106

List of Tables

Table 2.1	Summary of U.S. animal health industry market.....	7
Table 2.2	Some commonly-used polymers.....	16
Table 2.3	Properties of polystyrene	22
Table 2.4	Physical and chemical surface modification methods	24
Table 2.5	Microencapsulation techniques.....	34
Table 2.6	Sizes obtained from various bead-forming techniques.....	40
Table 4.1	Physicochemical properties of model drugs.	67
Table 5.1	Evaluation of prepared microcapsules	107

Abbreviations

AAc	Acrylic acid
APS	Ammonium peroxydisulfate
ASTM	American Society for Testing and Materials
BSA	Bovine serum albumin
DCM	Dichloromethane
DETA	Diethylene triamine
DMAa	<i>n,n</i> -Dimethylacetamide
EDA	Ethylene diamine
GRAS	Generally regarded as safe
O/W	Oil-in-water
PAA	Polyacrylic acid
PA	Polyamide
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PCI	Polycaprolactone
PLGA	Poly(lactic- <i>co</i> -glycolic acid)
PSf	Polysulfone
PSt	Polystyrene
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
SEM	Scanning electron microscope
TDC	Terephthaloyl dichloride
THF	Tetrahydrofuran
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water emulsion