

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.



**Massey University**

**Formation and Stability of Food-Grade Water-  
in-Oil-in-Water Emulsions**

**A thesis presented in partial fulfillment of the requirements for the  
degree of Doctor of Philosophy in Food Technology**

**By**

**Jiahong Su**

Riddet Institute

Massey University, Palmerston North, New Zealand

2008



## **Abstract**

The inherent thermodynamic instability of water-oil-water (W/O/W) emulsions restricts their applications in food systems. The objective of this study was to develop food grade W/O/W emulsions with both high encapsulation efficiency (EE) of entrapped compounds and long term stability using minimal concentrations of emulsifiers. Emulsions were characterised by phase separation, confocal microscopy, droplet sizing and EEs of macromolecules such as poly R-478 dye (a water-soluble dye) and small molecules such as sorbitol, NaCl and sodium ascorbate (SA).

Emulsions were prepared using soybean oil, polyglycerol ester of polyricinoleic acid (PGPR) as the emulsifier for the primary water-in-oil (W/O) emulsions and sodium caseinate (NaCN) as the sole emulsifier for the secondary W/O/W emulsions, in both buffered (0.1 M sodium phosphate buffer) and non-buffered (distilled water) systems. The concentration of PGPR had a significant effect on the EE of W/O/W emulsions. In non-buffered systems, the PGPR concentration could be reduced to 2% (w/v) to obtain an EE > 90%, whereas, in buffered systems, 4% (w/v) PGPR was required to maintain a similar EE. The ionic environment created in buffered systems had an effect on the emulsifying ability of PGPR. However, the concentration of PGPR in the internal aqueous phase could be reduced to 2% (w/v) without affecting the EE and the stability of the W/O/W emulsion by partially replacing the PGPR with 0.5% (w/v) NaCN, added to the aqueous phase of the primary W/O emulsion. The results indicate that there may be a possible synergistic effect between PGPR and NaCN, thus allowing the formulation of double emulsions with reduced surfactant concentration.

A modified gum arabic (Acacia (sen) SUPER GUM™) was investigated as an emulsifier to aid in the stabilisation of W/O/W emulsions. SUPER GUM™ at a range of concentrations was added as an emulsifier to replace NaCN in the external aqueous phase of W/O/W emulsions. The addition of 10% (w/v) SUPER GUM™ to the external aqueous phase allowed the PGPR concentration to be further reduced to 0.5% (w/v) while maintaining an EE > 90%. W/O/W emulsions stabilised with SUPER GUM™ were also found to be stable over a wide pH range, in the internal or external

aqueous phase, compared with W/O/W emulsions stabilised with NaCN; therefore, they may be suitable for applications over a wide range of pH values, as may occur during ingestion or incorporation into different food systems.

The encapsulation of small molecular compounds (sorbitol, NaCl or SA) in the internal aqueous phase had a significant effect on the stability of W/O/W emulsions, because osmotic pressure gradients were created by the addition of these compounds. With the addition of 0.5 M sorbitol to the internal aqueous phase, the osmotic pressure induced a water flux from the external aqueous phase to the internal aqueous phase, resulting in swelling of the internal water droplets. The resulting enlarged water droplets were more susceptible to coalescence and were expelled to the external aqueous phase. The EE of poly R-478 dye decreased significantly, mainly because of the expelling of internal water droplets. In contrast, the pulsed field gradient nuclear magnetic resonance (PFG-NMR) technique showed that sorbitol diffused through the oil phase; however, there was no evidence that sorbitol had an adverse effect on the emulsifying ability of PGPR.

The encapsulation of 0.5 M NaCl not only created an osmotic pressure gradient between the internal and external aqueous phases, but also reduced the emulsifying ability of PGPR, as phase separation occurred in W/O emulsions. The release of NaCl from W/O/W emulsions took place quickly; this was mainly due to diffusion of NaCl through the oil phase, because it was observed that release via expelling of internal water droplets took much longer to occur. The release of NaCl from W/O/W emulsions could be reduced by increasing the PGPR concentration.

For identically formulated W/O/W emulsions, the release of encapsulated SA was slower than the release of NaCl. Both diffusion and expelling of internal water droplets contributed to the release, depending on the experimental conditions. The release of SA could be slowed by adding macromolecules such as 0.5% (w/v) NaCN to the internal aqueous phase, by increasing the viscosity of the internal aqueous phase by the addition of 2% (w/v) carboxy methyl cellulose or by using a rotor-stator homogeniser instead of a high-pressure homogeniser to prepare the W/O/W emulsions. In addition, the replacement of 0.5% (w/v) NaCN with 10% (w/v) SUPER

GUM™ improved the EE of SA, possibly by acting as a rigid barrier on the surface of the oil droplets and therefore blocking both the diffusion of SA to the external aqueous phase and the expelling of internal water droplets.

## **Acknowledgements**

I could not have completed this PhD thesis alone. It is my pleasure to take this opportunity to thank the many people who made this thesis possible.

I was very fortunate with my chief supervisor, **Prof. Harjinder Singh**; his wide knowledge, his logical way of thinking and his professional guidance provided a good basis for the present thesis. He provided refreshing insight, critical questions and common sense, which were vital for this project. Thank you for your patience, for pushing me to see beyond the results and for teaching me to be independent in my research.

It is difficult to overstate my daily supervisor, **Dr. John Flanagan**, with his inspiration and his great efforts to help me to prepare this thesis. I greatly appreciate his detailed and constructive comments and his important support throughout this work. He always explained things clearly and simply, and offered encouragement, sound advice and lots of good ideas. I would have been lost without him. Thank you for helping me in the writing of this thesis in a professional way and for revising the English of my manuscript, particularly in your precious spare time after long days.

I warmly thank **Prof. Paul Moughan**, for his encouragement and inspiration of this study, and **Dr. Yacine Hemar**; his valuable advice and interesting explorations around my work were very helpful in this study.

I would like to thank **Dr. Jason Hindmarsh** wholeheartedly for setting up experiments and analysing data in magnetic resonance imaging studies; his kind support and guidance were of great value in this study.

I wish to extend my warmest thanks to all those people who helped me with my work in the laboratory and the office. Thanks to **Liz Nickless**, who helped me with confocal images, and **Matt Levin**, who come to the rescue during computer disasters. Thanks to **Michelle Tamehana, Janiene Gilliland, Steve Glasgow, Warwick Johnson** and

**Chris Hall**, who kindly helped me through the entire experimental period on a daily basis.

I am indebted to my officemates and friends, **Donjila** and **Angkana**. I enjoyed long chats with you and discussions about everything, which were fun and relaxing and which helped greatly through my study. I also appreciate that you shared your experiences in PhD study openly and offered me advice in many ways.

I wish to thank my family, my parents, **Chuli** and **Zhaoping**, and my sister, **Jiahua**, for their support during the years that I pursued my PhD. Last, but certainly not least, I give my loving thanks to my husband, **Hui**, and our daughter, **Emma**; your patience, love and encouragement are priceless to me and all I can say is that it would take another thesis to express my deep love for you; without your support, it would have been impossible for me to finish this work.

The financial support of the Riddet Institute is gratefully acknowledged.



## Table of Contents

<b>Abstract .....</b>	<b>i</b>
<b>Acknowledgements .....</b>	<b>iv</b>
<b>Table of Contents.....</b>	<b>vi</b>
<b>Chapter 1: Introduction.....</b>	<b>1</b>
<b>Chapter 2: Literature review.....</b>	<b>3</b>
<b>2.1. Introduction.....</b>	<b>3</b>
<b>2.2. Definition of a W/O/W emulsion .....</b>	<b>3</b>
<b>2.3. Formation of W/O/W emulsions.....</b>	<b>4</b>
<b>2.4. Process for the formation of W/O/W emulsions .....</b>	<b>6</b>
2.4.1. High-pressure homogeniser .....	9
2.4.2. Membrane emulsification.....	10
<b>2.5. Compositions of W/O/W emulsions .....</b>	<b>11</b>
2.5.1. Aqueous phase .....	11
2.5.2. Oil phase .....	11
2.5.3. Volume fraction of dispersed phase .....	12
2.5.4. Emulsifiers .....	13
<b>2.6. Instability of W/O/W emulsions .....</b>	<b>17</b>
2.6.1. Destabilisation of simple emulsions.....	17
2.6.2. Coalescence between the internal and the external aqueous phases .....	20
2.6.3. Transportation of water through the oil phase .....	21
2.6.4. Transportation of electrolyte through the oil phase .....	25
<b>2.7. Improvement of the stability of W/O/W emulsions.....</b>	<b>27</b>

---

*Table of contents*

---

2.7.1.	Control of the size of the internal water droplets .....	27
2.7.2.	Modification of the oil phase .....	28
2.7.3.	Addition of macromolecules to the internal aqueous phase .....	28
2.7.4.	Stabilisation of oil droplets in W/O/W emulsions .....	30
<b>2.8.</b>	<b>Characteristics of W/O/W emulsions.....</b>	<b>32</b>
2.8.1.	Droplet size distribution .....	32
2.8.2.	Confocal scanning light microscopy .....	35
2.8.3.	Encapsulation efficiency.....	36
<b>2.9.</b>	<b>Potential applications of W/O/W emulsions.....</b>	<b>37</b>
<b>2.10.</b>	<b>Conclusions.....</b>	<b>38</b>
<b>Chapter 3: Formation of W/O/W emulsions: selection of materials and processing conditions.....</b>		<b>40</b>
<b>3.1.</b>	<b>Introduction.....</b>	<b>40</b>
<b>3.2.</b>	<b>Materials and Methods.....</b>	<b>41</b>
3.2.1.	Materials .....	41
3.2.2.	Emulsion preparation.....	41
3.2.3.	Confocal laser microscopy .....	41
3.2.4.	Determination of average droplet size of primary W/O emulsions.....	42
3.2.5.	Determination of average droplet size of W/O/W emulsions.....	45
3.2.6.	Determination of encapsulation efficiency.....	45
<b>3.3.</b>	<b>Results and Discussion.....</b>	<b>46</b>
3.3.1.	Internal and external aqueous phase .....	46
3.3.2.	Oil phase.....	46
3.3.3.	Selection of hydrophobic emulsifier.....	48
3.3.4.	Selection of hydrophilic emulsifier .....	53
3.3.5.	Processing techniques.....	56

---

---

<b>3.4. Summary .....</b>	<b>65</b>
---------------------------	-----------

**Chapter 4: Effect of PGPR and macromolecular materials on the formation and stability of W/O/W emulsions..... 66**

<b>4.1. Introduction.....</b>	<b>66</b>
<b>4.2. Materials and Methods.....</b>	<b>66</b>
<b>4.3. Results and Discussion .....</b>	<b>67</b>
4.3.1. Effect of PGPR concentration on W/O emulsions.....	67
4.3.2. Effect of PGPR concentration on W/OW emulsions .....	69
4.3.3. Effect of ionic environment on emulsifying ability of PGPR.....	73
4.3.4. Effect of addition of macromolecular materials to the internal aqueous phase of W/O/W emulsions in buffered systems.....	81
4.3.5. Effect of addition of macromolecular materials to the internal aqueous phase of W/O/W emulsions in non-buffered systems .....	89
<b>4.4. Summary .....</b>	<b>94</b>

**Chapter 5: Effect of hydrophilic emulsifiers on the formation and stability of W/O/W emulsions ..... 95**

<b>5.1. Introduction.....</b>	<b>95</b>
<b>5.2. Materials and Methods.....</b>	<b>96</b>
<b>5.3. Results and Discussion .....</b>	<b>96</b>
5.3.1. Effect of NaCN concentration in the external aqueous phase on droplet sizes and EEs of W/O/W emulsions .....	96
5.3.2. Effect of SUPER GUM™ concentration in the external aqueous phase on droplet sizes and EEs of W/O/W emulsions.....	102
5.3.3. Comparison of NaCN and SUPER GUM™ as hydrophilic emulsifiers in non-buffered W/O/W emulsions .....	107
<b>5.4. Summary .....</b>	<b>111</b>

---

<b>Chapter 6: Characteristics of W/O/W emulsions containing hydrophilic encapsulants in the internal aqueous phase .....</b>	<b>112</b>
<b>6.1. Introduction.....</b>	<b>112</b>
<b>6.2. Materials and Methods.....</b>	<b>113</b>
6.2.1. Raw materials .....	113
6.2.2. Preparation of W/O/W emulsions.....	113
6.2.3. Determination of encapsulation efficiency (EE) .....	113
6.2.4. Determination of permeability of sorbitol through oil phase and the mean size of internal water droplets of W/O/W emulsions using the PFG-NMR technique. ....	114
<b>6.3. Results and Discussion.....</b>	<b>117</b>
6.3.1. W/O/W emulsions with sorbitol.....	117
6.3.2. W/O/W emulsions with NaCl in the internal aqueous phase.....	135
<b>6.4. Summary.....</b>	<b>156</b>
<b>Chapter 7: Encapsulation of sodium ascorbate in W/O/W Emulsion .....</b>	<b>157</b>
<b>7.1. Introduction.....</b>	<b>157</b>
<b>7.2. Materials and Methods.....</b>	<b>158</b>
<b>7.3. Results and Discussion.....</b>	<b>159</b>
7.3.1. SA in the aqueous phase of W/O/W emulsions with 0.5% (w/v) NaCN as hydrophilic emulsifier.....	159
7.3.2. Release of SA in W/O/W emulsions with 0.5% (w/v) NaCN or 10% (w/v) SUPER GUM™ as hydrophilic emulsifier.....	173
<b>7.4. Summary.....</b>	<b>179</b>
<b>Chapter 8: Conclusions and Recommendations .....</b>	<b>180</b>
<b>8.1. Formation of stable W/O/W emulsions.....</b>	<b>180</b>
8.1.1. The effect of the formation technique on W/O/W emulsions.....	180
8.1.2. The effect of hydrophobic emulsifiers on the stability of W/O/W emulsions.....	181

---

---

8.1.3.	The effect of hydrophilic emulsifiers on the stability of W/O/W emulsions .....	182
8.1.4.	The effect of encapsulated compounds on W/O/W emulsions .....	182
<b>8.2.</b>	<b>Potential applications of W/O/W emulsions for encapsulation .....</b>	<b>184</b>
<b>8.3.</b>	<b>Recommendations for future work .....</b>	<b>185</b>
8.3.1.	Internal aqueous phase .....	185
8.3.2.	Oil phase .....	186
8.3.3.	External aqueous phase .....	186
8.3.4.	Evaluation of interfacial properties .....	186
8.3.5.	Advanced model system .....	187
<b>8.4.</b>	<b>Conclusions.....</b>	<b>187</b>
<b>References.....</b>		<b>189</b>
<b>Appendix.....</b>		<b>213</b>