Investigation of polymeric biodegradable-biocompatible Janus particles in drug delivery systems and further augmentation via a facile synthesis method

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LIM YI GUANG, JEROME

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

12.7.2020

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Lim Yi Guang Jerome

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

13 Jul 2020

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Date

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Assoc Prof Joachim Loo

Authorship Attribution Statement

This thesis contains material from a paper published in the following peer-reviewed journal where I am listed as the first author.

Chapters 4, to 6 contains results from Lim, Y. G. J., Poh, K. C. W., & Loo, S. C. J. (2019). Hybrid Janus Microparticles Achieving Selective Encapsulation for Theranostic Applications via a Facile Solvent Emulsion Method. *Macromolecular rapid communications*, 40(7), 1800801 and Y. G. J., Tan, H. Y. J., & Loo, S. C. J. (2019). Synthesis of Polymeric Janus Superstructures via a Facile Synthesis Method. *Macromolecular rapid communications*, *Invited Publication*, 202000140.

The contributions of the co-authors are as follows:

- A/Prof Joachim provided the initial direction of the project and edited the manuscript drafts.
- I carried out the fabrication of Janus particles as well as dual-drug loaded Janus particles.
- All characterization works including sample preparation, microscopy, High Performance Liquid Chromatography, contact angle measurements and Energy Dispersive X-ray Analysis was carried out by me at the Facility for Analysis, Characterization, Testing and Simulation lab.
- Dr. Wilson Poh assisted in the fabrication of the Super-paramagnetic Iron Oxide Nanoparticles.
- Ms Jessalyn Low assisted in the fabrication of Janus/Core Shell hybrids.

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Abstract

Janus particles have been garnering considerable interest in recent times. Traditionally, Janus particles have been fabricated via Pickering Emulsions and Sputtering processes. Due to the harsh nature of these processes, the types of materials that could be used for Janus particles were primarily inorganic or metallic in nature. Polymeric Janus particles were generally not suitable for such methods due to the high temperatures and solvents used in these processes. To fabricate polymeric Janus particles, the predominant method had been microfluidics via co-jetting. Due to the extra care needed in the co-jetting process, this method usually suffers from extremely low throughputs. A breakthrough study in 2012 demonstrated the fabrication of polymeric poly(lactic-co-glycolic) acid (PLGA)/poly (caprolactone) (PCL) Janus particles via a solvent emulsion method, which could potentially circumvent the issue of low throughputs in microfluidics. However, various intrinsic issues such as the loss of Janus morphology upon drug addition had caused a halt in this research area. The work shown in this thesis attempts to address the issue of drug addition to polymeric biodegradable/biocompatible Janus particles via the solvent emulsion method. By understanding the thermodynamics behind the formation of Janus particles via the solvent emulsion method, various achievements such as dual-drug encapsulation, selective encapsulation of drugs and diagnostic materials can be carried out in a single-step synthesis. As will be discussed in detail of this thesis, the spontaneous and automatic partitioning behavior of drugs and diagnostic agents is found to be driven by thermodynamic factors, without requiring external intervention. Additionally, unique drug-release behavior depending on polymer combination is also demonstrated as a result of this selective encapsulation behavior. Various applications such as potential treatments to diabetes based on utilizing the selective encapsulation of drugs and diagnostic materials are also shown in a proof-of-concept model. Other additional derivatives such as Janus superstructure fabrication was also shown possible within this single-step synthesis method through the understanding of the formation mechanics of Janus particles.

Lay Summary

In ancient Greek mythology, Janus was known as a deity with two faces. It was believed in those times that Janus could look both into the past and future, and hence was seen as a deity of time and transitions. In the scientific world, this two-faced property of Janus finds itself uniquely in particles, where each individual particle has two distinct faces, and hence two distinct properties within this single particle. Some of the most common properties of Janus particles are particles that are both hydrophobic and hydrophilic. To achieve this, one face is modified to be hydrophobic and the other modified to be hydrophilic. The result is an amphiphilic particle that combines both of these hydrophobic and hydrophilic attributes. In this work, this two-faced property of particles is conferred to polymeric particles. The main significance of this is the ability to do all this within a single-step process that essentially simplifies and achieves similar results that more complicated methods have attempted to do. This is possible based on the understanding of the mechanism behind Janus particle formation that is governed by the balance of the interfacial tensions between the polymer and aqueous phases. By leveraging on this knowledge, it is demonstrated for the first time in this thesis on how drugs can be specifically localized and compartmentalized in different faces of the Janus particle via a single-step process. On top of this, an extension of the normal two-faced structure, also known as the Janus superstructure, can be fabricated within this single-step technique. Therefore, the works shown in this thesis revolve around the basic understanding of how Janus particles are formed and how the governing parameter of the polymer interfacial tension is a prime parameter that determines the degree of success in fabricating various novel structures.

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Table of Contents

Abstract	ii
Lay Summary	v
Acknowledgements	vii
Table of Contents	ix
Table Captions	xv
Figure Captions	xvii
Abbreviations	xxvii
 Chapter 1	 2
1.1 Hypothesis/Problem Statement	3
1.2 Hypothesis.....	4
1.3 Objectives and Scope	5
1.4 Dissertation Overview	6
1.5 Findings and Outcomes/Originality	8
Chapter 2	9
2.1 The Inception of Janus Particles	10
2.2 Applications of Janus Particles	10
2.2.1 Amphiphilic Janus Particles as Emulsifiers	11
2.2.2 Janus Particles as Micro-motors.....	12
2.2.3 Janus Particles as Bio-imaging Vectors	13
2.2.4 Polymeric Janus Particles in Drug Delivery Systems	14
2.3 Synthesis Methods for Janus Particles	15
2.3.1 Synthesis of Janus Particles via Pickering Emulsions	16
2.3.2 Janus Particle Fabrication via Surface Coating.....	18

2.3.3	Step-by-Step Synthesis.....	20
2.3.4	Polymeric vs Inorganic Janus Particles in Drug Delivery.....	21
	<i>Supposition #3: Microfluidic fabrication of Janus particles is inefficient due to the strict parameter requirements and low flow-rates needed for Janus droplet generation.....</i>	<i>23</i>
2.3.5	Fabrication of Polymeric Janus Particles via Microfluidics.....	23
2.3.6	Fabrication of Janus Particles via Solvent Emulsion	28
2.3.7	Synthesis of Janus Particles with Additional Functional and Structural Features	34
Chapter 3		41
3.1	Overview of the Approach.....	42
3.2	Polymeric Janus Particle Synthesis and the Selection of Polymers.....	42
3.2.1	Poly (lactic-co-glycolic) Acid	43
3.2.2	Poly (lactic) Acid	44
3.2.3	Poly (methylmethacrylate)	45
3.2.4	Ethyl co-vinyl-acetate	46
3.3	Solvent Emulsion Fabrication of Janus Particles.....	47
3.3.1	Micro-particle Fabrication of Janus Particles.....	48
3.4	Drug Encapsulation within Polymeric Janus Particles	48
3.4.1	Microparticle Fabrication of Drug-Loaded Janus Particles	49
3.4.1	Selective Dissolution.....	49
3.5	Drug Release Studies and Mathematical Model Quantification.....	50
3.6	The Higuchi Model for Drug Release.....	51
3.6.1	Ultraviolet Visible Spectroscopy (UV-Vis)	52
3.6.2	Calculation and Measurement of Calibration Curves of Drugs	53
3.6.3	Drug Encapsulation Efficiency (EE%) for Dox.....	54

3.6.4	Calibration Curves for Doxorubicin & Paclitaxel.....	54
3.6.5	Drug EE% for Rapamycin, Paclitaxel and Glybenclamide.....	55
3.7	Fabrication of Super-Paramagnetic Iron Oxide Nanoparticles (SPIONs)	55
3.8	Fabrication of SPION-loaded Janus Particles.....	56
3.9	Particle Characterization.....	57
3.9.1	Particle Morphology and Cross-Sectional Analysis via a Scanning Electron Microscope (SEM)	57
3.9.2	Particle Size Analysis.....	58
3.10	Reverse-Phase High Performance Liquid Chromatography (HPLC).....	59
3.11	Confocal Laser Scanning Microscopy (CLSM)	60
3.12	Measurements and Calculations of Interfacial Tensions Between Polymers and Aqueous Surfactants.....	61
3.12.1	Calculation of Polymer-to-Aqueous Phase Interfacial Tension..	62
3.12.2	Calculation of Polymer-to-Polymer Interfacial Tension.....	64
3.13	Materials	66
Chapter 4		71
4.1	Fabrication of Polymeric Janus Particles*	72
4.1.1	Establishing a Reference Point of Fabrication Parameters*	72
4.2	Mechanism of Janus Particle Formation.....	73
4.3	Interfacial Tension Measurements of the Respective Phases and the Calculation of the Spreading Coefficient*.....	75
4.3.1	Polymeric Janus Particles with Different Polymers and Their Selective Dissolutions*	78
4.4	Problems and Limitations with the Existing Harkin's Spreading Coefficient Theory	80

4.4.1	Effect of Polymer Weight Ratios in Janus Particle Formation with PLGA and EVA	82
4.4.2	Effect of Polymer Weight Ratios in Janus Particle Formation with PLLA and EVA.....	87
4.4.3	Effect of Polymer Weight Ratios in Janus Particle Formation with PLGA and PMMA	90
4.4.4	Comparisons and Discussions of Janus Particle Formation for PMMA/PLGA, EVA/PLGA and EVA/PLLA.....	93
4.4.5	Discussion on Polymer Portions in Janus Particles.....	94
4.5	Effect of Stirring Speed of Janus Particle Size and Morphology	96
4.5.1	Effect of Stirring Speed on the Size and Morphology of EVA/PLGA Janus Particles	97
4.5.2	Effect of Stirring Speed on the Size and Morphology of EVA/PLLA Janus Particles	99
4.5.3	Effect of Stirring Speed on the Size and Morphology of PMMA/PLGA Janus Particles	100
4.5.4	Statistical Analysis of the Effect of Stirring Speed on Particle Size	101
4.6	Structural Relationships of Size to Surface Morphology	103
4.6.1	Kinetic Trapping in the Fabrication of Janus Particles	105
4.7	Degradation Behavior of PMMA/PLGA, EVA/PLGA and EVA/PLLA	108
Chapter 5		113
5.1	Drug Loading and the Importance of Polymer Interfacial Tension	114
5.1.1	Drug Encapsulation with Doxorubicin*.....	115
5.1.2	Drug Encapsulation with Doxorubicin and Paclitaxel*	116
5.1.3	Confocal Mapping of Drug Encapsulation within EVA/PLGA..	119
5.2	Mechanism of Drug Selectivity	120

5.3	Mechanism of Drug Selectivity and Selective Encapsulation	121
5.3.1	Drug Release Studies for EVA/PLGA, EVA/PLLA and PMMA/PLGA.....	123
5.4	Discussions	127
Chapter 6		132
6.1	Justification for Design of Hybrid Janus Particles Encapsulated with SPIONs*	133
6.1.1	Encapsulation behavior of SPIONs in EVA/PLGA, EVA/PLLA and PMMA/PLGA*	134
6.2	SPION-Loaded Janus Particles as a Potential Treatment for Diabetes... 136	
6.2.1	Background	137
6.3	Optimization of Glybenclamide Release from EVA/PLGA Janus Particles + SPIONs	143
6.4	Selective Surface Functionalization of Janus Particles*	143
Chapter 7		150
7.1.1	Janus Superstructures*	151
7.1.2	Janus/Hollow Core Superstructures*	152
7.1.3	Varying of Osmogen Concentration in JHC Formation*	154
7.1.4	Janus-Coreshell Hybrid (JC) Superstructures*	155
7.1.5	Proposed Formation Mechanism of JC Superstructures*	157
7.2	Janus-Submicron Particle Hybrid (JS)*	160
Chapter 8		166
8.1	Conclusions	167
8.2	Future Recommendations	170
8.2.1	EVA/PLLA SPION + Dual-Drug Loaded Particles for Diabetes Management.....	170

8.2.2 Janus Coreshell Hybrid for Prolonged Drug Release.....	170
8.2.3 Calcium-Based Surface Coatings for Calcium-Targeted Mechanisms	171
8.2.4 In-vitro Testing of DOX and PTX Loaded Janus Particles in the Treatment of MCF-7 Spheroids	172
8.2.5 Janus-Submicron Particle Hybrid in Treatment of Biofilms.....	172

Table Captions

Table 1	Cost/benefit analysis of Microfluidics vs Solvent Emulsion for Janus particle synthesis.....	27
Table 2	Interfacial tension measurements of EVA/PLGA, EVA/PLLA and PMMA/PLGA particles along with their predicted and actual morphology	76
Table 3	Calculation of the Interfacial Tension Between (a) EVA and PLGA, (b) EVA and PLLA and (c) PMMA and PLGA based on DCM as a Solvent	77
Table 4	Formation ratios of Janus particles in EVA/PLGA, EVA/PLLA and PMMA/PLGA.....	82
Table 5	Weights of selectively dissolved polymer portions. Weights in mg.....	94
Table 6	Sizes in μm obtained for EVA/PLGA, EVA/PLLA and PMMA/PLGA Janus particles at different stirring speeds	101
Table 7	One-Way Analysis of Variance (ANOVA) of particle sizes for varying stirring speeds. MS is the mean-squared value. $\alpha = 0.05$	102
Table 8	p-value of two-tailed t-Test for each respective Janus particle combination....	103
Table 9	Encapsulation efficiencies of doxorubicin and Paclitaxel in whole particles as well as selective encapsulation efficiency calculations.....	118
Table 10	Encapsulation efficiency of Doxorubicin and PTX for individual particles of PLGA, PLLA, EVA and PMMA. Doxorubicin encapsulation in PMMA and EVA was negligible and hence not shown. *Encapsulation of doxorubicin + paclitaxel in EVA and PMMA not shown due to extremely low EE% of doxorubicin within both EVA and PMMA.....	121

Table 11	Correlation coefficient (r^2) and Rate Constant (K) of Dox and PTX from the respective Janus particle combinations after fitting with the Higuchi equation.....	125
Table 12	Encapsulation Efficiency and Selective Encapsulation of Rapamycin and Glibenclamide in PLLA/EVA Janus Particles	142
Table 13	Interfacial tensions, spreading coefficients and morphology predicted and observed at different PVA concentrations.	159

Figure Captions

- Figure 2.2.1-1** Colloid formation between oil and water with selectively coated gold/silica microparticles as emulsifiers. From [6] 12
- Figure 2.2.2-1** Schematic of Janus particle formation of platinum coated silica particles and its corresponding back-scattered electron image. 12
- Figure 2.2.4-1** Schematic of drug-loaded Janus particles via the microfluidic method. In this work, hydrophilic Doxorubicin and hydrophobic Paclitaxel were co-encapsulated within the same Janus particle..... 15
- Figure 2.3.1-1** Schematic of Janus particle fabrication via Pickering Emulsions. From [2] 17
- Figure 2.3.2-1** Fabrication schematic of bromine-functionalized silica particles and subsequent functionalization of fluorophores. From [25]..... 19
- Figure 2.3.5-1** Generation of droplet particles from microfluidics. From [35] 23
- Figure 2.3.5-2** Schematic representation of the fabrication of Janus particles via microfluidics. From [40] 24
- Figure 2.3.5-3** (a) Light microscope images of poly (acrylamide) and PMMA Janus particles. (b) SEM Image of the formed Janus particle. From [44] 26
- Figure 2.3.6-1** The formation of PLGA/PCL Janus particles via the solvent emulsion method; a first of its kind. From [20]..... 30
- Figure 2.3.6-2** The loss of anisotropy upon the addition of a drug to PLGA/PCL Janus particles fabricated via the solvent emulsion method. From [20]..... 32

Figure 2.3.6-3 (a) Rapamycin loaded PLGA/PCL Janus particles at a 19:11 ratio and (b) 20:11 ratio. The small change in the weight ratio causes a complete change in morphology. From [65].....	33
Figure 3.2.1-1 Chemical Structure of PLGA. From [10]	43
Figure 3.2.2-1 Chemical structure of PLA[15].....	44
Figure 3.2.3-1 Chemical Structure of PMMA. From [26].....	45
Figure 3.2.4-1 Chemical structure of EVA. From [37]	46
Figure 3.6.4-1 Calibration curves for (a) PTX and (b) Dox	55
Figure 3.9.2-1 Schematic of the working principle of an HPLC. Image obtained from [1]	59
Figure 3.9.2-1 Schematic of a single polymer coacervate droplet upon emulsion.....	62
Figure 3.12.2-1 Schematic representation of Young's equation of the interaction between the liquid and solid interface. From[59]	65
Figure 4.1.1-1 (a) An EVA/PLGA Janus particle subjected to selective dissolution by cyclohexane, leaving behind a PLGA half-face, (b) Neat EVA/PLGA Janus particles after fabrication	73
Figure 4.1.1-1 Schematic of the prediction model of final particle morphology[3].....	74
Figure 4.3.1-1 (a) EVA/PLGA, (b) PMMA/PLGA and (c) EVA/PLLA Janus particles fabricated via solvent emulsion	79
Figure 4.3.1-2 Selective dissolution of (a) EVAPLGA, (b)EVA/PLLA and (c) PMMA/PLGA.....	79

Figure 4.4.1-1 EVA/PLGA Janus particles formed with a ratio of 0.2 g : 0.1 g of EVA : PLGA. (a) x50, (b) x500, (c) x1000; EVA/PLGA Janus particles formed with a ratio of 0.18 g : 0.12 g of EVA : PLGA. (d) x50, (e) x200, (f) x500; EVA/PLGA Janus particles formed with a ratio of 0.16 g : 0.14 g of EVA : PLGA. (g) x50, (h) x200, (i) x500 83

Figure 4.4.1-2 EVA/PLGA Janus particles formed with a ratio of 0.14 g : 0.16 g of EVA : PLGA. (a) x50, (b) x200, (c) x500; EVA/PLGA Janus particles formed with a ratio of 0.12 g : 0.18 g of EVA : PLGA. (d) x50, (e) x200, (f) x200 of a different particle; EVA/PLGA Janus particles formed with a ratio of 0.1 g : 0.2 g of EVA : PLGA. (g) x50, (h) x200, (i) x200 of a different particle. 85

Figure 4.4.1-3 EVA/PLGA particles at the ratio of 0.2 g : 0.1 g EVA:PLGA. (a) SEM cross-sectional image, (b) Light microscope image of RhB loaded EVA/PLGA particles 86

Figure 4.4.2-1 EVA/PLLA Janus particles formed with a ratio of 0.2 g : 0.1 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.18 g : 0.12 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.16 g : 0.14 g of EVA : PLLA. (a) x50, (b) x200, (c) x500. 87

Figure 4.4.2-2 EVA/PLLA Janus particles formed with a ratio of 0.14 g : 0.16 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.12 g : 0.18 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.1 g : 0.2 g of EVA : PLLA. (a) x50, (b) x200, (c) x500 89

Figure 4.4.3-1 PMMA/PLGA Janus particles at a ratio of 0.2 g : 0.1 g PMMA : PLGA (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.18 g : 0.12 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500;

PMMA/PLGA Janus particles formed with a ratio of 0.16 g : 0.14 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500	90
Figure 4.4.3-2 PMMA/PLGA Janus particles formed with a ratio of 0.14 g : 0.16 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.12 g : 0.18 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.1 g : 0.2 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500	92
Figure 4.5.1-1 EVA/PLGA Janus particles and their corresponding light microscope images. (a) 200 RPM, (b) 400 RPM, (c) 800 RPM, (d) 1500 RPM	97
Figure 4.5.1-2 Close-up SEM images of EVA/PLGA Janus particles fabricated at (a) 200 RPM, (b) 400 RPM, (c) 800 RPM, (d) 1500 RPM	98
Figure 4.5.2-1 EVA/PLLA particles fabricated at (a) 200 RPM, (b) 400 RPM, (c) 800 RPM and (d) 1500 RPM	99
Figure 4.5.3-1 PMMA/PLGA particles fabricated at (a) 200 RPM, (b) 400 RPM, (c) 800 RPM and (d) 1500 RPM	100
Figure 4.5.4-1 Schematic explaining the formation of patchy EVA/PLGA and EVA/PLLA particles at low RPM and smooth particles at higher RPMs.	105
Figure 4.6.1-1 (a) Kinetically trapped EVA/PLGA particles and (b) Cross-sectional analysis of patchy EVA/PLGA. Large patches are found mostly on the surface, indicating that there is significant driving force for droplets to migrate to the surface as these droplets would satisfy the partial engulfment requirement	106
Figure 4.6.1-2 Thermodynamic behavior of polymer phases within the coacervate droplets during the emulsion process. Polymer droplets immediately aggregate into	

their opposing poles, creating a resemblance to the Janus morphology even before cloud point	107
Figure 4.6.1-1 Degradation behavior of EVA/PLGA, EVA/PLLA and PMMA/PLGA Janus particles over a 14 day period	110
Figure 5.1.1-1 (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA Janus particles encapsulated with Doxorubicin (d) Selective dissolution of Janus particles, leaving behind red hemispheres, indicating complete encapsulation of Dox within one face.	115
Figure 5.1.2-1 (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA Janus particles encapsulated with Dox and PTX. (d-f) Selective dissolution of each respective Janus particle combination. For EVA/PLGA and EVA/PLLA, EVA was selectively dissolved, leaving behind red crescents of PLGA and PLLA respectively. As for PMMA/PLGA, selective dissolution was done on the PLGA, leaving behind transparent crescents of PMMA.	116
Figure 5.1.3-1 Confocal mapping of RhB and DPTX in EVA/PLGA Janus particles. .	120
Figure 5.1.3-1 Contact angles with water on (a) EVA, (b) PLLA, (c) PLGA and (d) PMMA films	121
Figure 5.3.1-1 Release studies of Dox and PTX for (a) EVA/PLGA, (b) EVA/PLLA, (c) PMMA/PLGA Janus particles.	124
Figure 5.3.1-2 Fitting of the release profiles with the Higuchi Model	125
Figure 5.3.1-3 (a) Release studies for single polymers of EVA, PLGA, PMMA and PLLA over a period of 10 days. Comparison of PTX release based on calculations of the corresponding encapsulation percentage of single polymers vs the Janus particles of (b) EVA/PLGA, (c) EVA.	126

- Figure 6.1.1-1** (a) Schematic of SPION encapsulation in polymeric Janus particles, (b) Magnetic behavior of drug and SPIONs loaded EVA/PLGA Janus particles (c-f) SPION encapsulation in PLLA, EVA, PLGA and PMMA, (g) EVA/PLLA, (h) EVA/PLGA, (i) PMMA/PLGA Janus particles..... 135
- Figure 6.1.1-2** Selective dissolution of SPION-loaded (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA Janus particles 136
- Figure 6.2.1-1** Schematic of the working principle of RF-mediated delivery of both Rapamycin and Glibenclamide 140
- Figure 6.2.1-2** (a) EVA/PLLA and (b) PCL/PLGA Janus particles fabricated with Rapamycin, Glibenclamide and SPIONs 142
- Figure 6.2.1-1** Fabrication schematic for selective functionalization of Janus Particles with calcium-based compounds through and within the single-step solvent emulsion process 144
- Figure 6.2.1-2** (a-c) The formation of CaP onto one face of the Janus particle. From the light microscope images, the formations appear to be on the EVA face. (d-f) Similarly for CaCO_3 , the formation of the tiny spheres, which are confirmed by EDX to be calcium-based are formed entirely on the EVA face..... 145
- Figure 7.1.1-1** Formation of Janus superstructures through the facile solvent emulsion method. Only slight modifications are required within the polymer solution preparation. 152
- Figure 7.1.2-1** Schematic of JC formation through the addition of NaCl. The presence of NaCl within the coacervate phases causes osmotic pressure build up within the particle and causes water from the external aqueous phase to influx into the polymer droplet, creating a water pocket..... 153

Figure 7.1.2-2 (a) EVA/PLGA JHCs and (b) PCL/PLGA JHCs and the loss of Janus morphology upon the addition of osmogen for PCL/PLGA JHCs. The Janus morphology for EVA/PLGA however is well retained 154

Figure 7.1.3-1 Light microscope images of EVA/PLGA Janus particles formed when adding salt at (a) 20% w/w, (b) 15% w/w and (c) 10% w/w. Corresponding cross-sectional images of particles in (a), (b) and (c) are shown at (d), (e) and (f) respectively 155

Figure 7.1.4-1 Light microscope image of EVA/PLGA JC particles and various SEM images of the cross-section of the JC particles obtained through selective dissolution with cyclohexane (A), (B) and (C). (D) shows a cross sectional image of the JC particles through cutting 156

Figure 7.1.5-1 SEM images of (a-c) EVA/PLGA Janus particles fabricated with 2.5%, 1.0% and 0.5% PVA respectively. (d-f) Corresponding light microscope images of particles in (a-c) and (g-i) light microscope images of particles of each respective concentration selectively dissolved in cyclohexane, leaving behind pink PLGA components. Scale bar = 100 μ m. 158

Figure 7.1.5-2 Graph of polymer interfacial tension on decreasing PVA concentrations. 159

Figure 7.1.5-3 (a) Formation of EVA/PLGA Janus particles in 2.5% PVA. (b) During the initial point of particle fabrication in 0.5% PVA, the polymer droplet of EVA/PLGA faces a large increase in the interfacial tension of the polymer and aqueous phases. (c) To reduce this interfacial energy, the system attempts to reduce the total exposed surface area and in this process, causes some PLGA to be hidden within the core, away from the aqueous medium
160

Figure 7.1.5-1 Schematic of working principle of JS particles. EVA/PLGA particles having a higher IFT would split first, causing the release of the encapsulated

payload. PMMA/PLGA on the other hand having a lower IFT would stay intact for a longer period of time than EVA/PLGA..... 161

Figure 7.1.5-2Light microscpe images of (a) EVA/PLGA and (b) PMMA/PLGA JS particles with C6-PLLA as payload particles. SEM cross sectional images of (c) EVA/PLGA and (d) PMMA/PLGA C6-PLLA loaded particles... 163

Figure 7.1.5-3Degradation profile for (a) PMMA/PLGA and (b) EVA/PLGA. The presence of yellow microparticles within the surrounding media indicated that the encapsulated sub-micron particles had released into the surrounding media after the Janus particles split open 164

Figure 8.2.2-1Schematic of Enhanced Sustained Release of Doxorubicin from Janus Coreshell Hybrids 171

Abbreviations

ACE	Anterior Chamber of the Eye
ACN	Acetonitrile
C6	Coumarin 6
CLSM	Confocal Laser Scanning Microscopy
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
DOX	Doxorubicin
DPTX	Dansyl-Chloride tagged Paclitaxel
EDX	Energy Dispersive X-ray Spectroscopy
EE%	Encapsulation Efficiency
EVA	Poly (Ethylene-co-Vinyl Acetate)
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High-Performance Liquid Chromatography
PCL	Poly(caprolactone)
PLGA	Poly (Lactic-co-Glycolic Acid)
PLLA	Poly (L-Lactic Acid)
PMMA	Poly (Methyl Methacrylate)
PTX	Paclitaxel
PVA	Poly (Vinyl Alcohol)
RhB	Rhodamine B
RHRD	Radio-frequency Hyperthermia Regulated Delivery
SEM	Scanning Electron Microscopy
SEI	Secondary Electron Images
SPIONs	Super-Paramagnetic Iron Oxide Nanoparticles
THF	Tetrahydrofuran

Chapter 1

Introduction, Hypothesis, Scope and Novelty

This chapter will give a background on the existing landscape of particulate systems in drug delivery. More specifically, anisotropic particles, also known as Janus particles, will be described further in this section and their progress in the field. While this field of Janus particles have experienced progress especially in the area of synthesis, there still exists certain fundamental problems that will have to be addressed in order to substantiate the potential of Janus particles in the field of drug delivery.

1.1 Hypothesis/Problem Statement

In the field of drug delivery, particulate systems have been growing in popularity, owing to their versatility, cost effectiveness and potential to treat diseases in novel methods. Traditionally, drug delivery with these particulate systems have been done with neat, single species polymeric particles that are usually encapsulated with therapeutic drugs. In more recent times, particulate systems comprising of particles with unique anisotropy in their morphology have been garnering significant interest. These anisotropic particles, or Janus particles, are usually biphasic particulate systems comprising of particles that have two different faces made up of two different polymers. Each particle is comprised of two hemispheres meeting at a junction, otherwise known as a bifurcation.

Traditionally, the fabrication of Janus particles is done through more direct methods such as sputtering, microfluidics and toposelective surface modification. As the sputtering and toposelective processes are rather harsh in nature, they are typically unsuitable for polymeric materials. Additionally, they only address to surface modifications and not to encapsulation techniques. Hence, microfluidics has been a prime fabrication method that has been used for polymeric Janus particle synthesis. In this method, two polymer solutions are co-jetted together at a junction. At this junction, the polymer solutions fuse together and through careful control, the Janus particles are fabricated droplet by droplet. While microfluidics has potential, there are various intrinsic properties of microfluidics that inhibits its progress. One major downside to microfluidics is the low throughput. Due to the nature of the co-jetting process, the extra care needed to ensure droplet formation usually results in low flow-rates, which subsequently causes low throughputs. Flow-rates were usually around 3-100 $\mu\text{L/hr}$, which translated to several grams per day[1]. Additionally, polymer choices are limited due to the inability to use polymers of high intrinsic viscosity as the viscoelastic response from such polymers prevents droplet formation at this microscale.

It was later discovered in 2012 that these Janus particles could be fabricated through a cost-efficient method known as solvent emulsion. In this system, the polymers used would have to be weighed out in the appropriate ratios and the Janus particles would form automatically upon emulsion. Due to the simplicity of this process, its scalability can reach up to tens of grams per hour, notwithstanding that further optimization can greatly improve the scalability beyond the metrics used in lab-based research[2]. However, beyond this work, not much has been done to further validate the potential of these polymeric Janus particles. One major reason was because this method was only shown effective in fabricating these blank polymeric Janus particles. In the same report that demonstrated the fabrication of Janus particles through solvent emulsion, the particles lost their anisotropy upon the addition of a drug. Therefore, polymeric Janus particles fabricated via solvent emulsion had major gaps pertaining to its use in drug-delivery applications as there had been no established improvements for encapsulating drugs within these Janus particles. In order to harness the potential of polymeric Janus particles in the field of drug delivery, an understanding in the formation mechanism had to be re-analyzed. In doing so, a new approach would be required for the synthesis of polymeric Janus particles in drug delivery.

1.2 Hypothesis

Hypothesis #1: *Polymers with higher polymer-to-polymer interfacial tension are more independent to weight ratio changes and encapsulant addition in the formation of Janus particles.*

Sub-Hypothesis #2: *Drugs can be selectively encapsulated within each face of the Janus particles depending on the degree of hydrophilicity of the respective polymer faces.*

Hypothesis #3: *Existing parameters can be blended into the solvent emulsion method in the fabrication of Janus superstructures. The values of the interfacial tension data would be pivotal to the fabrication as well as the applications of these superstructures.*

1.3 Objectives and Scope

The first objective was to understand how different polymers would affect the polymer-to-polymer interfacial tension, and how this would influence the ability of these Janus particles to encapsulate small-molecule drugs. The second objective was to establish a platform system of fabricating Janus particles that could maintain Janus anisotropy with different polymeric combinations as well as through the addition of small-molecule drugs. With this understanding, the final objective was to develop a proof-of-concept to develop these Janus particles into Janus superstructures, where these additional structural features could confer unique applications to these Janus particles i.e. such as time-based payload delivery.

To achieve these objectives, the following work was done:

1. Fabricating a reference set of polymeric Janus particles to create a base-line and reference point for the variation of multiple parameters used, such as RPM and weigh ratios.
2. Fabricating Janus particles with varying weight ratios and encapsulating drugs to study the correlation of how a larger polymer-to-polymer interfacial tension allows Janus particles to maintain Janus anisotropy through these processes.
3. Encapsulating different drugs into each polymer combination as well as demonstrating the selective encapsulation of these drugs into specific faces of the Janus particle based on the respective degree of hydrophilicity of each polymer.
4. Showcasing the effect of different polymeric combinations of Janus particles in obtaining varied release profiles of two drugs i.e. sequential, simultaneous or in-between.
5. Demonstrating the fabrication of Janus superstructures via the single-step

solvent emulsion process by blending in existing technologies.

6. Obtaining and demonstrating how the interfacial tension data is pivotal in both the fabrication as well as potential applications for these superstructures.

To the best of my knowledge, this is the first attempt in showcasing the fabrication of Janus particles with different polymeric combinations through the analysis and study of interfacial tension data. All this while encapsulating drugs and other diagnostic materials all within a single-step process. In doing so, this work overcomes previously faced problems such as the loss of Janus anisotropy upon the addition of drugs as well as low throughputs from microfluidics (100 $\mu\text{L/h}$) compared to the throughputs achieved in this thesis (3 mL/h).

1.4 Dissertation Overview

This thesis describes the fundamentals of Janus particle formation and how this knowledge can be leveraged upon to develop new and unique structures that can be used for different applications. This thesis is organized into the following chapters:

Chapter 1 introduces the background of polymeric particulate systems in drug delivery from the beginning of neat single polymer particles to other more advanced conformations like double layered particles. Their advantages and disadvantages are briefly described and compared.

Chapter 2 reviews the existing literature in the field of Janus particles based on the most commonly use techniques such as microfluidics, toposelective surface modification as well as solvent emulsion. The various extent and strengths of these methods will be briefly described and how they have been utilized to generate Janus particles of various configurations. In addition, the limitations and shortcomings of these methods will also be described and wherein lies the gap that this thesis will attempt to bridge.

Chapter 3 discusses the various fabrication and characterization methods used for this thesis along with some basic principles of the equipment used in this thesis. It will outline the key fabrication method used in fabricating these Janus particles as well as other modifications of this technique to fabricate Janus particles of different configurations. In-vitro drug release studies will also be showcased.

Chapter 4 showcases the fabrication of various combinations of polymeric Janus particles through a single-step solvent emulsion method. It reports on various weight ratios used for each combination were shown to determine the best ratio for ideal Janus morphology. The underlying mechanism for Janus particle formation and the importance of the polymer interfacial tension will be highlighted.

Chapter 5 demonstrates the ability to selectively encapsulate drugs within each different polymeric combination of Janus particles. The ability to selectively encapsulate drugs to varying degrees depending on the polymer used is also showcased. The different drug release behaviors of each polymeric combination is also shown.

Chapter 6 expounds on the selective encapsulation of diagnostic materials along with the drugs within the respective Janus particles. The ability to selectively functionalize the surfaces of these Janus particles with calcium compounds, without the need for masking techniques will also be shown.

Chapter 7 provides deeper insight into further building upon the simple fabrication procedure to produce Janus superstructures. These superstructures retain the basic Janus morphology but have an additional structural attribute that could lead to promising applications.

Chapter 8 provides a conclusion of all the findings in the previous chapter as well as potential future works to further develop these technologies into practical applications.

1.5 Findings and Outcomes/Originality

This research led to several novel outcomes by:

1. Establishing and proving the effectiveness of a facile and scalable method for different polymeric combinations of Janus particle synthesis in a single-step process, which was otherwise shown in literature to be limited and non-robust.
2. Utilizing this simple method to selectively encapsulate drugs and other diagnostic materials within the different polymeric combinations of Janus particles. Other capabilities include being able to streamline the selective surface functionalization of these particles within the single-step process as well as to fabricate superstructures of Janus particles.
3. Demonstrating the effect of polymeric combination on the dual-drug release kinetics of these Janus particles in-vitro.
4. Conceptualize and demonstrate the proof-of-concept to utilize these selectively loaded Janus particles for therapeutic purposes for treatment in diabetes.
5. Developing Janus superstructures through the same facile fabrication method by simple tweaks to the fabrication method. These Janus superstructures are promising technologies that could have potential future practical application

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Chapter 2

Literature Review – State of the Art and Current Limitations

This chapter provides a detailed review on Janus particles and its origins. The different methods traditionally used for Janus particle synthesis will be discussed in detail and their shortcomings and limitations will be highlighted to show the need for better and more stream-lined processes. The introduction and role of Janus particles in the field of drug delivery will be reviewed and it will be outlined as to why traditional fabrication methods have become limitations within themselves for Janus particle applications. Hence, this chapter would be an important foreword to establish the gap and the significant missing link that this thesis aims to bridge.

2.1 The Inception of Janus Particles

In 1991, Pierre-Gilles de Gennes was awarded the Nobel Prize in Physics for his discovery in “Methods Developed for Studying Order Phenomena in Simple Systems can be Generalized to More Complex Forms of Matter, in Particular to Liquid Crystals and Polymers”. Pierre coined the term “Janus grains” first in his Nobel acceptance speech and proposed that such an entity would have one face apolar and the other polar, similar to the two-faced nature of the god Janus[1]. For example, one face of the particle can be hydrophilic, while the other hydrophobic, giving rise to particles that could behave like surfactants. These ideas were primarily left ignored for a number of years while most research efforts were concentrated on particles with homogeneous chemical properties[2]. A major reason for the lack of interest in Janus particles was the lack of efficient methods in Janus particle synthesis. Nevertheless, significant landmarks have been achieved since the inception of Janus particles and a wide range of Janus particles have been designed and successfully fabricated for a wide variety of applications. In the field of Janus particles, there are two important areas that must first be determined. The first is its intended application and the other is the synthesis route to achieve the desired properties needed for that application. In almost all instances, the route of fabrication is the deterministic factor that translates these ideas into practical and feasible products. If synthesis methods are highly laborious and inefficient, it would affect the practicality of the technology. Hence, a balance between novelty and feasibility has to be achieved in order to realize these Janus particles in practical applications.

2.2 Applications of Janus Particles

Before giving an overview on the synthesis methods, some applications of Janus particles will be showcased. This is to give an overview of the potential of Janus particles and the impetus to use its unique anisotropy for new applications that conventional isotropic particles are unable to do so.

2.2.1 Amphiphilic Janus Particles as Emulsifiers

Janus particles are structurally unique, where they are composed of two distinct hemispheres within a single unit. The bifunctional potential of these particles brings about broad possibilities and promising applications in the field of bioimaging, theranostics, sensors, catalysis, just to name a few[3]. The ability to combine two or more properties onto a single entity creates new possibilities that previously have not been done before.

An interesting application has been the development of Janus particles as amphiphilic particles. Traditionally, amphiphilic substances are usually chemical in nature whereby they possess both a hydrophobic and hydrophilic chain. At high enough concentrations known as the critical micelle concentration, these amphiphilic molecules can form micelles[4]. In comparison to dense films formed by surfactant molecule at the interface, amphiphilic Janus particles would form interfaces with large interstices, in which chemical exchange could potentially occur between the interface. This semi-permeable monolayer property has been realized by Faria et al. where amphiphilic Janus particles have been utilized to enhance phase-selectivity of catalytic processes in biofuel processing[5]. The use of amphiphilic Janus particles as emulsifiers has also been demonstrated by Fiji et al. In this work, they demonstrated that selectively coated silica particles with gold via sputtering exhibited amphiphilic properties (**Figure 2.2.1-1**). Due to the hydrophilic and hydrophobic properties of gold and silica respectively, this amphiphilic nature of these particles could be mediators at the interface of oil and water, giving rise to colloidal stability for an extended period of time[6].

Figure 2.2.1-1 Colloid formation between oil and water with selectively coated gold/silica microparticles as emulsifiers. From [6]

2.2.2 Janus Particles as Micro-motors

With careful design, Janus particles can move autonomously based on their response to the external chemical environment, with relation to their anisotropic chemical make-up. While these autonomous motions can operate on a variety of mechanisms such as chemotactic, bubble-propulsion and self-electrophoresis, they are fundamentally based on the anisotropic chemical properties of the Janus particle.

Archer et al. demonstrated the ability to fabricate Janus particles with capability of bubble-propelled movement (**Figure 2.2.2-1**). This was done by partially entrapping silica particles onto wax and depositing platinum on the exposed surface. Rather than traditional vapor deposition techniques, seed and growth techniques were employed in this method where formed platinum seed solution was added to a Pickering solution of silica particles. The bond of platinum and silica was electrostatically mediated due to the functionalization of charged amino-propyl silane on the exposed surface of the silica particles[7]. Through the catalytic decomposition of H_2O_2 , these Janus particles exhibited non-Brownian motion, demonstrating a link between translational propulsion velocity and reaction rate.

Figure 2.2.2-1 Schematic of Janus particle formation of platinum coated silica particles and its corresponding back-scattered electron image.

Similarly, Guix et al. had demonstrated environment-influenced non-Brownian motion of

calcium-based Janus particles selectively coated with cobalt within tumor environments. Due to the acidic conditions from lactic acidosis from tumor cells, these calcium-based Janus micromotors undergo controlled dissolution, generating effervescence and hence driving their motion. The translation of Janus micromotor concepts from non-biomedical into biomedical applications indicates a paradigm shift in the usage of Janus particles in biomedical applications that have previously not been thought possible[8].

2.2.3 Janus Particles as Bio-imaging Vectors

In the field of oncological research, various treatments such as cell-based therapies, chemotherapy and even photothermal therapy has been garnering considerable interest. A fundamental pivot in oncology is the swift initial detection and diagnosis of abnormalities within patients for effective treatments[9]. While various existing diagnostics like magnetic resonance imaging (MRI), Optical Imaging (OI) and Computed Tomography (CT) scans have been widely employed as diagnostic tools for oncology, there are various drawbacks to these methods. For example, OI lacks spatial resolution and tissue flux intensity mediation while MRI and CT have difficulties in the detection of tumors smaller than 0.5cm. Hence, the development of multi-functional agents for multi-imaging techniques would prove to be advantageous for cell-labelling and in-vivo imaging. Janus particles comprised of several functional materials into one entity have been shown to be highly effective in multi-modality imaging[10]. A good example of such a concept is the work by Schick et al. They had demonstrated the formation of Au@MnO@SiO₂ Janus particles and their capability of fluorescence tracking, MRI and CT imaging simultaneously[11].

In addition to bio-imaging, the combination of triple-modal imaging and photothermal therapy has been promising in theranostic treatments for cancer. Ju et al. had created Au-Fe₂C Janus nanoparticles shown to have broad absorption at the near-infrared region, capable of both triple-modal imaging and photothermal therapy. Additionally, the functionalization of Au-Fe₂C Janus nanoparticles with affibody proteins showed

significantly improved selectively towards HER2 tumor cells as opposed to non-targeted Au-Fe₂C Janus nanoparticles[12].

2.2.4 Polymeric Janus Particles in Drug Delivery Systems

For drug delivery applications, polymeric drug delivery systems have been found to be highly versatile systems[15-17]. The ability to encapsulate small molecule drugs within its polymeric matrix gives rise to the ability for sustained and controlled drug release[16]. Additionally, the use of biodegradable and biocompatible polymers as drug carriers have been a widely used and attractive preposition for drug delivery applications. Implantability of such devices gives rise to better patient compliance and the use of biodegradable carriers facilitates their removal from the body through natural metabolic means, omitting the need to subsequent surgery for implant removal[17].

One of the first reports of that comprised of both the fabrication of Janus particles as well as its application in drug delivery was carried out by Xie et. al[18] (**Figure 2.2.4-1**). In this report, PLGA Janus particles encapsulating both DOX and PTX were fabricated using this co-jetting procedure and was done via a single-step process. The way that the fabrication was carried out involved the dissolution and dispersion of PTX and DOX respectively into separate solutions of PLGA. Upon dissolution of these drugs, the polymer solutions are carefully co-jetted and due to the use of a volatile solvent, the emulsion process would evaporate the solvent and the Janus particle would be formed. Subsequent drug release studies with these Janus particles showcased the co-delivery of two drugs with disparate solubilities. The significance of this work portrayed a significant advantage of Janus particles over conventional particles, where drugs with contrasting solubilities could be co-encapsulated within the same particle without suffering from low encapsulation efficiencies[19]. Furthermore, the combination of two or more drugs could potentially simplify the release kinetics greatly as opposed to a mixture of individual batches particles containing each type of drug. The combinational drug release from two individual batches of particles cannot be simply calculated through taking into account their proportions from drug delivery kinetics individually. This is due to possible influence between the particle batches. Instead, release kinetics have to be studied for each proportion mixture and then

used accordingly. This greatly complicates the co-delivery of more than one drug with individual particle batches[20].

Figure 2.2.4-1 Schematic of drug-loaded Janus particles via the microfluidic method. In this work, hydrophilic Doxorubicin and hydrophobic Paclitaxel were co-encapsulated within the same Janus particle

Albeit not fully polymeric in nature, Garbuzenko et al. demonstrated the creation of a PLGA/lipid Janus particle hybrid capable of encapsulating two agents with contrasting solubilities; DOX and curcumin. In this work, the fabrication of ‘ice-cream’ shaped Janus particles consisting of a spherical PLGA side and a cone-shaped lipid side were fabricated along with co-encapsulation of DOX within the PLGA and curcumin within the lipid region. In-vivo work showed a significant reduction of tumor volume in Athymic mice of mice exposed to the drug-loaded Janus particles as opposed to the control groups.

2.3 Synthesis Methods for Janus Particles

***Supposition #1:** Existing efficient methods for Janus particle synthesis are usually unsuitable for polymeric Janus particles, especially for drug delivery applications.*

While Janus particles have been shown to have promising applications and unique capabilities due to their Janus morphology, the main barrier of its full potential has been its fabrication technique. As their size range of interest is usually in the micro to nano scale, strictly controlled, multi-step processes are usually needed to ensure proper Janus particle

synthesis. Despite having various efficient and relatively simple methods of Janus particle synthesis, they are mostly only suitable for inorganic materials such as silica. The synthesis of polymeric Janus particles is primarily lacking in terms of an efficient and scalable method. In this section, an overview of various commonly used methods for Janus particle synthesis will be described and why they add little value in the development of polymeric Janus particles for drug delivery.

2.3.1 Synthesis of Janus Particles via Pickering Emulsions

Pickering emulsions have been one of the most common methods for Janus particle synthesis. This is mainly because it is a highly efficient and relatively simple method as many kinds of functionalization can be achieved through attaching a variety of attachments onto the particle surfaces in bulk. Particles are first grafted at a biphasic interface comprised of two immiscible fluids at the interface, most commonly at the interface of molten paraffin and water (**Figure 2.3.1-1**). In this way, one side of the particle is embedded within one phase and the other side of the particle in the other. Hong et al. first demonstrated the synthesis of such Janus particles by fixating silica particles at the interface between molten wax and water[21]. Upon the solidification of the wax, one half of the silica particles were embedded within the wax, while the other face that was not embedded within the wax was exposed to the surface. Subsequently, the collidosomes were exposed to (amino-propyl)triethoxysilane and the wax dissolved away with toluene. Epifluorescent images indicated the partial coating of the (amino-propyl)triethoxysilane on the surface of the particle. In this method, one side of the particle is protected with a coating, leaving the other side exposed and susceptible to chemical modification. After modification is completed on the exposed face, the coating on the protected area is removed, leaving behind a selectively modified particle.

Figure 2.3.1-1 Schematic of Janus particle fabrication via Pickering Emulsions. From [2]

In both of these examples, the heat involved to melt the wax as the masking layer would usually melt the polymers that are of interest. Secondly, the solvents used in these multi-step processes such as toluene would dissolve away any of the polymers. This leaves Pickering emulsions specifically useful only for silica or metallic based materials, where the required operating conditions as well as precursors used would not affect the materials used as particles. Janus particles that would be fabricated with polymers would not be suitable for this process. Most importantly, this method of synthesis usually involves surface functionalization and modification. In drug delivery, drugs or other encapsulants should ideally be entrapped within the matrix of the carrier so as to provide a means of sustained and controlled release. Therefore, despite Pickering emulsions being an efficient and simple method, its surface functionalization features does little to help realize polymeric Janus particles as drug delivery vehicles.

2.3.2 Janus Particle Fabrication via Surface Coating

Selective surface coating of isotropic particles for the synthesis of Janus particles has been another commonly scalable and efficient method for Janus particle synthesis. The basic principle of creating Janus particles through surface coating works by causing the flow of target materials from one direction to the exposed surfaces of particles facing this flow. Because the opposite side of the particles are not in direct line of this flow, the natural shadowing effect creates a particle where one side is coated, while the unexposed side neat. This method is more specifically known as sputtering and usually involves metallic or even ceramic material targets as choice materials for selective coating[23, 24].

The use of metallic coatings has been a popular choice for the creation of Janus particles. Particularly in the field of self-propelling particles, the use of metallic catalysts such as platinum is an attractive means of creating Janus particles. Unlike Pickering emulsions, sputtering is more scalable due to the single step process of coating. Brown et al. demonstrated the partial coating of polystyrene particles with platinum on one face. Due to this selective coating, self-propelling motion was shown and proven to be driven by diffusiophoresis, where the particle is propelled by a concentration gradient due to the interactions of the solute and particle[24]. Toposelective coating can also be done on particles for other applications. Chen et al. had demonstrated a plasma deposition method using 1-bromopropane as the deposition plasma (**Figure 2.3.2-1**). With further modification through a series of steps, fluorophores could be attached to the bromine-functionalized surface.

Figure 2.3.2-1 Fabrication schematic of bromine-functionalized silica particles and subsequent functionalization of fluorophores. From [25]

While the fabrication of Janus particles via sputtering offers potential in developing Janus particles with a wide variety of applications as shown, sputtering is often a costly process[26]. This process usually involves costly machinery and can be a potential barrier to commercialization. More importantly however, the process of sputtering usually involves harsh conditions such as high vacuum, temperature and high kinetic impact from target ions. The biodegradable and biocompatible polymers that are accepted and traditionally used in the field of drug delivery are generally not suitable for such harsh synthesis conditions. Even if polymeric particles could be successfully sputtered anisotropically, it does not address the issue of drug encapsulation as these sputtered materials only function as exterior coatings. Therefore, while sputtering has been an effective means to create Janus particles for other applications, sputtering has little impact in the improvement on the fabrication methods of Janus particles in the field of drug delivery. Hence, the sputtering technique does not bridge the gap between in the fabrication of polymeric Janus particles as drug delivery vehicles.

2.3.3 Step-by-Step Synthesis

A more direct way of fabricating Janus particles is through a step-by-step process. In this process, a seed, usually inorganic in nature like Fe_2O_3 or mesoporous silica, is first prepared. Subsequently, this seed is partially masked in a protective layer and the exposed side is prepared and subjected to polymerization. Yin et al. demonstrated the use of mesoporous silica in the synthesis of silica-polystyrene Janus particles. In their work, mesoporous silica seeds were first synthesized via a sol-gel method known as the Stöber Method. The silica seeds were then partially masked within a 1-Vinylimidazole droplet and then functionalized with potassium persulfate. Then, polystyrene was polymerized on the functionalized surface to create a polystyrene-silica Janus particle[27]. Feyen et al. showed another derivative of polystyrene-silica Janus particles but through the use of a magnetic seed Fe_3O_4 . Subsequently polymerization of polystyrene on the Fe_3O_4 seed created a particle of polystyrene and a partially exposed surface of the Fe_3O_4 seed. Finally, silica was grown on the exposed the Fe_3O_4 seed through an asymmetric growth and controlled with the use of different surfactants[28].

Other examples pertaining to polymer-polymer Janus particles or even inorganic-inorganic Janus particles have been demonstrated. Tanaka et al. fabricated polymer-polymer Janus particles through ground-up polymerization and self-assembly of polystyrene-poly(methylmethacrylate) Janus particles. Monomer units of methylmethacrylate and styrene were dissolved into a toluene solution, followed by the addition of azo-initiators for polymerization. The phase separation of these polymers was done through slow evaporation of toluene from the homogeneous polystyrene and polymethylmethacrylate toluene-dispersed droplets. Additionally, these particles were shown to have pH-responsive properties[29]. Li et al. demonstrated the fabrication of inorganic-inorganic Janus particles that were done through silica seeded growth and subsequent growth of a secondary hydrophobic silica component. This created an amphiphilic particle capable of passive loading of hydrophilic DOX and hydrophobic PTX within the pores of the particles[30].

The common theme shared amongst these examples is the complex and laborious steps involved to achieve the final product. In these examples, strictly controlled protocols had to be followed and synthesis times often exceeded beyond 48 hours. Additionally, the yields rarely exceeded several milligrams. This is notwithstanding that these described methods are only for the blank vehicles that are not drug-loaded. Hence, further steps would need to be developed for drug loading within these methods, further adding to the complexity involved.

2.3.4 Polymeric vs Inorganic Janus Particles in Drug Delivery

***Supposition #2:** Polymeric Janus particles are more easily tunable and simpler to fabricate than inorganic Janus particles for drug delivery applications.*

Polymeric materials such as biodegradable polyesters like poly(lactic-co-glycolic) acid (PLGA) and biocompatible polymers like poly(methylmethacrylate) (PMMA) have numerous advantages in the field of drug delivery. They are approved by the Food and Drug Administration (FDA) and are predictable in drug release and degradation for biodegradable polyesters[31]. Commonly used inorganic materials such as mesoporous silica have been shown to be potential candidates as drug delivery carriers. The main encapsulation mechanism of mesoporous silica is through the containment of drug within the pore network[32]. Because of the rigid nature of the silica, a secondary passive-loading of the desired drugs is needed for drug encapsulation. Additionally, pore-sizes within silica have been shown to be difficult to control, further posing disadvantages to controlled drug release[33].

A major advantage of Janus particles in the field of drug delivery is the ability to combine more than one drug, particularly two drugs with contrasting chemical properties such as hydrophilic/hydrophobic drugs. While multi-drugs can be loaded onto isotropic particles, drug-to-drug interactions makes the independent release of each drug almost impossible to control[33]. Hence, having bi-compartmental configurations for the encapsulation of each drug respectively would prove to be advantageous in ensuring minimal interactions

between these drugs. While there currently are no studies that directly compare inorganic vs organic Janus particles within similar conditions, various studies pertaining to similar drugs with inorganic and polymeric Janus particles can be examined to determine a more suitable system.

For inorganic Janus particles, Li et al. created mesoporous silica based Janus nanoparticles (upconversion nanoparticles(UNCP)@SiO₂@mSiO₂&Periodic mesoporous silica(PMO)). These nanoparticle were comprised of hydrophilic (UNCP@SiO₂@mSiO₂&PMO) and hydrophobic(PMO) domains. DOX and PTX were used as model small-molecule drugs with DOX representing a hydrophilic drug and PTX representing a hydrophobic drug. Additionally, the release of each drug was shown to be controllable via heat and and near-infrared (NIR) light and was found to be 50% more effective in cancer cell destruction as opposed to a single-triggered release system[33].

Xie et al. demonstrated the first demonstration of the fabrication of dual-drug loaded Janus particles via a microfluidic approach (**Figure 2.2.4-1**). In this study, similar drugs of PTX and DOX were used as model drugs and their encapsulation within Janus particles. While there are no in-vivo studies within this work, it was the first demonstration of release kinetics of both hydrophobic and hydrophilic drugs from polymeric Janus particles. In contrast to the previous work by Li et al., the fabrication steps involved in the inorganic drug delivery Janus particles were considerably more complicated. The silica based Janus nanoparticles required a combination of layer-by-layer synthesis steps and strictly controlled heterogeneous nucleation and anisotropic growth to fabricate the nanoparticles. In addition, subsequent steps were needed to entrap the hydrophobic PTX and hydrophilic DOX into their respective compartments through passive diffusion. In comparison, the work by Xie et al. only required simple pre-dissolution of drugs and polymers within a solvent solution and the co-extrusion of the polymer solutions created these drug loaded Janus particles.

As a result, the fabrication of polymeric Janus particles for drug delivery applications appears to be considerably simpler and more convenient as opposed to the fabrication

approach that is often required in inorganic Janus particles for drug delivery. Additionally, the tunability of polymeric materials such as PLGA give it a key advantage over inorganic materials. For example, the molecular weight, rates of drug release, rate of degradation and degree of hydrophobicity can be controlled through the manipulation of the ratio between the glycolic and lactide groups (G:L). For these reasons, polymeric Janus particles were proposed to be more versatile and worth further investigation due to the lack of its undiscovered potential.

Supposition #3: Microfluidic fabrication of Janus particles is inefficient due to the strict parameter requirements and low flow-rates needed for Janus droplet generation.

2.3.5 Fabrication of Polymeric Janus Particles via Microfluidics

Due to the incompatibility of Pickering emulsions and sputtering for polymeric Janus particle fabrication, the pre-dominant means to fabricate Janus particles has been done through microfluidics. Microfluidics involves a system of microchannels that have been shown to be compatible with many chemical and biological precursors. This technology involves the generation of discrete droplets that are monodisperse in nature[34].

Figure 2.3.5-1 Generation of droplet particles from microfluidics. From[35]

For microparticle fabrication via microfluidics, various infrastructure have to be in place. The simplest way is through using a syringe pump into a microfluidic chip comprised of an outer channel with a continuous phase and the inner channel as the polymer phase. In this method, passive droplet generation is carried out through controlling the steady-state flow velocity of the continuous phase and the inner polymer phase. This steady-state flow of the continuous phase exerts a drag force on the droplet from the inner polymer channel, generating a droplet. Parameters such as the flow velocity, viscosity and channel angles have to be precise in order for successful droplet generation. In recent years, the integration of ultrasound with microfluidics has garnered considerable interest. The main advantage of ultrasound integration is enhanced droplet handling through ultrasonic acoustophoresis. However, the added cost of these equipment (several \$1000s) usually hinder them from pervasive use[36-38]. Additionally, the material of the microchip has to be considered for its processing and customization through lithographic techniques such as micro-milling, photoresist coating, chemical etching and UV cross-linking[38].

Prior to Janus particle fabrication, microfluidics have been used in a wide variety of applications with regards to the synthesis of monodisperse particles. For example, Xu et al. had created Bupivacaine-encapsulated PLGA via a microfluidics method and demonstrated the in-vitro drug release kinetics of these particles. The particles generated from this method were highly monodisperse and the specific sizes could be controlled by adjusting different parameters[39].

Figure 2.3.5-2 Schematic representation of the fabrication of Janus particles via microfluidics. From [40]

While seemingly straightforward to fabricate microparticles via this technique, the fabrication of Janus particles through microfluidics requires a different approach. Instead of the conventional single channel system, Janus particle synthesis via microfluidics requires a dual-channel system that co-jets two polymer solutions[42, 43]. At the junction of the two channels, both polymer solutions flow and forms a droplet, creating a Janus particle. This is otherwise known as a side-by-side capillary system(**Figure 2.3.5-2**)[40]. In certain cases, an external UV source would be needed for the cross-linking of the extruded droplets to form these Janus particles[43]. Due to the drop-by-drop generation, consistent monitoring is also needed and hence adds significant processing efforts to Janus droplet generation as the flow of both polymer phases have to be carefully controlled.

While most efforts for Janus particle fabrication were conceptualized through proof-of-concept models, most of the work did not delve specifically into drug delivery applications[45-47]. It was only from the work from Xu et al., that the potential for polymeric Janus particles was realized (**Section 2.2.4**).

Other groups had also reported on the fabrication of such particles for drug delivery. For example, Ikram et al. reported on the fabrication of poly (acrylamide) and PMMA Janus particles, in the first demonstration of a Janus particle comprising of different polymers via this technique. In this work, the drugs ketoprofen and sodium fluorescein could be conveniently dissolved into their respective polymer channels and be encapsulated into the respective face upon solidification of the extruded droplet. The simultaneous delivery of ketoprofen and sodium fluorescein also demonstrated the versatility of this system in being able to accommodate two different polymer species and two different types of drugs[44].

Figure 2.3.5-3(a) Light microscope images of poly (acrylamide) and PMMA Janus particles. (b) SEM Image of the formed Janus particle. From [44]

While microfluidics has shown tremendous potential in harnessing Janus particles as viable candidates in drug delivery, various intrinsic limitations within this process inhibit the progress of Janus particles. One fundamental issue with microfluidics is the low throughput[36, 48, 49]. Typically, the microfluidic process requires strict control over the flow rate in order for the droplets to form properly. As a consequence, the flow rate is usually relatively low, causing the throughput of particles to be low as well (3μL/min for Janus particle fabrication[18]). Various methods currently employed today for Janus particle fabrication typically fall in the order of tens of grams per day[20]. This significantly hinders the production of Janus particles and its translation to an industrial scale. It was also argued that the production limitation could be expedited through the introduction of multi-channel microfluidic systems that could potentially increase the production of Janus particles. While this may be true in principle, the added cost and infrastructure required to do so would often times outweigh the benefits[50, 51](further cost/benefit analysis in **Table 1**).

Another intrinsic problem of microfluidics is the challenge in using viscous fluids.[51]. Fluids that are more viscous than water are more resistant to flow within the continuous phase. This is especially problematic since it is more difficult for viscous fluids to form a

droplet. Additionally, both extruded fluids from the respective channels must exhibit similar viscoelastic responses to form a droplet. If not, the incompatible flow behavior would convolute the polymer flow, preventing the formation of Janus particles[53, 54]. As expected, this severely limits the versatility of microfluidics as it limits the tunability in designing drug delivery vehicles. A direct consequence of this is the final drug release profile. In cases where microfluidics was employed for Janus particle fabrication, the release study duration rarely exceeded 24-48 hours before complete release of drugs. For example in the work by Xie et al., PLGA Janus particles with an IV of 0.19g/mL had achieved near complete release in 48 hours[18]. This is fundamentally due to the requirement of the low viscosity of polymer solutions needed, which usually indicates that polymers of low molecular weight are needed, for example the use of PLGA in microfluidics rarely exceeds 30kDa[54]. In general, the lower the molecular weight, the higher the rate of bulk erosion. This is due to the increased permeability of the surrounding medium to penetrate into the polymer matrix, which causes the breaking down of the polymer chains and hence the release of the entrapped drug within the polymer matrix[56-58]. Hence if a higher molecular weight variant is desired for prolonged release, the entire microfluidic system would need to be re-designed in order to develop compatible flow dynamics between both channels. All in all, the microfluidic process, while having potential for developing applications, faces intrinsic problems that hinders it from realizing the true potential of Janus particles. Coupled with the fact that microfluidics usually requires expensive machinery and infrastructure, the cost to benefit ratio (**Table 1**) is usually unfavorable using microfluidics[58].

Table 1 Cost/benefit analysis of Microfluidics vs Solvent Emulsion for Janus particle synthesis.

	Microfluidics	Solvent Emulsion
Advantages	<ul style="list-style-type: none"> Narrow size distribution 	<ul style="list-style-type: none"> Cost efficient <ul style="list-style-type: none"> Overhead Stirrer costs S\$700-S\$1000 Rapid synthesis time (1-2 hrs)

		<ul style="list-style-type: none"> • Large throughput (3-30g/hr) and potential for industrial scale-up • Simple operation (Over-head stirrer, beakers, polymer solutions and surfactants) • Suitable for a wide range of polymers
Disadvantages	<ul style="list-style-type: none"> • Costly <ul style="list-style-type: none"> ○ Microfluidic pump (\$\$1000-\$8000) ○ Microfluidic chips (material costs on top of fabrication customization costs) • Low-throughputs (~3 $\mu\text{L}/\text{min}$ polymer flow rate for Janus particle fabrication) • Complex operation(<i>in considerations</i>) • Limited polymer choices (i.e. polymers with high inherent viscosity are generally not preferred) 	<ul style="list-style-type: none"> • Relatively large size distribution
Considerations	<ul style="list-style-type: none"> • Choice of microfluidic chip material (PMMA, PDMS, TFE/PDD etc.)* • Fabrication of microchannels on microchips (etching processes i.e. micro-milling, chemical etching, photoresists used, UV cross-linking) • Angle of microchannels for polymer flow and aqueous phase flow • Optimized flow rates within channels (ratio of flow rate between polymer and aqueous phase) for droplet generation • Subsequent steps for solvent removal from formed micro-droplets • Other additional steps for particle solidification needed such as UV-crosslinking, polymerization etc 	<ul style="list-style-type: none"> • Paddle size • Height of paddle from base of beaker • Volume of polymer and aqueous solutions • Beaker size

*Polydimethylsiloxane (PDMS), tetrafluoroethylene-perfluorodimethyldioxole-fluorocopolymer(TFE/PDD)

2.3.6 Fabrication of Janus Particles via Solvent Emulsion

Supposition #3: *Despite current knowledge on the thermodynamic emulsion system of Janus particle formation, drug addition into polymeric Janus particles is still in question.*

The fabrication of microparticles via solvent emulsion is a well-known method due to its efficiency and scalability[59]. In this method, two immiscible phases, usually a water/oil mixture is emulsified using an over-head stirrer. For the fabrication of microparticles, the

oil phase is comprised of a volatile organic solvent in which the evaporation of this organic solvent during solvent emulsion would cause the precipitation of solid polymeric microparticles[60]. In this method, various parameters have to be considered, such as the initial polymer concentration, paddle length and surface area, paddle height within beaker, beaker volume, surfactant volume and concentration and the stirring speed[61]. As these parameters can be easily controlled and pre-determined with standardized paddles, beaker sizes, surfactant volume and concentrations, the independent parameters can be narrowed down to the stirring speed, polymers and encapsulants used and weight ratios. While the size distribution of microparticles fabricated in solvent evaporation is known to vary, this size variation does not necessarily limit the application of these particles. For example, Fan et al. demonstrated the fabrication of rapamycin-loaded PLGA and PCL polymeric particles via solvent emulsion to be within the size range of 50-100 μm for implantation within the anterior chamber of the eye (ACE). From this implantation, the controlled release of rapamycin showed a marked increase of 70% in islet volume growth between day 3 and day 20. In context, the mean volume within the ACE is approximately 200 μL and the allowable implantation volume usually falls within the range of tens of micro-liters. Despite this narrow allowable volume, the fabrication PLGA and PCL particles were still successfully implanted along with the islet cells[62]. Baek et al. showcased the effectiveness of multi-drug loaded PCL/PLLA particle blends in the treatment of Parkinson's Disease. The particles fabricated in this work was done via solvent emulsion with sizes ranging from 800 – 1400 μm . Despite the relatively large sizes and the large size variation, the standard deviations of the drug release from each time point was only $\pm 5\%$ [63].

In 2012, it was reported by Romanski et al. of the first ever report of the fabrication of Janus particles via the solvent emulsion method (**Figure 2.3.6-1**)[20]. PLGA/poly(caprolactone)(PCL) Janus particles were fabricated within a single-step solvent emulsion process that only required pre-determination of the initial polymer ratio prior to fabrication. Various size regions were demonstrated in this work using various levels of shearing such as manual shaking (low energy), rotor-stator homogenization (moderate energy) and high-pressure homogenization (high energy).

Figure 2.3.6-1 The formation of PLGA/PCL Janus particles via the solvent emulsion method; a first of its kind. From [20]

From the lenses of microfluidics, this method appeared to bridge the gap over the many challenges posed by microfluidics. Additionally, the use of two different types of polymers with large chemical differences was a breakthrough in giving rise to a versatile system capable of incorporating different polymers of different properties. The consequence of such a discovery had several important implications. One of which was that these polymeric Janus particles could be fabricated in a simple and cost-effective means, without the complexity that has traditionally been involved with in microfluidics. Potentially, Janus particles could be fabricated and functionalized with different capabilities such as drug delivery and diagnostics in a simple and cost-effective method.

From **Figure 2.3.6-1**, the formation of clear bifurcations within the particles was observed. Based on surface analysis of individual particles of PCL and PLGA respectively, the faces could be identified based on their textural differences. It was also deduced through the

images of the formed emulsions prior to solvent dissolution that the emulsion droplets had in fact formed not from nucleation but rather segregation of the polymer droplets sheared during solvent evaporation. This observation coupled with the high yield of Janus particles obtained implied theoretically that upon the shearing of the polymer solution, each emulsion droplets formed would have similar polymer weight ratios as with the initial polymer solution. The progressive formation from polymer phases into Janus particles spontaneously also indicated that the interaction of this triphasic system of two polymers and an aqueous phase formed a thermodynamic relationship.

In fact, various groups have made progress in the deduction of this thermodynamic process via solvent emulsion. The deduction of this thermodynamic process hinges on the work by Harkins[64]. Briefly, Harkins had studied the behavior of liquids and their spreading behavior on solids and immiscible liquids. Through his observations, he had postulated and derived the spreading coefficient, where if a liquid had a positive spreading coefficient, it would spread and if it had a negative coefficient, it would contract[65]. Using this, Torza and Mason established the thermodynamic relationship of the interfacial tensions of the polymers and aqueous phase to their respective spreading coefficients, which predicted particle morphologies[66]. Building upon their models, Zarzar et al. showcased the reconfigurable nature of complex emulsions via temperature and their relation to the interfacial tension between the droplets and the external phase.[66] Ge et al. also demonstrated the variation of Janus topology with immiscible aqueous phases in vegetable oils. By altering the mass fractions of the aqueous phases, various topologies corresponding to the size of the respective aqueous hemispheres were observed[67]. Despite the progress in this aspect of emulsion formation and Janus particle formation, the study of the behavior of polymer solutions and the addition of drugs was still in question.

Since the first report by Romanski et al.[20], little progress has been achieved with regards to Janus particles and drug delivery. Another report in 2016 by Zhou et al. had demonstrated the fabrication of a light-responsive polymeric Janus particle via this solvent emulsion method, but did not address issues pertaining to drug delivery[68]. Ironically, the

major clue pertaining to the reason behind this silence was subtly shown in the very first report.

Figure 2.3.6-2 The loss of anisotropy upon the addition of a drug to PLGA/PCL Janus particles fabricated via the solvent emulsion method. From [20]

While it was shown that PLGA/PCL Janus particles could be fabricated from this solvent emulsion method, the anisotropy of the Janus particles appeared to be significantly altered upon the introduction of the drug. Essentially, the classic Janus morphology comprised of its hemispheres was lost. From **Figure 2.3.6-2**, it can be seen that addition of the drug Grisofulvin into the polymer solution used in the emulsion process resulted in patchy particles that did not bear any resemblance to the traditional Janus morphology. This issue presents a conundrum, that in order to make these particles useful for drug delivery, drug encapsulation would be vital for this technology. Coupled with the fact that fabricating Janus particles with different biodegradable and biocompatible polymers has been challenging[69], this disruption of the anisotropy upon the addition of a drug becomes

counter-intuitive to the development of this technology as viable drug carriers for drug release.

Some years later in 2018, Fan et al. documented on the effects of drugs with different properties and its effect on Janus particle formation. It was shown that the net charge of drug molecules and polymer weight ratio were key determining factors in Janus particle formation[65]. While this work is a useful framework tool and the first report to establish a comprehensive understanding for drug incorporation in Janus particle formation, there were still various fundamental issues left unanswered.

Figure 2.3.6-3(a) Rapamycin loaded PLGA/PCL Janus particles at a 19:11 ratio and (b) 20:11 ratio. The small change in the weight ratio causes a complete change in morphology. From [65]

For example, it was shown that the addition of different drugs with known net charge would significantly affect the final particle morphology (core-shell or Janus). However, a slight change in the polymer weight ratio from 19:11 to 20:11 PLGA:PCL (**Figure 2.3.6-3**) completely altered the particle morphology. This implied that precise weight fractions needed to be ensured in order for Janus particle synthesis. While it was also shown that the charge of the drug would affect Janus particle formation, various questions such as the effect of different variations of the same drug on Janus particle formation were still unknown. This is also not accounting the addition of two drugs that would be co-encapsulated within the Janus particles. The compounded and combined effect of two drugs on Janus particle morphology has not been studied and poses additional challenges and questions as to successfully forming Janus particles. Additionally, it also introduces more

complexity as specific drug properties must first be made known before the success of Janus particle formation can be guaranteed. The existence of drugs in many modulations and potential permutations and combinations of drugs for Janus particle encapsulation would be impractical to fully document. Ideally, Janus particle formation via solvent emulsion should be independent of drug properties to reduce the amount of background work and complexity there would be to encapsulate them within Janus particles. Hence, there is a significant need to develop a further understanding into Janus particle formation that would allow not only Janus particle formation, but for Janus particles to be formed independent from the drugs and their properties within a facile synthesis method. This would help to realize the potential of polymeric Janus particles for various applications in drug delivery.

2.3.7 Synthesis of Janus Particles with Additional Functional and Structural Features

***Supposition 4:** Commonly employed methods to synthesize Janus particles with additional features such as magnetic and hollow-cores requires complicated methods and steps.*

Apart from traditional Janus particle fabrication, the creation of other derivatives such as magnetic Janus particles and hollow-core Janus particles have been employed. Magnetic Janus particles comprising of one polymeric and one in-organic face have been done previously. For example, Prasad et al. fabricated mono-disperse inorganic-organic Janus particle hybrids comprising of a perfluoropolyether face and a carbosilane magnetic face. In this work, side-by-side jetting of solution of the polyether and another of carbosilane was done simultaneously to initiate the droplet generation of this hybrid Janus particles. Upon the application of the magnetic field, the hybrid particles migrated to the corresponding magnetic pole with the inorganic magnetic component spear-heading the movement. Jeong et al. also demonstrated the use of microfluidics in the fabrication of fully polymeric based magnetic Janus particles that were done through the phase separation of both poly(methylmethacrylate) (PMMA) and poly (dimethylsiloxane). But despite these

advances, their fabrication rates were no more than 100 $\mu\text{L/hr}$. In some scenarios, additional cross-linking by UV irradiation was needed[70, 71].

In most of the previous microfluidic reports, almost all of them share an intrinsic problem: There is little flexibility in controlling the morphology of the resultant particles as the microfluidic droplet template and the subsequently polymerization are usually coupled within a single step. More recently Seiffert et al. had demonstrated a means to overcome this limitation. An additional third continuous phase of oil was channel inbetween the two converging channels of the microgels dyed with two different stains. In doing so, hollow Janus microgels could be fabricated. By modulating and regulating the flow rate of this inner oil channel, the inner morphology of the particle could be controlled. However, this method was only limited to about 100-500 $\mu\text{L/hr}$. Within these two microgel channels, each channel was stained with different fluorescent dyes for quick and easy identification of each phase. However, in the fluorescent images, it appeared that some cross-mixing had occurred while having a higher internal oil flow-rate to create a larger pore. This indicated a limit to the partitioning of the Janus faces via this method. Hence, there still exists significant gaps in addressing the synthesis issues in Janus particles, particularly addressing the complexity involved in fabricating these unique structures.

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Chapter 3

Experimental Methodology

This chapter will expound in detail the methods used for the fabrication of Janus particles, as well as various methods used in characterizing these particles. The first section details the materials used as well as the fabrication of these Janus particles with all three different polymeric combinations. The integration of drugs as well as other diagnostic materials will also be explained in this section. Other miscellaneous fabrication steps such as the fabrication of diagnostic and ceramic materials will also be discussed. Next, the characterization methods as well as methods used for in-vitro drug release studies will also be documented. Finally, the drug encapsulation as well as surface tension measurements will be documented in this section.

3.1 Overview of the Approach

To test the hypotheses proposed various aspects were targeted in the following chapters:

Chapter 4 & 5 focuses on the fabrication of polymeric Janus particles with different types of biocompatible and biodegradable polymers. This is to test the first hypothesis of the ability of polymeric combinations with relatively high polymer-to-polymer interfacial tensions to form Janus particles even with large weight ratio changes and addition of more than one drug. **Chapter 6** tests the second hypothesis of selective-drug encapsulation within these polymeric Janus particles. Briefly, two drugs of contrasting hydrophilicities were chosen. By studying the degree of hydrophilicity of each polymer based on contact angle measurements, the partitioning behavior of each drug can be determined and quantified from the selective encapsulation efficiency of each drug within each polymeric combination. **Chapter 7** tests the hypothesis that various existing technologies, such as single-step hollow particle[2] and sub-micron particle encapsulation[3] can be blended into the solvent emulsion for Janus superstructure synthesis.

3.2 Polymeric Janus Particle Synthesis and the Selection of Polymers

For the synthesis of polymeric Janus particles, polymers that have been well known for their biocompatibility and biodegradability were chosen. Most studies currently focus on single species PLGA/PLGA[4] Janus particles while the others have only focused on PLGA/PCL[5-7]. The basis of choosing various polymeric combinations was to create a library of potential polymeric combinations that could be used. Additionally, the polymer-polymer interaction during Janus particle could also be studied and measured to form an understanding on how the polymer-polymer interaction affects Janus particle formation. Given this limited selection, coupled with problematic drug encapsulation, the investigation of different polymeric combinations for drug delivery applications would considerably improve the versatility of the fabrication of Janus particles.

3.2.1 Poly (lactic-co-glycolic) Acid

In the current landscape, there is no shortage on research that has gone into poly(lactide-co-glycolide) (PLGA) as a viable and safe candidate as a drug carrier. To date, PLGA remains to be the most researched biodegradable polymer, owing to being FDA-approved as well as its superior operating characteristics such as being biocompatible and biodegradable[8]. In its chemical backbone, PLGA is comprised of lactide and glycolide monomers in its backbone (**Figure 3.2.1-1**). Depending on the ratio of lactide to glycolide component, different types of PLGA can be obtained, ranging from different forms of crystallinity, glass transition temperature and degree of hydrophilicity. The degradation of PLGA is typically bulk erosion and occurs from the hydrolysis of its ester bonds and varies depending on its lactide to glycolide ratio. PLGA degrades into its precursor components lactic acid and glycolic acid. These chemicals are naturally found from various metabolic pathways in the human body. Hence, the ability of the human body to metabolize these byproducts allows them to be removed safely from the body without surgical intervention[9].

Figure 3.2.1-1 Chemical Structure of PLGA. From [10]

The hydrolytical instability of various forms of PLGA causes such derivatives of PLGA to be degraded in a relatively short span of time i.e. 1 month. Microparticles made out of PLGA 50:50 exhibit low hydrolytical stability and its rapid degradation has been proven to be useful in short term applications that require rapid release[11-13]. In contrast, increasing the ratio of lactide to glycolide to 75:25 for example reduces the bulk erosion of

PLGA, creating longer term release. Therefore, PLGA is a versatile drug carrier that can be optimized for various applications through tunable drug release kinetics based on the polymer used[14-17]. For the work demonstrated here, PLGA 50:50 inherent viscosity (IV) 1.0dL/g (corresponding to 153 000 g/mol) was used due to its prevalence and effectiveness in various drug delivery studies, showing sustained release up to even 30 days[18, 19].

3.2.2 Poly (lactic) Acid

Poly (lactic acid) belongs to a group of aliphatic polyesters originating from α -hydroxy acids (**Figure 3.2.2-1**). Its basic building block, lactic acid, was first isolated in 1780 and commercially produced in 1881[20]. Lactide comes in two optically active isomers, D and L lactide and can undergo hydrolytic degradation through the hydrolysis of its ester bonds. PLA has also been known to be a generally regarded as safe (GRAS) polymer and hence has found itself in multiple biomedical applications[21]. Unlike PLGA, PLA is generally regarded to be more hydrolytically stable, resulting in a longer shelf-life and its longevity has been found

Figure 3.2.2-1 Chemical structure of PLA[15]

advantageous for applications where a prolonged residence of PLA would be beneficial[15]. For example, PLA has been a favored material in the use of biodegradable heart stents due to its relatively long degradation time of up to 12 months[22]. For practical applications, PLA is usually used with a mixture of its active D and L isomers. This is done through the addition of the opposite isomer into the bulk material, which causes a melting point depression (for ease of manufacturing) and is also found to cause an improvement in

mechanical properties[23]. In the fabrication of polymeric Janus particles, PDLLA IV 2.36 (corresponding MW ~460 000 g/mol) was used as this polymer was seen as a viable candidate due to prevalent use[24, 25]. Additionally, the relatively high IV of this PLLA would help to validate the fabrication of polymeric Janus particles through solvent emulsion, which has shown to be challenging through microfluidics[7].

3.2.3 Poly (methymethacrylate)

Figure 3.2.3-1 Chemical Structure of PMMA. From [26]

Poly (methyl methacrylate) (PMMA) has been an extensively used polymer both in the biomedical field as well as conventional day-to-day applications(**Figure 3.2.3-1**)[26]. Due to its high resistance to sunlight exposure, ease of manufacturability and optically clear properties[27, 28], PMMA was traditionally used in the windshields of fighter planes during the second World War[29]. Incidentally, the pilots whose eyes were pierced by flying shards of PMMA from the shattered windshields experienced significantly less infections than those who were injured by standard glass. This was the inception of the safe implantation of polymers in the human body and is the first known and documented biocompatible polymer. Since then, a wide variety of uses for PMMA as a biocompatible material has been devised[30, 31].

PMMA is a synthetic polymer made from methyl methacrylate monomers via radical or anionic polymerization. Within the confines of physiological conditions, PMMA remains inert, thereby making it an attractive material capable of implantation within the human body. PMMA has been used extensively as contact and intraocular lenses[32, 33] as well as having multiple uses in dental applications like bone cements and screw fixations in bones[34, 35]. Pertaining to drug delivery applications, PMMA with IV of 0.2 dL/g (~120 000 g/mol) have been used successfully for drug delivery applications and were hence chosen for this study[36].

3.2.4 Ethyl co-vinyl-acetate

Figure 3.2.4-1 Chemical structure of EVA. From [37]

Ethylene co-vinyl acetate (EVA) is a copolymer of ethylene and vinyl acetate monomers (**Figure 3.2.4-1**). While traditionally used in packaging and wire cable insulation, EVA eventually found its way into biomedical applications[37]. Owing to its favorable inflammatory characteristics and good release properties based on its low molecular weight[38], EVA was became increasingly popular in biomedical applications, especially for transdermal applications[39-42]. The monomers of ethylene and vinyl acetate are polymerized in solution to produce EVA copolymers, typically for copolymers containing 20-50 wt% of vinyl acetate[37]. Within the field of drug delivery, EVA with 40% vinyl acetate (MW ~120 000g/mol) was chosen primarily due to its use in drug delivery studies.

Lee et al. showcased the fabrication of multi-layered particles using PLLA, PLGA and EVA and subsequently showcased the drug release of these triple layered particles with DOX and PTX as model drugs[43, 44]. Therefore based on the drug delivery potential of these polymers, these polymers were used in this work.

3.3 Solvent Emulsion Fabrication of Janus Particles

The primary method for Janus particle synthesis is the solvent emulsion process. This process is attractive because of its simplicity and scalability. Simple laboratory equipment such as an over-head stirrer and a beaker containing the aqueous dispersive phase are needed. This method of solvent emulsion involves first dissolving the polymers in a suitable solvent, followed by adding the desired components into the polymer solution and is then emulsified in an aqueous surfactant before harvesting the particles. The added components to the polymer solution need not be dissolved in the polymers as they just need to be dispersed in the polymer solution in order to be encapsulated into the formed particles. Hence, this is an attractive method as a wide variety of small molecule drugs or other diagnostic materials as this in conjunction with the scalability of this method provides a means to scale up the production of these particles industrially to meet market demands.

The primary means of emulsion generation through solvent evaporation usually requires some form of shear force that breaks up the immiscible oil phase within the aqueous phase. Various independent variables such as the paddle surface area, paddle shaft, beaker size and volume and paddle height within beaker were standardized throughout these experiments. The paddle used is a 305mm length by 32mm diameter. Beakers were standardized to 250 mL beakers with a 3.5cm base radius. All volumes of surfactants used were standardized to 100mL, which translated to a height of 5cm within the beaker. The paddle height from the beaker was standardized at 1cm from base of beaker. From this baseline, the rounds per minute (RPM) were varied to obtain different sized particles, where the higher RPM translates to smaller sized particles due to the increase in shear forces. With these independent variables standardized, the variation of other variables such as surfactant concentration and RPM can be studied. Morphological analysis coupled with

other characterization methods such as interfacial tension data can be correlated empirically to explain various phenomena observed such as the formation of Janus core-shells with a reduction in surfactant concentration and the variation of particle sizes by varying the RPM of stirring.

3.3.1 Micro-particle Fabrication of Janus Particles

An oil/water solvent emulsion technique was used for the fabrication of Janus microparticles. A 10% (w/v) polymer solution was prepared for EVA/PLGA, PMMA/PLGA, EVA/PLLA and EVA/PCL and each was dissolved in three mL of DCM. For PMMA/PLGA, EVA/PLGA and EVA/PLLA, the weight ratios experimented with were in the ratio of 0.2 g : 0.1 g, 0.18 g : 0.12 g, 0.16 g : 0.14 g, 0.14 g : 0.16 g, 0.12 g : 0.18 g, and 0.1 g : 0.2g. All ratios were dissolved in three mL of DCM to obtain a 10% (w/v) polymer solution. After polymer dissolution, the polymer solution was poured and emulsified in a PVA solution (100 mL, 2.5% w/v). An overhead stirrer (Calframo BDC1850-220) was used to form the emulsion at 400 rpm for two hrs at room temperature (25 °C). The formed particles were centrifuged, rinsed three times with deionized water and lyophilized overnight.

3.4 Drug Encapsulation within Polymeric Janus Particles

As mentioned in **Chapter 2**, the ability to load drugs within these Janus particles via solvent emulsion has been shown to be challenging. Despite the economical and scalable nature of the solvent emulsion process, the addition of drugs within these Janus particles is still in question. In this experimental design, two drugs of contrasting solubilities were chosen. Theoretically, it had been postulated by various groups that the bicompartamental structure of the Janus particle could be advantageous in encapsulating two drugs of disparate solubilities[45, 46]. However up till now, no study had been able to demonstrate the selective encapsulation into each face of the Janus particles via solvent emulsion. DOX and PTX were chosen as the model small-molecule drugs for encapsulation within the various Janus particle polymeric combinations. These two drugs made excellent candidates

for a variety of reasons. Firstly, both DOX (logP 0.71) and PTX (logP 3.96) have contrasting solubilities[47]. The logP of the drug describes its partitioning ratio in an octanol/water mixture. The more hydrophobic the drug, the greater its partitioning within the octanol phase and hence the greater the logP ratio. Hence, the higher the logP, the more hydrophobic the drug is. Secondly, both DOX and PTX are well-known anti-cancer drugs that have substantial evidence for their synergistic effects[47, 48]. On top of this, there is also substantial evidence that demonstrates how different polymers can produce different release profiles[44]. This in combination with dual-drug loading will therefore help us to not only determine the encapsulation behavior of the drugs within the Janus polymeric combinations, but to also ascertain any differences or characteristics in their release profiles. For example, a sequential release profile of DOX followed by PTX from PMMA/PLGA particles can provide safer and more efficacious treatment of metastatic breast cancer.

3.4.1 Microparticle Fabrication of Drug-Loaded Janus Particles

20 mg of Dox was added into PMMA/PLGA, EVA/PLGA and EVA/PLLA polymer solutions and left to dissolve and disperse for 30 mins. For dual-drug loaded Janus particles, Dox (20 mg) and PTX (6 mg) were dissolved into a 10% (w/v) polymer solutions of EVA/PLGA, EVA/PLLA and PMMA/PLGA for 30 mins. Subsequently, all three polymer solutions were emulsified in a PVA aqueous solution (100 mL, 2.5% w/v) with an overhead stirrer (Calframo BDC1850-220) for two hrs at room temperature (25 °C). The theoretical drug loading for Dox and PTX was set to 66.6% and 2% respectively. The precipitated particles were centrifuged and rinsed for three cycles and lyophilized overnight.

3.4.1 Selective Dissolution

As the partitioning behavior of both DOX and PTX needs to be determined, each face of the Janus particle should ideally be dissolved selectively, followed by the addition of another solvent to dissolve the drugs found in that face of the Janus particle. Unlike standard single polymer species particles, the integrity of the opposing face needs to be maintained and undissolved. Hence, the use of a selective solvent would need to be employed In the

fabrication of these Janus particles of different polymeric combination, a simple method was derived to further study and confirm that the formation of these Janus particles was indeed two hemispheric portions meeting at a bifurcation junction within the Janus particle.

Selective dissolution of the three respective Janus particle combinations was done by choosing a solvent that dissolves one polymer and not the other in a particular Janus particle polymer combination. For the three respective Janus particle combinations of PMMA/PLGA, EVA/PLGA and EVA/PLLA, DMSO, cyclohexane and THF were used respectively. In the PMMA/PLGA combination, DMSO selectively dissolves PLGA and not PMMA. While cyclohexane and THF dissolves EVA in the EVA/PLGA and EVA/PLLA combination respectively. The remaining undissolved components were hence PLGA, PLGA and PLLA in the PMMA/PLGA, EVA/PLGA and EVA/PLLA combinations respectively. First, particles of each combination were placed into a glass vial. The respective selective solvents were then added to each of the respective polymeric combinations and left to dissolve for one hr. After one hr, the remaining components were carefully removed with a syringe and were placed onto a carbon tape. The carbon tape containing the remaining particle components were placed into a vacuum oven and left overnight to remove any excess solvents. After solvent removal, the particles were then ready for characterization.

3.5 Drug Release Studies and Mathematical Model Quantification

The goal of drug delivery studies is to model the release behavior of these drug-loaded particles in physiological conditions. While it is ideal to conduct and study the effectiveness of these particles in-vivo, the release behavior of these particles in-vitro plays a vital role. This is to study its behavior and to perform any tweaks or adjustments to the parameters before progressing to in-vivo studies. In the drug release studies performed in this work, the particles were suspended in a release media comprised of phosphate-buffered solution (PBS) along with the addition of surfactants such as Tween 80. The main role of PBS is to mimic the buffering properties of physiological conditions within the human body while Tween 80 acts as a model for amphiphilic molecules within the human body,

such as enzymes and proteins[49]. The particles are usually portioned and placed within this release media that is stored in a shaking incubator at 37°C. This is to mimic the temperature of the human body while also mimicking movements within the human body.

The use of mathematical models to quantify drug release provides useful information into the governing mass transport and chemical processes involved in the release of drugs. With this information, the optimization of the drug carrier can be carried out for optimal performance in a systematic approach.

3.6 The Higuchi Model for Drug Release

The Higuchi model has been the most popular mathematical model used in the study of drug release kinetics. This is due to the great advancement of the Higuchi model in representing the mathematical relationship between particulates dispersed in homogeneous matrices while suspended in a diffusing medium. While there are other models such as the zero and first order kinetics, they generally do not model realistically to real-life drug release kinetics. For example, zero order kinetics assumes a steady state release where the rate of release is constant and independent of drug concentration within the matrix. This is usually unrealistic since the diffusion nature of drug release is governed by both matrix effects and the concentration of drug[50]. First order kinetics are also difficult to define as first-order states that the change in concentration with respect to change in time is only dependent on the concentration, without factoring in matrix effects.

In 1962, Higuchi proposed an improved equation for a matrix saturated with a drug, where Q is the amount of release drug at time t quantified by the unit area, C_0 is the concentration of the diffusing liquid within the polymer matrix, ε is the porosity of the matrix, t is time and r is the capillary tortuosity factor:

$$Q = \sqrt{2C_0\varepsilon \frac{Dt}{\pi}}$$
Eq. 1

Higuchi further went on to conclude that the amount of drug release is proportion to the square root of time, simplifying the equation to, where K_H is the release constant of Higuchi:

$$Q = K_H\sqrt{t}$$
Eq. 2

By applying this improved relation, the release kinetics can be simplified to a linear function, where the gradient of the linear graph can be quantified as the release constant while also calculating the correlation function. Despite this, there are several critical assumptions for Higuchi's model that have to be met in order to accurately model drug release with this model:

1. The initial concentration of drug within the matrix is significantly higher than the solubility of the drug.
2. The diffusion is unidirectional, indicating that the changes at the boundary of the matrix to the external phase are negligible.
3. The drug carrier is significantly larger than the size of the drug molecules
4. The polymer matrix swells negligibly.
5. The drug diffusivity remains constant.
6. Perfect sink conditions are maintained throughout the release study[51].

Up till now, there has been no report that conducted the modelling of the drug release from these polymeric Janus particles and hence this work serves to fill in this gap through detailed theoretical analysis of the drugs for the first time.

3.6.1 Ultraviolet Visible Spectroscopy (UV-Vis)

UV-Vis is an attractive method for determining the optical properties of both solids and liquids. The ability of UV-Vis to analyze the analyte concentration of drugs is a useful method, particularly due to the simplicity of its application. This technique measures the absorption of light across the desired range by placing a dispersed sample in a cuvette in between the light source and detector. By using the Beer-Lambert law the distance of the light path length and the absorption coefficient of the desired analyte, which is known through the absorption wavelength, the concentration of the compound can be determined through the amount of light absorbed at that wavelength.

Due to the good solubility of Dox in H₂O, the concentration of Dox can be easily measured through UV-vis. For the measurement of Dox samples, 100 µL of sample solution was extracted and placed in a 96-well Corning flat-bottom culture plate. The concentration of this Dox solution was measured by placing the culture plate into a Tecan infinite M200 plate reader with the absorbance wavelength at 480 nm.

3.6.2 Calculation and Measurement of Calibration Curves of Drugs

To quantify drug release, calibration curves of each respective drug is required to form a mathematical relationship between the concentration of the drug to the magnitude of the absorption readings from HPLC and UV-Vis. To generate this mathematical relationship, known concentrations of each drug are aliquoted into ascending concentrations and then characterized.

In each of the respective drugs (Dox, PTX, Rapa and Gly), the dissolution medium chosen for the calibration curve would be the respective mobile phase used in its analysis for HPLC or in absorbance analysis. Nine concentrations were used to derive the calibration curve (700, 300, 100, 70, 30, 10, 7, 3 and 1 µg/mL) for each of the respective drugs. Dissolution medium for PTX, Rapa and Gly were 70 ACN: 30 H₂O, 80 MeOH: 20 H₂O and 60 ACN: 40 H₂O respectively.

3.6.3 Drug Encapsulation Efficiency (EE%) for Dox

For the EE% of Dox, 3 mg of particles from each drug-loaded Janus particle combination (EVA/PLGA, EVA/PLLA and PMMA/PLGA) were measured and 2 mL of DCM was added to fully dissolve the particles. 2 mL of DI H₂O was added to the DCM-suspended Dox and vortexed for 30 s to allow the dissolution of Dox into the H₂O. 100 µL of Dox solution was removed and placed into a 96-well Corning flat-bottom culture plate. The concentration of this Dox solution was measured by placing the culture plate into a Tecan infinite M200 plate reader with excitation and emission wavelengths at 488 nm. This absorbance reading was correlated into the equation of the Dox calibration curve obtained and the EE% was calculated with the following formula:

$$EE\% = \frac{\text{Actual Drug Loading}}{\text{Theoretical Drug Loading}} \times 100 \quad \text{Eq. 3}$$

To account for matrix effects, the amount of drug measured in the drug-loaded Janus particles was portioned in another sample with the same weight of polymer used. The measured drug from these samples was also found to be ~99% similar and hence validated the amount of measured drug within the particles.

3.6.4 Calibration Curves for Doxorubicin & Paclitaxel

In order to be able to quantify the amount of drugs being encapsulated within these Janus particles, calibration curves of both Doxorubicin and Paclitaxel were done. The range of concentrations for the calibration curves were done from 1 mg/mL to 7 µg/mL for both drugs. In both graphs of Doxorubicin and Paclitaxel, the R values were >0.999 (**Figure 3.6.4-1**). This indicates a strong correlation for the concentration equation graph for each of these respective drugs and were used in the calculation for drug concentration in the following section.

Figure 3.6.4-1 Calibration curves for (a) PTX and (b) Dox

3.6.5 Drug EE% for Rapamycin, Paclitaxel and Glybenclamide

3 mg of particles containing either Rapamycin, Paclitaxel or Glybenclamide were weighed out and dissolved with 2 mL of DCM to fully dissolve the drug carrier and release the drugs into the DCM. 1 mL of methanol was added to precipitate out the polymers and left to vortex for 30 s. Subsequently, the resulting solution was centrifuged and the supernatant was extracted and left to vacuum dry overnight to remove the DCM solvent and leave behind the drug precipitates. Subsequently, the respective mobile phase for the drug to be analyzed was added and vortexed for 2 mins to allow complete dissolution of the drugs into the mobile phase solution. This solution was then vialled for HPLC. For samples that contain polymers other than PLGA, the methanol addition would not precipitate out the polymers. Instead, the resulting solution was vacuum dried immediately and the respective mobile phase was added and to dissolve the drug and then vialled for HPLC.

3.7 Fabrication of Super-Paramagnetic Iron Oxide Nanoparticles (SPIONs)

The selection of SPIONs as the diagnostic material of choice was due to the studies on the use of SPIONs in multiple applications such as hyperthermia-mediated treatment and

magnetic-control capability. The encapsulation of SPIONs within this work adopted technology from Poh et al.[52], where oleic-acid functionalized SPIONs are dispersed within the polymer solutions prior to emulsion. Based on Poh et al.'s findings, the SPIONs were found to be entrapped within the interstitial network of the polymers. Hence, it is predicted that they would also function as plasticizers and could potentially increase the rate of drug release due to the loosening of the polymer chains[53]. Furthermore, these SPIONs have been shown to be safe to the human body throughout its duration of use and they are easily discharged from the body through normal metabolic functions. In addition to that, the SPIONs are also easy to fabricate and can be done so in a short amount of time.

Oleic acid-capped SPIONs were fabricated with the technique reported by Cahyana et.al[54]. Briefly, FeCl_2 (0.30 g) and FeCl_3 (0.82 g) were dissolved in 20 mL of deoxygenated deionized water. This solution was then heated at 80 °C for three mins before one mL of ammonium hydroxide was added and heated for another 30 mins. Subsequently, five mL of oleic acid was added to the black precipitates and was heated at 95 °C for 90 mins. After cooling, the mixture was centrifuged and rinsed three times before storing in DCM.

3.8 Fabrication of SPION-loaded Janus Particles

10% polymer solutions of PMMA/PLGA, EVA/PLGA and EVA/PLLA were first prepared with weight ratios 0.14 g : 0.16 g, 0.12 g : 0.18 g and 0.14 g : 0.16 g respectively and dissolved in three mL of DCM. After being left to dissolve for 30 mins, 0.5 mL of the SPIONs solution (1 mg/mL) were added to the 10% polymer solution. After being shaken for 30 mins, the SPION + polymer solution were emulsified in a PVA aqueous solution (100 mL, 2.5% w/v) with an overhead stirrer (Calframo BDC1850-220) for two hrs at room temperature (25 °C). The precipitated particles were centrifuged and rinsed for three cycles and lyophilized overnight.

3.9 Particle Characterization

3.9.1 Particle Morphology and Cross-Sectional Analysis via a Scanning Electron Microscope (SEM)

SEM is a common method used in morphological as well as compositional analysis. The main concept of the SEM is using high energy electron beams as a visual ‘paintbrush’ for objects and surface features within the micron size range. This feature works on the principle of the wavelength of the electron, which is a function of the acceleration voltage used in the SEM. As the wavelength of the electron is inversely proportional to the acceleration voltage of the electron, a higher electron voltage would naturally yield images of higher resolution. However, the consequence of using a higher acceleration voltage would be generation of more kinetic energy when the primary electron beam hits and interacts with the sample. Due to the polymeric nature of most samples used in this thesis, a high acceleration voltage would not be suitable as the samples would start to melt. Hence, the optimal acceleration voltage was set at five kV. Within the chamber of the SEM, a primary electron beam is first emitted from the top by an electron gun and is focused through a series of electromagnetic condenser lenses onto the sample. As this primary electron beam interacts with the sample, multiple signals are generated and can be utilized to analyze different aspects of the sample. This is also known as the interaction volume. Most commonly, the secondary electron signal is used primarily for imaging purposes as it is collected through a photo-multiplier and projected as a visual image of the sample. Due to the low penetration depth of the secondary electrons, only surface morphologies can be observed in the SEM. Other signals of interest generated in the interaction volume which are useful for this thesis are the k-alpha x-rays generated within the interaction volume. As each element in the periodic table has a unique atomic structure, the ionization of elements from the primary electron beam and the replacement of high energy electrons to these vacancies from the respective orbitals causes the generation of elementally unique k-alpha x-rays. These x-rays can be collected and used for elemental analysis of samples. This is also known as Energy Dispersive X-ray Spectroscopy (EDX).

Particles were imaged with a Scanning Electron Microscope (SEM, JEOL JSM 6360A) at five kV for surface morphology, cross-sectional and selective dissolution studies. For surface morphology analysis, particles were harvested from the lyophilization steps and a small amount was removed with a spatula and placed on a carbon tape. Subsequently, the carbon tape containing the particles were mounted onto the metallic specimen stubs and coated with gold using a gold sputter (SPI-module) for 45 seconds in a vacuum environment. The particles were then removed from the sputter and were placed into the SEM for analysis.

For cross-sectional analysis, particles were first mounted onto a carbon tape. The carbon tape containing the particles were subsequently flash-frozen in a container of liquid-nitrogen for 60 seconds before being removed. The frozen particles were then incised and cross-sectioned with a surgical blade and the particles were then imaged under the SEM.

For analysis using EDX, carbon-coating in place of gold coating was used. A fresh strand of carbon string was attached onto both electrodes of the carbon coater (SPI-module). After samples were mounted onto a carbon tape fixated on the sample stub, the stub was placed within the carbon coater and pumped down to vacuum condition. The carbon filament was heated up to 3V for 10 s for degassing purposes before being brought back down to 0V. After degassing, the filament was brought to 7.5 V and engaged until filament was severed. Samples were then removed and analyzed with the EDX module on the SEM.

3.9.2 Particle Size Analysis

The particle size was measured through the obtained SEM images via the ImageJ software. 3 different batches of polymers from the same fabrication batch were imaged and at least 10 particles were randomly measured to obtain the size averages and standard deviation.

3.10 Reverse-Phase High Performance Liquid Chromatography (HPLC)

Figure 3.9.2-1 Schematic of the working principle of an HPLC. Image obtained from [1]

In the analysis of the concentration of drug molecules within the field of drug delivery, HPLC is a common technique used due to its low detection limit as well as the ability to separate and analyze analytes released within the release media. In reverse-phase HPLC, the mobile phase is the polar compound while the stationary phase is the non-polar or hydrophobic components. As the stationary column is modified with compounds to make it non-polar, lipophilic compounds will have affinity to the hydrophobic ligands on the column and interact with them within the column for a certain amount of time as the mobile phase passes through (**Figure 3.9.2-1**).

In this technique, a small sample volume from the drug-containing samples are extracted and injected into the flow system. As the mobile phase flows through the system and through the stationary column, the mobile phase carries along the samples solution. Upon entering the column, the substances within the sample solution interact with the ligands within the column and are retained for a certain amount of time before being eluting out. By using a consistent flow rate and formulation of mobile phase, a calibration curve relating the retention time, absorbance and concentration of the drug can be obtained. This calibration curve can then be used as a form of measurement to the concentration of the drug release studies. As the standards created for the drugs involve the initial and pure form of the drug, any particular variation in the retention time during the study can also give clues about the degradation behavior and changes within the drug. This insight can be used

to further optimize the drug release studies for accurate results. In this study, several drugs were used and analyzed with HPLC.

For PTX, the concentration of PTX for all samples was determined using HPLC with an Agilent 1100 HPLC system using a Zorbax SB-C18, 5 μ m, 4.6 x 250 mm column. Column temperature was set at room temperature (25 °C) and the retention time for PTX was between 3.9-4.1 mins. All concentrations of PTX was measured at 227 nm. The mobile phase was a 70% ACN 30% water mixture with a flow rate of 1.0 ml/min.

For Glybenclamide, the concentration of Glybenclamide was analyzed with HPLC with an Agilent 1100 HPLC system using a Zorbax SB-C18, 5 μ m, 4.6 x 250 mm column. Column temperature was set at room temperature (25 °C) and the retention time for Glybenclamide was between 5.8-6.0 mins. All concentrations of Glybenclamide was measured at 237 nm. The mobile phase used was a 60%ACN 30% H₂O mixture with a flow rate of 1.0 mL/min.

For Rapamycin, its concentrations were analyzed with an Agilent 1100 HPLC system with an Agilent Poroshell 120 column, 2.7 μ m, 2.1 x 150 mm. Column temperature was set to room temperature (25 °C) and the retention time for Rapamycin was between 8.9-9.1 mins. All concentrations were measured at 278 nm. The mobile phase used was an 80% Methanol 20% H₂O mixture with a flow rate of 0.7 mL/min.

3.11 Confocal Laser Scanning Microscopy (CLSM)

In recent times, the popularity of CLSM has grown particularly in the field of cell studies. This is mostly due to the ease of being able to obtain high quality images of both fixed and living cell specimens. One major advantage of the use of the CLSM is the ability to eliminate the out-of-focus light or glare that are out of the focal plane. This is because the out-of-focus signals are not confocal with the small pin-hole aperture preceding the detector and hence do not reach the detector. This allows for precision imaging of the specific plane and even allows for 3D imagery through raster scanning of multiple planes.

In this work, CLSM was used for the detection of the drug distribution of fluorescent molecules encapsulated within the Janus particles to mimic the encapsulation behavior of the drugs. Rhodamine B (RhB) and PTX chemically tagged with dansyl chloride (DPTX) were encapsulated into these Janus particles and the resulting particles were analyzed with CLSM. The excitation/emission peaks for RhB and DPTX was 540/625 nm and 372/518 nm respectively. As both RhB and DPTX have contrasting solubilities, the mapping of drug loading of DOX and PTX can be visualized within the particles via CLSM.

3.12 Measurements and Calculations of Interfacial Tensions Between Polymers and Aqueous Surfactants

In order to successfully predict Janus particle morphology based on Harkin's equation and the prediction extension made by Torza and Mason[53], the respective interfacial tensions between the polymers used in Janus particle fabrication and that of the surfactant have to be measured and calculated. At the start, the polymer precursors are homogeneously dissolved within a solvent, which ensures that for a given unit volume of the polymer-containing solvent, the original ratio of polymers used would be constant within that unit volume. Upon the emulsification process, the shear forces from the emulsion system breaks up the polymer solution into tiny coacervate droplets and the surfactants within the surfactant solution stabilizes these fluid polymer droplets. It is therefore also assumed that each polymer droplet would contain the same ratio as initially measured and used during polymer dissolution. Because Janus particle formation is significantly thermodynamically driven based on the equations and predictions from Harkin, it is therefore also assumed that within each emulsified droplet, all the droplets would fulfill these thermodynamic requirements as the initial measured ratios were found to have formed Janus particles. Hence as the solvent evaporates, the precipitated particle would form a Janus particle.

At the instance upon emulsification of the polymer solution within the surfactant solution, there is still a large amount of solvents within each droplet such that the phases can migrate

and diffuse throughout the dimensions of the droplet. Because of this, 3 surfaces of contact become of interest when calculating and predicting Janus particle formation. From **Figure 3.9.2-1**, the three surfaces are γ_{ab} , γ_{bc} and γ_{ac} , where a and c are the two respective polymers used and b is the aqueous phase. Hence, γ_{ab} is the interfacial tension between polymer a and the surfactant solution b , γ_{bc} the interfacial tension between polymer c and surfactant solution b and γ_{ac} the interfacial tension between polymers a and c .

Figure 3.9.2-1 Schematic of a single polymer coacervate droplet upon emulsion.

3.12.1 Calculation of Polymer-to-Aqueous Phase Interfacial Tension

As the final particle morphology indicates the formation of two hemispheres, with each face being joined at an interface, it is therefore also assumed that the Janus particle in essence is comprised of two droplets of polymers a and b fused at the interface. To calculate this, the pendant-drop method is used.

The discovery and evolution of the Pendant-drop method is by no means trivial. The theory behind the pendant drop method hinges on the balance between the surface tension of the droplet, which strives to minimize its surface tension by becoming spherical, and the force of gravity, which elongates and deforms the droplet. According to Pascal's Law of hydrostatic pressure, there exists a pressure difference between the inside and outside of

the droplet. Hence, the Young-Laplace quantifies with the principle radii of curvature of the droplet R_i in relation to this pressure change (R_1 is the radius of curvature of the horizontal axis of the drop while R_2 is the radius of curvature from the vertical axis, Δp the change in pressure and σ is the surface tension)[55]:

$$\Delta p = \sigma \left(\frac{1}{R_2} + \frac{1}{R_1} \right) \quad \text{Eq. 4}$$

In the 1880s, Bashforth and Adams had further extended the Young-Laplace equation to take into account the elongation of the droplet due to gravitational forces and correlated the interfacial tension of the droplet and external environment to the shape of the droplet. At the vertex of the droplet, the radii of curvature are all equal ($R_1 = R_2 = R$), which makes it a good reference point for the radius of origin. Unlike a sphere, the dimensional relations of the 3-axes in its 3 dimensional conformation are not uniform. Hence, there are 3 first-order differential equations with 3 boundary values that can quantify for each respective axis at different origin points based on the radius of curvature for both the horizontal and vertical axis[56].

Coupled with today's imaging technology, present day contact angle measuring equipment function by calculating the shape of the imaged droplets through complex algorithms to derive their respective interfacial tensions[57].

For the work used in this thesis, the measurements were done on an OCA 15Pro, Dataphysics contact angle measurement apparatus. A PVA solution of known concentration was placed into a quartz cuvette. Polymer solutions were prepared and were drawn into 1 mL syringe needles and placed into the syringe holder perpendicular to the light and camera direction. The ideal injection volume was determined through trial and error to obtain the most accurate and feasible droplet. 20 droplets for each polymer solution was carried out and the obtained values were obtained through the profiling of the droplet shape by the DataPhysics OCA software.

3.12.2 Calculation of Polymer-to-Polymer Interfacial Tension

Unlike calculating the interfacial tensions of the polymers to aqueous phase, the calculation of polymer-to-polymer interfacial tensions required a different approach. For the polymer to aqueous phase interaction the polymer solution is immiscible to the aqueous phase. For the polymers however, both polymers are dissolved in the same solvent and hence the pendant droplet method cannot be used. To resolve this issue, the calculation of polymer interfacial tension was hypothesized and demonstrated by Cao et. al[5].

Based on Young's equation[58], there is a relationship between the contact angle, surface tension of the liquid, interfacial tension between liquid and solid and the surface free energy of the solid (**Figure 3.12.2-1**). To calculate the surface free energy, the unknown interfacial tension σ_{sl} has to be derived. This can be done through Fowkes method, where σ_{sl} can be substituted with (σ_s is the surface energy of the solid, σ_l the surface energy of the liquid and θ the contact angle):

$$\sigma_{sl} = \sigma_s - \sigma_l \cdot \cos \theta \quad \text{Eq. 5}$$

Figure 3.12.2-1 Schematic representation of Young's equation of the interaction between the liquid and solid interface. From[59]

By substituting **Eq. 5** to the Owens-Wendt-Kaelble equation, the following relationship can be derived (σ_s^D and σ_l^D are the disperse components of the solid and liquid respectively, σ_s^P and σ_l^P are the polar components of the solid and liquid respectively):

$$\sigma_l(1 + \cos \theta) = 2 \left(\sqrt{\sigma_s^D \cdot \sigma_l^D} + \sqrt{\sigma_s^P \cdot \sigma_l^P} \right) \quad \text{Eq. 6}$$

From **Eq. 6**, there exists two sets of unknowns; the polar and dispersive components of a liquid (σ_l^P & σ_l^D) and the polar and dispersive components of a solid (σ_s^P & σ_s^D). In this setting, the solid would be the polymers used in Janus particle fabrication while the liquids would be liquids of known polar and disperse parts. By using two probe liquids with known dispersive (σ_l^D) and polar (σ_l^P) components, their values can be substituted into **Eq. 6**. Their contact angles were also recorded and substituted into **Eq. 6**. Through this, the polar and dispersive components of the polymers (σ_s^P & σ_s^D) could be obtained and their surface energies calculated through the summation of their dispersive and polar components.

For the measurements made in this thesis, polymer films (PLGA, PLLA, PMMA and EVA) used in the fabrication of Janus particles were drop-casted onto glass slides and left to dry

in the open to form polymer films. Two liquids, ethanol and H₂O were used in the contact angle experiments on an OCA 15Pro with Dataphysics OCA software. Droplets were formed on the surface of the polymers through optimized dispersion volumes. 20 drops were made on each polymer film and its values were averaged. For contact angles too small to quantify, a value of 5° was used[60].

3.13 Materials

Poly (D-L-lactic acid) (PLLA) (intrinsic viscosity (IV): 2.38 dL/g, Bio. Invigor), Poly (vinyl alcohol) (PVA, mol wt 30 000 – 70 000, Sigma), poly(ethylene-co-vinyl acetate) 40% wt vinyl acetate (EVA, Sigma)), Poly(DL-lactide-co-glycolide) (50:50, IV: 0.8-1.2 dL/g, Purasorb)), poly(methyl methacrylate) weight-average molecular weight (M_w) 120 000 (PMMA, Sigma), poly(ethylene-alt-maleic anhydride) (PEMA, Sigma) were used without further purification. Rapamycin and Glybenclamide were purchased from LC Laboratories. Tween® 80, calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$), ammonium bicarbonate (NH_4HCO_3), ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), HPLC Grade Methanol, Rhodamine B, Coumarin 6 were purchased from Sigma. Doxorubicin and Paclitaxel were purchased from Xingchen Chempharm Co. Ltd. Dichloromethane (DCM), cyclohexane, HPLC grade Acetonitrile (ACN) and Tetrahydrofuran (THF) and Dimethyl Sulfoxide (DMSO) were purchased from Tedia.

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Chapter 4

Polymeric Janus Particle Fabrication via Solvent Emulsion & the Underlying Mechanism of Formation

This chapter highlights the key important initial findings of the fabrication of Janus particles via the solvent emulsion method. Different polymeric combinations of Janus particles, including those which were previously deemed unsuitable in literature for Janus particle formation, were carried out and showcased in this section. The effect of polymer weight ratio as well as increasing stirring speeds was explored in this section to determine the most optimal parameters for Janus particle fabrication. While the synthesis is simple, the underlying mechanics of this formation is a complex process that involves a balance of multiple forces and factors. This chapter also aims to establish how these factors are influenced and how the polymer selection is an important step in Janus particle fabrication. The role of kinetic factors involved in Janus particle formation are also described and observed in the fabrication of Janus particles. Furthermore, the results obtained in this chapter would allow further exploitation of this fabrication method to further build upon basic synthesis of blank Janus particles into useful particles loaded with drugs.

*This section published substantially as [1]

4.1 Fabrication of Polymeric Janus Particles*

***Hypothesis #1:** Polymers with higher polymer-to-polymer interfacial tension are more independent to weight ratio changes and encapsulant addition in the formation of Janus particles.*

This section will investigate the effect of different processing parameters on the formation of Janus particles to address to **Hypothesis #1**. The parameters include polymer mass ratios and the addition of drugs. Based on these results, more insight can be obtained to determine how these parameters can affect the final particle morphology. Hence, it would allow for tunability and better understanding of formation of these Janus Particles via a single-step solvent emulsion process. Additionally, synthesis of Janus particles with different biodegradable and biocompatible polymers were investigated in the fabrication of polymeric Janus particles. While the synthesis of Janus particles through solvent evaporation has previously been done, there is limited exploration into the use of different polymers, particularly for polymers suitable for use in biomedical applications. This lack of data was primarily due to the unsuccessful attempts by previous researchers to do so[2]. To investigate this, various polymeric combinations of biodegradable and biocompatible polymers were used and their formation mechanisms were investigated through interfacial tension data.

4.1.1 Establishing a Reference Point of Fabrication Parameters*

A first reference set of Janus particles were made using PLGA and EVA (**Figure 4.1.1-1**) to establish a baseline on the particle morphology and size as described by the independent parameters in **Section 3.3**. The stirring speed was set at 400 RPM and emulsified for two hours. The polymer weight ratio used was 0.18g: 0.12g of PLGA:EVA and set at a concentration in DCM at 10%. Based on these results, the size of the particles obtained are approximately 200 μm . From a close inspection of the particle morphology from the SEM, the particles formed appear to have two faces, joined together at the bifurcation junction between them. To determine if in fact the Janus particles are formed by two different

polymers, selective dissolution was carried out with these particles. Cyclohexane was used to selectively dissolve EVA and the resulting structure can be seen from the SEM. The remaining structure from the dissolution was hence inferred to be PLGA and concluded that the particle is formed by two different polymers. This sets the initial reference point for the fabrication parameters used.

Figure 4.1.1-1 (a) An EVA/PLGA Janus particle subjected to selective dissolution by cyclohexane, leaving behind a PLGA half-face, (b) Neat EVA/PLGA Janus particles after fabrication

4.2 Mechanism of Janus Particle Formation

Before delving more into the experimental data, the mechanism of Janus particle formation would be described to further substantiate the preceding results. In the solvent emulsion system, the polymer solution is broken up into many tiny droplets that are stabilized by the surfactants within the aqueous phase. Within each coacervate polymer droplet in the aqueous phase, interfacial tensions exist between the respective polymers and the aqueous phase as well as the interfacial tension between both polymers where both polymer faces meet (schematic shown in **Figure 3.9.2-1**).

The mechanism of Janus particle formation in solvent emulsion has a few assumptions:

1. Emulsion system has to be comprised of three phases; aqueous phase and both polymer phases.
2. The polymers used have to be immiscible beyond cloud point.
3. Solvent of choice for both polymers has to be the same.
- 4.

More importantly however, these interfacial tensions determine the spreading coefficients between the phases and determines the final particle morphology. According to Harkin, the spreading coefficient equation is as follows (S is the spreading coefficient, γ_{jk} , γ_{ik} and γ_{ij} are the respective interfacial tensions between the three phases) :

$$S_i = \gamma_{jk} - (\gamma_{ij} + \gamma_{ik}) \quad \text{Eq. 7}$$

From this spreading coefficient, three possible combinations can be derived (**Figure 4.1.1-1**):

Figure 4.1.1-1 Schematic of the prediction model of final particle morphology[3]

$$\text{Full Engulfment: } S_1 < 0, S_2 < 0, S_3 > 0 \text{ or } S_1 > 0, S_2 < 0, S_3 < 0 \quad \text{Eq. 8}$$

$$\text{Partial Engulfment: } S_1 < 0, S_2 < 0, S_3 < 0 \quad \text{Eq. 9}$$

$$\text{No Engulfment: } S_1 < 0, S_2 > 0, S_3 < 0 \quad \text{Eq. 10}$$

(S_1 & S_3 are the spreading coefficients of the polymer phases and S_2 for the aqueous phase)

The spreading coefficient is an empirical measurement of the ability of a particular phase to spread. If the spreading coefficient is positive, the phase will spread and if it is negative, the phase will contract[4]. By first measuring and calculating the different interfacial tensions, the derivation of the spreading coefficients allows us to predict the final particle morphology. For the full engulfment condition, a positive spreading coefficient of either phase 1 or 3 indicates that a coreshell structure would form, with the phase having the positive spreading coefficient to be the outer layer (i.e. $S_3 > 0$, phase 3 is the outer layer). For the partial engulfment or Janus configuration, all spreading coefficients of each phase are negative. This indicates that the phases are in equilibrium and do not have preferential spreading over one another. For the no-engulfment condition, the spreading coefficient of the aqueous phase is positive, indicating that the aqueous phase spreads over each polymeric phase individually, separating the two polymeric phases from one another to become particles of each individual species.

4.3 Interfacial Tension Measurements of the Respective Phases and the Calculation of the Spreading Coefficient*

Prior to Janus particle formation with EVA/PLLA and PMMA/PLGA, interfacial tension data were measured for three polymeric combinations of Janus particles, namely EVA/PLGA, EVA/PLLA and PMMA/PLGA, and are shown in **Table 2**. The respective interfacial tension of the different polymers that would be used in the fabrication of Janus particles, along with the parameters used for EVA/PLGA in **Figure 4.1.1-1**, were obtained

using the pendant-drop method (Section 3.12). The Owens-Wendt-Kaelble method was employed to calculate the interfacial polymer tension and steps are shown in Table 3. In all three combinations, the calculated spreading coefficients at chosen weight ratios predicted a Janus morphology. As a point of comparison, various extreme ratios of EVA/PLGA and PMMA/PLGA were also measured and were predicted to be core-shell particles.

Table 2 Interfacial tension measurements of EVA/PLGA, EVA/PLLA and PMMA/PLGA particles along with their predicted and actual morphology

	Ratio*	Interfacial Tension ^{&}			Spreading Coefficient [#]			Predicted ⁺	Observed
		γ_{12}	γ_{23}	γ_{13}	S_1	S_2	S_3		
EVA/PLGA	12:18	9.91	10.39	15.323	-14.83	-4.97	-15.99	Janus	Janus
EVA/PLGA	20:10	6.59	23.78	15.323	1.09	-15.04	-32.513	Core-shell	Core-shell
EVA/PLLA	14:16	12.08	12.59	22.66	-22.15	-2.01	-23.19	Janus	Janus
PMMA/PLGA	14:16	11.08	11.59	3.34	-3.86	-19.55	-3.85	Janus	Janus
PMMA/PLGA	20:10	7.37	15.67	3.34	4.96	-19.70	-11.64	Core-shell	Core-shell

*The respective emulsion systems each contain three phases; Phase 1: Polymer A/DCM, Phase 3: Polymer B/DCM and Phase 2: PVA/H₂O. The denoting of Phase 2 as the aqueous phase was established as common nomenclature in the emulsion systems studied in other literature[2, 5].

[&]The interfacial tensions between the polymer phases and the aqueous phase was measured using a pendant drop method which is based on the Young-Laplace equation. The polymer interfacial tension i.e PLGA/EVA, PLLA/EVA and PLGA/PMMA were determined through the Owens-Wendt method. All interfacial tension values were expressed in mN/m.

[#]The spreading coefficient was calculated through Harkin's equation: $S_i = \gamma_{jk} - (\gamma_{ij} + \gamma_{ik})$. All values are expressed in mN/m.

⁺Particle morphology was predicted based on the classic spreading and wetting theory[6]. The calculated spreading coefficients of each respective phase can be correlated to the following: $S_1 < 0$, $S_2 < 0$, $S_3 < 0$ for partial engulfment or Janus particles, $S_1 < 0$, $S_2 < 0$, $S_3 > 0$ or $S_1 > 0$, $S_2 < 0$, $S_3 < 0$ for full engulfment with either phase 1 as the outer shell and

phase 3 the inner shell and vice versa and $S_1 < 0$, $S_2 > 0$, $S_3 < 0$ as no engulfment where each polymer phase exists as its own.

Table 3 Calculation of the Interfacial Tension Between (a) EVA and PLGA, (b) EVA and PLLA and (c) PMMA and PLGA based on DCM as a Solvent

(a)	Water-PLGA	Ethanol-PLGA	Water-EVA	Ethanol-EVA
Contact Angle(°) *	75.55	4.97	111.6	4.88
Surface Energy (mN/m)&	39.59		24.267	
Interfacial Tension (mN/m)#	15.323			

(b)	Water-PLLA	Ethanol-PLLA	Water-EVA	Ethanol-EVA
Contact Angle(°)	101.58	5.10	111.6	5.67
Surface Energy (mN/m)	46.93		24.267	
Interfacial Tension (mN/m)	22.66			

(c)	Water-PLGA	Ethanol-PLGA	Water-PMMA	Ethanol-PMMA
Contact Angle(°)	75.55	4.73	73.76	5.88
Surface Energy (mN/m)	39.59		36.253	
Interfacial Tension (mN/m)	3.34			

*Contact angle of polymers was done on polymer films casted on a glass slide with a drop of H₂O and ethanol. The contact angle of the droplet and polymer film was measured using a static sessile drop technique. All measurements in $^{\circ}$.

[&]The surface energy of the respective polymers was calculated through the interfacial equation derived by Owens and Wendt. In this equation, there are two components, the dispersive and polar component. The dispersive and polar components for each liquid, ethanol and H₂O are well known parameters. Based on **Eq. 11**, two equations with two unknowns for each respective polymer film can be obtained and then solved for γ_s^d , γ_s^p and

γ_t^d , γ_t^p . The surface energy was then calculated with by summing the polar and disperse components.

$$1 + \cos\theta = 2 \left[\frac{\sqrt{\gamma_s^d \gamma_L^d}}{\gamma_L} + \frac{\sqrt{\gamma_s^p \gamma_L^p}}{\gamma_L} \right] \quad \text{Eq. 11}$$

#The polymer interfacial tension was calculated to be the difference between their surface tensions[7].

4.3.1 Polymeric Janus Particles with Different Polymers and Their Selective Dissolutions*

Using the ratios from **Table 2** and the ratios for predicted Janus morphology, EVA/PLGA, EVA/PLLA and PMMA/PLGA Janus particles were fabricated via solvent emulsion and the particles were shown in **Figure 4.3.1-1**. For EVA/PLLA, the weight ratio used was 0.16g : 0.14g of EVA:PLLA. For PMMA/PLGA, the polymeric weight ratio chosen was 0.16g: 0.14g PLGA:PMMA. For both of these combinations, it was observed that the formation of Janus particles are similar to EVA/PLGA, where both polymer faces are formed and joined at a junction demarked by a bifurcation. This also corresponds with the predictions in **Table 2**. In previous studies from other groups, it was concluded that the formation of PMMA/PLGA was not possible. However, the fabrication of PMMA/PLGA Janus particles was demonstrated here, indicating some underlying factors that are currently unknown[2]. In all three polymeric combinations, the yields obtained were above 90%.

Figure 4.3.1-1 (a) EVA/PLGA, (b) PMMA/PLGA and (c) EVA/PLLA Janus particles fabricated via solvent emulsion

In order to determine which polymer forms the Janus particle face, selective dissolution was employed (**Figure 4.3.1-2**). In this method, a solvent that selectively dissolves one polymer, but not the other, was used. For example in EVA/PLGA, cyclohexane was used. Cyclohexane selectively dissolves EVA but not PLGA. Hence, the remaining polymer would be determined to be PLGA and not EVA. For EVA/PLLA, THF was used, where THF selectively dissolves EVA but not PLLA. Similarly for PMMA/PLGA, DMSO was used, where DMSO selectively dissolves PLGA but not PMMA. In all three polymer combinations, the polymers that remained were having a crescent shape. This therefore confirms that within the Janus particle, each face forms a crescent or semi-circular shape and both faces meet together at the bifurcation.

Figure 4.3.1-2 Selective dissolution of (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA

It can be concluded that each of the Janus particles formed in the respective combination is formed by two distinct faces made up of each polymer in the respective polymer combinations.

4.4 Problems and Limitations with the Existing Harkin's Spreading Coefficient Theory

While the system of particle morphology prediction has been well established with Harkin's spreading coefficient theory in **Section 4.3**, there are various limitations within this classical model. Upon analysis of this model, the prediction mode of this model appears to be computational, where it only denotes the morphology that would be formed by inputting interfacial tension data. It does not indicate any degree of tolerance for each morphology within this prediction model. This becomes problematic as this model is only able to quantify for changes in the interfacial tension data based on the use of different polymers or encapsulants and outputs a prediction. By considering the tunability aspect of drug delivery vehicles, it is common to vary polymer weight ratios, molecular weights, drugs and drug amounts to optimize the release profile of the carrier[8-11]. As a result, a lot of background work would need to be done with these parameters to determine how they would affect the interfacial tension and subsequent morphology obtained. This would be highly impractical considering the number of parameters that have to be considered. Since parameters such as drug properties and weight ratios have been shown to alter the interfacial tensions of the polymers, having a model that indicates the 'tolerance' of a polymeric combination to these parameters would be ideal in synthesizing drug-loaded Janus particles. In doing so,

Based on the analysis of the large amount of interfacial tension data obtained by Fan et al., their mechanistic study of the effect of drugs on the polymer interfacial tension indicated a postulation that had potential in quantifying this tolerance (γ_{12} and γ_{23} are the interfacial tensions between the respective polymers and aqueous phase, γ_{13} is the interfacial tension between the polymers:

$$|\gamma_{12} - \gamma_{23}| < \gamma_{13} \quad \text{Eq. 12}$$

In this postulation, they mentioned that in addition to the spreading coefficient requirements, **Eq. 12** is an additional indication for successful Janus particle formation. For example, it was more recently noted that from the interfacial tension values obtained for successful Janus morphologies formed, the interfacial tension between the polymers is always higher than the difference of the interfacial tension of each polymer phase to the surrounding aqueous medium[5]. This could then be used as a simpler means of determining the success of Janus particle formation. However, the underlying significance of the polymer-to-polymer interfacial tension (γ_{13}) was not tested in their study. Based on **Hypothesis 1**, our hypothesis was that the larger the interfacial tension of the polymers used in the formation of Janus particles, the larger the tolerance the particle has to varying weight ratios and added substances into the polymer, which would alter its interfacial tension values.

From **Table 2**, EVA/PLGA and EVA/PLLA is noted to have relatively large polymer-polymer interfacial tension values (γ_{13} : 15.323 and 22.66 mN/m respectively) whereas that of PMMA/PLGA is relatively small (3.34 mN/m). Even in the study by Fan et al., the γ_{13} value of the PCL/PLGA combination used was measured to be 0.65 mN/m and had showed a high sensitivity to weight ratio changes[5]. This therefore means that due to the smaller interfacial tension of PMMA/PLGA, this combination had a smaller tolerance window to interfacial changes caused by the weight ratios. From **Hypothesis 1**, we also postulate that this tolerance can also be applied to the addition of drugs.

As a large library of drugs would need to be obtained and categorized according to their properties, it would be impractical to do in this study due to drug availability. Hence to test **Hypothesis 1**, weight ratio variations were used instead. EVA/PLGA, EVA/PLLA and PMMA/PLGA were fabricated with varying weight ratios and their yields are summarized

in **Table 4**. Detailed analysis of each polymeric combination to the weight ratios were also shown in the following sections.

Table 4 Formation ratios of Janus particles in EVA/PLGA, EVA/PLLA and PMMA/PLGA.

Weight Ratio	0.2 g : 0.1 g	0.18 g : 0.12 g	0.16 g : 0.14 g	0.14 g : 0.16 g	0.12 g : 0.18 g	0.1 g : 0.2 g
EVA/PLGA	No (<5%)	No (<10%)	Yes (>80%)	Yes (>80%)	Yes (>90%)	No (<20%)
EVA/PLLA	No (<5%)	No (<10%)	Yes (>80%)	Yes (>90%)	Yes (>80%)	No (<20%)
PMMA/PLGA	No (<5%)	No (<5%)	No (<5%)	Yes (>90%)	No (<10%)	No (<5%)

4.4.1 Effect of Polymer Weight Ratios in Janus Particle Formation with PLGA and EVA

For consistency in Janus particle fabrication, the stirring speed was set to 400 RPM for all samples. Six different weight ratios were used in order to determine the effect of polymer mass ratios on the Janus particle formation.

In the first three sets of ratios used, the proportion of EVA was more than the amount of PLGA used. In the 0.2 g : 0.1 g of EVA : PLGA ratio used (**Figure 4.4.1-1a-c**), the Janus particles formed were sparse and were only found in particles that were smaller in the range of sizes of particles formed. The larger sized particles (> 300 μm) appeared to be spherical with round patches formed on its surface. There was no significant Janus morphology observed for the larger particles. Similar to the above ratio, the ratio of 0.18g : 0.12 g EVA : PLGA (**Figure 4.4.1-1d-f**) had similar morphology and results as the previous ratio of 0.2 g : 0.1 g of EVA : PLGA. The number of Janus particles formed were almost negligible, with the smaller particles showing some Janus morphology. However upon reaching the ratio of 0.16 g : 0.14 g EVA: PLGA, the yield of Janus particles increased significantly (> 90%) and were found in almost every particle across the range of sizes produced. Despite some round patches on the surface of the Janus particle, the Janus morphology is distinct

Figure 4.4.1-1 EVA/PLGA Janus particles formed with a ratio of 0.2 g : 0.1 g of EVA : PLGA. (a) x50, (b) x500, (c) x1000; EVA/PLGA Janus particles formed with a ratio of 0.18 g : 0.12 g of EVA : PLGA. (d) x50, (e) x200, (f) x500; EVA/PLGA Janus particles formed with a ratio of 0.16 g : 0.14 g of EVA : PLGA. (g) x50, (h) x200, (i) x500

in this ratio. Furthermore, one face of the Janus particle appears to be significantly bigger than the opposing face.

The subsequent three ratios used comprise of PLGA being in a larger ratio than EVA. As the ratio of PLGA is increased to 0.14 g : 0.16 g EVA : PLGA, the Janus particle yield dramatically increases (>80%). However, the degree of patches forming on the Janus

particle appears to increase, with some particles showing dual bifurcations on its surface (**Figure 4.4.1-2a-c**). For the 0.12 g : 0.18 g EVA : PLGA ratio (Figure 4.4.1-2d-f), the Janus particle yield also remains high (>90%) and the Janus particles formed appear to be relatively even, where both faces of the particle appear to be of the same size. Despite this, there are also patches that are visible on the particle surface. Finally for the ratio of 0.1 g : 0.2 g EVA:PLGA (**Figure 4.4.1-2g-i**), the Janus particle yield drops significantly (~50%) and the size of the faces appear to be partial where one side is larger than the other. Furthermore, one side of the particle appears to be porous with many patches on its surface.

From these results, it was determined that the ratio of 0.12 g : 0.18 g of EVA : PLGA is the ideal ratio for Janus particle formation in this solvent emulsion method. For the other parameters, either the yield is affected or the morphology of the Janus particles becomes less ideal. It was assumed that the larger amount of polymer in the weight ratio would be the predominant polymer in the Janus particle. Hence for the extreme ratios where a low yield of Janus particles were formed, the particles formed were dual-layered particles with the polymer used at a higher ratio being the outer core.

For example in the 0.2 g : 0.1 g EVA : PLGA ratio, the formation of Janus particles were sparse. Within the fabrication step, Rhodamine B (RhB) was added into the polymer solution. It was also determined that RhB had preference to be encapsulated complete into the PLGA face as opposed to the EVA face (more details will be expounded on this phenomenon in **Chapter 5**). In this ratio, the particles formed are dual-layered coreshell particles with the PLGA component in the core and the EVA as the outer component. This is confirmed in **Figure 4.4.1-3** where the particle formed contains a pink core (PLGA) and a transparent outer layer (EVA), along with the dual-layered cross-sectional image. These findings are also further validated from the morphology predictions in **Table 2** where core-shell particles were predicted for EVA/PLGA particles made at a 20:10 ratio. The mechanics of selective staining of RhB on the PLGA will be further discussed in **Chapter 5**.

Figure 4.4.1-2 EVA/PLGA Janus particles formed with a ratio of 0.14 g : 0.16 g of EVA : PLGA. (a) x50, (b) x200, (c) x500; EVA/PLGA Janus particles formed with a ratio of 0.12 g : 0.18 g of EVA : PLGA. (d) x50, (e) x200, (f) x200 of a different particle; EVA/PLGA Janus particles formed with a ratio of 0.1 g : 0.2 g of EVA : PLGA. (g) x50, (h) x200, (i) x200 of a different particle.

Figure 4.4.1-3 EVA/PLGA particles at the ratio of 0.2 g : 0.1 g EVA:PLGA. (a) SEM cross-sectional image, (b) Light microscope image of RhB loaded EVA/PLGA particles

4.4.2 Effect of Polymer Weight Ratios in Janus Particle Formation with PLLA and EVA

Figure 4.4.2-1 EVA/PLLA Janus particles formed with a ratio of 0.2 g : 0.1 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.18 g : 0.12 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.16 g : 0.14 g of EVA : PLLA. (a) x50, (b) x200, (c) x500.

The ideal mass ratio for EVA/PLLA Janus particles was done to determine which ratio would be most effective and suitable for producing Janus particles. Six different mass ratios were used and the results are shown below. For consistency, all particles were emulsified at 400 RPM.

The first three ratios studied have EVA higher in proportion to the PLLA used. Based on the particles formed in this ratio (**Figure 4.4.2-1a-c**), the yield of Janus particles appeared to be extremely low. There was virtually no Janus particle formation and the particles that were formed were comprised of spheres with many patches on its surface. In the ratio of 0.18g : 0.12 g EVA: PLLA (**Figure 4.4.2-1d-f**), the yield of Janus particle formation was significant (>80%). However in some cases, the Janus morphology is not obvious and the particles that were formed have large convoluted patches. For the ratio of 0.16 g : 0.14 g EVA:PLLA (**Figure 4.4.2-1g-i**), there appears to be some resemblance of particles with the Janus morphology. However, the patches on the particles are large and in some cases, there are dual bifurcations on some particles, indicating that the Janus structure was not achieved.

In the subsequent three ratios, the PLLA component was at a higher ratio than the EVA component. The ratio of 0.14g: 0.16g EVA : PLLA (**Figure 4.4.2-2a-c**) yielded the highest amount of Janus particles (~100%) with distinct Janus morphology on the particles. The particles appeared to be relatively smoother with less patches than the other ratios and were also more uniform in the surface distribution of each face. Similar to the previous ratio, the ratio of 0.12 g : 0.18 g EVA : PLLA (**Figure 4.4.2-2d-f**) yielded almost 100% of particles as Janus particles. The particles were well defined with the Janus morphology. The particles were also smooth on the surface with minimal patches. Finally for the ratio of 0.1 g : 0.2 g EVA : PLLA (**Figure 4.4.2-2g-i**), the Janus particle yield drops drastically (<10%). The particles produced were also highly patchy with little to no Janus morphology.

All in all for EVA/PLLA Janus particles, it was observed that three of the six ratios used were able to produce Janus particles. This indicates that there is a degree of tolerance with regards to mass ratios for the formation of the Janus particles. Despite the morphologies in

some ratios such as 0.18 g : 0.12 g and 0.16 g : 0.14 g EVA : PLLA showing some particles with convoluted morphologies, the Janus morphology is maintained with relative uniformity.

Figure 4.4.2-2 EVA/PLLA Janus particles formed with a ratio of 0.14 g : 0.16 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.12 g : 0.18 g of EVA : PLLA. (a) x50, (b) x200, (c) x500; EVA/PLLA Janus particles formed with a ratio of 0.1 g : 0.2 g of EVA : PLLA. (a) x50, (b) x200, (c) x500

4.4.3 Effect of Polymer Weight Ratios in Janus Particle Formation with PLGA and PMMA

Next, the optimal mass ratio for PMMA/PLGA Janus particles were determined. An RPM of 400 was fixed for all particles and obtained particles were observed. The first three ratios comprise of PMMA at a higher proportion than PLGA.

Figure 4.4.3-1 PMMA/PLGA Janus particles at a ratio of 0.2 g : 0.1 g PMMA : PLGA (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.18 g : 0.12

g of PMMA : PLGA. (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.16 g : 0.14 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500

For PMMA/PLGA Janus particles at the ratio of 0.2 g : 0.1 g PMMA : PLGA, there are no Janus particles formed, further validated by the measurements in **Table 2**. Furthermore, the morphology of the Janus appears to be smooth without any bifurcations. Interestingly for the 0.18 g : 0.12 g PMMA : PLGA ratio, there is a formation of some Janus particles with some particles showing partial Janus morphology where the opposite face comprises of a smaller patch than the traditional Janus morphology. Despite this, the yield still remains to be low (<5%). When the ratio was changed slightly to 0.16 g : 0.14 g PMMA : PLGA, the formation of Janus particles dropped dramatically with the presence of very few Janus particles (<5%). Non-Janus particles appear to have a smooth surface.

In the subsequent three ratios (**Figure 4.4.3-1**), the ratio of PLGA used is higher than that of PMMA. The first ratio of 0.14 g : 0.16 g PMMA : PLGA yielded the best morphology with the highest yield of Janus particles (>90%). The particles formed in this ratio showed a clear bifurcation within the particle. The faces comprised of one smooth face and another face that had a patchy morphology. Moving to the 0.12 g : 0.18 g PMMA : PLGA ratio however, the yield of Janus particles drops dramatically (<10%). The few Janus particles formed in this ratio however appear to be similar in morphology to those Janus particles formed in the 0.14 g : 0.16 g PMMA : PLGA ratio. Finally for the 0.1 g : 0.2 g PMMA: PLGA ratio, the formed particles have a very low yield of Janus particles (<5%). Furthermore, the particles formed are highly patchy with tiny round droplets on the particle surface.

Based on the results obtained, the most feasible weight ratio for Janus particle formation for PMMA/PLGA is 0.14 g : 0.16 g PMMA : PLGA. In contrast to EVA/PLGA and EVA/PLLA, the PMMA/PLGA combination appears to be more restricted and hence is determined to be the less versatile polymer combination with regards to weight ratio changes.

Figure 4.4.3-2 PMMA/PLGA Janus particles formed with a ratio of 0.14 g : 0.16 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.12 g : 0.18 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500; PMMA/PLGA Janus particles formed with a ratio of 0.1 g : 0.2 g of PMMA : PLGA. (a) x50, (b) x200, (c) x500

4.4.4 Comparisons and Discussions of Janus Particle Formation for PMMA/PLGA, EVA/PLGA and EVA/PLLA

From the formation of Janus particles from the three polymeric combinations, it was determined that all three combinations could form Janus particles. However, it was noted that the EVA/PLGA and EVA/PLLA polymer combinations showed greater independence from the polymer ratio variations as denoted by the green rows in **Table 4**. Comparatively, PMMA/PLGA Janus particles were mainly restricted to just one weight ratio (0.14 g : 0.16 g PMMA : PLGA) and any other weight ratio did not yield Janus particles at a high yield. This increased degree of tolerance of EVA/PLGA and EVA/PLLA and decreased degree of tolerance for PMMA/PLGA is not quantified in the current paradigm of particle morphology predictions. This coincides with several earlier findings in **Table 2** where it was found that amongst the three polymeric combinations chosen, PMMA/PLGA has the smallest tolerance window of the three with regards to the weight ratios used. Hence, this proves **Hypothesis #1**, where polymer combinations with higher polymer-to-polymer interfacial tension have a higher degree of tolerance and can form Janus particles more readily.

While it may be intuitive to assume that **Eq. 12** would entirely replace the Harkin's Spreading Coefficient Theory and render it obsolete, this is not the case. In **Eq. 12**, it implies that a γ_{13} value that is as high as possible would confidently ensure Janus particle formation and hence the only requirement would be to find suitable polymers with γ_{13} to be as high as possible. However, there is an upper limit to this postulation. Assuming γ_{13} approaches to an exceedingly large value, the spreading coefficients S_1 and S_3 would be highly negative while S_2 would approach to become a positive value. In turn, this satisfies the no-engulfment condition and both polymer phases would separate from one another, forming their own particles. This is also validated by the fact that an exceedingly large γ_{13} indicates that a very large amount of energy would be needed for both polymers, phases 1 & 3, to form a surface with one another[12]. Hence, this energy requirement would not be favourable for these polymer phases to form surfaces with one another and is more energetically favorable for them to form single-species particles (**Eq. 10**). This therefore

shows a need for both models where the classical Harkin's Spreading Coefficient Theory can be used as a means to validate the formation of polymeric combinations to form Janus particles, while **Eq. 12** can be used to evaluate their degree of tolerance in forming Janus particles.

4.4.5 Discussion on Polymer Portions in Janus Particles

In the formation of Janus particles through solvent emulsion, the morphology of the particles are left to be decided by thermodynamic processes as discussed in **Chapter 4**. Because of this, certain variations in the interfacial tension values can cause the formation of Janus particles with different appearances. For example, various reports show that within the Janus configuration, there are multiple variants such as perfect-Janus or dumb-bell Janus. In the perfect Janus configuration, the polymer portions appear to be similar in their morphology in terms of shape. Whereas for dumb-bell Janus, there are some morphological variations between the two-faces of the Janus particle[13]. But despite these variations, they should not be confused with the actual weight portions of the Janus particle. While it could be argued that the final weight portions in the Janus particle differs significantly from the initial portioned weight ratios based on their seemingly increased volumes, there are several evidences that substantiate the fact that the portions are identical to the initial weight ratio.

Table 5 Weights of selectively dissolved polymer portions. Weights in mg.

30mg of Polymers	EVA/PLGA	EVA/PLLA	PMMA/PLGA
Ratios	12:18	14:16	14:16
Polymer 1 (EVA or PMMA)	11.13	13.63	13.21
Polymer 2 (PLGA or PLLA)	17.41	15.12	15.17

The first reasoning is from simple experiments of weighing the remaining faces of each polymer combination from their selective dissolution studies shown in **Figure 4.3.1-2**. It is found from **Table 5** that the polymer portions follow very closely to that of the initial starting ratios shown in **Table 4**. For example, the ideal weight ratios of EVA/PLGA was shown to be 0.12g of EVA and 0.18g of PLGA and the weight portions of 30mg of selectively dissolved EVA/PLGA was found to be approximately 11.13mg and 17.41mg.

Secondly, various mathematical modelling done by Jennifer et al. had also showcased these portions were equal to their starting ratios. For example, PCL/PLGA Janus particles were fabricated in a 1:1 ratio with each portion at 25mg. Mathematical modelling of their ratios for PVA concentrations between 1 and 2% were almost identical[2].

Finally, this can also be explained from a theoretical point of view. Prior to the emulsion process, the polymers from their respective weight portions were homogenized and dissolved completely. This also infers that upon the emulsion process, the polymer droplets being emulsified would also have a similar ratio to the starting portions. Due to the high yield of Janus particles formed, even from combinations that had a low weight tolerance[5], it indicates that the weight portions should have been similar. If the weight ratios were to have differed from the initial portions, then the yields would have been significantly affected and Janus particles would not have consistently been formed. Additionally from **Figure 4.6.1-2**, it was observed that the emulsion droplets were already beginning to show Janus morphology almost immediately. For there to be weight ratio variations, some driving force would have to be present to extract out a certain polymer weight from each emulsion droplet and inter-particle diffusion would be the only possible way for this change. Given the chaotic nature of the solvent emulsion process, this inter-particle diffusion can be ruled out and would be more logical to accept that the final polymer portions are equal to the initial portions.

4.5 Effect of Stirring Speed of Janus Particle Size and Morphology

In the synthesis method of solvent emulsion, there are two important parameters that determine the final size of the particles. The first is the concentration of surfactant. Surfactants are added to the aqueous phase in order to prevent the coalescence of the formed polymer droplets. Upon formation of the emulsion droplets, the surfactants stabilize the hydrophobic polymer droplets through the interaction of the hydrophobic tail of the surfactants while the hydrophilic heads interact with the aqueous phase, protecting the polymer droplet from coalescence. With a higher concentration of surfactants, the emulsion droplets can be stabilized with more surfactant molecules, which makes the emulsion droplets smaller[14]. The resulting particles would therefore be smaller as opposed to particles fabricated in a surfactant of lower concentration. The other parameter that could be changed is the speed of stirring during the emulsion process[15]. As the stirring speed increases, the shear forces present in the emulsion to break down the polymer solution into smaller droplets increases. Hence, smaller emulsion droplets form, which result in smaller particles. Of the two methods, the most cost-effective parameter to change would be to increase the stirring speed. While possible to control the size with the surfactant concentration alone, more surfactants would be needed to make a higher concentration of surfactants. Furthermore, there is a limitation on the concentration of surfactant to the particle size due to the solubility limit of the surfactants. Hence, the change in the stirring speed was the parameter that was studied to determine its effect on the size and morphology of the particles. In the following section, the variation in RPM is based on the standardized parameters of beaker size, paddle surface area, paddle height from base of beaker, surfactant concentration and surfactant volume (**Section 3.3**).

4.5.1 Effect of Stirring Speed on the Size and Morphology of EVA/PLGA Janus Particles

Figure 4.5.1-1 EVA/PLGA Janus particles and their corresponding light microscope images. (a) 200 RPM, (b) 400 RPM, (c) 800 RPM, (d) 1500 RPM

As determined previously that the most effective weight ratio for EVA/PLGA Janus particles is 0.12 g : 0.18 g, this ratio was hence used. In this study, the stirring speeds used were 200, 400, 800 and 1500 RPM. It was noted that there is an inverse relationship between the stirring speed and particle size where the higher the stirring speed, the smaller the particles. In all cases, Janus particles were successfully formed at high yields (> 90%). Even at relatively small sizes (~25 μm), the Janus particles were formed and retained their characteristic morphology (**Figure 4.5.1-1**).

The range of sizes formed from 200, 400, 800, and 1500 RPM in **Figure 4.5.1-1** are $359 \pm 159 \mu\text{m}$, $201 \pm 74 \mu\text{m}$, $72 \pm 31 \mu\text{m}$ and $24 \pm 13 \mu\text{m}$ respectively. It is notable that the size distribution decreases as the RPM used increases.

Figure 4.5.1-2 Close-up SEM images of EVA/PLGA Janus particles fabricated at (a) 200 RPM, (b) 400 RPM, (c) 800 RPM, (d) 1500 RPM

It can hence be established that for the EVA/PLGA combination, the Janus morphology is relatively tolerant to the changes in the parameters that affect its size. Throughout all the different stirring speeds used, it can be concluded that this combination forms Janus particles at high yields. This can be attributed to the polymer interfacial tension between EVA and PLGA, which will be expounded more in **Section 4.7**.

4.5.2 Effect of Stirring Speed on the Size and Morphology of EVA/PLLA Janus Particles

Figure 4.5.2-1 EVA/PLLA particles fabricated at (a) 200 RPM, (b) 400 RPM, (c) 800 RPM and (d) 1500 RPM

EVA/PLLA Janus particles were fabricated at different stirring speeds and the results are shown in **Figure 4.5.2-1**. Intuitively, there is an inverse relationship in the particle size to the stirring speed with smaller particles at higher RPMs while larger particles are formed at lower RPMs. All particles regardless of stirring speed showed a high yield (>90%) of Janus particles. Similar to the EVA/PLGA combination, this combination of polymers also demonstrated a high yield of Janus particles across all stirring speeds used here. This indicates that this ratio has a high tolerance, similar to EVA/PLGA, to parameters that would affect its size and can be attributed to its polymer interfacial tension. More will be discussed in **Section 4.7**.

4.5.3 Effect of Stirring Speed on the Size and Morphology of PMMA/PLGA Janus Particles

For the PMMA/PLGA combination (**Figure 4.5.3-1**), the trend of more uniform Janus faces was not observed as compared with EVA/PLGA and EVA/PLLA. As the RPM was increased, the particles from 200 to 800 RPM showed similar morphology while particles were decreasing in size.

Figure 4.5.3-1 PMMA/PLGA particles fabricated at (a) 200 RPM, (b) 400 RPM, (c) 800 RPM and (d) 1500 RPM

However, the patchiness of the particles does not appear to change. In the batch that was fabricated at 1500 RPM, the yield of Janus particles formed had also dropped drastically

with little to no formed Janus particles. In the 1500 RPM batch, the particles produced did not exhibit any Janus morphology. Instead, the particles were highly patchy in nature. In an earlier report, it was shown and discussed that PMMA/PLGA particles were not feasible for Janus particle formation[2]. Based on these results however (**Figure 4.5.3-1**), it is shown that PMMA/PLGA particles could be fabricated but would require specific requirements, such as the optimal weight ratio as well as stirring speed. Keeping this in mind, more insight into why PMMA/PLGA is more sensitive to these size-affecting parameters than the other two combinations of EVA/PLGA and EVA/PLLA will be explained in **Section 4.7**.

A possible explanation as to why PMMA/PLGA particles fabricated at high RPMs would significantly decrease the yield of Janus particles formed lies in its γ_{13} value. Due to the high RPM, the rate of DCM evaporation is increased greatly. While yet to be determined, it is postulated that the rapid evaporation rate alters the interfacial tension of the polymer phases and a large majority of the coacervate polymer droplets are affected kinetically and non-Janus structures are formed as a result. Whereas for EVA/PLGA and EVA/PLLA, the high interfacial tension between the polymers gives them the tolerance to the minute changes in the parameters, allowing them to form Janus particles even at very small sizes and rapid evaporation rates.

4.5.4 Statistical Analysis of the Effect of Stirring Speed on Particle Size

Table 6 Sizes in μm obtained for EVA/PLGA, EVA/PLLA and PMMA/PLGA Janus particles at different stirring speeds

	RPM			
(Size in μm)	200	400	800	1500
EVA/PLGA	361 ± 53	214 ± 39	78 ± 26	23 ± 11
EVA/PLLA	794 ± 79	211 ± 76	178 ± 65	119 ± 32
PMMA/PLGA	343 ± 88	176 ± 39	67 ± 24	27 ± 13

Table 7 One-Way Analysis of Variance (ANOVA) of particle sizes for varying stirring speeds. MS is the mean-squared value. $\alpha = 0.05$.

		<i>MS</i>	<i>F-value</i>	<i>p-value</i>	<i>F-crit</i>
EVA/PLGA	Between Groups	229189.82	175.34	1.61x10 ⁻²¹	2.89
	Within Groups	1307.41			
EVA/PLLA	Between Groups	934226.34	216.09	4.57x10 ⁻²³	2.89
	Within Groups	4323.25			
PMMA/PLGA	Between Groups	199647.52	77.65	8.75x10 ⁻¹⁶	2.89
	Within Groups	2571.21			

Table 6 shows the mean and standard deviation values of the respective particle sizes. To validate the significance of the obtained values in **Table 6**, one-way ANOVA test was applied to these results. The null-hypothesis is denoted as H_0 , where the means of all the samples in each respective stirring speed are equal and that stirring speed has no effect on the particle sizes. From **Table 7** the F-value obtained for all three polymeric combinations was significantly greater than F-crit i.e. $F \gg F\text{-crit}$. This indicates that for the given $\alpha = 0.05$, there is sufficient evidence to reject the null-hypothesis, where at least one of the samples have significantly different size means and form a completely different population. Generally speaking it, can be concluded that the size variations between samples for each stirring speed varies significantly and formed their own size populations. To investigate this further, a two-tailed t-Test was carried out on each particle species between their respective stirring speeds i.e. 200 vs 400, 400 vs 800 and 800 vs 1500 RPM.

From **Table 8**, it is noted that for the given probability of $\alpha = 0.05$, only the EVA/PLLA combination showed a probability higher than 0.05 between particles at 400 vs 800 rpm ($p = 0.11$). This indicated that the particles fabricated with 400 and 800 RPM for EVA/PLLA are within the same size population and can be concluded that not much size variation

occurs between these conditions. For the remaining comparisons, all groups show a significant difference between one another, validating the use of RPM to create different populations of different sized particles.

Table 8 p-value of two-tailed t-Test for each respective Janus particle combination

	200 vs 400	400 vs 800	800 vs 1500
EVA/PLGA	1.42×10^{-6}	3.61×10^{-8}	1.17×10^{-5}
EVA/PLLA	1.2×10^{-11}	0.11	1.10×10^{-4}
PMMA/PLGA	3.79×10^{-5}	6.70×10^{-7}	3.11×10^{-4}

4.6 Structural Relationships of Size to Surface Morphology

Interestingly, it was noted that the higher the RPM used and hence the smaller the particle, the Janus morphology appeared to be more uniform with less and less patches. This is particularly true for EVA/PLGA and EVA/PLLA particles. For the EVA/PLGA and EVA/PLLA particles fabricated at 200 RPM (**Figure 4.5.1-2(a)** & **Figure 4.5.2-1(a)**), the sizes obtained were rather large ($\sim 400 \mu\text{m}$) and the surface of the particles had large patches. For the particles made at 400 and 800 RPM, the particle sizes were smaller and the degree of the amount of patches reduces significantly. Finally for the particles made at 1500 RPM, the sizes obtained were the smallest with the morphology of the particle being the more uniform and smooth in its morphology.

Initially, it was predicted that the particles fabricated under 200 RPM would yield particles with the best Janus morphology. This is because the slow stirring speed of the emulsion system would decrease the rate of evaporation of DCM due to the least amount of agitation and shear forces from stirring. Hence, the immiscible droplets of the polymers will have a larger timing window for diffusion across the polymer droplet into their respective face before the DCM evaporates and immobilizes the tiny droplets, leading to the formation of patches. Conversely, the particles that are fabricated at 1500 RPM would yield the particles with the worst Janus morphology as the rapid shearing forces from stirring would cause rapid evaporation of DCM and immobilize the tiny polymer droplets within the whole

polymer droplet quicker. However, the findings were opposite from the initial hypothesis where the particles fabricated at 200 RPM yielded particles with the worst morphology as opposed to those fabricated at 1500 yielding the best Janus morphology.

One possible explanation for this phenomenon can be related to the diffusion distance of the polymer droplets during the emulsion process. Upon emulsification, the polymer droplets that enter the aqueous system are uniformly mixed with both species of polymer, PLGA and EVA. Due to the immiscibility of both polymers and the Oswald ripening effect, the polymer faces of PLGA and EVA begin to grow bigger and bigger as more of the smaller droplets start to fuse with the larger polymer droplets. As determined from the SEM images in **Figure 4.5.1-2**, the size of the formed particles with 200 RPM average at $329 \pm 159 \mu\text{m}$ whereas in contrast, the particles fabricated at 1500 RPM yielded particles in the size range of $24 \pm 13 \mu\text{m}$. Therefore, the smaller distance in the smaller particles allows for quicker and easier diffusion of the coacervate phases and subsequent ripening before the solvent evaporates(**Figure 4.5.4-1**).

Therefore in comparison, the diffusion distance for the polymer droplets fabricated in 200 and 1500 RPM would have a difference of at least $300 \mu\text{m}$. This larger diffusion distance contributes to the formation of patches on larger particles, while the smaller particles, such as those fabricated at 1500 RPM have smoother and more ideal Janus morphologies. However, this only applies to the EVA/PLGA and EVA/PLLA combination and does not seem to apply to the PMMA/PLGA combination. One possible explanation would be the interaction between PMMA and PLGA. More details will be explored in **Section 4.7**.

Figure 4.5.4-1 Schematic explaining the formation of patchy EVA/PLGA and EVA/PLLA particles at low RPM and smooth particles at higher RPMs.

As for the particle size trend across each combination, it was noted that the EVA/PLLA combination had the particles with the largest size, followed by EVA/PLGA and PMMA/PLGA. Among the 4 polymers used, PLLA has the highest viscosity (IV 2.36). Therefore, this possibly suggests that the higher viscosity of PLLA causes larger particles to form along with higher size deviations. This corresponds to various findings in literature about the relation of viscosity and particle size[16].

4.6.1 Kinetic Trapping in the Fabrication of Janus Particles

As the polymer solution containing both polymers is poured and emulsified within the aqueous phase, the solvent begins to evaporate. Due to the rapid shear force of the stirring, the DCM evaporates rapidly. This causes the polymers to precipitate and gradually buildup

a polymeric network that causes any phases that have not fused to their correct faces to be trapped within the opposite face. To simulate this, two polymer solutions at 10% and 15% concentration of EVA/PLGA were prepared and emulsified. The higher concentration of 15% was to simulate this effect of evaporating solvents and the accelerated formation of the polymer network due to a lesser amount of solvent added.

Figure 4.6.1-1 (a) Kinetically trapped EVA/PLGA particles and (b) Cross-sectional analysis of patchy EVA/PLGA. Large patches are found mostly on the surface, indicating that there is significant driving force for droplets to migrate to the surface as these droplets would satisfy the partial engulfment requirement

In **Figure 4.6.1-1**, particles of EVA/PLGA showed Janus particles with a large number of big patches on the particle. Interestingly from cross-sectional analysis, it was observed that within the Janus structure itself, there are close to no patches or incomplete coalescence droplets within the particle but instead all occur at the surface of the particle. Hence, this indicates that the thermodynamic driving force would be significant enough for the phases that have not fused with their correct phases to be driven to the surface. Essentially, each individual patch satisfies the partial engulfment theory and form ‘mini-Janus’ structures and come in the form of patches.

It is important to note that during particle fabrication, both thermodynamic and kinetic factors are present. While the thermodynamics of this system can be predicted through the

spreading coefficients, it does not take into account the evaporation of the solvent during the fabrication step, which could affect the interfacial tensions and ultimately the spreading coefficient[17]. Due to the relatively rapid evaporation of the solvent in this process, there could be instances where the particles are kinetically trapped in a thermodynamically unfavorable conformations due to the rapid evaporation of the solvent. Therefore, it would be important to determine the speed with which the phases would phase separate in accordance to the spreading coefficients and be kinetically trapped in a thermodynamically favorable conformation before the solvent evaporates.

Figure 4.6.1-2 Thermodynamic behavior of polymer phases within the coacervate droplets during the emulsion process. Polymer droplets immediately aggregate into their opposing poles, creating a resemblance to the Janus morphology even before cloud point

Emulsion droplets were removed from the emulsion container during the initial stage of synthesis (**Figure 4.6.1-2**). In this batch, two dyes which would dye both faces distinctly was added to the mixture in order to recognize the different polymer phases. It was observed that immediately after emulsion, the droplets phase separated within the droplets while the polymer phases are still mobile due to the large amount of DCM. This observation confirms that the effects of the thermodynamic factors come into play immediately as the polymer solution is being emulsified. As the DCM solvent evaporates, the polymers reach cloud point and the polymers gradually precipitate and solidify into their respective poles. However, the gradual precipitation of the polymer phases creates a network that solidifies and prevents any diffusion of the stray droplets that have not joined with their correct faces. In this example, the observation of the gradual solidification and phase separation of the polymer droplet might not fully model that within the emulsion system as the droplets here

were left in situ without any other agitation. This would reduce the evaporation rate of DCM greatly, and allow the polymer phases to diffuse and fuse with their appropriate phases. Despite this, these results give insight into the formation mechanics of Janus particles, where both the thermodynamic factors and Ostwald ripening are driving forces that cause the particles to form Janus particles.

4.7 Degradation Behavior of PMMA/PLGA, EVA/PLGA and EVA/PLLA

According to **Table 2**, the interfacial tensions of the polymers (γ_{13}) were measured. From Kruss' definition, the interfacial tension is the amount of energy required per unit for two immiscible fluids to form a surface[12]. For example in EVA/PLGA Janus particles, the EVA/DCM and PLGA/DCM phases meet at the bifurcation interface. The EVA phase prefers to associate with its own DCM/EVA molecules and likewise for DCM/PLGA. At the interface between these two polymers, the molecules at this surface have an exposed surface where there are no more molecules of its own kind to bind to. Hence, these molecules at the surface would need to form surfaces with a phase not containing its preferred molecules to bind to. Because of this, energy would be required to form the surfaces between EVA and PLGA. Hence, the higher the interfacial energy, the more energy would be required for them to form surfaces, and hence could be inferred that Janus particle polymer combinations with high interfacial tensions are less adhesive at their interface. In order to test this concept, EVA/PLGA, EVA/PLLA and PMMA/PLGA particles were placed in PBS and left in a shaking incubator over a period of 14 days. The particles were sampled and observed under SEM to evaluate any changes in morphology of the particles throughout the degradation duration.

At day one to four, all three polymeric combinations were still intact with the particles still joined at their interface. Some particles for EVA/PLGA appeared to have one face shrinking slightly within the Janus particle but Janus morphology was still intact. At day seven, the particles of EVA/PLGA appeared to have split at the center into two hemispheric portions while the EVA/PLLA particles showed on face of the particle shrinking, similar

to the trend observed in day four for EVA/PLGA. At day 10, particles of EVA/PLLA have mostly begun to split at the centre, leaving behind two hemispheric faces. On the other hand, the PMMA/PLGA polymeric combination appeared to be stable and remained intact throughout the 14 day study.

Based on these results, it can be concluded that the effect of polymer interfacial tension is an important parameter in the usage of such particles in drug delivery applications where the particles are subject to sink conditions for an extended period of time. For the EVA/PLGA and EVA/PLLA combination, their relatively high interfacial tension values (15.32 and 22.66 mN/m respectively) would indicate that the bond at the interface between both polymers is relatively weak. Because of this, the particles begin to split completely upon reaching day seven of the degradation studies. Whereas for PMMA/PLGA, the lower interfacial tension of 3.34 mN/m indicates that its bond at the interface is relatively stronger than that of EVA/PLGA and EVA/PLLA. In congruence to the results, the PMMA/PLGA Janus particles exhibit greater resilience in its bond at the interface and stay intact even up to day 14. Previously in literature, it was shown that PCL/PLGA Janus particles with measured γ_{13} values near ~ 0 mN/m, showed varying degradation rates between each face but remained intact beyond 35 days[7]. The previously measured interfacial tension values of PCL/PLGA was found to be 0.65 mN/m[5]. However, it had not been yet established until now that the effect of using different polymer combinations for Janus particles fabrication would yield such a trend of face separation of the Janus particles depending on its interfacial tension.

Additionally, the findings of these results converges on how the formation of Janus is first dependent on the interfacial tension where the higher the interfacial tension, the higher the likelihood of Janus particle formation and higher tolerance to changing parameters. While on the other hand, Janus particles formed with a high interfacial tension at its interface would yield particles that split relatively quickly at its interface. While it was first assumed that for Janus particles to function properly, they would need to exist as a single entity joined at its interface, this ability to control the duration of face splitting can also be useful. More details are expounded in **Chapter 7**.

Figure 4.6.1-1 Degradation behavior of EVA/PLGA, EVA/PLLA and PMMA/PLGA Janus particles over a 14 day period

In conclusion, the mechanism of formation of Janus particles through a solvent emulsion process can be explained through Harkin's spreading theory and the various

thermodynamic requirements for Janus particle formation and otherwise. The individual interfacial tension and polymer-to-polymer interfacial tension were also measured and were successfully correlated to the models used to predict particle morphology. In addition, the role of the polymer-to-polymer interfacial tension was proven to be a highly important parameter that affects the tolerance of a specific polymeric combination to varying weight ratios. This indicates that the initial polymer choice for Janus particle is an important requirement prior to Janus particle synthesis and validates **Hypothesis 1**. Other parameters such as stirring speed would affect the particle morphology via kinetic factors. The role of kinetic factors in Janus particle was also demonstrated where PMMA/PLGA particles fabricated at high stirring speeds cause a low yield of Janus particles despite being at the correct weight ratio. The results of kinetic trapping was also described where particles with patchy surfaces were observed due to incomplete coalescence of polymer phases to their respective poles. Finally, the way that the polymer interfacial tension affects the degradation behavior of these Janus particles has also not been exclusively studied until now and its importance in developing other applications that utilize this concept (**Chapter 7**).

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Chapter 5

The Loading of Drugs within Different Combinations of Polymers for Janus Particles

This chapter leverages on the knowledge established in the previous chapter for Janus particle formation, and the importance of the polymer interfacial tension to load drugs into different polymeric combinations of Janus particles. The fabrication of dual-drug loaded Janus particles is demonstrated in this chapter and also the ability to selectively encapsulate these drugs within different faces of the Janus particles is shown. Additionally, the underlying mechanics of how this selective encapsulation occurs is proposed. The drug release behavior of each of this combinations is also showcased and how different combinations can give rise to different types of release profiles.

*This section published substantially as [1]

5.1 Drug Loading and the Importance of Polymer Interfacial Tension

***Hypothesis # 1:** Polymers with higher polymer-to-polymer interfacial tension are able to resist weight ratio changes and encapsulate drugs and other encapsulants without losing anisotropy.*

***Sub-Hypothesis #2:** Drugs can be selectively encapsulated within each face of the Janus particles depending on the degree of hydrophilicity of the respective polymer faces.*

It has been shown in **Chapter 4** of how Janus particles of different polymeric combinations can be fabricated through a facile synthesis method. It was also established in its mechanics that the polymer interfacial tension is an important parameter to not only predict the formation of Janus particles but also the degree of tolerance that each Janus particle polymer combination has.

The next step beyond Janus particle fabrication would be drug encapsulation within these Janus particles. The ability to selectively encapsulate more than one drug into these Janus particles is non-trivial. Usually, the Janus morphology is lost once drugs are encapsulated and in turn, patchy particles are formed. While Xie et al. had demonstrated the selective encapsulation of DOX and PTX within PLGA/PLGA Janus particles, this process was carried out via microfluidics and was only able to achieve throughputs of approximately 3 $\mu\text{L}/\text{min}$ [2]. In one of the first reports of polymeric Janus particle synthesis through solvent emulsion, the same report shows the loss of Janus morphology in PLGA/PCL Janus particles the moment drugs are added within the fabrication step. Patchy particles are formed as a result of this addition of drug[3]. Other work has also gone into establishing how the properties of drugs would affect the formation mechanics of these Janus particles[4]. While this report is useful in documenting the effect of drugs on the formation of Janus particles, the report only studies the effect of one drug. Simultaneous addition of two drugs was not studied. Additionally, with this approach, it would be highly laborious to evaluate the feasibility of every permutation and combination of each drug on the formation of Janus particles. In order to resolve this conundrum, the significance of **Eq. 12** as a second layer of validating the success of Janus particle formation through quantifying the tolerance of a polymeric combination to interfacial changes empirically. In other words,

the higher the γ_{13} , the more tolerant and easier it is for that particular polymeric combination to form Janus particles.

It is hypothesized in **Sub-Hypothesis #2** that this additional criterion of validation can be extended to the success of Janus particle formation with the addition of drugs. This equation is the cornerstone for drug encapsulation in Janus particles, where the high interfacial tension of the polymer used in the Janus particle would create a higher ‘tolerant window’ for Janus particles to encapsulate the drug while maintaining the Janus structure. While different drugs would affect the polymer to aqueous phase interfacial tensions in different ways, the high interfacial tension between the polymers would ensure sufficient ‘cushion’ for simultaneous drug encapsulation and Janus structural integrity. In order to further demonstrate the versatility of these particles, it would be of interest to determine the encapsulation behavior of Janus particles with these different Janus particle combinations as well as the partitioning behavior of the drugs into the respective faces of each Janus particle combination.

5.1.1 Drug Encapsulation with Doxorubicin*

Figure 5.1.1-1 (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA Janus particles encapsulated with Doxorubicin (d) Selective dissolution of Janus particles, leaving behind red hemispheres, indicating complete encapsulation of Dox within one face.

The encapsulation of Doxorubicin was done with each of these Janus particle combinations by adding Dox to the initial polymer solution. While initially predicted that there would be a certain degree of drug partiality between both polymer faces, it was found that each of these Janus particle combinations shows a distinct encapsulation of red on one face, leaving the other side transparent. Selective dissolution of EVA in the EVA/PLGA (**Figure 5.1.1-1**) was carried out and it was found that the remaining parts would be PLGA hemispheres. Separate solutions of PLGA and EVA were made with the addition of Dox to either the PLGA or EVA solution and subsequently mixed. Similarly for EVA/PLLA and PMMA/PLGA, Dox was first dissolved in separate solutions of EVA, PLLA and PMMA respectively and regardless of whichever solution Dox was first dissolved in, the encapsulation of Dox was still found to be in the aliphatic polymers i.e. PLGA or PLLA, with further confirmation shown in **Figure 5.1.2-1**.

5.1.2 Drug Encapsulation with Doxorubicin and Paclitaxel*

Figure 5.1.2-1 (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA Janus particles encapsulated with Dox and PTX. (d-f) Selective dissolution of each respective Janus particle combination. For EVA/PLGA and EVA/PLLA, EVA was selectively dissolved, leaving behind red crescents of PLGA and PLLA respectively. As for PMMA/PLGA, selective dissolution was done on the PLGA, leaving behind transparent crescents of PMMA.

Encapsulation of more than one drug within Janus particles through the solvent emulsion process is non-trivial. Unlike microfluidics, directing the drug loading to be in a specific face of the polymer cannot be directly controlled as precursors have to be prepared before fabrication. While microfluidics has been shown to be able to encapsulate these drugs[5], microfluidics are limited to the polymer type and cannot be used for polymers with high viscosity. Furthermore, the throughput of microfluidics is very low. Here, we demonstrate the ability to load these drugs within two different polymers, with some at high viscosities (PLLA I.V. 2.36).

To further investigate the drug encapsulation behavior of these Janus particles, Paclitaxel (PTX), which is another cancer drug was chosen. Dox and PTX each have disparate solubilities (logP of 0.71 and 3.96 respectively). LogP is the partitioning ratio of the drug of interest in an octanol-water mix. The higher the logP, the larger the proportion of the drug dissolving in the octanol component and hence the higher the hydrophobicity of the drug. Hence, it could be inferred that doxorubicin was the more hydrophilic drug and PTX was the significantly more hydrophobic drug. Furthermore, Doxorubicin and Paclitaxel have been well-studied in combinational therapy for cancer treatment due to their synergistic effects[6-8]. While the synergy of DOX and PTX can potentially help with reducing the dosage requirements, another key feature is the sequence of drug infusion. A phase I study showed tremendous reduction in side effects in a treatment group with initial infusion of DOX for 24 hours followed by infusion of PTX for 24 hours in comparison with a treatment group that had DOX followed by PTX infusion[9]. The contrasting logP values of Dox and PTX indicate that co-encapsulation of these drugs into a single entity would affect drug loading and encapsulation efficiency due to the contrasting properties of these drugs[2].

Janus particles of each polymer combination with both Doxorubicin and Paclitaxel were fabricated (**Figure 5.1.2-1a-c**). Similar to **Figure 5.1.1-1**, the particles obtained appeared to be of a similar appearance with one hemisphere stained red. Due to the colorless nature of PTX it is impossible to determine the disparity of PTX visually. The particles loaded with Dox and PTX were subjected to selective dissolution. For EVA/PLGA and EVA/PLLA, the selective solvents used dissolved away the EVA component, leaving

behind the red hemispheres which were concluded to be either PLGA or PLLA. As for PMMA/PLGA, the selective solvent used was selective to the PLGA, leaving behind the PMMA component. Hence, the presence of the transparent hemispheres indicate that the red hemispheres which were dissolved was PLGA.

To determine the drug encapsulation of paclitaxel within each of these Janus particles, the drug encapsulation efficiency of each drug was done for each Janus particle combination. Based on **Table 9**, the overall encapsulation efficiencies were determined to be approximately 90% for doxorubicin in each of the Janus particles. As for paclitaxel, the encapsulation efficiencies were approximately 70% for each Janus particle combination. As for the selective encapsulation, it was determined that 100% of doxorubicin had encapsulated into either PLGA or PLLA of each combination, with negligible amounts in the opposing side. This therefore further confirms that the semi-circular red crescents from **Figure 5.1.2-1** were indeed complete encapsulation of doxorubicin within the PLGA or PLLA region.

For paclitaxel, the drug partitioning in each Janus particle showed varying degrees of partitioning. For the EVA/PLGA combination, paclitaxel showed significant partitioning with the EVA face (~86%) while the PMMA/PLGA combination showed relatively even distribution of paclitaxel within each face. As for EVA/PLLA, the partitioning is in-between that of EVA/PLGA and PMMA/PLGA, where a majority of the paclitaxel could be found (~70%) but a still a sizable portion of paclitaxel was found in the PLLA face (~30%).

Table 9 Encapsulation efficiencies of doxorubicin and Paclitaxel in whole particles as well as selective encapsulation efficiency calculations.

	EE% of Each Drug in Whole Janus Particle [%]		
	EVA/PLGA	EVA/PLLA	PMMA/PLGA
Dox	93.59 ± 13.75	92.09 ± 20.46	84.25 ± 8.88
PTX	64.30 ± 0.78	72.11 ± 12.66	71.54 ± 14.20
	Loading % of Drug in Each Face of Janus Particle [%]		

	EVA	PLGA	EVA	PLLA	PMMA	PLGA
Dox	0	100	0	100	0	100
PTX	86.14 ± 7.64	13.86 ± 7.64	68.92 ± 5.32	31.08 ± 5.32	45.27 ± 2.84	54.73 ± 2.84

To further prove the effectiveness of these Janus particles in co-encapsulating Dox and PTX within a single particle, single-species polymers encapsulating each respective drug was done. From **Table 10**, it is noted that the overall EE% of Dox in PLLA and PLGA is lower than that of the overall EE% in Janus particles while the encapsulation of PTX in each polymer is higher than that of the Janus particle. However, co-encapsulation of Dox and PTX in PLLA and PLGA showed that the overall EE% of each individual drug is lower than the overall EE% in a Janus particle (~ 40% for each drug in PLGA and PLLA respectively) (**Table 10**). Hence this proves that the efficiency of encapsulating more than one drug of disparate solubilities is more effective and efficient in a Janus particle than a conventional single species polymer particle system.

5.1.3 Confocal Mapping of Drug Encapsulation within EVA/PLGA

Based on the contrasting selective encapsulation of both DOX and PTX in PLGA and EVA respectively, it would be of interest to visualize their selective encapsulation within these Janus particles. In this demonstration, dansyl-chloride tagged PTX (DPTX) was used as the fluorescent agent to mimic the properties of PTX. Various authors have shown the validity of DPTX as a good means to model the behavior of PTX in regards to its encapsulation behavior within different polymers[10, 11]. For more prominent fluorescence, RhB was used in place of DOX. This replacement was validated in **Figure 4.4.1-3** of the preferential encapsulation of RhB within PLGA, similar to the encapsulation behavior of DOX.

Figure 5.1.3-1 Confocal mapping of RhB and DPTX in EVA/PLGA Janus particles.

In **Figure 5.1.3-1**, the confocal mapping images show a contrasting image of RhB on one face and DPTX on the opposing face. Additionally, PLGA droplets that did not completely diffuse and fuse with the PLGA face were also found to be selectively encapsulated with RhB within the EVA regions. This showcases that the drugs were indeed selectively encapsulated within each polymer face and validates this solvent emulsion process to fabricate drug-encapsulated Janus particles with drugs within each specific face.

5.2 Mechanism of Drug Selectivity

The significance of such drug encapsulation is that by being able to load two drugs within a particle selectively, any drug to drug interactions which could otherwise affect release kinetics can be reduced. While each individual drug can be loaded into two batches and the drug-loaded polymer subsequently being mixed in principle, this fabrication of dual drug loaded Janus particles can all be done in just one step. More importantly however, the mixing of two batches of particles containing two different drugs requires more calculations for optimal drug ratio and suffers from complications during its administration. For example, the usual administration of these particles through a needle syringe to the target site cannot guarantee that the particles within the target site itself is in the correct proportion due to the dynamics of particle loss[12]. This is more pronounced when drug ratios are of importance. For applications which require small and strict volume control for

administration of particles (such as the anterior chamber of the eye), it would be much simpler to have a single batch of particles than for particles to be mixed as the allowable volume for administration limits the number of injections.

Table 10 Encapsulation efficiency of Doxorubicin and PTX for individual particles of PLGA, PLLA, EVA and PMMA. Doxorubicin encapsulation in PMMA and EVA was negligible and hence not shown. *Encapsulation of doxorubicin + paclitaxel in EVA and PMMA not shown due to extremely low EE% of doxorubicin within both EVA and PMMA

	PLGA + Dox + PTX	PLLA + Dox + PTX	EVA + PTX + Dox	PMMA + PTX + Dox
Dox EE (%)	40.04 $\pm$ 1.65	41.43 $\pm$ 0.20	NA*	NA*
PTX EE(%)	48.13 $\pm$ 3.02	46.21 $\pm$ 1.62	68.2 $\pm$ 3.4	88.5 $\pm$ 2.41

5.3 Mechanism of Drug Selectivity and Selective Encapsulation

Figure 5.1.3-1 Contact angles with water on (a) EVA, (b) PLLA, (c) PLGA and (d) PMMA films

In order to further understand this partitioning behavior of both drugs, it was hypothesized that the degree of hydrophilicity of the respective polymers and the interaction of the drug based on its intrinsic hydrophilic/hydrophobic property has an influential role in the drug partitioning mechanism. It has also been previously shown in other studies that due to this

interaction between polymer and drug, different drugs can be selectively encapsulated within different layers of the same particle through a similar solvent emulsion process[11]. The hydrophilicity of each respective polymer was done through contact angle measurements with water via a static sessile drop technique (**Figure 5.1.3-1**). As it has been established that there is a correlation with the contact angle to the degree of hydrophilicity of the polymer[13], the contact angles obtained from the neat polymer films would give insight to the intrinsic polymer hydrophilicity, which would affect the drug encapsulation. From the contact angle studies on the respective polymer films, it was found that the contact angle was in the order $EVA > PLLA > PMMA > PLGA$. Hence it was concluded that EVA was the most hydrophobic polymer, followed by PLLA, PMMA and then PLGA.

This correlated well with the EE% results obtained in **Table 9**. Looking at the selective EE% of EVA/PLGA for both Doxorubicin and PTX, the encapsulation of Doxorubicin was almost 100% in the PLGA face. Between EVA and PLGA, PLGA is significantly more hydrophilic than EVA based on the contact angle results. The difference in contact angles between EVA and PLGA are also the largest amongst the three polymeric combinations ($\sim 46^\circ$). Due to the hydrophilic nature of Doxorubicin and PLGA, Doxorubicin is partitioned into PLGA. As for PTX, the large contact angle disparity and hence hydrophilicity difference between EVA and PLGA, PTX being a hydrophobic drug, would be predominantly encapsulated into the EVA component ($\sim 86\%$) and only 14% in the PLGA component. As for EVA/PLLA, the contact angle difference is $\sim 14^\circ$. Therefore, the partitioning of PTX between both of these polymer faces is less pronounced with $\sim 70\%$ in EVA and $\sim 30\%$ in PLLA. Finally for PMMA/PLGA, the contact angle measurements are almost identical with PLGA being slightly more hydrophilic than PMMA. Hence, a slightly larger proportion $\sim 55\%$ of PTX is found in PLGA and the remaining 45% is found in PMMA. However, this mechanism is not fully understood. For example, the predominant preference of Doxorubicin into the aliphatic polymers PLGA and PLLA without any partiality is not fully understood.

5.3.1 Drug Release Studies for EVA/PLGA, EVA/PLLA and PMMA/PLGA

Drug release studies were conducted for EVA/PLGA, EVA/PLLA and PMMA/PLGA dual-drug loaded Janus particles. Release studies were conducted up to 11 days or when one of the drug reaches complete release. The significance of this study would be to compare the release profiles of single polymers to Janus particles and determine any different behaviors of both types of particles despite similar drug loading and encapsulation efficiencies.

Figure 5.3.1-1 shows the drug release profiles for each of the Janus particles. For the EVA/PLGA combination, the drug release appears to be sequential, where both PTX and Doxorubicin reach complete release on day 11. For the PMMA/PLGA combination, the release of Doxorubicin reaches near completion on day 11 while the release of PTX is significantly hampered. PTX shows no release from day zero to day four in PMMA/PLGA and only commences from day four onwards. Due to the low amount of release of PTX, the kinetics were not calculated for PTX from PMMA/PLGA. Finally, for the EVA/PLLA combination, Doxorubicin was released completely at day 11 while PTX showed about half the release as compared to EVA/PLGA. Hence, the release profile of EVA/PLLA lay somewhere in-between that of EVA/PLGA and PMMA/PLGA.

Based on the calculation of the rate kinetics of drug release for Doxorubicin, it was concluded that the release rates for Doxorubicin in all three particle combinations were similar. The rate constants for drug release in each of the three combinations was $5.7 \pm 0.4 \text{ h}^{-1}$ for all three Janus particles ($R^2 > 0.99$). As for the release behavior of PTX, all three Janus particle combinations showed contrasting release rates (

Table 11). To account for these contrasting release rates, it was hypothesized that the proportionality of the drugs within each face of the polymers in each polymeric combination had a significant role to play in affecting the release. Based on **Table 9**, the proportion of encapsulated PTX in the EVA/PLGA combination was predominantly within

EVA (86%). Hence, it could be inferred that most of the release via diffusion would be coming from EVA. In contrast, the diffusion of PTX from PMMA/PLGA would likely be from both faces due to the relatively equal distribution of PTX within the particle. As for the EVA/PLLA combination, the release of PTX would still be predominantly from EVA but due to the larger proportion of PTX inside PLLA, most PTX would be released as well.

Figure 5.3.1-1 Release studies of Dox and PTX for (a) EVA/PLGA, (b) EVA/PLLA, (c) PMMA/PLGA Janus particles.

Figure 5.3.1-2 Fitting of the release profiles with the Higuchi Model

Table 11 Correlation coefficient (r^2) and Rate Constant (K) of Dox and PTX from the respective Janus particle combinations after fitting with the Higuchi equation

	Correlation Coefficient r^2		Rate Constant K [h^{-1}]	
	Dox	PTX	Dox	PTX
EVA/PLGA	0.9898	0.9869	5.9916	5.7610
EVA/PLLA	0.9898	0.9751	5.2282	3.2744
PMMA/PLGA	0.9874	<i>Release too low</i>	5.8595	1.5376

Figure 5.3.1-3 (a) Release studies for single polymers of EVA, PLGA, PMMA and PLLA over a period of 10 days. Comparison of PTX release based on calculations of the corresponding encapsulation percentage of single polymers vs the Janus particles of (b) EVA/PLGA, (c) EVA

To further substantiate this theory, single-polymer microparticles of similar sizes (100-120 μm) were fabricated and encapsulated with one drug (i.e. PTX in EVA, PLGA, PMMA and PLLA). Subsequently, their respective ratios were calculated by calculating the summation release based on the drug distribution ratio in each particle.

From **Figure 5.3.1-3**, the drug release profile of PTX from each individual polymer is shown for a duration of 10 days. It could be noted that PTX release from PLLA and PMMA showed negligible release of only up to 10% at day 10. For PLGA, the release of PTX is relatively low from day one to seven but experiences a burst release subsequently after day seven. Finally for EVA, there is a burst release of PTX at day one and has relatively

consistent drug release from day one onwards. Through these release profiles, the summation release was approximated based on the drug ratio in each polymeric combination. The calculated drug release of PTX based on individual polymers can be seen from **Figure 5.3.1-3 (b), (c) and (d)** and compared based on the actual release of PTX from the respective Janus particles. For the EVA/PLGA combination, the trend of the release of PTX can be correlated to the release of PTX in EVA alone due to the high partiality of PTX in EVA. As for PMMA/PLGA and EVA/PLLA, the release of PTX based on the calculations of the single-polymers closely correlated to the release of PTX from the Janus particles. Hence, it can be concluded that the release of PTX from Janus particles would be determined by the % encapsulation proportion as well as the individual property of the polymer.

5.4 Discussions

DOX and PTX have been popular drugs administered for breast cancer due to their well-known effectiveness in combinational therapy[14]. For single dose drugs, the drug accumulation near tumor sites are usually too low to achieve an effective dose. Therefore, higher drug concentrations are usually administered to compensate. However, the administration of a high concentration of these drugs usually results in the onset of severe side-effects. Some types of side-effects involve nausea, vomiting, loss of appetite and even prolonged heart damage for the case of DOX[15]. While it is also known that those dosage of drug administered can be lowered with combinational therapy, the specific reduction in dosage is usually debatable[16]. Another point of view about the combinational therapy of DOX and PTX can be found in the sequence with which the drugs are being administered. In two separate clinical studies conducted, improved synergy and reduction in side-effects was reported when DOX was infused first followed by PTX. Holmes et al. reported a dramatic reduction in the onset of stomatitis in patients receiving 24 hours DOX infusion followed by infusion with PTX. 7 in 10 patients reported grade 2 or 3 stomatitis when PTX was administered first followed by DOX. Only 1 in 10 reported grade 2 stomatitis when DOX was administered followed by PTX[9]. Gehl et al. had also reported on the improved

synergistic effects when DOX was first administered followed by PTX with an overall response rate of 83% [14].

For the EVA/PLGA, EVA/PLLA and PMMA/PLGA Janus particles fabricated, the release profiles showed characteristic differences in terms of their sequence of release. By comparing the rate constants in

Table 11, it can first be noted that both DOX and PTX have similar values (5.9916/h and 5.7610/h respectively). This data indicates that both DOX and PTX are released simultaneously throughout the release study. In contrast, the release from PMMA/PLGA particles indicated a sequential release profile due to the contrasting release constants of DOX and PTX (5.8595/h and 1.5376/h respectively). For EVA/PLLA, the release rates (3.2744/h) of PTX appeared to be in-between that of those released from EVA/PLGA and PMMA/PLGA. With regards to synergistic treatment with DOX and PTX, demonstrating the different types of release profiles with each of these polymeric combinations shows that polymer choices for fabricating Janus particles are important for their applications. It is predicted that the PMMA/PLGA combination would show better synergistic effect with reduced onset of side effects over the other three combinations in the treatment of breast cancer cells due to the sequential release profile of DOX followed by PTX.

In addition, the modelling of the release profiles with the Higuchi model could provide several explanations to the discrepancies in the cumulative release amounts found in **Figure 5.3.1-1**. For example in the EVA/PLGA combination, the EVA/PLGA Janus particles were found to have almost double the cumulative release amounts as opposed to the calculated individual ratio of EVA and PLGA individual particles. This difference is also observed in EVA/PLLA and PMMA/PLGA to a certain degree. In the Higuchi model, the fundamental basis is the pseudo-steady state of the release of entrapped drug molecules. This pseudo-steady state results from the concentration gradient of the drug which

increases from the outer surface to the inner core of the particles. From the curve fitting of the release profiles in the Higuchi model in **Figure 5.3.1-2**, it was concluded that the release profiles follow the Higuchi model. In **Figure 4.6.1-1**, it is observed that EVA/PLGA Janus particles split at their bifurcation first, followed by EVA/PLLA and PMMA/PLGA showing no splitting. This splitting for EVA/PLGA is most pronounced at day 4, where almost all the particles have dissociated. In relation to the Higuchi model, the release of drugs from the EVA/PLGA Janus particles from the first four days most likely occurs in the external spherical region and hence a concentration gradient would be formed from the outer to inner regions of the particle. Upon the splitting of the EVA/PLGA Janus particles, a fresh unexposed surface that was formerly the interface between EVA and PLGA is now exposed and subjected to drug release. Because of this, the drug release is accelerated by a second stage burst release. This hence results in the considerably higher cumulative release of PTX from the EVA/PLGA Janus particles as opposed to the individual particles. As the EVA/PLLA Janus particles take longer to split at the bifurcation, this second stage release is less pronounced and similarly for PMMA/PLGA.

All in all, this further validates **Hypothesis #1** and **Sub-Hypothesis #2** where drugs can be loaded within Janus particles of high γ_{13} without losing the Janus anisotropy. Additionally, the partitioning of drugs is also governed by the degree of hydrophilicity of the polymer in relation to that of the drug. It was demonstrated that depending on the Janus particle combination, drugs can be selectively encapsulated within a single particle. In doing this, this would minimize or prevent any drug to drug interactions that could otherwise affect drug release kinetics. Furthermore, the ability to load two drugs into a single particle can improve the encapsulation efficiencies of each respective drug by using a Janus particle, as opposed to encapsulating two drugs into one particle. This is more notable when the chosen drugs have disparate solubilities. The release kinetics behavior of both drugs Doxorubicin and PTX were found to be significantly different depending on the type of polymeric combination used in the Janus particle. Sequential, simultaneous or a combination of sequential and simultaneous release profiles can be obtained based on the chosen polymer combination.

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Chapter 6

Selective Encapsulation of Superparamagnetic Iron Oxides (SPIONs), Selective Surface Functionalization and Potential Applications

In this chapter, the ability to selectively encapsulation diagnostic materials as well as selectively modifying the surfaces of these Janus particles will be explored. The significance of the results shown here demonstrate that in conjunction with drugs, diagnostic materials can also be selectively encapsulated, which gives these Janus particles the potential to be viable as theranostic agents, where they can confer both diagnostic as well as therapeutic effects. The selective surface functionalization of these Janus particles with calcium-based compounds also demonstrates how the surfaces of these Janus particles can be selectively functionalized without the need for masking or any other external intervention as previously needed by other methods. This is all done through surface-charge mediation and the interaction of the calcium ions to the charge on the surface of the polymer. All in all, the ability to realize these Janus particles as potential theranostic agents is hinged on how all these features can be added through the single-step solvent emulsion process.

*This section published substantially as [1]

6.1 Justification for Design of Hybrid Janus Particles Encapsulated with SPIONs*

Sub-Hypothesis #2: *Drugs can be selectively encapsulated within each face of the Janus particles depending on the degree of hydrophilicity of the respective polymer faces.*

Superparamagnetic iron oxide nanoparticles (SPIONs) are single-domain magnetic particles without magnetic memory. On its own, these particles have no magnetization but only become magnetized upon being introduced into an external magnetic field. These particles are known to be MRI-contrasting agents, biocompatible and easy to fabricate. Hence, they make excellent candidates for use in a wide variety of medical applications[2].

In the current landscape, magnetic polymeric Janus particles exhibiting magnetic properties in one face have been showcased before. These are generally known as inorganic-polymer Janus particle hybrids where the magnetic face is usually inorganic while the polymer face has been synthesized with a wide range of polymers[3-6]. In these hybrids, an inorganic seed, usually magnetic in nature, has to first be synthesized usually through sol-gel or co-precipitation methods. Subsequently, part of this magnetic seed needs to be masked selectively followed by polymerization of polymers on the exposed face. In the perspective of the fabrication process, these processes required are usually highly laborious, as polymerization reactions and crystal-seeded growths have to be carefully controlled. Additionally, subsequent steps such as UV-crosslinking are often needed.

Prior to our published work[1], there were no existing methods that could create hybrid magnetic Janus particles within a single-step process that was easily scalable. In this section, the ability to selectively encapsulate diagnostic materials such as SPIONs was demonstrated with each of the three Janus particles (EVA/PLGA, EVA/PLLA and PMMA/PLGA). This demonstration utilizes the preferential interaction of SPIONs with the more hydrophilic polymer and facilitates selective encapsulation of SPIONs within

a Janus particle face. In doing so, it is hoped that this demonstration and understanding can serve as building blocks for future research involve hybrid magnetic Janus particles.

6.1.1 Encapsulation behavior of SPIONs in EVA/PLGA, EVA/PLLA and PMMA/PLGA*

The independent parameters used in the encapsulation of SPIONs was the optimal polymer weight ratios as shown in **Table 4** and the amount of SPIONs. The SPIONs were standardized at 100mg for each polymer batch. The amount of SPIONs chosen corresponds to the work by Poh et al., approximately 100 mg of SPIONs were used in samples of 200-300mg of polymer by weight. This amount of SPIONs was also found to be optimal for directed movement with an external magnetic field due to the sufficiently high magnetic saturation measured from encapsulated SPIONs within a polymer matrix[7].

SPIONs were added to the initial polymer solution of each respective combination prior to fabrication and left to emulsify. **Figure 6.1.1-1g-i** shows the encapsulation behavior of SPIONs within each respective Janus particle combination. Additionally, both DOX and PTX were added into the polymer solution to determine if the Janus particle could encapsulate all three entities. Based on **Figure 6.1.1-1g-i** it was discovered that the SPIONs demonstrated selective encapsulation similar to that of DOX where there was distinct partiality in one face of the Janus particle. Hence, it had a significant preference for one polymer as opposed to the other. Interestingly, no SPIONs were detected and harvested from the aqueous solution through sweeping with a magnet. This hence indicates that virtually all the SPIONs had been entrapped within the polymers and were similar with the findings from Poh et al.[7].

Initially, it was predicted that the SPIONs would be homogeneously dispersed within the Janus particle. But the findings in **Figure 6.1.1-1** indicated that there was a preference of the SPIONs to encapsulate within one face of the Janus particle and not the other. It was assumed that the reason behind this selectivity was due to the preference of SPIONs being encapsulated in one polymer and not the other i.e. in PLGA and not EVA. However, upon fabricating SPION-loaded single polymer particles, it was shown that each individual polymer could encapsulate the SPIONs with virtually 100%

encapsulation of all SPIONs. It was only in the presence of the other polymer that this partiality occurs. In addition, it indicates an internal driving force that causes the SPIONs to prefer one type of polymer to the other. To further understand this driving force, the degree of hydrophilicity of each polymer would need to be taken into account. With reference from **Figure 5.1.3-1**, the contact angle of water to each respective polymer film was measured. It was determined in the previous section that the higher the contact angle with water, the more hydrophobic the surface. Hence between EVA/PLGA for example, the PLGA polymer would be significantly more hydrophilic than the EVA polymer.

Figure 6.1.1-1 (a) Schematic of SPION encapsulation in polymeric Janus particles, (b) Magnetic behavior of drug and SPIONs loaded EVA/PLGA Janus particles (c-f) SPION encapsulation in PLLA, EVA, PLGA and PMMA, (g) EVA/PLLA, (h) EVA/PLGA, (i) PMMA/PLGA Janus particles

Additionally, it was also noted in previous literature that the fabrication of these SPIONs in an aqueous medium causes the formation of OH- groups on the surface of these SPIONs[8]. Hence, these SPIONs would be agents capable of significant H-bonding with the carbonyl groups on the respective preferred polymer. To further confirm this

hypothesis, selective dissolution was conducted on the SPION-loaded Janus particles to determine which polymer the SPIONs have encapsulated.

Figure 6.1.1-2 Selective dissolution of SPION-loaded (a) EVA/PLGA, (b) EVA/PLLA and (c) PMMA/PLGA Janus particles

From **Figure 6.1.1-1**, the results further confirm the selective encapsulation of SPIONs with the more hydrophilic polymer. The remaining dark colored hemispheres are confirmations of the encapsulation of the SPIONs in PLGA and PLLA respectively (**Figure 6.1.1-2a&b**). For **Figure 6.1.1-2c** the presence of transparent hemispheres in the PMMA/PLGA combination further confirm the encapsulation of these SPIONs into PLGA, leaving behind transparent PMMA.

This demonstration shows that **Sub-Hypothesis #2** can also be extended to the selective encapsulation of magnetic materials. It also serves as the building blocks to possibility use other diagnostic tools from different classes of materials within these Janus particles. Drug encapsulation studies will be shown in the following section.

6.2 SPION-Loaded Janus Particles as a Potential Treatment for Diabetes

Before showcasing further studies of drug encapsulation into SPION-loaded Janus particles, a bit of background behind the rationale of this work is shown in this section.

6.2.1 Background

Diabetes has been one of the most challenging diseases to treat in the 21st century. It is estimated that diabetes affected 415 million people in 2015 and is expected to rise to 642 million by the year 2040[9]. While a wide variety of treatments exist for diabetes, one of the most attractive therapeutic methods is the implantation of functional islets into a patient[10]. Islet transplantation allows for the restoration of normal carbohydrate metabolism and has potential to restore a patient's normal way of life. However, islet transplantation faces a high rate of failure, particularly because of immune rejection and a lack of vascularization. Additionally, the inability to observe the development of pancreatic islet development due to islets being implanted within the body creates additional barriers to understanding and potentially improving the development of implanted islets. This hampers the understanding of successful implantation and continues to be a barrier to having islet implantation as a reliable means of treatment[11]. More recently, the implantation of islet cells into the anterior chamber of the eye (ACE) has been a promising means of successful implantation of islets within the human body. Firstly, implantation of islets into the ACE allows for non-invasive and direct observation of islet cell development in-vivo. Secondly, the ACE has been traditionally known to be an immune privileged part of the human body, and hence allows for more sustained growth of foreign implanted cells and sometimes even permanent residence of foreign cells within the ACE[12].

It was shown by Ali et al, that the implantation of islets cells within the ACE showed rapid and complete vascularization within 10 days. Furthermore, these islets that developed within the ACE could respond to both glucose and forskolin, a drug that stimulates glucose responsiveness[13]. Therefore, the ACE has been proven to be a superior site for islet implantation, that could work towards the ideal of the restoration of normal carbohydrate metabolism and normal patient way of life. While implantation of islets cells within the ACE confers immune privilege to the islet cells, there still exists a certain degree of immune response from the body to these implanted cells. Hence, there is still a need for the presence of immunosuppressant drugs to help curb the immune response upon the implanted cells and would hence prolong the lifespan of these implanted cells[14]. Due to the enclosed environment of the ACE, localized

immunosuppression is also advantageous. Conventional intravenous administration of immunosuppressive drugs causes systemic circulation throughout the entire body and potentially compromises the body's immune system. This greatly reduces the body's defence mechanism against a wide variety of common infections[15]. With localized immunosuppression, this reduces the effects of immunosuppressant drugs onto the entire body and instead localizes this effect to the ACE where the implanted islets would be located.

One of the most commonly used immunosuppressive drugs is Rapamycin. This is due to the ability of Rapamycin to inhibit the mammalian target of Rapamycin (mTOR) signaling pathway. The mTOR pathway is an important and prime regulator for most mammalian metabolism and physiology[16], giving Rapamycin a variety of uses including being used as an anti-cancer agent[17]. However, Rapamycin is a highly hydrophobic agent, with a logP of 4.85, and hence has poor water solubility[18]. This causes Rapamycin to have low bioavailability and remains to be a challenge to administer it via oral or intravenous administration. To circumvent this, previous work by Fan et al. demonstrated the encapsulation of Rapamycin into PLGA and PCL microparticles and have been reported to show sustained release of up to 30 days. This sustained release of Rapamycin over the 30 day period was hypothesized to increase the bioavailability of Rapamycin and hence extend the duration and effectiveness of immunosuppression to further sustain the implanted islets. Furthermore, the prevention of immune rejection was demonstrated to be effective throughout the 30 day study for implanted islet cells and Rapamycin loaded microparticles, as opposed to cells implanted with blank particles which showed a rapid decline of islet numbers by 14 days[19].

While the addition of an immunosuppressive drug has been shown to sustain and further prevent immune rejection of islet cells, it would also be advantageous to simultaneously deliver drugs that would stimulate the endogenous production of insulin from the islet cells. These class of drugs are known as sulfonylureas and have been common modes of treatment used for Type II diabetes patients. Sulfonylureas such as Glibenclamide function by binding to ATP-sensitive K^+ channels that are on the cell membrane surfaces of pancreatic beta cells. This binding causes the channels to close and eventually causes depolarization of the beta cells as surplus K^+ ions are unable to exit

the cell. This depolarization triggers the opening of the voltage-gated Ca^{2+} channels and this rise in the intracellular concentration of Ca^{2+} causes the exocytosis of granulated insulin and hence the increased secretion of insulin[20]. Hence, it would be an attractive proposition to design a drug carrier that would incorporate both Glibenclamide and Rapamycin for its simultaneous release within the ACE for both the preservation of implanted islets as well as its stimulation for the release of insulin. Additionally, it would be even more advantageous to have the carrier capable of triggered release, which would only cause Glibenclamide to be released on demand and further prevent various side-effects such as hypoglycemia.

For successful treatment and management of diabetes through this method, several goals were envisaged:

1. The sustained release of Rapamycin is an important aspect so as to improve the survivability of islets cells within the ACE by reducing occurrences of immune rejection.
2. Simultaneous delivery of both Glibenclamide and Rapamycin would be essential in order to fulfill the first goal, as well as to sensitize and increase the production of insulin on-demand i.e. after a meal.
3. Selective encapsulation of both drugs within Janus particles would help realize the goal of mediated delivery of Glibenclamide as the means of mediation would not interfere with the release of Rapamycin.

To realize these goals, Janus particles of EVA/PLLA selectively encapsulated with Rapamycin, Glibenclamide and SPIONs was devised. It is predicted that the Rapamycin (logP 4.85) would be selectively encapsulated within EVA while the more hydrophilic Glibenclamide (logP 3.78) would be selectively encapsulated within PLLA. This deduction was made based on the contact angle measurements in **Figure 5.1.3-1** where the higher contact angle found with water and EVA indicates its higher degree of hydrophobicity, and hence the preference of Rapamycin in the EVA. Similarly, the lower contact angle in PLLA would favor the encapsulation of Glibenclamide within PLLA. The addition of SPIONs as investigated in relation to **Figure 6.1.1-2** would also indicate that the encapsulation of SPIONs would be within PLLA and would also contain majority of the Glibenclamide. An important additional advantage of these Janus particles in this application is the ease of quantification of drugs within the ACE.

While it would be similar to encapsulate two different batches of Rapamycin and Glibenclamide in two particle batches, calculations for particle mixing would be required. More importantly, the colorless nature of both Rapamycin and Glibenclamide would render it almost impossible to quantify accurately the actual proportion of particles that enter the ACE as this is normally done through manual counting of particles that make it to the ACE. Due to the potent nature of Glibenclamide, this could further complicate matters as inaccurate quantification of Glibenclamide within the ACE could cause an excessive or inadequate release for islet sensitization and subsequently insulin release. With the use of Janus particles, this greatly reduces the complexity for quantifying the available reservoir of drugs that have entered the ACE. As it was shown previously that the ratio of drugs within the Janus particles are all similar within each batch, simple counting of particles would suffice for drug quantification. Furthermore, the ease of particle identification visually would be advantageous to this process.

The use of SPIONs in hyperthermia-regulated delivery is by no means a novel method[21]. However, the usage of SPIONs for selective drug release while simultaneously releasing both drugs would be a novel and a potentially effective means with which to manage diabetes. The added advantage of triggered release would be highly attractive due to the ability to cause increased insulin secretion upon meal ingestion and suppressing insulin secretion when it is not needed. This would reduce the possibility of hypoglycemia caused from excessive insulin within the body. Additionally, the means of hyperthermia regulated delivery allows for reusability throughout the duration of the particle's implantation within the body.

Figure 6.2.1-1 Schematic of the working principle of RF-mediated delivery of both Rapamycin and Glibenclamide

In this mode of treatment, Glibenclamide and Rapamycin would be selectively encapsulated within PLLA and EVA respectively. SPIONs would also be found in the PLLA face. Upon the application of a specific radio-frequency, the encapsulated SPIONs within PLLA would vibrate and generate heat. This heat would cause a localized increase in temperature in the PLLA face and with sufficient heating, this temperature would reach beyond the glass transition (T_g) of PLLA, which is found to be around 60°C[22]. By reaching the T_g for PLLA, we hypothesize that this polymer chain relaxation would cause an increase in the diffusion of Glibenclamide from the particles to the surrounding medium of the ACE. The removal of radiofrequency would cease the vibration of SPIONs and cause the polymer to cool down and reharden, which would cease the increased diffusion of Glibenclamide from PLLA. Hence, this creates a possibility to only release Glibenclamide upon demand by inducing the radiofrequency after the patient has consumed a meal (**Figure 6.2.1-1**).

As a point of reference, EVA/PLLA Janus particles were encapsulated with 6mg of Rapamycin, 20mg of Glybenclamide and 100mg of SPIONs. In comparison, PCL/PLGA Janus particles containing the same amount of all the respective drugs and diagnostic agents were fabricated. Regarding the morphology, the particles formed with EVA/PLLA are much more resembling of Janus particles as opposed to the highly patchy particles formed by PCL/PLGA. Hence, this also shows the importance of the polymer interfacial tension in being able fabricate Janus particles as the patchy particles might cause inconsistencies due to the possible heating within the opposing EVA side, which could release the drugs encapsulated within EVA instead.

Figure 6.2.1-2 (a) EVA/PLLA and (b) PCL/PLGA Janus particles fabricated with Rapamycin, Glibenclamide and SPIONs

Table 12 Encapsulation Efficiency and Selective Encapsulation of Rapamycin and Glibenclamide in PLLA/EVA Janus Particles

	Overall EE%	Selective EE%	
	EVA/PLLA	EVA	PLLA
Rapamycin	41.5 ± 1.5	75.7 ± 5.7	24.3
Glibenclamide	65.4 ± 6.5	66.5 ± 8.2	33.5

From **Table 12** it was also determined that the majority of Rapamycin (80%) was found to be encapsulated within EVA. However, the encapsulation of Glibenclamide was mostly found to be encapsulated within the EVA face. While these findings for Glibenclamide are not as expected, the encapsulation of SPIONs within the PLLA face could have affected the selective EE% of Glibenclamide within the PLLA face due to hydrophilic-hydrophobic interactions between Glybenclamide and the SPIONs. It is reasoned that since the triggered release of Glybenclamide is based on the heating action of the SPIONs, most of the release of Glybenclamide triggered by the RF will be released from the PLLA. On the other hand, passive release of Glybenclamide and Rapamycin would come from the EVA. This passive release of Glybenclamide would help to maintain a basal level of insulin, which is required for consistent glucose levels during fasting[23]. This in conjunction with the selective encapsulation of SPIONs in PLLA could potentially facilitate the impulse delivery of Glibenclamide on-demand through a RF-mediated treatment.

6.3 Optimization of Glybenclamide Release from EVA/PLGA Janus Particles + SPIONs

In the ideal scenario, the background release of Glybenclamide should be minimized such so as to prolong the delivery life-span of the particles. On the other hand, the release of Glybenclamide should be high enough such that a basal level of insulin can be maintained. Preliminary drug release studies of Glybenclamide from EVA/PLLA Janus particles in **Figure 6.2.1-2** indicated reaching ~50% cumulatively while approaching 80% within the next 24 hours. As Glybenclamide is known as a potent sulfonylurea, over-exposure to this drug can lead to hypoglycaemia, which is the depletion of glucose within the blood. This condition is potentially life-threatening and needs to be prevented. Based on the EE% studies of Glybenclamide, the approximated daily dose of Glybenclamide released through the Janus system is ~7mg. This is well above the recommended dose of 2.5mg daily[24]. Hence, there still is a need to lower the daily dose by achieving an overall lower and sustained release of Glybenclamide.

It is predicted that by lowering the amount of SPIONs within the Janus particle, the overall release of Glybenclamide can be managed. As more SPIONs are encapsulated within the particles, the SPION clusters act as plasticizers or fillers within the polymer matrix. It has been shown by previous authors that materials with a plasticizing effect increases the amount of drug released. This is because these molecules fill in the gaps in between the polymer chains and promotes diffusion of drugs due to a greater influx of water from the surrounding medium[25]. As these particles do not need to be magnetically controlled for this application, a lower magnetic saturation due to the lowering of the concentration of SPIONs would not be critical.

6.4 Selective Surface Functionalization of Janus Particles*

In the previous sections, selective encapsulation of different drug molecules and magnetic materials was demonstrated. While most of these works involved intrinsic encapsulation within the Janus particles, other interesting behavior of these Janus particles include being able to be selectively functionalized externally without requiring direct control. In previous literature, the method of selectively coating a surface of a material usually requires more direct methods such as sputtering and pickering emulsions[26, 27]. While these methods have potential, these methods require

Figure 6.2.1-1 Fabrication schematic for selective functionalization of Janus Particles with calcium-based compounds through and within the single-step solvent emulsion process

complicated steps and strict control. Furthermore, the conditions (high temperature and use of solvents) that these techniques are carried out under are usually not suitable for such biodegradable and biodegradable polymers.

In this section, the ability to selectively functionalize these Janus particles by utilizing the previously used solvent emulsion method is demonstrated. The distinct advantage of such a method is the cost-effectiveness as well as the simplicity. In this embodiment, the ability to coat different kinds of calcium compounds such as CaCO_3 and CaP are shown. This is done through a co-precipitation method, where two solutions that are calcium and carbonate or phosphate based are mixed together, causing the precipitation of either CaCO_3 or CaP . Upon precipitation of these compounds within the solution during the solvent evaporation step, the precipitated chemicals are then deposited selectively onto the Janus particle. As a form of demonstration, both PTX and Doxorubicin were also added ad before in the polymer solution to demonstrate the potential for an all-in-one synthesis step (**Figure 6.2.1-1**).

Figure 6.2.1-2 (a-c) The formation of CaP onto one face of the Janus particle. From the light microscope images, the formations appear to be on the EVA face. (d-f) Similarly for CaCO_3 , the formation of the tiny spheres, which are confirmed by EDX to be calcium-based are formed entirely on the EVA face.

Figure 6.2.1-2 shows the selective functionalization of EVA/PLGA Janus particles with both CaCO_3 and CaP. Both these batches of particles were analyzed under EDX for Ca and P elements on the surface of the particle. For the particles coated with CaP, strong peaks for Ca and P were detected from the EDX and are shown in the mapping image in **Figure 6.2.1-2b**. The highlighted portions of each element overlap with one another as well as the crystal formations on the particle themselves. As for the CaCO_3 coated particles, the structure of the coating appears more spherical in nature and similarly to the CaP particles, the coating appears on one face of the particle distinctly.

To identify which face the coating is forming on, light microscope images were done on the particles. Basing the previous establishment of the selective encapsulation of Doxorubicin within the EVA/PLGA Janus particle to be in PLGA, the presence of the tiny spheres and Ca crystals on the transparent face of the Janus particle indicate that this formation occurs selectively on the EVA face. Interestingly as well, the PLGA droplets that appear to be kinetically trapped within the EVA face that are found on the

surface do not appear to have any CaCO_3 on their surface. This hence indicates that the coating is indeed preferentially deposited onto the EVA face.

This toposelective coating of calcium based compounds on the unmasked Janus particles can be attributed to the zeta potential of the respective faces. In the EVA/PLGA Janus particles, it is hypothesized that the zeta potential of EVA[28] as opposed to PLGA[29] is more negative when in a PVA surfactant solution. Because of this, the Ca^{2+} ions first attach to the more negative EVA and upon the addition of the secondary compound, the insoluble calcium compounds precipitate on that polymer as a result[30]. Due to explorative nature of this work, more research will be expanded into this area and is shown in **Chapter 8**.

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Chapter 7

Janus Superstructures

A superstructure is defined as a structure that is an extension of an existing structure. In this chapter, the fabrication of Janus superstructures with augmented structural features on top of the traditional dual-face morphology are demonstrated. Additionally, the fabrication of these Janus superstructures are done within this simple single-step process without requiring additional steps for the fabrication. While some of these structures have been done previously in literature, they often require multi-step processes that need to be highly controlled and further complicates reproducibility. It is hypothesized that the interfacial tension of the polymers used are an important parameter in creating tolerance to the thermodynamic changes that these modifications could potentially introduce. The goal of this chapter is to showcase the fabrication of these Janus superstructures within this facile method and showcase the multiple applications for them.

7.1.1 Janus Superstructures*

Hypothesis #3: *Existing parameters can be blended into the solvent emulsion method in the fabrication of Janus superstructures. The values of the interfacial tension data would be pivotal to the fabrication as well as the applications of these superstructures.*

It was demonstrated succinctly in previous chapters of the fabrication of traditional Janus particles that are comprised of two polymer faces joined at a bifurcation. Janus particles comprised of various polymeric combinations were shown, as well as the ability to load drugs and other diagnostic materials selectively. By showing how the polymer-polymer interfacial tension data is critical in producing Janus particles, more confidence in Janus particle exploration with different parameters can be done. As it is not practical to study every thermodynamic effect that each parameter would have, understanding the polymer-polymer interfacial tension gives sufficient confidence for future work pertaining to these parameters. In literature, the blending of parameters such as addition of osmogens and sub-micron particles to the solvent emulsion step has created particles with additional structural features. For example, the addition of osmogens by Kharal et al. showed the formation of hollow-core particles, with the modulation of core size through the amount of osmogen added[1]. Khung et al. also showcased the encapsulation of dye-loaded submicron particles into neat PLGA particles as a means for secondary drug release. This was done through the use of a water-oil-water emulsion as opposed to an oil/water emulsion[2]. By blending some of these parameters in, it was hypothesized in **Hypothesis #3** that Janus superstructures can be formed. These superstructures would have the original Janus morphology but having an additional structural feature. In this chapter, the superstructures are Janus/Hollow Core (JHC), Janus /Coreshell hybrid (JC) and Janus/Submicron particle hybrid (JS).

Figure 7.1.1-1 Formation of Janus superstructures through the facile solvent emulsion method. Only slight modifications are required within the polymer solution preparation.

7.1.2 Janus/Hollow Core Superstructures*

The formation of JHCs was done by first making a polymer solution of the appropriate weight ratio of both polymers and subsequently adding and homogeneously dispersing an osmogen to the polymer solution. Upon emulsification of this osmogen-added polymer solution, each polymer droplet contains a certain amount of NaCl. This salt creates osmotic pressure within the polymer droplet due to the higher concentration of salt within the particle as opposed to the aqueous phase. Because of this concentration difference, there is an influx of water from the external aqueous phase into the core of the polymer droplet in order to balance out the salt concentration. As the polymer solvent (DCM) evaporates, the polymers phase separate into their respective faces and forms around the inner water pocket, creating a hollow core when the harvested particles are lyophilized.

Figure 7.1.2-1 Schematic of JC formation through the addition of NaCl. The presence of NaCl within the coacervate phases causes osmotic pressure build up within the particle and causes water from the external aqueous phase to influx into the polymer droplet, creating a water pocket

To first demonstrate the importance of the interfacial tension between the two polymer choices, JHCs made of PCL/PLGA and EVA/PLGA were made. As a point of comparison, the interfacial tension of EVA/PLGA is $\gamma_{eva/plga} = 15.323 \text{ mN/m}$ whereas that of PCL/PLGA is $\gamma_{pcl/plga} = 0.65 \text{ mN/m}$. It can be seen that the interfacial tension of PCL/PLGA is dramatically lower than that of EVA/PLGA. Fabrication of PCL/PLGA and EVA/PLGA JHCs showed that the resulting particles of PCL/PLGA have lost resemblance to the Janus morphology while the EVA/PLGA particles demonstrate Janus morphology (**Figure 7.1.2-2**). Hence, this demonstrates the importance of the interfacial tension in Janus superstructure fabrication and how the criteria of the interfacial tension is a simple and elegant means to decide the success outcome of Janus superstructure synthesis.

Figure 7.1.2-2 (a) EVA/PLGA JHCs and (b) PCL/PLGA JHCs and the loss of Janus morphology upon the addition of osmogen for PCL/PLGA JHCs. The Janus morphology for EVA/PLGA however is well retained

7.1.3 Varying of Osmogen Concentration in JHC Formation*

For the formation of JHCs, the amount of osmogen added to the initial polymer solution is an important parameter in the formation of the inner hollow core. In principle, the more osmogens are added, the more osmotic pressure buildup in the particle occurs. Therefore, there would be a greater influx of water into the core of the particle and a larger pore results.

Figure 7.1.3-1a-c shows the effect of varying salt amounts based on w/w that was added to the polymer solution and their corresponding SEM cross-sectional images. For identification purposes, RhB was added as well to the polymer solution in order to confirm the formation of the Janus morphology. Starting at 10% w/w of salt, the pores within the particle appear to be sparsely dispersed around the particle. Increasing the amount of 15% w/w shows the formation of a larger pore within the center of the particle but still having numerous smaller pores around the vicinity of the large pore. Finally, adding in 20% w/w of salt causes the formation of a large uniform pore in each individual particle. From these results, it can be concluded that a 20% w/w of salt within

the polymer solution is the most ideal amount of salt for pore formation in these Janus particles.

Figure 7.1.3-1 Light microscope images of EVA/PLGA Janus particles formed when adding salt at (a)20%w/w, (b) 15% w/w and (c) 10% w/w. Corresponding cross-sectional images of particles in (a), (b) and (c) are shown at (d), (e) and (f) respectively

Hence, this demonstrates the ability to simply create JHC through a simple tweak of adding salt to the polymer solution and being able to optimize its morphology through varying the salt concentration added to the polymer solution.

7.1.4 Janus-Coreshell Hybrid (JC) Superstructures*

As described in **Section 3.14** on the work by Torza and Mason, their extension of the spreading coefficient theory describes three possible thermodynamically stable particle conformations concerning a two immiscible phase system. Namely, these conformations are no engulfment, partial engulfment and full engulfment. In the full engulfment configuration, it is usually comprised of a core-shell structure, with either polymer as the outer layer and the other polymer as the inner layer. The partial

engulfment structure is known as the Janus morphology, where both phases are exposed to outer environment while both phases meet at a junction (Section 4.2). Based on the current paradigm of emulsion morphology, there is no described observation of a meta-stable state integrating two morphologies i.e. Core-shell and Janus. The work here describes a method in which to combine both the core-shell and Janus structure into a single entity, thereby combining both the core-shell and the Janus particle. By simply altering the concentration of the external surfactant concentration, this meta-stable JC hybrid can be formed. Here, we demonstrate the formation of these JC particles within this single step process at a high yield.

Figure 7.1.4-1 Light microscope image of EVA/PLGA JC particles and various SEM images of the cross-section of the JC particles obtained through selective dissolution with cyclohexane (A), (B) and (C). (D) shows a cross sectional image of the JC particles through cutting

Figure 7.1.4-1 shows the light microscope image of EVA/PLGA Janus particles. RhB was added to the polymer solution for identification of the PLGA component within the particle. The particles have the expected Janus morphology, with two distinct faces as PLGA is dyed by the RhB. Within the core of the particle is a spherical component that is determined to be PLGA due to the pink stain from RhB. Selective dissolution with cyclohexane was employed by dissolving the EVA component while preserving the PLGA. From **Figure 7.1.4-1c**, the remaining PLGA components show an outer hemispheric hollow core with a spherical PLGA particle sitting within the pore. This further confirms the formation of an inner core within the Janus particle of EVA/PLGA.

7.1.5 Proposed Formation Mechanism of JC Superstructures*

For the formation of JC superstructures, a simple adjustment of lowering the surfactant concentration from 2.5% to 0.5% was done. To the formation mechanism, systematic reduction of the surfactants was done and particles were fabricated (**Figure 7.1.5-1**). With concentrations of PVA at 2.5% and 1%, it was noted that the normal EVA/PLGA Janus particles were formed, with selective staining of RhB on PLGA. Upon reaching 0.5% PVA, it was noted that JC particles formed at almost 100%. This sharp change in morphology indicated that a critical point in the interfacial tension data was reached, causing this sudden change in morphology.

To further understand this phenomenon, the interfacial tension measurements were done for this EVA/PLGA polymer solution and the 0.5 % PVA solution. From **Figure 7.1.5-2**, the polymer-aqueous phase interfacial tensions appeared to be gradually increasing upon reducing the surfactant concentration. Additionally, the interfacial tensions obtained were processed with the spreading coefficient theory and in all concentrations, Janus morphologies were predicted (**Table 13**). This discrepancy in the prediction model also establishes that the current models may not be entirely sufficient in particle morphology predictions.

Figure 7.1.5-1 SEM images of (a-c) EVA/PLGA Janus particles fabricated with 2.5%, 1.0% and 0.5% PVA respectively. (d-f) Corresponding light microscope images of particles in (a-c) and (g-i) light microscope images of particles of each respective concentration selectively dissolved in cyclohexane, leaving behind pink PLGA components. Scale bar = 100 μ m.

Figure 7.1.5-2 Graph of polymer interfacial tension on decreasing PVA concentrations.

Table 13 Interfacial tensions, spreading coefficients and morphology predicted and observed at different PVA concentrations.

PVA conc. (wt/v%)	Interfacial Tension (mN/m)			Spreading Coefficient (mN/m)			Morphology	
	γ_{12}	γ_{23}	γ_{13}	S_1	S_2	S_3	Predicted	Observed
2.5	21.04	23.42	11.25	-8.87	-33.22	-13.63	Janus	Janus
1.0	26.61	25.75	11.25	-12.11	-41.11	-10.39	Janus	Janus
0.5	30.88	30.13	11.25	-11.99	-49.76	-10.51	Janus	Janus core-shell

It was postulated that as the interfacial tensions of γ_{12} and γ_{23} increased upon the reduction of the surfactant concentration to 0.5%, it gradually became less energetically favorable for the droplet to maintain a Janus morphology. To compensate for this increase in interfacial tension, some of the polymer was driven into the core of the opposing polymer (i.e. PLGA in EVA) as it is energetically more favorable to form a surface with the opposing polymer as compared to forming a surface with the external aqueous phase ($\gamma_{13} \ll \gamma_{12} \text{ \& } \gamma_{23}$)(**Figure 7.1.5-3**). The resultant structure is therefore a JCS particle, where both the Janus and core-shell morphology were observed. However,

the reasons why PLGA forms the core, rather than EVA, is still not fully understood. Upon visual counting, it was noted that a very high proportion (~100%) of particles showed this structure. This indicates that this is a process highly driven by a thermodynamic driving force and not a kinetic occurrence. Furthermore, it confirms that the interfacial tension has a large role to play in causing this formation. However, we do not enough evidence at this point to establish why PLGA forms the core into EVA and not EVA into PLGA.

Figure 7.1.5-3 (a) Formation of EVA/PLGA Janus particles in 2.5% PVA. (b) During the initial point of particle fabrication in 0.5% PVA, the polymer droplet of EVA/PLGA faces a large increase in the interfacial tension of the polymer and aqueous phases. (c) To reduce this interfacial energy, the system attempts to reduce the total exposed surface area and in this process, causes some PLGA to be hidden within the core, away from the aqueous medium

7.2 Janus-Submicron Particle Hybrid (JS)*

JS particles are traditional Janus particles but with a hollow core that is filled with other smaller sub-micron particles. This is done through microencapsulation of smaller submicron particles within the Janus particle core. Previously, JC particles were demonstrated to be fabricated by adding osmogens to the polymer solution. For the JS particles, a W/O/W emulsion was employed. In W/O/W emulsions, a small amount of water is first added into the initial polymer solution and then emulsified. This creates tiny droplets of water that are dispersed within the polymer solution. Upon emulsification of the primary emulsion into the aqueous phase, the polymer solution is broken up into tiny coacervate phases and within each coacervate droplet are water droplets within the polymer solution. As the solvent evaporates, the surrounding polymer solution reaches cloud point and the polymer precipitates. Due to the presence of the water within the polymer, the water forms a cavity within the particles when they are lyophilized.

It was previously shown possible to be able to microencapsulate smaller submicron particles within a larger outer shell. Therefore, this idea is to further expound this microencapsulation into Janus particles in order to create unique release profiles.

Figure 7.1.5-1 Schematic of working principle of JS particles. EVA/PLGA particles having a higher IFT would split first, causing the release of the encapsulated payload. PMMA/PLGA on the other hand having a lower IFT would stay intact for a longer period of time than EVA/PLGA

The fundamental principle of the JS particles operates on the understanding of the degradation behavior of these Janus particles. More specifically, the effect with which

the polymer combination has on the degradation behavior. In **Figure 4.6.1-1**, it was demonstrated that EVA/PLGA, EVA/PLLA and PMMA/PLGA showed varying amounts of time before the two faces begin to split from the centre. The EVA/PLGA and EVA/PLLA particles were broken in half within four to seven days while PMMA/PLGA particles were still well intact up to 14 days and beyond. This behavior was attributed to the interfacial tension of the polymers used where the lower the interfacial tension, the stronger the bond between the two faces and hence the retention of the Janus structure for a longer period of time. By using this concept, we hypothesized that it would be possible to design a time-controlled payload release system where the microencapsulated submicron particles could be release to their target environment after a certain amount of time. This would be achieved through a selection of the polymeric combination for microencapsulation based on their interfacial tension. We hypothesize that the smaller the interfacial tension, the longer it takes for the Janus structure to split and vice versa.

As a proof-of-concept, the payload material chosen was C6-loaded PLLA submicron particles which were then loaded into PMMA/PLGA and EVA/PLGA particles. **Figure 7.1.5-2** shows light microscope and cross-sectional SEM images of EVA/PLGA and PMMA/PLGA particles with loaded C6. While the size of the payload particles were first measured to be approximately one to three μm , the appearance of significantly larger droplets of C6-PLLA within both EVA/PLGA and PMMA/PLGA could be due to the coalescence of the C6-PLLA particles during fabrication. However, it could still be confirmed that the payload particles were still loaded within the core of the particles despite the change in size.

Figure 7.1.5-2 Light microscope images of (a) EVA/PLGA and (b) PMMA/PLGA JS particles with C6-PLLA as payload particles. SEM cross sectional images of (c) EVA/PLGA and (d) PMMA/PLGA C6-PLLA loaded particles

A time release study for these JS particles made of EVA/PLGA and PMMA/PLGA was carried out over a period of five days (**Figure 7.1.5-3**). At day one, it was noted that PMMA/PLGA was well intact while EVA/PLGA was showing signs of splitting on some particles. Moving to day two, 100% of the EVA/PLGA particles had completely dissociated and release its payload. However, PMMA/PLGA was still intact. It was only from day three where PMMA/PLGA particles started to split and release their payload into the surrounding environment. Upon day five, close to 100% of the PMMA/PLGA particles had dissociated. This shows the ability to utilize the degradation behavior of two different types of Janus particle polymer combinations for time-based payload release.

Figure 7.1.5-3 Degradation profile for (a) PMMA/PLGA and (b) EVA/PLGA. The presence of yellow microparticles within the surrounding media indicated that the encapsulated sub-micron particles had released into the surrounding media after the Janus particles split open

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Chapter 8

Conclusions and Future Recommendations

This chapter gives an overall summary of the results obtained in the previous chapters of this thesis and gives insight into the extent with which the objectives were fulfilled. All the results would be culminated and framed to give an overall yet detailed view of the obtained results, their current implications as well as any possible future recommendations and works that could be done to further the work of this thesis. Briefly, this thesis showcases the production of Janus particles through a cost-efficient yet efficient method of synthesis known as solvent emulsion. While there have been reports in literature for the production of Janus particles through this method, these studies often halt at the fabrication component. Various exploration and work into drug encapsulation and drug release was not expounded into. This was soon found to be caused to the intrinsic lack of insight and understanding as to how Janus particle anisotropy could be maintained despite the addition of drugs or diagnostic materials. In this thesis, the various important parameters for successful Janus particle fabrication with drug encapsulation were showcased along with drug release studies. Finally, applications for these Janus particles through the treatment of diabetes as well as Janus Superstructures were also demonstrated.

8.1 Conclusions

In this work, some of the major gaps in the field of Janus particle and drug delivery were addressed in **Chapter 2**. While it was previously demonstrated in previous reports that these Janus particles could be easily fabricated through a cost-efficient single-step solvent emulsion process, the very same report also showcased an inherent issue where the Janus morphology was lost after the addition of drugs during fabrication. Hence, there was a need to understand and overcome this inherent issue in order for Janus particles to be relevant in the field of drug delivery.

In this thesis, it was first demonstrated that Janus particles which were comprised of different polymeric combinations could be fabricated (**Section 4.3.1**). By tweaking the weight ratios of the polymers in each respective combination, Janus particles could be produced at specific weight ratios (**Table 4**). Some polymeric combinations exhibited a high tolerance of vary weight ratios for successful Janus particle formation while other combinations were highly sensitive to only one single ratio. To establish a further understanding of this, the mechanics of Janus particle formation was investigated in **Section 4.2**. Through Harkin's equation and the Spreading Coefficient theory the mechanics of Janus particle formation were determined to be thermodynamically driven, where the satisfaction of various thermodynamic factors would be the deciding indication of the end particle morphology. Additionally, it was also determined that the formation of Janus particles were kinetically driven, where varying certain parameters, such as the stirring speed, would induce quicker solvent evaporation and cause kinetic trapping and non-Janus morphologies despite the fulfilment of the theoretical thermodynamic requirements (**Section 4.5 & 4.6.1**). Within the mechanics study of Janus particles, it was importantly addressed that the polymer interfacial tension was a deciding factor for the tolerance of a particular polymer combination to the formation of Janus particles. The higher the interfacial tension, the higher the tolerance of Janus particle formation. This aspect was particularly demonstrated when the PMMA/PLGA polymeric combination, which has a low polymer interfacial tension, was fabricated at a high RPM of 1500. The particles that were produced from this high RPM did not produce Janus particles, despite satisfying the theoretical spreading coefficient requirements.

Subsequently, drugs were then incorporated into each Janus particle combination through the single-step solvent emulsion process. By adding both Doxorubicin and Paclitaxel, it was shown that the particles produced still maintained their Janus morphology (**Section 5.1.2**). Additionally, it was also determined that drugs were selectively encapsulated at varying degrees within each respective polymer combination. This understanding was explained through contact angle measurements of the respective polymers, where the more hydrophilic polymer (lower contact angle) would have a higher affinity to the more hydrophilic drugs (low logP) and vice versa (**Section 5.3**). In the drug release studies carried out with each of these dual-drug encapsulated Janus particle combinations, it was found that different drug release profiles could be obtained. The drugs could either be simultaneously, sequentially or partially sequential and simultaneously released. This unique ability was attributed to both the degree of encapsulation of each specific drug in each specific polymer face (**Section 5.3.1**).

On top of drug encapsulation, it was also demonstrated that various diagnostic materials such as Super Paramagnetic Iron Oxides could be selectively encapsulated into these Janus particles (**Chapter 6**). This selective encapsulation was similarly attributed to the high degree of hydrophilicity of the iron oxides due to the presence of –OH groups on its surface and its ability to form H-bonds with the carbonyl groups found on the polymers (**Section 6.1.1**). These SPION-loaded Janus particles were then further expounded to have potential in managing diabetes through hyperthermia-regulated delivery of Glibenclamide and passive delivery of Rapamycin to implanted islets within the ACE for triggered insulin release. Additionally, selective surface functionalization of these Janus particles with calcium-based compounds was also demonstrated and could be streamlined into the solvent emulsion process through simple co-precipitation (**Section 6.2**).

Finally, Janus Superstructures were also demonstrated as a derivation from the facile synthesis method. Through the understanding of the polymer interfacial tension, various derivatives from the Janus morphology as Janus Superstructures could be realized as the confidence of the particles maintaining Janus morphology despite various adjustments to the fabrication parameters were done. In summary,

Janus/Coreshell hybrid particles were fabricated through the lowering of the surfactant concentration, and hence increase in polymer/aqueous phase interfacial tension. While not completely understood, it was hypothesized that the drastic increase in interfacial tension values causes the system to be driven to reduce the interfacial tension and hence hides a droplet within the core, which results in a coreshell (**Section 7.1.4**). Janus/Hollow-core hybrids were also shown to be possible through the addition of an osmogen, NaCl, within the polymer solution prior to fabrication. This osmogen creates osmotic pressure within each coacervate droplet and causes the aqueous medium from the external phase to flux into the polymer droplet. As a result, a hollow-core is formed. It was also shown that the understanding of the polymer interfacial tension was important in the success of this Janus/Hollow-core formation as a polymeric combination PCL/PLGA that had a low polymer interfacial tension could not form the hybrid (**Section 7.1.3**). Janus particles encapsulated with sub-micron particles within its core was also demonstrated to be possible. This was done through a W/O/W emulsion process of suspending the sub-micron particles within the water emulsion droplets and subsequently emulsifying this polymer solution. This created a pocket containing the submicron particles and resulted in the presence of submicron particles within the Janus particles. It was also demonstrated that based on the understanding of the polymer interfacial tension, the splitting of Janus particle, and hence release of sub-micron particles would be different for both EVA/PLGA and PMMA/PLGA. Hence, this creates a platform to design payload-based Janus particles that have a tweakable duration of containment and release (**Section 7.2**).

All in all, this thesis demonstrates various aspects of Janus particles in drug delivery that were not shown in previous reports. The understanding behind drug encapsulation in Janus particles and successful Janus morphology would be important platforms to help augment present particle technologies for more challenging applications. For example, the combination of drug encapsulation and anisotropy within the Janus particle is a platform to help realize hyperthermia-mediated drug delivery for diabetes management. In addition, it is hoped that this information would help the realization of new technologies that were made possible through particle anisotropy.

8.2 Future Recommendations

8.2.1 EVA/PLLA SPION + Dual-Drug Loaded Particles for Diabetes Management

The combination of both triggered and sustained release of two drugs simultaneously has been shown in **Section 6.3** to be a promising means to manage diabetes. While it was shown possible to materialize the concept through the fabrication of the dual-drug + SPIONs loaded particles, more testing and optimization would be needed to realize this concept to be a clinically-substantiated technology.

Presently, some issues faced with the current particles are having a low proportion of Glibenclamide encapsulated in the PLLA face, where the SPIONs are located. It is hypothesized that the amount of SPIONs has a direct correlation to the amount of drug that would be encapsulated into the PLLA face. While Glibenclamide would theoretically have a higher preference to be encapsulated in PLLA due to PLLA being more hydrophilic than EVA, and the logP of Glibenclamide being lower than Rapamycin, the encapsulation of SPIONs reduces the maximum drug loading within PLLA. Hence, the amount of SPIONs encapsulated can be tested systematically with different amounts of SPIONs within the Janus particle. The optimal amount of SPIONs can hence be tested to see which concentration would give the best balance of low background release as well as sufficient response to radiofrequency for hyperthermia-regulated delivery.

8.2.2 Janus Coreshell Hybrid for Prolonged Drug Release

In **Section 5.4.1**, the release behavior of the respective Janus particle combinations were demonstrated. While it was shown that both drugs could be released in different manners depending on the polymer combination, a consistent trend noticed between all three particles was that Doxorubicin was releasing rapidly. This is mostly due to Doxorubicin being a hydrophilic drug and the burst release of hydrophilic drugs in

particulate systems is a common issue [1, 2]. In order to circumvent this, various measures such as dual-layered particles were fabricated with the aim of reducing the burst release as well as prolonging the release of the hydrophilic drug over a longer period of time. Usually, the hydrophilic drug would be encapsulated within the inner layer. The outer shell behaves as a protective layer to prevent the rapid diffusion of the drug to the surrounding environment. This was demonstrated by Lee et al.[3] where multilayered particles encapsulated with Doxorubicin showed significantly longer release of Doxorubicin as the inner polymeric core containing Doxorubicin was sustaining the release of Doxorubicin despite the depletion of Doxorubicin in the outer layer.

Figure 8.2.2-1 Schematic of Enhanced Sustained Release of Doxorubicin from Janus Coreshell Hybrids

8.2.3 Calcium-Based Surface Coatings for Calcium-Targeted Mechanisms

The ability to coat calcium selectively onto Janus particles via the single step process is the first step in realizing multi-purposed targeting and drug release vehicles. In work by Bastari et al.[4], the coating of calcium phosphate was demonstrated on PLGA particles. After subsequent coating with calcium phosphate, bone-targeting agents such as bisphosphonates could be conjugated onto the calcium phosphate compounds, giving rise to bone-targeting capabilities for these drug delivery applications. With the studied drug delivery capabilities of these Janus particles along with the selective coating, the ability to use these particles as both drug delivery and calcium-targeting agents could be useful in applications that help to relieve problems from atherosclerosis. Due to the

calcium build up within arteries, the gradual accumulation of these deposits narrows the blood vessels, potentially being lethal when the arteries are fully blocked[5]. While still in its infancy, it is proposed that one side of the Janus particle can be made with calcium phosphate and functionalized with bisphosphonates. While the opposing side of the Janus particle would be loaded to contain statins, which are commonly used drugs to reduce low-density lipoproteins.

8.2.4 In-vitro Testing of DOX and PTX Loaded Janus Particles in the Treatment of MCF-7 Spheroids

As shown in **Chapter 5**, the ability to create dual-drug loaded Janus particles is shown in three polymeric combinations. Between these three polymeric combinations, the drug release profiles of DOX and PTX were found to be different in terms of their sequence of release. For the EVA/PLGA Janus particles, both DOX and PTX were found to be released simultaneously, while that of PMMA/PLGA was found to be sequentially. EVA/PLLA Janus particles were found to be somewhere inbetween where the release of PTX is modulated to be slower than DOX, but still quicker than that of PTX from PMMA/PLGA. It was also established that the synergistic effects of DOX and PTX can be effective based on the sequence of the administration of each drug. For example, it was cited that infusion with DOX followed by PTX had a significantly lesser onset of side effects. Hence, these three drug loaded Janus particle combinations could be tested in-vitro with 3D spheroids of MCF-7 cells, where both DOX and PTX are most commonly used in their treatment.

8.2.5 Janus-Submicron Particle Hybrid in Treatment of Biofilms

The rise of virulent strains of multi-drug resistant bacteria is a rising concern in the world today. Due to the lack of development of new anti-biotics and the increasing resistance of bacteria to presently available anti-biotics, this issue remains to be highly urgent. Recently, Baek et al. had demonstrated the use of lipid-polymer nanoparticle (LPN) hybrids capable of improved targeting and efficacy towards a range of Gram-positive and Gram-negative bacteria[6]. The encapsulation of these LPNs within Janus particles could be the next step in bacterial treatment. By encapsulating a host of

antibiotics within the outer Janus shells, the release of these antibiotics could work to disrupt the biofilms, leaving the bacteria previously enmeshed within the biofilms to be exposed. Upon the cracking of the Janus cells, the release of the encapsulated LPNs could more effectively target and attack the bacteria cells, making this to be more effective in the treatment of biofilms.

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