Synthesis, Characterization and Applications of Polymeric Emulsions for Dual-Drug Delivery

By

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Abstract of the Thesis

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Developing a novel smart material with tunable properties and multiple functionalities is of great interest in scientific community. Janus particles exhibit many unique chemical and physical properties due to their two distinct surfaces. They are in great demand for diverse applications across many fields including pharmaceutical, electronics, biomedical engineering (affinity with human endothelial cells was reported), magnetolythic therapy, etc. As a drug delivery carrier, Janus particles offer a platform for co-encapsulating drugs with different solubility and release kinetics. This study investigated the effects of solvents and surfactants on nanosuspensions formulation by emulsion-diffusion method. Model Biopharmaceutical Classification System (BCS) class II drug, ibuprofen was used in the preparation of nanosuspensions using three different water miscible solvents and blends of different nonionic surfactants. Surfactants with similar chemical structures but opposing hydrophilicities act synergistically. This study shows that for any set of low HLB and high HLB surfactants systems, combination of surfactants with HLB value near the mid-point produces most efficient and stable nanosuspensions. It is observed that for any combination

of nonionic surfactants, the smallest particle size is achieved when surfactants with equal amounts are incorporated.

We have also performed a different study focused on a novel method of double emulsion for coencapsulation and staggered release of hydrophobic and hydrophilic drug from PLGA/PCL Janus particles were investigated. Acetaminophen (APAP) and Naproxen (NPX) were chosen as the model hydrophilic and hydrophobic drug pair for encapsulation method and drug release. Due to its poor oil solubility and tendency to escape to the outer aqueous phase, it needs a special modification during the emulsification process. Three different strategies were employed for incorporating hydrophilic drugs: a) O/W emulsion with partially-water miscible solvent, b) O/W emulsion with methanol as a co-solvent, and c) W/O/W double emulsion. Encapsulation efficiencies, percent drug loading and differential drug release kinetics were measured and compared for different methods of synthesis. It was observed that the double emulsion method resulted in the highest encapsulation efficiency, drug loading of the hydrophilic drug and highest concentration of drug release over the period of time.

Acknowledgement

This thesis was made possible by the great support of some phenomenal people who I can call as my family and friends, colleagues and alumni. First and foremost, I would like to thank my thesis advisor, Dr. M. Silvina Tomassone, for her constant support of my research, for giving me encouragement in my research work. I would like to thank her for her patience and enthusiasm and putting a lot of trust in me in doing my research work.

I would like to thank Jennifer Winkler, alumnus of Rutgers whose Phd research I extended in my Master's thesis. In this thesis, the conceptual idea was based on the dissertation done by Jennifer Winkler. So I would like to thank her for providing me authorization of extending her work, putting details of her dissertation in my thesis and giving me feedback and guidance from time to time.

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1. Introduction

Data and content from this topics has been included in the following dissertation:

Winkler, Jennifer Sherri. **Emulsion-based synthesis and characterization of biphasic Janus particles for dual drug delivery.**

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1.1. Effects of Surfactants on the formation of Nanosuspensions

Poor aqueous solubility is a problem faced by an increasing number of new chemical entities as well as current drug compounds. Nanosuspensions have emerged as an effective and versatile means of improving the bioavailability of poorly water-soluble drugs. The high surface area-to-volume ratio and increased saturation solubility of nanosized drugs leads to an increase in dissolution rate, and, consequently, more efficient drug delivery [1,2]. Nanosuspensions offer many advantages over other nanotechnology-based drug delivery systems, including increased chemical stability, drug loading, and reduced toxicity and side effects [3,4]. Moreover, these suspensions can be further processed into conventional dosage forms for oral, parenteral, pulmonary, dermal, or ocular administration.

Nanosuspension manufacturing processes are broadly classified as “top-down” if large particles are broken down into the nano-regime or “bottom-up” if dissolved compounds are grown into nanoscale crystals from solution [5,6]. Top-down technologies such as high pressure homogenization and media milling utilize shear forces, communition, and cavitation to achieve particle size reduction [7]. Although these methods produce nanosuspensions with narrow size distributions and little batch-to-batch variation, they are time-consuming and require the use of expensive equipment. Alternatively,

nanosuspensions can be created using a bottom-up approach by emulsion-diffusion. In this method, the drug is dissolved in a partially water-miscible solvent with low toxicity such as ethyl acetate, methyl ethyl ketone (MEK), or n-butyl lactate so that an emulsion is formed. The solvent is extracted from the O/W emulsion droplets by simply adding water. After adding an excess volume of water, the partially water-miscible solvent readily diffuses to the external aqueous phase, resulting in instantaneous precipitation of the drug particles and the formation of a nanosuspension.

Regardless of the preparation method, surfactants are needed to prevent aggregation during synthesis, storage, and administration [10]. Despite the critical role of surfactants in the creation and stabilization of nanosuspensions, surfactant selection remains a largely empirical process guided by trial-and-error experimentation [11]. The hydrophile-lipophile balance (HLB) was designed in 1949 by William C. Griffin as a tool to assist in the selection of surfactants. In the HLB system, the emulsifying tendency of a nonionic surfactant is quantified based on the size and strength of its hydrophilic and lipophilic moieties [12]. The HLB is an arbitrary scale that ranges from 0 to 20 but extends up to 50 for ionic surfactants. The more dominant the hydrophilic portion, the higher the HLB value. Generally, surfactants with an HLB value in the 10-18 range are used to form stable O/W emulsions like the ones used in the emulsion-diffusion method [13]. Despite the vast amount of work done on emulsion diffusion techniques there has not been a systematic study to evaluate the effect of surfactant synergism, HLB value, and solvent properties on the characteristics of poorly water-soluble drugs. We focus on a model Biopharmaceutical Classification System (BCS) class II drug, ibuprofen nanosuspensions which are three poorly soluble drugs with very despair solubilities ranging from very insoluble (0.000937mg/ml) to insoluble (0.25 mg/ml). Surfactant synergism is studied by comparing

the performance of blends to that of chemically similar surfactants with low, medium, and high HLB for each of the surfactant pairs studied.

Nonionic surfactants are preferred for pharmaceutical applications due to their low toxicity profiles. The nonionic surfactants studied here represent two major chemical classes: sorbitan esters (Spans and Tweens) and linear block copolymers (Poloxamers). Spans are fatty acid esters of sorbitol and Tweens are ethoxylated derivatives of Spans. Poloxamers comprise a central chain of either the hydrophobic Spans, Tweens, and Poloxamers were chosen because they are commonly used as inactive ingredients in suspensions and a multitude of other pharmaceutical products [20,21].

The selected compound with varying physicochemical properties, ibuprofen, were used as model drug to evaluate the role of surfactant synergism via HLB values. The solubility of the model drugs in water is ranged from 0.000937 mg/mL to 0.25 mg/mL. The low solubility and high permeability of BCS Class II drugs make them ideal candidates for solubility enhancement techniques. Solvents were selected based on their low toxicity, water miscibility, and solvency power for the model drugs. Ibuprofen is highly soluble in ethyl acetate. Ibuprofen is soluble in a range of solvents, allowing for direct examination of the effect of solvent properties on particle size.

1.2 Janus particles with dissimilar dual drug loading and differential release kinetics

Data and content of this chapter has been submitted to the Experimental Biology and Medicine Journal:

Dual drug-loaded Biodegradable Janus Particles for Simultaneous Co-delivery of Hydrophobic and Hydrophilic Compounds by *Jennifer S. Winkler, Mayur Barai and Maria S. Tomassone**

Current approaches to nanoparticle-based combination therapy include encapsulating multiple drugs into a single nanoparticle core [1-6,7,8], conjugating one drug to the particle surface while encapsulating the other inside of the core [9], and covalently conjugating multiple drugs to the same polymer backbone [10]. While innovative, these strategies fail to provide uploading of two drugs with strongly dissimilar aqueous solubility (i.e. hydrophobic and hydrophilic), segregation of potentially reactive drug compounds, and sequenced drug release all simultaneously. Janus particles offer a platform for the co-encapsulation and staggered release of drugs with widely disparate solubility as well as independent release kinetics[11,12]. Staggered release profiles are especially desirable in treating certain diseases that require exposure to one active agent at a specific rate, followed by exposure to another active agent at a different rate. Combination therapy is especially useful in cancer treatment, where co-administration of multiple drugs targeting different pathways has been shown to reverse multidrug resistance, increase therapeutic efficacy, and reduce side effects [4,13,14]. Recent works have presented the synthesis of Janus particles without drug encapsulation, using microfluidic devices[19],[20] and single emulsion polymerization [21]. Dual drug encapsulation with Janus particles was recently reported using graphene oxide with a thermoresponsive method for drug delivery[22].

The synthesis and characterization of biphasic poly (lactic-co-glycolic acid) (PLGA) / polycaprolactone (PCL) Janus particles from O/W emulsions were studied previously[23,24]. In this work we present novel drug loading strategies for the simultaneous

inclusion of hydrophilic and hydrophobic drugs in Janus particles, measuring their encapsulation efficiency and drug release kinetics. Although single and double emulsions were previously used in preparing polymer nanoparticles for the delivery of hydrophilic compounds[25,26],[27] none of the previous work have studied dual encapsulation of disparate solubility drugs in biodegradable Janus particles. To the best of our knowledge this work is the first of its kind to simultaneously encapsulate two disparate solubility drugs into biodegradable and biocompatible Janus particles, measure their effective encapsulation efficiencies and release kinetics. This work describes three novel synthetic routes and formulations for the inclusion of hydrophilic compounds into Janus particles. Acetaminophen (APAP) and Naproxen (NPX) were chosen as the model hydrophilic-hydrophobic drug pair. Acetaminophen and naproxen are often used in combination due to their additive effects in pain management and treatment of rheumatoid arthritis [32,33].

In this work, hydrophobic compounds were encapsulated into biocompatible and biodegradable Janus particles by including them in the oil phase prior to emulsification. To incorporate the hydrophilic compound, we compared three different methods:

(i) Single oil-in-water (O/W) emulsion containing a partially water-miscible solvent, (ii) O/W emulsion using a co-solvent (O/W-S), and (iii) water-in-oil-in-water (W/O/W) double emulsion. The O/W single emulsion method is not suitable for microencapsulation of water-soluble compounds due to rapid partitioning into the outer aqueous phase. The double emulsion method requires two surfactants: one for the inner aqueous phase and one for the outer aqueous phase. The hydrophilic drug is dissolved in the inner aqueous phase, which is emulsified into a polymer solution and hydrophobic drug in organic solvent to form the primary emulsion. The primary emulsion is then added to the outer aqueous phase

containing surfactant and homogenized to produce the double emulsion. The solvent is allowed to evaporate, leaving an aqueous suspension of particles. The same factors that influence particle formation from single emulsions discussed in the previous chapter also apply to double emulsions. There are more variables related to the internal W/O emulsion to take into consideration, such as W/O emulsifier type and concentration and internal water phase volume and composition[34,35]; however, these factors have been widely studied and thus are not discussed in this work.

2. Experimental

2.1 Materials

2.1.1 Effects of Surfactants on the formation of Nanosuspensions

Name	Supplier
Ibuprofen	VWR International (USA)
Fenofibrate	Sigma-Aldrich (USA)
Indomethacin	Fisher Scientific (USA)
Ethyl Acetate	Fisher Scientific (USA)
n-Butyl Lactate	Fisher Scientific (USA)
Methyl Ethyl Ketone (MEK)	Fisher Scientific (USA)
Tween 80	Fisher Scientific (USA), Croda (Edison, NJ)
Tween 61	Fisher Scientific (USA), Croda (Edison, NJ)
Span 80	Fisher Scientific (USA), Croda (Edison, NJ)
Poloxamer 124	VWR International (USA), BASF Corporation
Poloxamer 181	VWR International (USA), BASF Corporation
Poloxamer 188	VWR International (USA), BASF Corporation

All materials used in this study are biocompatible, biodegradable and of analytical grade.

2.1.2 Janus particles with dissimilar dual drug loading and differential release kinetics

Name	Supplier
Poly(lactide-co-glycolide)(PLGA, lactide: glycolide =65:35, M.W.=40,000-75,000)	Sigma Aldrich (St. Louis, MO, USA)
Polycaprolactone (PCL, M.W.=42,500-65,000)	Sigma Aldrich (St. Louis, MO, USA)
Poly(ethylene glycol) (PEG, M.N.=400)	Sigma Aldrich (St. Louis, MO, USA)
Curcumin (CUR)	Sigma Aldrich (St. Louis, MO, USA)
Quercetin (QCT)	Sigma Aldrich (St. Louis, MO, USA)
Naproxen (NPX)	Sigma Aldrich (St. Louis, MO, USA)
Acetaminophen (APAP),	Sigma Aldrich (St. Louis, MO, USA)
Dichloromethane (DCM)	Sigma Aldrich (St. Louis, MO, USA)
Methanol	Sigma Aldrich (St. Louis, MO, USA)
Tetrahydrofuran (THF)	Sigma Aldrich (St. Louis, MO, USA)
Span 80	Fisher Scientific (Waltham, MA, USA)
Tween 80	Fisher Scientific (Waltham, MA, USA)
Polyvinyl alcohol (PVA, 98 mol% hydrolyzed, M.W.=9,000-10,000)	Polysciences (Warrington, PA, USA)
Phosphate Buffer Saline (PBS)	
Slid-a-lyzer mini dialysis (20K MWCO)	Thermo-Fisher Scientific

All materials used in this study are biocompatible, biodegradable and of analytical grade.

2.2 Methods

2.2.1 Effects of Surfactants on the formation of Nanosuspensions

2.2.1.1. Drugs selection

The poorly water-soluble drug, Ibuprofen, were used as the model drugs for this study. Basic chemical and physical properties of the drugs are presented in Table 1. The molecular weights of the compounds ranged from 206 g/mol for ibuprofen to around 360 g/mol for both fenofibrate and indomethacin. The logP values varied between 3.97 for ibuprofen and 5.3 for fenofibrate, while water solubility ranged from 0.000937 for indomethacin mg/mL to 0.25 mg/mL for fenofibrate. Due to wide difference in their

properties, they are ideal for screening studies. Out of these three drugs, Ibuprofen was chosen for this study.

Property	Ibuprofen	Fenofibrate	Indomethacin
Molecular formula	$C_{13}H_{18}O_2$	$C_{20}H_{21}O_4Cl$	$C_{19}H_{16}ClNO_4$
Molar mass (g/mol)	206.29	360.83	357.79
Melting point (°C)	76	80.5	158.96
Water solubility (mg/mL)	0.021	0.25	0.000937
LogP	3.97	5.3	4.27
Refractive Index	1.436	1.546	1.74

Table 1: Properties of ibuprofen, fenofibrate, and indomethacin.

2.2.1.2 Selection of Surfactants

A set of poloxamers and a set of sorbitan esters commonly used in pharmaceutical applications were chosen to study the formation of nanosuspensions. The surfactants were selected to cover a wide range of hydrophilicity and hydrophobicity, and their chemically similar structures allowed for optimal interfacial packing.

Poloxamer 181 and Poloxamer 188 were used in combination because they are similar in chemical structure and their mixtures offer HLB values ranging from 3.5 to 29. Poloxamer 181 and Poloxamer 188 are both PEO-PPO-PEO triblock copolymers. Poloxamer 188 is much more hydrophilic than Poloxamer 181 due to its higher EO molar percentage (and thus has a higher HLB value); Span 80 and Tween 80 were also selected because they are similar in chemical structure but dissimilar in hydrophobicity. The Span 80/Tween 80 surfactant series gives HLB values ranging from 4.3 to 15. Additionally, chemically similar surfactants with midrange HLB values were chosen for each surfactant

pair in order to separate the effect of surfactant synergism from that of HLB value. All of the surfactants studied here are FDA approved for use in pharmaceutical products [23]. Properties of the surfactants used in this study are shown in Table 2.

Poloxamers	HLB	Average Molecular Weight
Poloxamer 124 (Pluronic® L44)	16	2090–2360 g/mol
Poloxamer 181 (Synperonic® PE/L61)	3.5	2000 g/mol
Poloxamer 188 (Pluronic® F68)	29	7680–9510 g/mol
Sorbitan Esters		
Span® 80	4.3	428.62 g/mol
Tween® 65	10.5	1842 g/mol
Tween® 80	15	1310 g/mol

Table 2: Properties of surfactants

2.2.1.3 Selection of Solvents

Solvents were selected based on their low toxicity, water miscibility, and solvency power for the model drugs. Ibuprofen has good solubility in ethyl acetate and n-butyl lactate. The properties of these solvents are displayed in Table 3.

Property	***n*-butyl lactate**	**Ethyl acetate**	**Methyl ethyl ketone**
Molecular formula	$C_7H_{14}O_3$	$C_4H_8O_2$	C_4H_8O
Molar mass (g/mol)	146.19	88.11	72.11
Water solubility (g/100 mL)	7.7	8.3	27.5
Density (g/mL)	0.984	0.897	0.805
Viscosity (cP)	3.58	0.45	0.426
Boiling point (°C)	189.4	77.1	79.64

Table 3: Properties of solvents

2.2.1.4 Selection of homogenizer or sonication

Two types of homogenization methods are used: Ultra Turrax or Ultra-Sonication

Ultra Turrax: They are composed of coaxial intermeshing rings with radial openings. The fluid enters in the center of the systems and is accelerated by the rotor. As it passes through the system, the fluid is accelerated and decelerated multiple times which results in high tangential forces.

Ultra-Sonication: Associated pressure gradients cause deformation of droplets. Cavitation caused by drop of local pressure below the vapor pressure of the solvent generates turbulent flow and high shear

Depending upon the particle size and homogeneity required we can choose either of the two. If more uniform distribution and smaller particle size is required, Ultra-Sonication is preferred.

2.2.1.4 Preparation of surfactant solutions

Span 80/Tween 80 and Poloxamer 181/Poloxamer 188 mixtures with a range of HLB values were prepared. The amount of each surfactant A and B needed to reach the desired HLB value was determined using the following equation:

$HLB_{Mix} = (X_A*HLB_A + X_B*HLB_B)$, where X_A is the molar fraction of A and X_B is the molar fraction of B

2.2.1.5 Preparation of nanosuspensions

Nanosuspensions were prepared by the emulsion-diffusion method. First, a stable emulsion is formed by applying shear to the crude emulsion. Emulsion droplets containing the active ingredient are stabilized by surfactant(s). After adding an excess volume of water, solvent diffuses out of the droplets into the continuous phase, leading to drug precipitation. The final result is a solution of drug crystals stabilized by surfactants.

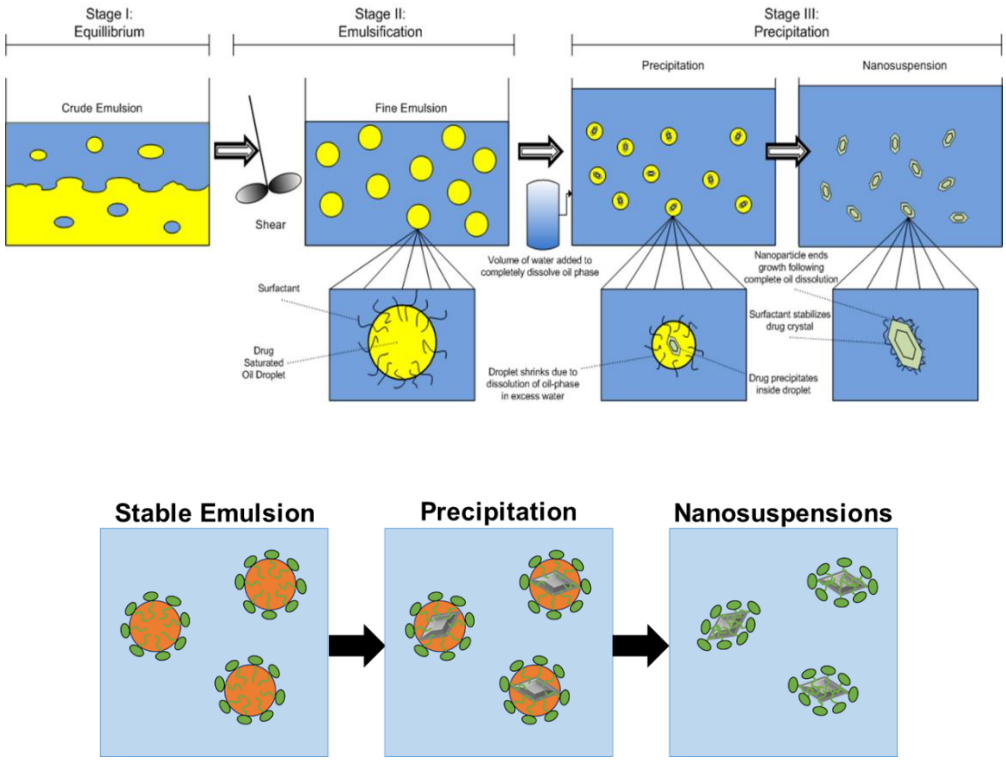


Figure 1: Schematic of emulsion-diffusion process

A 100 mg/mL solution of ibuprofen in ethyl acetate or n-butyl lactate (20 mL) was added to a 4% w/w aqueous surfactant solution (80 mL) and emulsified with the Ultra Turrax rotor-stator homogenizer for 5 minutes at 12,500 rpm. An excess volume of water (200 mL) was added to the O/W emulsion at a rate of 200 mL/min while still under homogenization, resulting in the precipitation of drug particles. Solvent selection was based on the solubility of the drug. The phase volume ratio and the volume of added water in the final step were chosen based on the miscibility of the solvent with water. Experiments were performed at 25º C because the HLB system does not take into account the effect of temperature, which may have an effect on the size of emulsion droplets [24]. Temperature increase during homogenization was determined to be negligible (< 2ºC).

2.2.1.6 Particle size distribution

Volume size distribution was determined by laser diffraction using a Beckman-Coulter LS-13320. Samples were run with a combined obscuration and polarization intensity differential scattering (PIDS) using 1.486 as the refractive index for ibuprofen, and 1.333 for the dispersion medium which is water [25]. All data are presented as the mean particle diameter (d50) and standard deviation of three independent samples produced under identical conditions.

2.2.2 Janus particles with dissimilar dual drug loading and differential release kinetics

2.2.2.1 Single Oil in Water Emulsion Method (O/W)

(i) Preparation of PLGA/PCL Janus Particles for Hydrophilic and Hydrophobic drugs pair

A schematic of the Janus particle formation process is shown in Figure 1.

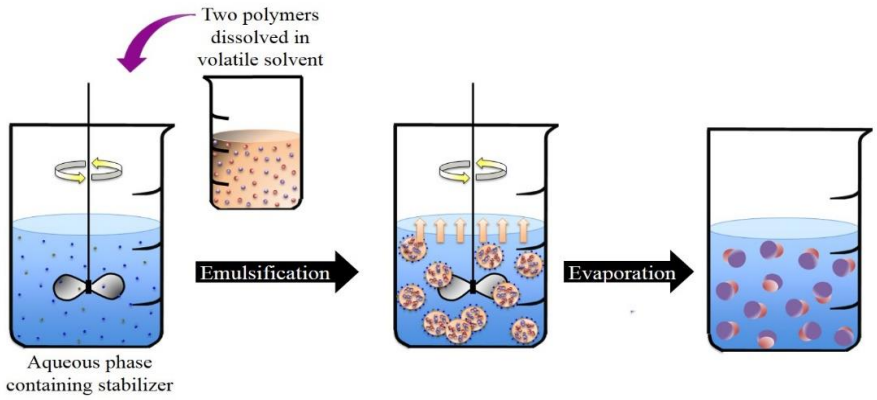


Figure 2: Overview of the modified emulsification solvent evaporation method for producing biodegradable Janus particles. Step 1: Polymer solution is added to an aqueous solution containing stabilizer. Step 2: The two-phase system is homogenized to form an oil-in-water emulsion. Step 3: The solvent evaporates or diffuses out of the saturated oil droplets, leading to co-precipitation of the two polymer species into Janus particles.

Janus particles containing the hydrophilic and hydrophobic compounds APAP and NPX were synthesized using a single O/W emulsion-solvent evaporation method, but with some modifications to accommodate the loading of the hydrophilic APAP. Since APAP is

poorly soluble in the chlorinated hydrocarbon solvents that are typically used for O/W emulsions (i.e., DCM and chloroform), two different strategies were employed:

a) Single oil-in-water (O/W) emulsion containing a partially water-miscible solvent, where ethyl acetate was used as the solvent and

b) O/W emulsion using a co-solvent (O/W-S), where a mixture of DCM and Methanol was used as the solvent

a) Single oil-in-water (O/W) emulsion containing a partially water-miscible solvent:

The oil phase was created by dissolving 2.5% w/v of each PLGA (lactide:glycolide=65:35, M.W.=40,000-75,000) and Polycaprolactone (PCL, M.W.=42,500-65,000) in 4 mL of ethyl acetate. After that 2.5% APAP and 2.5% NPX is dissolved in oil phase. Separately, a 10 mL solution of 1% w/v PVA in deionized water was prepared. The oil phase was added to the water phase and emulsified. The O/W emulsion was further homogenized using either an Ultra Turrax T-25 rotor-stator for 5 minutes at 12k rpm. Post- homogenization, the O/W emulsion was magnetically stirred and kept in an open beaker to allow for solvent evaporation. Upon complete solvent removal, the size distribution is done by Beckman Coulter's Laser Diffraction module. Later, particles were harvested by centrifugation at 20,000 rpm for 30 minutes. The supernatant was discarded, and the remaining powder bed was washed with deionized water. Particles were stored in a vacuum desiccator for further analysis.

b) O/W emulsion using a co-solvent (O/W-S):

The oil phase was comprised of 5% w/v 50:50 PLGA/PCL, 2.5% w/w APAP, and 2.5% w/w NPX. For the O/W-S method, methanol was added at methanol-to-DCM ratios of 1:1. The water phase was comprised of 1% w/v PVA solution. Typically, 4 mL of oil

was added to 10 mL water and emulsified using an Ultra Turrax T-25 rotor-stator for 5 minutes at 12k rpm. The resultant O/W emulsion was magnetically stirred until complete solvent evaporation. Upon complete solvent removal, same procedure was followed for harvesting particles as used in previous methods.

2.2.2.2 Preparation of PLGA/PCL Janus Particles by a novel Double Emulsion Method

Double W/O/W emulsions are commonly used to encapsulate hydrophilic compounds into particles. As with the single emulsion method, particles are formed from a single O/W emulsion template. However, in the double emulsion method hydrophilic compounds are entrapped inside of W/O emulsion droplets which reside in the core of the particles. This is necessary for compounds that are insoluble in the solvent used as the oil phase.

PLGA/PCL with a dual encapsulation were created by a novel double emulsion method by modifying the single emulsion approach. The inner aqueous phase consisted of 20% w/v APAP dissolved in 75:25 PEG400/water. The primary water in oil emulsion indicated by W1/O was formed by adding 500 µL of the PEG400/water solution to the oil phase, which consisted of 0.25 g PLGA, 0.25 g PCL, 0.025 g NPX, and 0.2 g Span 80/Tween 80 (HLB 5) dissolved in 5 mL DCM. The W/O emulsion was homogenized using an Ultra Turrax T-25 rotor-stator for 5 minutes at 16k rpm. Finally, the W/O emulsion was added to the outer aqueous phase (12.5 mL 1% PVA w/v solution with 10% w/v NaCl) and emulsified at 6k rpm for 2 minutes. The resultant W/O/W emulsion was magnetically stirred in an open beaker to allow solvent evaporation to proceed. A schematic showing the steps of the W/O/W technique is shown in Figure 3.

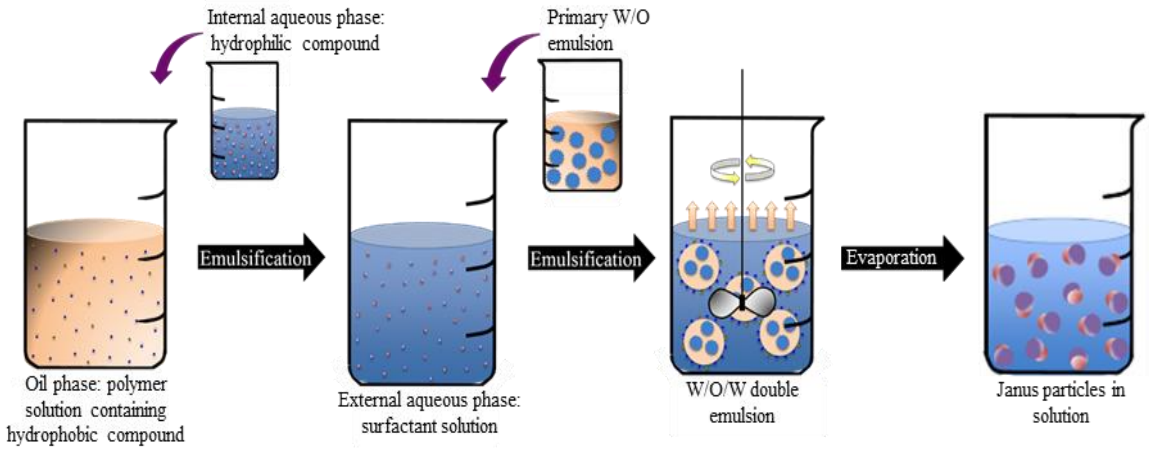


Figure 3: Overview of the double emulsion solvent evaporation method used to encapsulate hydrophilic compounds inside Janus particles.

Encapsulation Efficiency studies:

After centrifugation, the supernatant was analyzed for drug content. The amount of each drug present in the samples was calculated by deconvoluting the NPX and APAP spectra using the Excel solver function. Wavelengths chosen were 230, 243, 252, 272, 318, and 331 nm. The encapsulation efficiency (E.E.) was calculated using the following equation:

$$\text{E.E. (\%)} = \frac{Initial\ drug\ loaded - Free\ drug}{Initial\ drug\ loaded} * 100 \qquad [1]$$

The drug loading was calculated using the following equation:

$$\text{D.L. (\%)} = \frac{Actual\ drug\ content\ of\ particles}{Amount\ of\ drug + polymer} * 100 \qquad [2]$$

Drug Release Kinetics

The release profile of two actives, Naproxen and Acetaminophen, from Janus particles was studied using Slid-a-lyzer mini dialysis devices with 20K MWCO. The

devices were loaded with 500μL of the formulation and the receptor medium contained 14mL of phosphate buffer saline at pH 7.4. The devices were kept in a water bath at 37°C with constant shaking. The release study was allowed to run over a 24h period with sampling done at 2h, 4h, 6h, 21h, 22h, 23h and 24h. At each time point, 3mL of the receptor media was collected from each tube and an equal amount of fresh PBS was used to replenish. The samples collected at each time point were quantified using US-Vis Spectroscopy and concentrations were plotted against time to get the profile.

3. Results and Discussion

3.1 Effects of Surfactants on the formation of Nanosuspensions

3.1.1 Effect of surfactants with different HLB values with different types of solvents

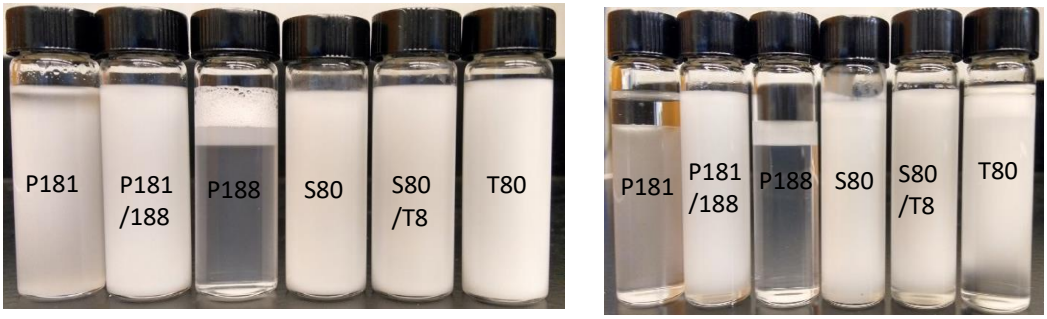


Figure 4: Stabilizing effect of Poloxamers and Sorbitons with Ethyl Acetate at time t=0 and after t=48 hrs

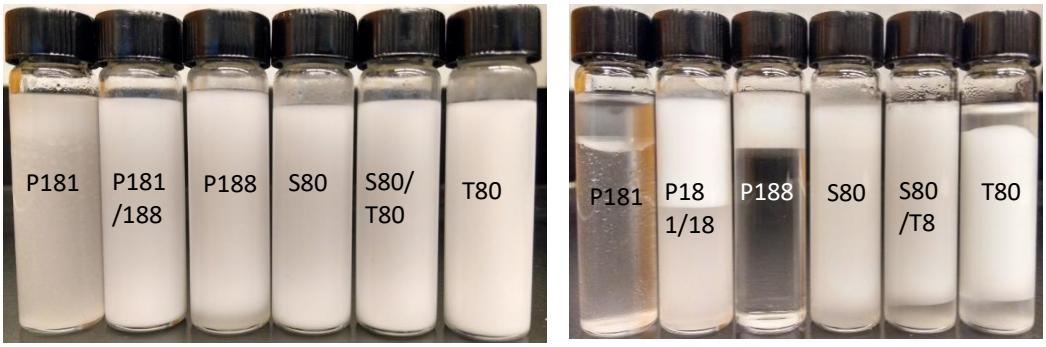


Figure 5:Stabilizing effect of Poloxamers and Sorbitons with Butyl Lactate at time t=0 and after t=48 hrs

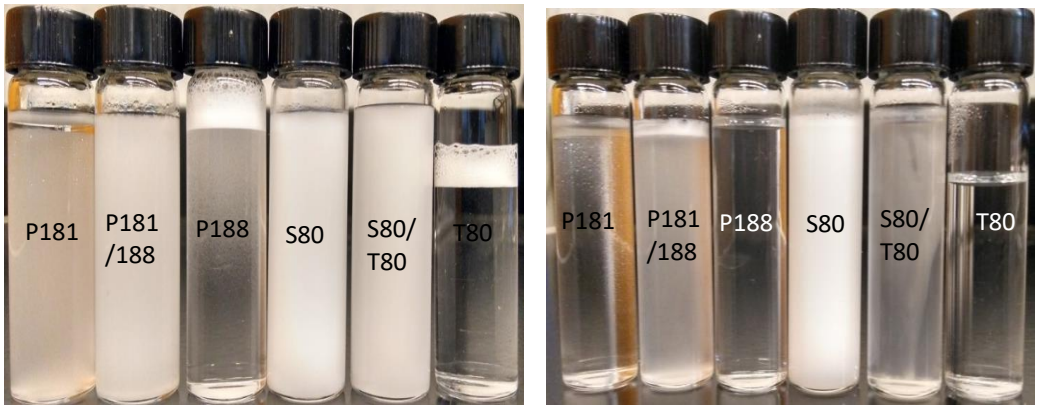


Figure 6: Stabilizing effect of Poloxamers and Sorbitons with MEK at t=0 and after t=48 hrs

Figure 4,5 6 shows HLB kits produced from ethyl acetate, butyl lactate and methyl ethyl ketone (MEK) used as the solvent and several individual Poloxamers and tweens.

The stability of the emulsion significantly impacts the resulting nanosuspensions extracted from the emulsion, as small, stable droplets are required in order to create small particles, however, it should be noted that the individual surfactant, or combination of surfactants must also stabilize the resulting suspension to work adequately for this process, and therefore, the appropriate balance of surfactants with a strong affinity for stabilizing the ibuprofen particles in conjunction with a stable emulsion would result in the suspension with the most desirable small particles on the order of a few hundred nanometers.

Effects of rotor stator and sonicator on a particle size distribution for combination of Poloxamers can be observed from figures 9 and 10. When only rotor stator is applied, wider distribution in particle size is observed as compared to sonication. There is a secondary peak which shows bimodal distribution and particle size on a higher side. When sonication is applied, the particles are more homogenized and narrow distribution from 0.2 to 0.6 microns is achieved.

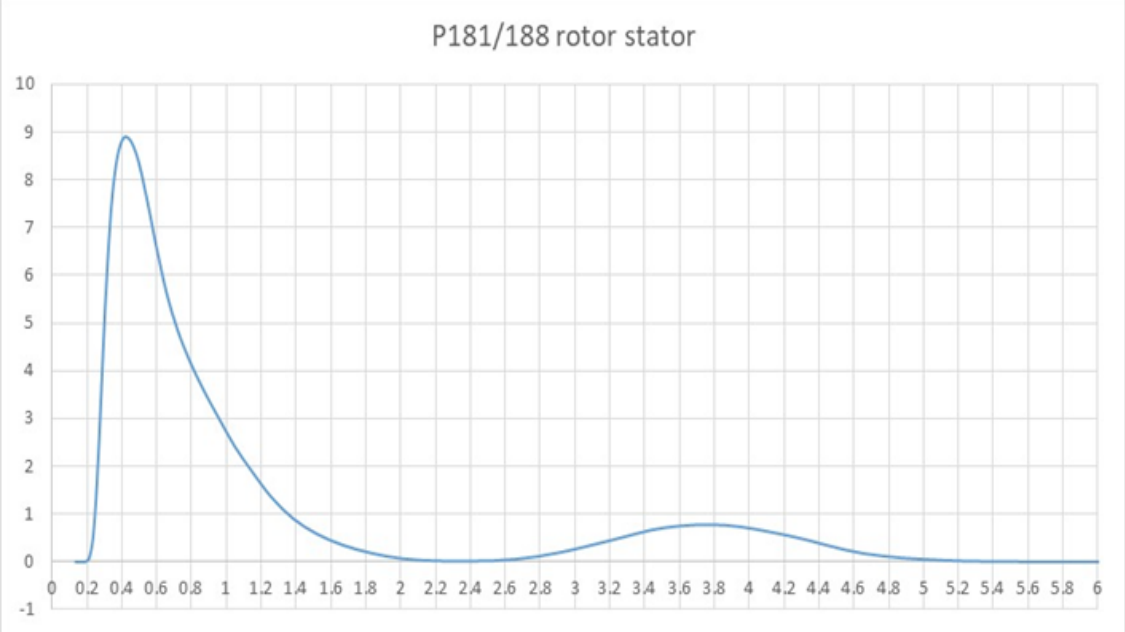


Figure 7: Particle Size Distribution for combination of synergistic Poloxamers with rotor stator with a wider distribution as indicated by the amount in microns. There is a secondary peak suggesting that there are some smaller particles formed (bimodal)

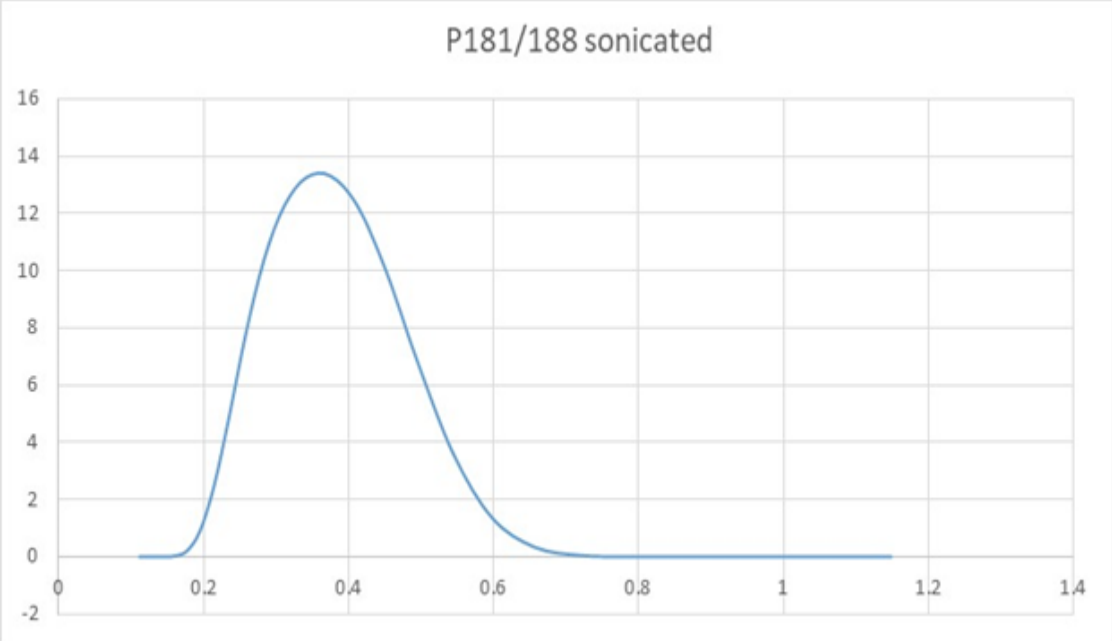


Figure 8: Particle Size Distribution for synergistic Poloxamers with sonicator showing a more narrow distribution from 0.2 to 0.6 microns.

For ibuprofen, all Span 80/Tween 80 combinations, including pure Span 80 and pure Tween 80, resulted in nanosuspensions when ethyl acetate was used as the solvent. Using n-butyl lactate instead of ethyl acetate resulted in slightly larger ibuprofen nanosuspensions.

Figure 9 shows the particle size distributions of the ibuprofen suspensions containing the smallest particle size and lowest polydispersity for comparison for n-butyl lactate and for ethyl acetate formulations. The observable differences in the mean particle size, as well as the span of the distribution are indicative of the importance of both the surfactant type and the ability of the solvent to diffuse through the surfactant layer which is 4% of the aqueous solution.

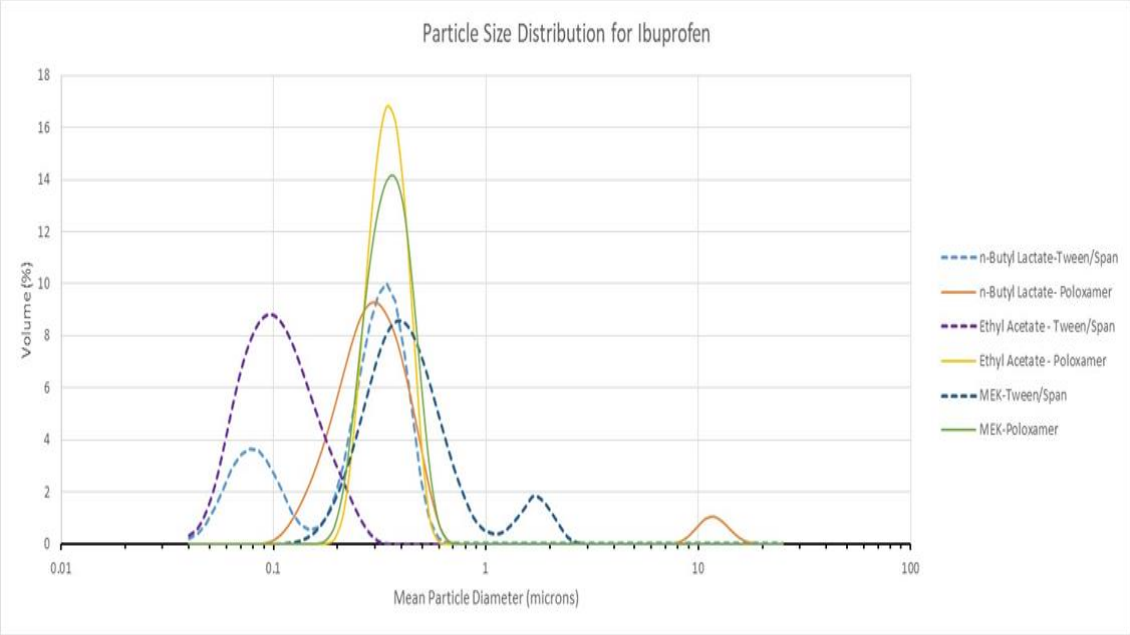


Figure 9: Particle size distributions of the ibuprofen suspensions with Butyl Lactate, Ethyl Acetate and MEK as a solvent

These results suggest that for any combination of nonionic surfactants the smallest particle size will be achieved when equal parts of each surfactant are incorporated with Ethyl Acetate as a solvent as shown by the purple curve in fig. 9.

3. Results and Discussion

3.2 Janus particles with dissimilar dual drug loading and differential release kinetics

3.2.1 PLGA/PCL Janus Particles Containing APAP and NPX using the O/W Emulsion Method with a partially water-miscible solvent

Ethyl acetate was used as the partially water-miscible solvent for co-encapsulation of APAP and NPX. Although the solubility of APAP in ethyl acetate is quite low, it is higher than that in DCM (10.73 g/kg vs. 0.32 g/kg). Optical images of PLGA/PCL Janus particles containing APAP and NPX are shown in Figure 11. These Janus particles appear to have holes on the surface as result of the slow evaporation rate and long residence time of the solvent ethyl acetate. UV-Vis spectra for APAP-NPX in EA is shown in Figure 10.

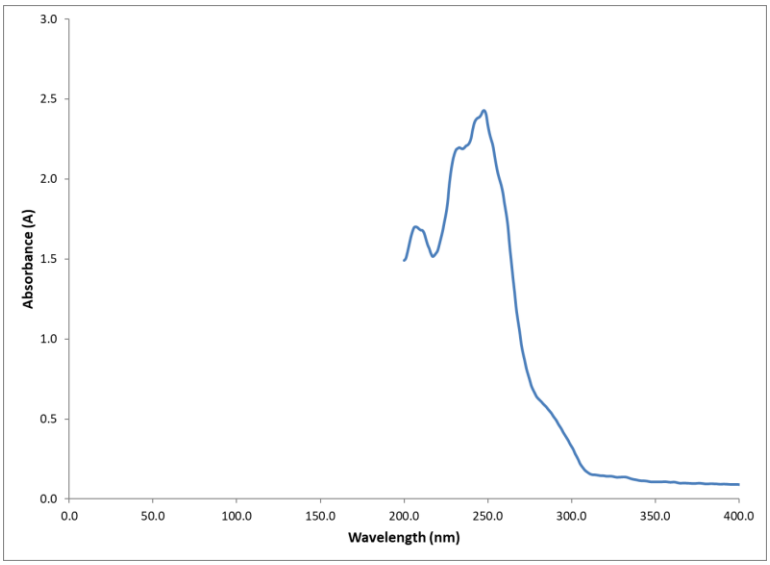


Figure 10: UV spectra of APAP and NPX with Ethyl Acetate in 50:50 methanol/water

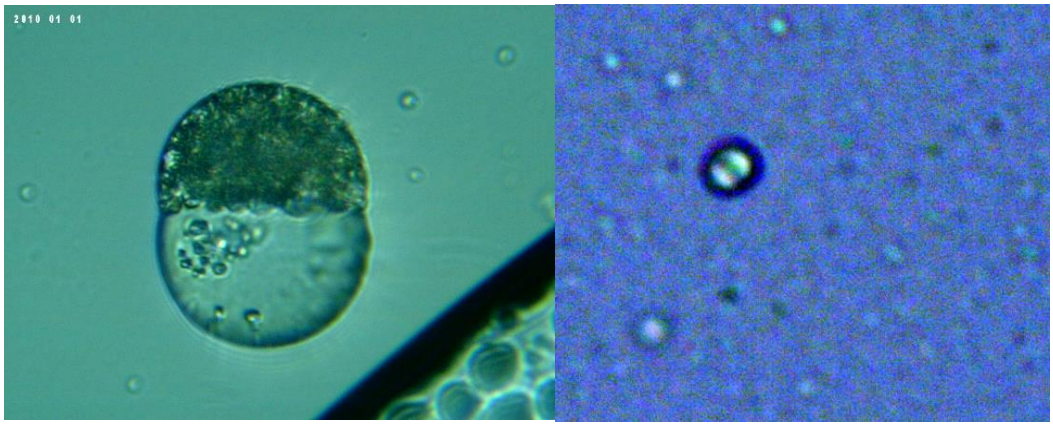


Figure 11: PLGA/PCL Janus particles containing APAP and NPX.

3.2.2 PLGA/PCL Janus Particles Containing APAP and NPX using the O/W Emulsion Method with a Co-solvent

Janus particles containing APAP and NPX were prepared using the co-solvent method using methanol as the co-solvent. Optical images of resulting PLGA/PCL Janus particles containing APAP and NPX are shown in Figure 12. These Janus particles appear

to be protruding as result of the insufficient solvent and the longer residence time of methanol.

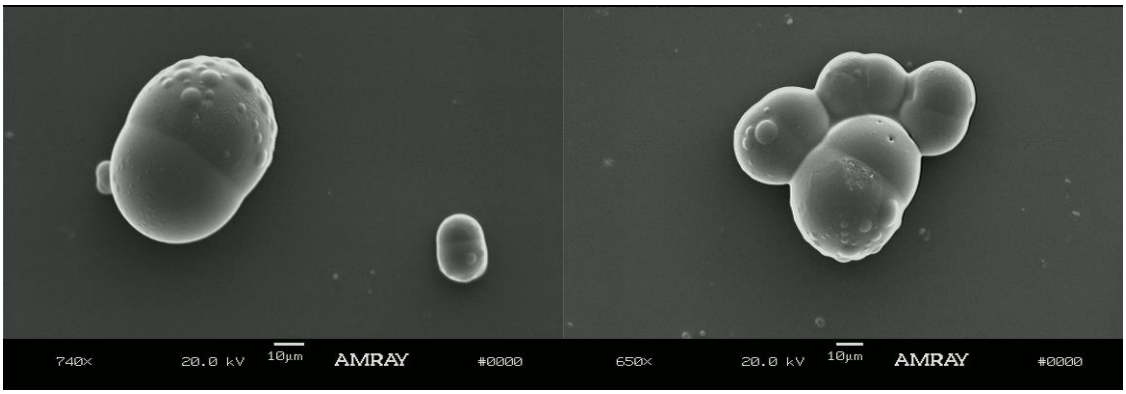


Figure 12: PLGA/PCL Janus particles containing APAP and NPX prepared by the O/W-S method using methanol.

3.2.3 PLGA/PCL Janus Particles Containing APAP and NPX using the W/O/W Emulsion Method

The double emulsion method was used to encapsulate APAP in inner water droplets within Janus particles containing NPX. Using a 75:25 v/v mixture of PEG 400/water as the inner aqueous phase instead of water greatly increased the amount of APAP that could be incorporated into the particles. The solubility of APAP in a 75:25 blend of PEG 400/water is ~220 mg/mL, compared to only approximately 12 mg/mL in water. It is important to minimize the volume of W1 because smaller internal water phase volume has been shown to reduce porosity and burst release[40,41]. Thus, a W1/O/W2 ratio of 1/10/30 was used. PLGA/PCL Janus particles containing APAP and NPX prepared by the double emulsion method are shown in 10. These particles exhibit an oblong shape compared to the standard PLGA/PCL biphasic Janus particles normally obtained with single emulsions.

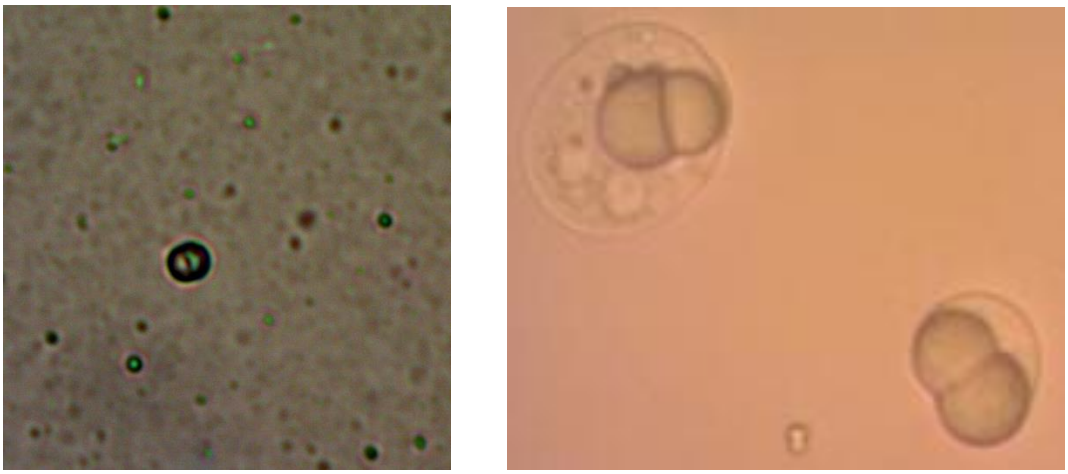


Figure 13: PLGA/PCL Janus particles containing APAP and NPX prepared by the double emulsion method.

The complexity of W/O/W emulsions renders the formulation and process variables much more important and less flexible than a standard O/W emulsion process. For example, it was found that the primary W/O droplets should be significantly smaller in diameter than the outer O/W emulsion droplets to prevent coalescence and rupture of inner droplets. Surface protrusions due to large W/O emulsion droplets are shown in Figure 11. Additionally, NaCl was added to the external aqueous phase in order to balance the osmotic pressure gradient, leading to greater emulsion stability[40,42]. This allows the W/O emulsion droplets to remain small and prevents destabilization of the W/O/W emulsion.

3.2.4 Particle Size Distribution for Single and Double Emulsions

The solvent plays an important role in crystal growth and morphology. Ultimately, solvent selection is dictated by the solubility of the drug. Most poorly water-soluble drugs exhibit high solubility in at least one partially water-miscible solvent suitable for emulsion-precipitation. Ethyl acetate has the largest solvent-to-water diffusion coefficients. This corresponds to faster dissolution of emulsion droplets and rapid drug precipitation, theoretically resulting in smaller particles. The size distribution data is gathered from the

Beckman Coulter's laser diffraction liquid module. The suspension is used in its original form in the laser diffraction to get the distribution.

It is clear from Figure 13 that a nanoemulsion containing APAP-NPX with ethyl acetate as a solvent gives the minimum mean particle size. Double Emulsion also shows unimodal distribution which is significant.

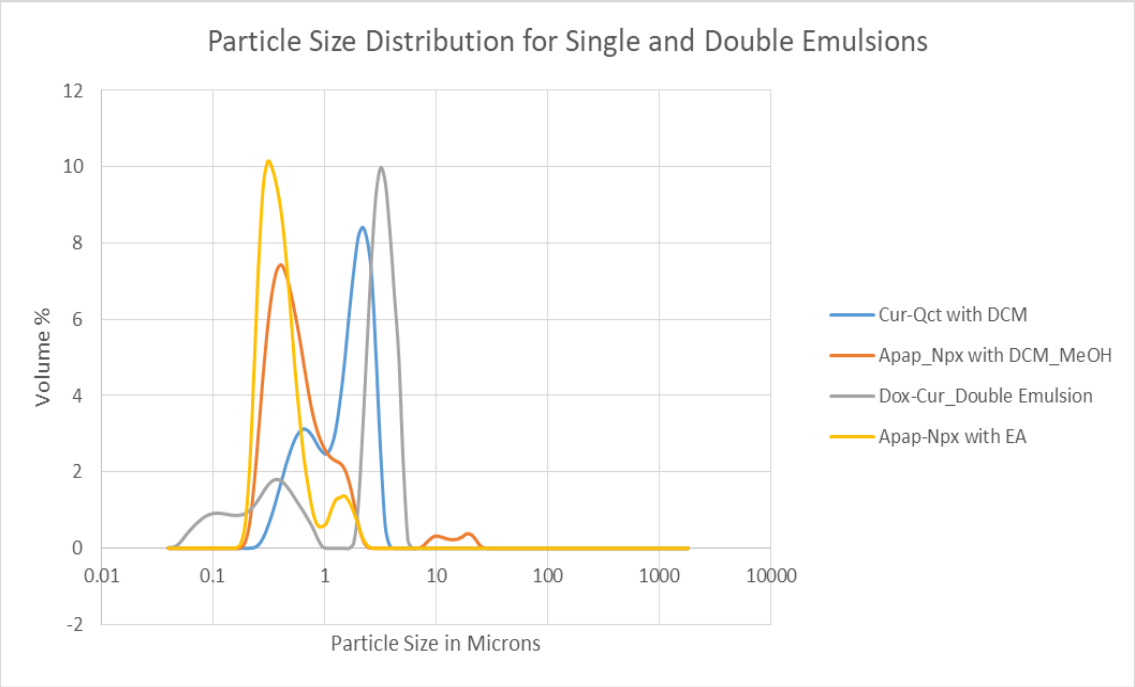


Figure 14: Particle Size Distribution for Single and Double Emulsions

3.2.5 Encapsulation Efficiency of Janus Particles

The encapsulation efficiency of the hydrophilic and the hydrophobic drugs, APAP and NPX, can only be measured indirectly from the supernatant due to interference from the polymers' spectra. The EE and DL of Janus particles containing APAP and NPX synthesized via the ethyl acetate-in-water single emulsion method, the O/W emulsion method using DCM as the solvent and methanol as a co-solvent, and the W/O/W emulsion method are contained in 3.

	APAP		NPX	
Synthesis Method	**EE (%)**	**DL (%)**	**EE (%)**	**DL (%)**
O/W with Ethyl Acetate	54.90 ± 16.01	4.26 ± 0.49	93.98 ± 0.45	7.22 ± 0.83
O/W with DCM + Methanol	21.04 ± 0.72	1.69 ± 0.28	91.88 ± 1.00	7.36 ± 0.86
Double Emulsion	68.29 ± 3.04	15.93 ± 4.39	85.49 ± 0.20	9.14 ± 2.50

Table 4: Encapsulation efficiencies of APAP and NPX in Janus particles synthesized via single and double emulsions.

Table 6 shows that the EE of naproxen, a hydrophobic drug, was very high with all the emulsion techniques, ranging from 85-94%. This translated to total drug loadings ranging from 7.22% for the single O/W emulsion with ethyl acetate to 9.14% for the double emulsion. The loading of naproxen was comparable for both single emulsion techniques: 7.22% when ethyl acetate was used as the oil phase and 7.36% when a mixture of DCM and methanol was used. The double emulsion method resulted in the highest EE of 68.29% for the hydrophilic drug APAP, while the single emulsion methods gave EE's of 21.04% using DCM+Methanol as the oil phase and 54.90% using ethyl acetate as the oil phase. The drug loading for APAP was considerably lower at 4.26% and 1.69% for the O/W-EA and O/W-DCM emulsions respectively, and 15.93% in the double emulsion batch. Such a high drug loading was achieved by the double emulsion method due to the high solubility of APAP in the PEG400/water internal water phase.

Double emulsions are frequently used for the entrapment of hydrophilic compounds. A very high concentration of APAP is possible using the W/O/W emulsion technique with PEG400/water as the inner water phase despite the small volume of W1. For example, even with an inner water phase only $1/10^{th}$ of the volume of the oil phase that contains NPX, there is a higher content of APAP than NPX (15.93% w/w total formulation vs. 9.14%). The O/W-S method using methanol resulted in the lowest EE despite APAP's

high solubility in methanol. This is due to the fact that methanol is completely miscible with water, causing most of the APAP dissolved in methanol to escape to the water phase during evaporation since APAP is soluble in water and practically insoluble in DCM. Using ethyl acetate as the solvent resulted in a moderate EE of APAP. All three methods resulted in relatively high EE of NPX, which is expected for the encapsulation of hydrophobic compounds using O/W emulsion-based techniques.

3.2.6 Drug release kinetics

Janus Particle formulation of three different methods for APAP and Naproxen were subjected to drug release studies and the release curves of each drug were then plotted in Figure 15. From Figure 15, it can be seen that double emulsion is having a higher and longer cumulative drug release with naproxen having higher concentrations than APAP which is in accordance with the data reported for the encapsulation efficiency and drug loading studies discussed in the previous section. The higher the encapsulation efficiency, the higher the amount of drug encapsulated and the higher the concentrations that are released. Also, Naproxen is released starting at 2hrs and continues to release until 24 hrs, while APAP is released at 21hrs showing a much smaller release kinetics (i.e. a differential release).

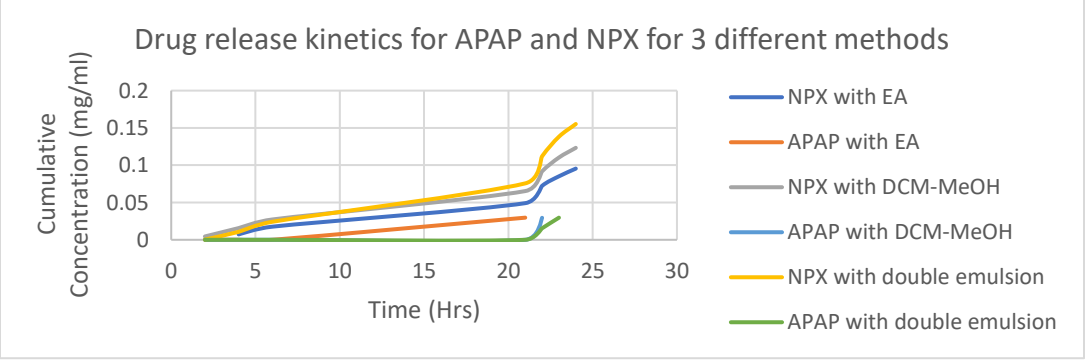


Figure 15: Drug release concentration for Naproxen and APAP for 3 different synthesis method

4. Conclusions

4.1 Effects of Surfactants on the formation of Nanosuspensions

This study demonstrates the effect of surfactant synergism, HLB, and solvent properties on nanosuspension formation by emulsion-diffusion for three case studies. There is a wide range of pharmaceutically acceptable nonionic surfactants to choose from with little guidance for formulators. In particular, the widely used sorbitan esters (Spans and Tweens) and high molecular weight PEO-PPO-PEO block copolymers (Poloxamers) yielded stable nanosuspensions. Blending these surfactants in equal proportions results in smaller and more stable nanosuspensions than using individual surfactants of the same HLB value. This is due to the optimal interfacial packing provided by chemically similar surfactants with contrasting hydrophilicities, indicating that the synergism provided by two chemically similar surfactants is more critical to control the size of the nanosupension than their HLB value. For any set of low HLB and high HLB surfactant, the most efficient combinations for producing nanosuspensions are obtained by using combinations near the mid-point of HLB values. All nanosuspensions prepared using surfactant mixtures at the mean HLB value had excellent physical stability.

In addition, it was shown that an important consideration for utilizing the emulsion-diffusion method is the choice of solvent. Overall, the result obtained for the three case studies indicate that the emulsion-diffusion method yields stable nanosuspensions for drugs with vastly different physicochemical properties given the right formulation variables.

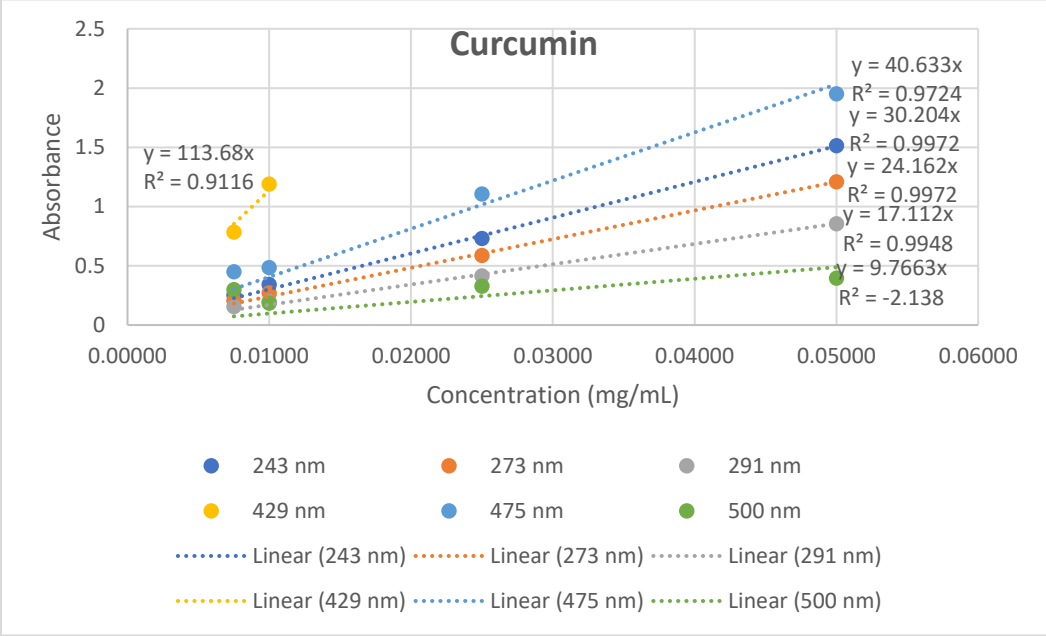
4.2 Janus particles with dissimilar dual drug loading and differential release kinetics

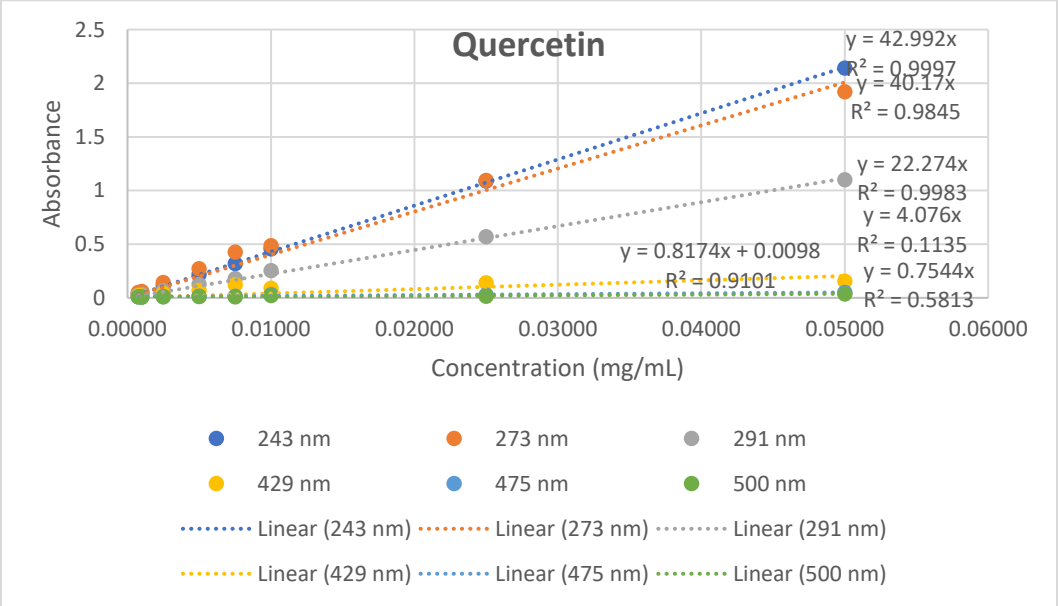
This study demonstrates three methods for incorporating a hydrophilic and hydrophobic drug, as well as a UV detection method for measuring the encapsulation efficiency and drug release of two different compounds from PLGA/ PCL Janus particles. Subtracting the drug content in the supernatant from the initial amount of drug loaded was validated as an accurate method by which to measure encapsulation efficiency in Janus particles. This is necessary in some cases where the polymeric matrix is insoluble in the solvent being used for UV analysis or if it is desired to measure drug release at discrete timepoints.

Unlike hydrophobic drugs which are readily encapsulated by a single O/W emulsion, encapsulating hydrophilic compounds requires more complex processing. NPX was encapsulated into the particles at a reasonably high encapsulation efficiency regardless of the synthesis method owing to its high oil solubility. The W/O/W double emulsion showed the highest encapsulation efficiency and overall DL of APAP out of the three synthesis methods tested. Double emulsions are inherently more complex than single emulsions with the addition of another phase, thus there are more variables that need to be taken into consideration. For example, if inner W/O droplets are too large, this can result in surface protrusions. In single emulsion O/W-S system, it is observed that low API solubility due to lack of solvent power can result in the formation of free drug needles due to the partitioning of the drug to the aqueous phase where it is more soluble. Janus particles have the potential to meet the ever-growing demand for multifaceted drug delivery systems capable of targeting and treating complex diseases.

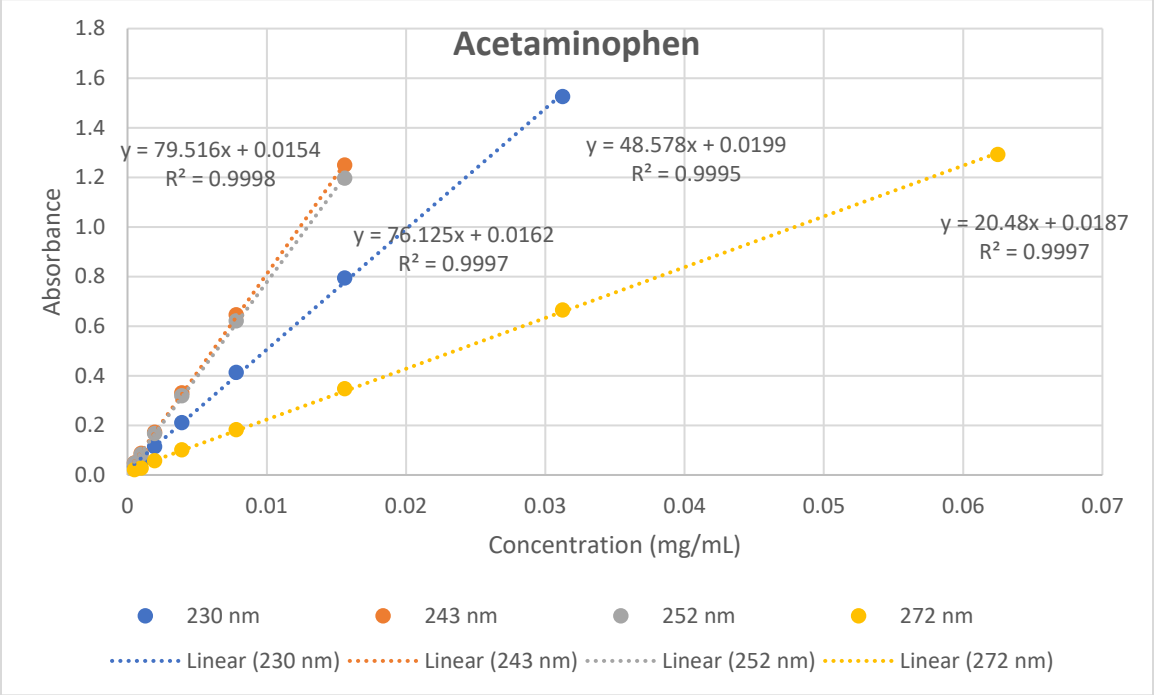
APPENDIX

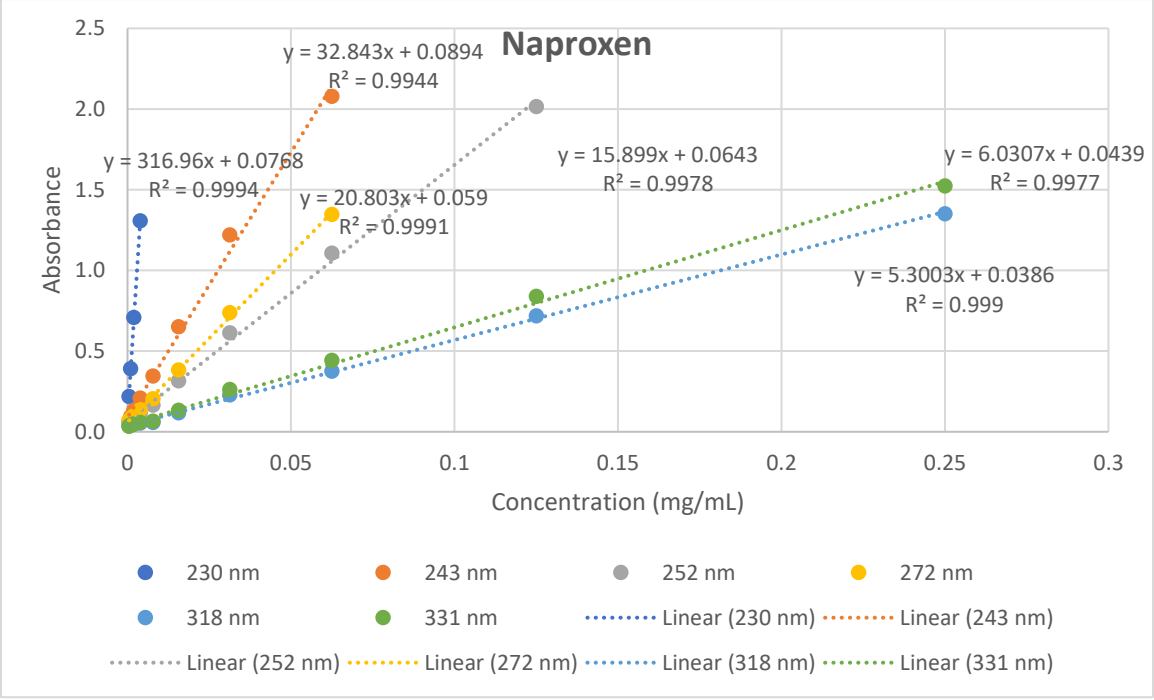
Calibration curves for curcumin and quercetin in 50:50 methanol/water are provided below.





Calibration curves for acetaminophen and naproxen in 50:50 methanol/water are provided below.





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