MEDIUM-CHAIN-LENGTH POLY(3-HYDROXYALKANOATE) NANOPARTICLE SUSPENSIONS

by

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A thesis submitted to the Department of Chemical Engineering
In conformity with the requirements for
the degree of Master of Applied Science

Queen's University

Kingston, Ontario, Canada

(August, 2016)

Abstract

The main goal of this thesis was to prepare medium-chain-length poly-3-hydroxyalkanoate (mcl-PHA) nanoparticle suspensions at high solids content (≥ 10 % w/v). A two-stage emulsification-solvent evaporation process was employed to produce poly-3-hydroxydecanoate (PHD) suspensions. The formulation and processing conditions including ultrasonication time and amplitude, selection of solvent, and selection of surfactants and their concentrations were investigated to make concentrated suspensions (10 and 30 % (w/v)) of PHD with particles less than 300 nm. Among the ionic surfactants tested to stabilize the suspension, the anionic, sodium dodecyl sulphate (SDS), and the cationic, dodecyltrimethylammonium bromide (DTAB) surfactants produced the smallest particle sizes (~100 nm). However, more stabilized nanoparticles were obtained when the ionic surfactant, SDS, was combined with any of the non-ionic surfactants tested, with polyoxyethylene octyl phenyl ether (Triton X-100) or polyoxyethylene (20) sorbitan monooleate (Tween 80) resulting in a slight increase in zeta potential over 30 days while the zeta potential with other non-ionic surfactants decreased.

Mcl-PHA containing 11 and 18 % of carboxyl groups was synthesized via free radical addition reaction of 11-mercaptoundecanoic acid to the pendant double bonds of unsaturated poly-3-hydroxynonanoate (PHNU). Colloidal suspensions prepared by ultrasonication needed a surfactant to maintain stability, even at 0.4 % solids of mcl-PHA containing 11 % carboxylation (PHNC-1) unlike the stable suspensions prepared without surfactants by the titration method. Similar particle sizes (155.6 ± 8.4 to 163.4 ± 11.3 nm) and polydispersity indices (0.42 ± 0.03 to 0.49 ± 0.04) were obtained when several non-ionic surfactants were tested to minimize particle agglomeration, with the smallest particles obtained with Triton X-100. When Triton X-100 was combined with a variety of ionic surfactants, smaller nanoparticles (97.1 ± 1.1 to 121.7 ± 5.7 nm)

with a narrower particle size distribution (0.21 ± 0.001 to 0.25 ± 0.003) were produced. The SDS and Triton X-100 combination was chosen to evaluate other mcl-PHAs at 10 % (w/v) solids content. Slightly smaller nanoparticles were formed with carboxylated mcl-PHAs compared to mcl-PHAs having aliphatic pendant side chains. Mcl-PHA consisting of 18 % carboxylation (PHNC-2) formed a much smaller nanoparticles and higher zeta potential.

Co-Authorship

The author was supervised by Dr. Bruce A. Ramsay and Dr. Juliana A. Ramsay, who contributed to the preparation of the manuscripts presented in this thesis. Hang Li produced the poly-3-hydroxyalkanoates (PHAs) used in Chapter 3 and 4.

Acknowledgements

First, and most importantly, I would like to thank Almighty God because he gave me the strength and determination to carry on throughout this very difficult but rewarding phase of my life. There are several people that must be acknowledged for their contributions in helping me complete my thesis over the past two years.

I am extremely grateful to my supervisors, Dr. Bruce A. Ramsay and Dr. Juliana A. Ramsay for continuously helping me improve my skills and the quality of my work. Their inspiration and great efforts to explain things in a clear and simple way have greatly influenced my way of thinking and approach to this research. Their advice on doing the experiments and constructive comments on each draft directly contributed to this study. They taught me how to think in a more organized and systematic fashion which will benefit me greatly in my new career. They challenged me to think critically and persevere through the many challenges I encountered in my work. They will be my mentor for a life time.

My special thanks also goes to my labmate, Hang Li for his technical support and involvement in discussions on my project.

I wish to express my sincere gratitude to Dr. Cunningham for always being there to answer my questions. I am also grateful to Dr. Parent for his assistance with the synthesis of the carboxylated poly-3-hydroxynonanoate (PHNC).

I would like to give my heartfelt thanks to my parents and brother (Antwi Boasiako K. Peprah) in Ghana. Thank you, Mr. and Mrs. Peprah Mensah, for your unconditional love. You have done your best to better educate me, and have always encouraged me to pursue my goals and assured

me that you are just a phone call away. Your caring, giving, patience, and understanding helped me through every difficult and important moment. To you I owe a profound debt.

My thankfulness also goes to Nana Aba Konamah Lartey for her love and care. I wish to express my sincere gratitude to all my thesis committee members Dr. Docoslis, Dr. Hutchinson, and Dr. Xiang.

I am grateful to my uncles Kofi Acheampong and Stephen Ntoni. It is blessing having you both as uncles. May God richly bless your families.

Finally, I would like to dedicate this thesis in memory of my aunty, Esther Ntoni. Each time I encountered a roadblock, your advices gave me the strength to surmount all obstacles. You will forever be remembered.

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List of Symbols and Abbreviations

^{1}H-NMR	Proton-Nuclear magnetic resonance
Brij 58	Polyoxyethylene (20) cetyl ether
$CDCl_3$	Deuterated chloroform
CMC	Critical micelle concentration
CTAB	Cetyltrimethylammonium bromide
DLS	Dynamic light scattering
DLVO	Derjaguin, Landau, Verwey, and Overbeek
Dowfax 2A1	Sodium dodecyldiphenyl oxide sulfonate
DTAB	Dodecyltrimethylammonium bromide
GPC	Gel permeation chromatography
HLB	Hydrophilic-lipophilic balance
mcl	Medium-chain-length
mcl-PHA	Medium-chain-length Poly(3-hydroxyalkanoate)
mins	Minutes
mL	Milliliter
mM	Millimolar
M_n	Number- average molecular weight
M_w	Weight- average molecular weight
NMR	Nuclear magnetic resonance
O/W	Oil-in-water
PDI	Polydispersity index
PHA	Poly(3-hydroxyalkanoate)
PHB	Poly(3-hydroxybutyrate)
PHD	Poly(3-hydroxydecanoate)
PHN	Poly(3-hydroxynonanoate)
PHNC-1	Poly(3-hydroxynonanoate) with 11 % carboxyl groups in the pendant side chains

PHNC-2	Poly(3-hydroxynonanoate) with 18 % carboxyl groups in the pendant side chains
PHNU-1	Poly(3-hydroxynonanoate) with 11 % unsaturation (vinyl groups) in the pendant side chains
PHNU-2	Poly(3-hydroxynonanoate) with 18 % unsaturation (vinyl groups) in the pendant side chains
PIT	Phase inversion temperature
scl	Short-chain-length
scl-mcl-PHA	Short-chain-length-medium-chain-length Poly(3-hydroxyalkanoate)
scl-PHA	Short-chain-length Poly(3-hydroxyalkanoate)
SDBS	Sodium dodecylbenzenesulfonate
SDS	Sodium dodecyl sulfate
SOR	Surfactant to oil ratio
Span 80	Sorbitan monooleate
Span 85	Sorbitan trioleate
T_g	Glass transition temperature
Triton X-100	Polyoxyethylene octyl phenyl ether
Tween 20	Polyoxyethylene (20) sorbitan monolaurate
Tween 80	Polyoxyethylene (20) sorbitan monooleate
W	Work
W/O	Water-in-oil

Chapter 1
Introduction

1.1 Background

Plastics have increasingly become a major raw material since their introduction in the 1930s. It is an essential component of many products in sectors such as agriculture (Smit and Nasr, 1992), automobiles (Donahue et al., 2003), electronics (Alston et al., 2011), packaging materials (Lange and Wyser, 2003), and several other applications (Pinto et al., 1999). Plastics are durable, inexpensive, and versatile. Annual plastic usage in the world has increased about 20 times from 5 million tons in the 1950s to nearly 100 million tons in 2001. In 2013, the global production of plastics was about 299 million tons and has increased by 4 % since 2012 (Jambeck et al., 2015). Despite the merits derived from these petroleum-based materials, environmental problems arise. Over 60 % of the total solid plastic produced is disposed of in landfills throughout the world with less than 10 % being recycled (Williams and Slaney, 2007). This is of major concern due to potential health risks and its impact on environmental pollution (Yamamoto et al., 2001). Poly-3-hydroxyalkanoates (PHAs) are promising biomaterials that could reduce these concerns.

PHAs are biodegradable and biocompatible polyesters produced from renewable resources. They are synthesized by a wide variety of bacteria, often as an energy storage product under nutrient limited conditions. Depending on the carbon chain length of its repeating units, PHAs may be classified as short-chain-length (scl) or medium-chain-length (mcl). Scl-PHAs are thermoplastics that are somewhat brittle due to its high degree of crystallinity, and contain typically four to five carbon subunits. They are commercially available with current applications focusing on films, packaging, food service products, and agriculture (Wu et al., 2009).

Mcl-PHAs are of special interest due to their elastomeric properties. They contain six to fourteen carbon subunits. They are generally less crystalline than scl-PHAs with a relatively low melting temperature (Witholt and Kessler, 1999). Mcl-PHAs are not commercially available but may find applications in paint, coatings, adhesives (Van der Walle et al., 2001), drug delivery (Lu et al., 2011), toners (Sacripante et al., 2012), tissue engineering, and other biomedical applications (Chen and Wu, 2005). Many of these applications require use of mcl-PHA as a suspension, with specific nanoparticle size. For example, applications in toners require solids content in the range of 25-30 % (w/v) and particles sizes of 25-300 nm (Sacripante et al., 2012). A few studies have reported the behavior of mcl-PHA in aqueous suspension up to 0.4 % (Marchessault et al., 1995; Gaucher et al., 2010; Beauregard et al., 2016), but none has investigated the dense aqueous suspensions required commercially. Since mcl-PHA nanoparticle suspensions agglomerate without surfactant above 0.4 % solids (Beauregard et al., 2016), surfactants are needed at high solids content. Due to the hydrophobic nature of mcl-PHAs, they may require high surfactant concentrations to retain stability at high solids (30 % (w/v)). However, a higher surfactant concentration could introduce undesirable levels of impurities that may have a negative effect on the application. Thus, the processing conditions and surfactants needed to make stable mcl-PHA nanoparticles at high solids content require study.

Mcl-PHAs usually contain aliphatic side chains making them more hydrophobic. Depending on the mode of production and post-production modification, the long pendant side chain in mcl-PHAs can contain functional groups such as olefins (Fritzsche et al., 1990), halogens (Doi and Abe, 1990), epoxides (Bear et al., 1997), and esters (Scholz et al., 1994). Carboxylic acid groups are of great interest as they can increase the material's hydrophilicity and consequently reduce the stabilizer requirement for mcl-PHA latexes. Carboxylated mcl-PHAs

have been synthesized via chemical modification of the double bonds in long pendant side chains of unsaturated mcl-PHAs (Lee and Park, 2000; Kurth et al., 2002; Hany et al., 2004). Carboxylated mcl-PHA nanoparticle suspensions have only been prepared by Kurth et al. (2002) and Beauregard et al. (2016), at a final solids content of 0.2 % (w/v) and 0.4 % (w/v), respectively. The data of Kurth et al. (2002) suggest that mcl-PHA nanoparticle size had no significant decrease with increasing carboxyl content. Beauregard et al. (2016) obtained smaller particles with carboxylated than non-carboxylated mcl-PHA at low concentration without use of surfactant, suggesting that less surfactant may be required to produce high solids carboxylated mcl-PHA nanoparticle suspensions. Making carboxylated mcl-PHA suspensions at high solids content (up to 10 %) has not been previously studied.

1.2 Objectives

The two main objectives of this thesis are:

(1) To determine the processing conditions and the surfactants needed to prepare dense (≥ 10 % w/v), stable mcl-PHA (specifically, poly-3-hydroxydecanoate, PHD) nanoparticle suspensions in a two-stage emulisification-solvent evaporation method, and

(2) To produce carboxylated mcl-PHA and to study the processing conditions and the surfactants needed to prepare dense (10 % w/v), stable carboxylated mcl-PHA nanoparticle suspensions and to determine whether carboxylation affects particle size.

The organization of this thesis consists of five distinct chapters. Chapter 1 describes the background, motivation for the study, and a brief outline of the work completed. Chapter 2 covers the literature review. This chapter provides a basic introduction to emulsions, classification of emulsion, various components of emulsions, key components that can be

controlled to produce smaller droplets, and description of nano-emulsions. A brief introduction to mcl-PHA suspensions and carboxylated mcl-PHAs is included. In chapter 3, the two-stage emulsification procedure used to prepare dense stable mcl-PHA suspensions is discussed. The effects of nano-suspension formulation and processing parameters including ultrasonication time and amplitude, type and concentration of surfactant, and choice of organic solvent on mcl-PHA particle size and stability are investigated. Chapter 4 covers the procedure to produce carboxylated mcl-PHAs and its suspension. In chapter 5, general conclusions and recommendations for future work are discussed.

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Chapter 2
Literature Review

2.1 Emulsion

An emulsion is a dispersed system comprising of two immiscible liquid phases (McClements, 2015). However, emulsions usually refer to systems where both the dispersed phase and dispersion medium are liquids, with one of the liquids being dispersed in the other. Because of the differences in attractive interactions between the molecules of the two immiscible phases, an interfacial tension, γ, exists between the two immiscible phases. Therefore, emulsions usually contain an appropriate or a suitable emulsifying agent to aid stability aside from the dispersed phase and continuous medium.

Water has become a common choice of solvent for the continuous phase for several reasons. First, water is less expensive. It is non-toxic, has a high heat capacity and a low viscosity, which are good characteristics for heat transfer. Many emulsion properties such as stability, rheology, and color, depend on the emulsion droplet size and size distributions (McClements, 2015). Emulsions can be macro-, mini (nano)-, and micro- emulsions based on the emulsion droplet size (Jafari et al., 2008). Table 2-1 shows their characteristics.

Table 2-1 Characteristics of macro-, nano-, and micro-emulsions (Jafari et al., 2008)

Property	**Macro-emulsion**	**Nano-emulsion**	**Micro-emulsion**
Appearance	Formulation-dependent	Transparent to milky	Transparent
	Classic	High energy	Low energy

Preparation method	homogenization	(pressure)	emulsification
Surfactant concentration	Fairly low	Medium (<10 %)	Fairly high (10-20 %)
Droplet size	0.5-100 μm	20-500 nm	10-100 nm
Thermodynamic stability	Unstable; kinetically stable	Unstable; kinetically stable	Stable

Emulsions are classified not only with respect to composition, but also with respect to morphology. A system in which water act as a continuous phase and oil as a dispersed phase is termed oil-in-water (O/W) emulsion. In this case, the oil droplets are dispersed throughout the aqueous phase and the emulsifier is generally soluble in the aqueous phase. By contrast, in a water-in-oil (W/O) emulsion, water is dispersed throughout the continuous oil phase and the emulsifier is generally soluble in the oil phase (Mason et al., 2006).

2.2 Nano-emulsions

Nano-emulsions are considered as emulsion systems with uniform and extremely small droplet size mostly covering the size range 20-500 nm (Solans et al., 2003). This class of emulsions is also referred to as mini-emulsions, finely dispersed emulsions, ultrafine emulsions, submicrometer-sized emulsions etc. The term nano-emulsion is usually preferred because it gives a clear idea of the nanoscale size range of the droplets. Furthermore, it is concise and it avoids

misinterpretation with the term micro-emulsions which are thermodynamically stable systems (Solans et al., 2005).

A micro-emulsion is another type of disperse system representing one liquid phase dispersed in the other and forms spontaneously (Table 2-1). The major difference between micro-emulsion and nano-emulsion is that the former is thermodynamically stable, while the latter is not but kinetically stable (Koroleva and Yurtov, 2012). Nano-emulsions usually appear transparent or translucent to the naked eye because of their droplet size (Fig. 2-1). This property together with a viscosity similar to that of water makes them of special interest for practical applications (Solans et al., 2005).

Fig. 2-1 Image of a macro-emulsion (left) and a nano-emulsion (right)

Tadros et al. (2004) reviewed the advantages of nano-emulsions. First, due to the small droplet size phenomenon such as creaming or sedimentation is prevented upon storage. Second,

the submicrometer droplet size helps prevent any flocculation of the droplets and this keeps the system dispersed without any separation occurring. Third, the nano-size droplets aid to inhibit their coalescence, since these droplets are non-deformable and hence surface fluctuations are prevented. Low surfactant concentration is needed to prepare stable (O/W) nano-emulsion, contrast to micro-emulsion which require high surfactant concentration. In addition, sub-micron droplets enhance uniform deposition on substrate (Tadros et al., 2004).

Regardless of the advantages of nano-emulsions, they have only attracted interest in recent years. Nano-emulsion preparation requires special techniques, such as ultrasonics and high pressure homogenizers. Microfluidizer is quite expensive. Hence, there is a perception in the pharmaceutical, cosmetic, and chemical industry that nano-emulsions are more expensive to produce (Tadros et al., 2004). Furthermore, there is a lack of understanding regarding the mechanism of producing submicrometer-sized droplets, the role of surfactants and the interfacial chemistry that is involved. In addition, there is little knowledge on the mechanism of Ostwald ripening, which may be considered the most common and difficult instability problem with nano-emulsions. There is also the fear of introducing new systems without a full evaluation of the cost involved and merits (Tadros et al., 2004). Nano-emulsions are used in the pharmaceutical industry as drug delivery systems, in cosmetics as personal care formulations, in the chemical industry for the preparation of latex particles (Ishikawa et al., 2005; Solans et al., 2003).

2.3 Preparation of Emulsions

Emulsions can be prepared by pre-mixing a fluid phase that contains surfactants and other components, then mechanically mixing to deform and disrupt large droplets. Surfactants usually adsorb at the newly formed interfaces to stabilize the droplets. There are several

preparation techniques, but the simplest may be mechanical stirring such as with ultra-turax, which is often used in the academic and industry fields (Antonietti et al., 2002). Nano-emulsions may be difficult to prepare with this equipment, since it consists of stable emulsion with droplet size in the nanometric scale. Nano-emulsions, being non-equilibrium systems, do not form spontaneously. As a result, much higher energies are required as the viscous resistance during agitation adsorbs most of the energy and creates heat. Usually, high force dispersion devices, such as an ultrasonicator and high pressure homogenizer, are applied to obtain extremely small and homogeneously distributed droplets. Thus, nano-emulsions may be prepared by utilizing either high energy emulsification methods with suitable choice of surfactants or low energy emulsification methods like phase inversion temperature (PIT) principle (Tadros et al., 2004).

2.3.1 Use of High Energy Emulsification Methods

Various high energy emulsification methods have been reported in the literature, including ultrasonic emulsification, high pressure homogenization, and high-shear stirring. Rotor-stator systems were initially used for the preparation of nano-emulsions, but preparation of emulsions with the droplet size less than 200-300 nm becomes hard to accomplish. Sub-micrometer-size emulsion droplets are formed under ultrasonic emulsification and high pressure homogenization (Koroleva and Yurtov, 2012).

2.3.2 Use of Phase Inversion Temperature (PIT) Principle

Nano-emulsions can be prepared by low-energy emulsification methods such as PIT. The transitional PIT method changes factors that affect the hydrophilic-lipophilic balance (HLB) of the system, such as temperature and/or electrolyte concentration or changing the HLB value of

the surfactant at a constant temperature when using surfactant mixtures. The second PIT method, catastrophic inversion, increases the volume fraction of the dispersed phase (Tadros et al., 2004). PIT method is successfully used for the preparation of nano-emulsions stabilized by ethoxylated surfactants because the HLB of these surfactants is highly sensitive to changes in temperature. This method may not be applicable when using ionic and non-ionic surfactants whose HLB is significantly less sensitive to temperature variations (Koroleva and Yurtov, 2012).

2.4 Mechanisms of Emulsion Formation

To disperse one liquid in another in the form of an emulsion, an amount of work (W) in erg must be applied to the system. W is equal to the product of the interfacial tension (γ) in erg/cm^2 and ΔA (cm^2), which is the increase in surface area of dispersed phase as the emulsion droplets are formed. This is mathematically expressed as;

$$W = \gamma \Delta A \quad \text{……..Equation 1}$$

To produce small droplets with a reduced amount of work during emulsification, the interfacial tension between water and oil must first have a sizeable decrease. A suitable emulsifying agent is usually used to lower the interfacial tension. Typically, W must be small to achieve thermodynamic stability, indicating that both the surface area and interfacial tension must be decreased. To obtain a lower specific surface area, the droplets must be larger. Consequently, it could lead to aggregation or coalescence of the droplets and quick breakdown of the emulsion. Hence, there must be a balance between the thermodynamic and physical stability that is maintaining both W and emulsion droplets as minimal as possible. The formation of large droplets of usually a few micrometers may be quite easy, but it could be difficult to

produce sub-micron droplets. This indicates that the most significant process in emulsion formation is the droplet breakup but not the droplet formation (Walstra, 1993).

2.5 Stability of Emulsions

Unlike micro-emulsions, nano-emulsions will ultimately separate into their component phases, since they are thermodynamically unstable. The main mechanisms of instability that are involved in leading to emulsion breakdown are illustrated schematically in Fig. 2-2. By considering the mechanism of emulsion breakdown, the mechanism for emulsion stabilization may be obtained. The five processes leading to emulsion instability or emulsion breakdown (Tadros, 2009; McClements, 2007) are summarized in Fig. 2-2.

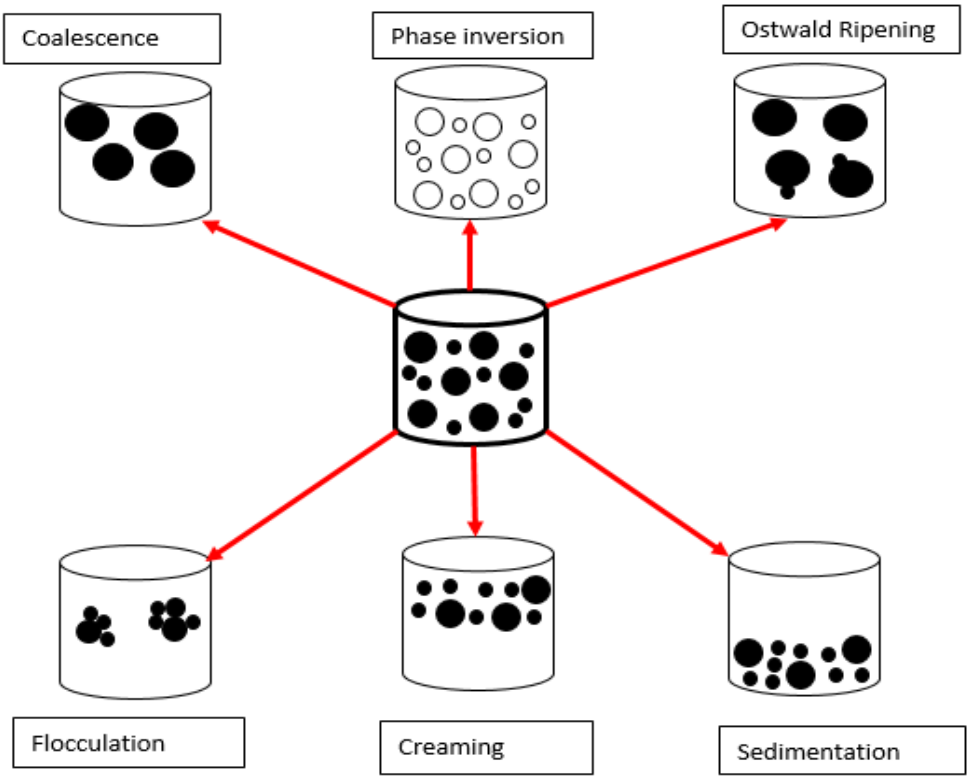


Fig. 2-2 Different breakdown processes of emulsions (modified from Tadros, 2009)

2.5.1 Creaming and Sedimentation

These phenomena are the result of an external force field applied to the emulsion, usually gravitational or centrifugational. Creaming is typically the upward movement of dispersed droplets relative to the continuous phase, moving more rapidly either to the top if their density is less than that of the medium or to the bottom of the container, if their density is greater. There is no change in droplet size, but a buildup of an equilibrium droplet concentration gradient within the emulsion.

2.5.2 Flocculation

There is no change in basic droplet size in flocculation, but there is a buildup of aggregates of droplets within the emulsion. Flocculation in emulsion occurs as a result of the existence of attractive forces between the droplets.

2.5.3 Coalescence

Coalescence occurs when there is a change in the basic droplet size due to the coalescence of aggregated flocs of droplets or closely packed droplets. The limiting case for this phenomenon is the complete separation of the emulsion into separate phases.

2.5.4 Ostwald Ripening

In Ostwald ripening, there is a change in the basic droplet size and distribution due to diffusion of oil through the continuous phase. This phenomenon results from the finite solubility of the liquid phases. Larger emulsion droplets are formed at the expense of the smaller droplets due to the difference in chemical potential. The process will proceed until an equilibrium droplet size is achieved.

2.5.5 Phase Inversion

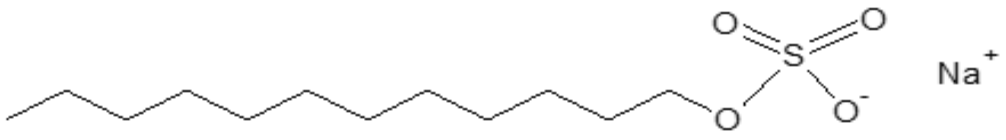
In phase inversion, an emulsion changes for example from an oil-in-water (O/W) emulsion to water-in-oil (W/O) emulsion. This process may occur due to the change in temperature or concentration of one of the components or by adding a new component to the system.

2.6 Surfactants

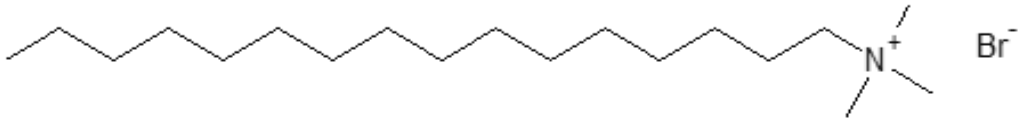
Surfactants are amphiphilic compounds consisting of a non-polar media (hydrophobic tail) and a polar media (hydrophilic head). They dissolve in both organic solvents and water due to their amphiphilic properties (Mishra et al., 2009). In mixtures of immiscible solvents, surfactants exist at the liquid-liquid interface thereby reducing the interfacial tension between dissimilar phases. In the same way, by adsorbing at the liquid-gas interface, surfactants also reduce the surface tension of liquids. Amphiphiles exhibit properties other than tension lowering and this is why they are often labelled according to their main use such as soap, detergent, wetting agent, emulsifier, foaming agent, dispersant etc.

Surfactants are divided into categories based on the charge of the polar head groups when dissolved in water at neutral pH: anionic, cationic, non-ionic and zwitterionic (amphoteric). Surfactants that possess negative charge head groups are referred to as anionic. Such surfactants include sodium dodecylbenzenesulfonate, sodium dodecyl sulfate etc. If the head group is positively charged, it is referred to as cationic surfactant, such as dodecyltrimethyl ammonium Br and cetyltrimethyl ammonium Br. If the head group possesses no charge, it is referred to as non-ionic surfactant, such as polyoxyethylene octyl phenyl ether and sorbitan monooleate. When

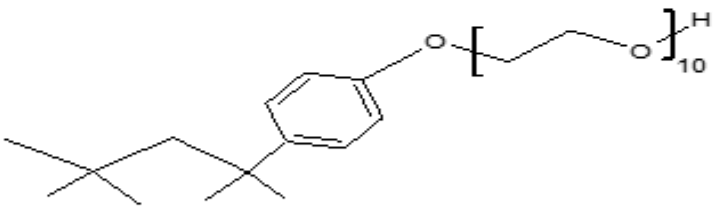
the head group contains both a positive and a negative charge, it is referred to as zwitterionic or amphoteric surfactant, for example amidobetaine and amine oxide (Tadros, 2009).



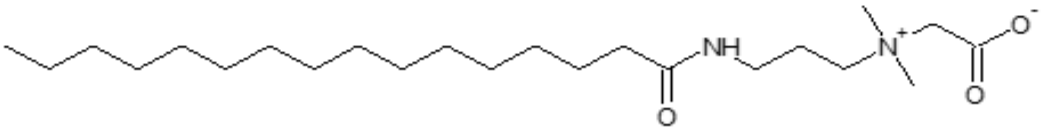
Sodium dodecyl sulfate



Cetyltrimethyl ammonium Br



Polyoxyethylene octyl phenyl ether



Amidobetaine

Fig. 2-3 Chemical structures of four kinds of surfactants (Housaindokht and Pour, 2012; Schmalstieg et al., 2002)

A fundamental property of surfactants is their ability to form micelles in solution. This property is due to the presence of both hydrophobic and hydrophilic groups in each surfactant molecule. Upon dissolving, after they reach a certain value of concentration, their molecules or ions begin to associate and to organize themselves into more complex units referred to as micelles. The characteristic concentration at which this process starts, is referred to as critical micelle concentration (CMC). When micelles form in water, their hydrophobic tails form a core that adsorb on oil droplets, whereas hydrophilic heads remain in contact with the water as an outer shell.

The various surfactants used in the preparation of O/W or W/O emulsions are frequently chosen on an empirical basis. One such semi-empirical scale for choosing surfactants is the hydrophilic-lipophilic balance (HLB) value developed by Griffin (Griffin, 1949). The concept of HLB was introduced as a measure of the polar character of the surfactants. For an O/W emulsion droplet, the hydrophobic tail lies in the oil phase, whereas the hydrophilic head lies in the aqueous phase. In contrast, for a W/O emulsion droplet, the hydrophilic portion lies in the water droplet, whereas the lipophilic portion lies in the hydrocarbon phase. Table 2-2 is a guide for the selection of surfactants for particular applications.

Table 2-2 HLB of surfactants and corresponding applications (Tadros, 2009)

HLB range	Application
3-6	W/O emulsifier
7-9	Wetting agent
8-18	O/W emulsifier

13-15	Detergent
15-18	Solubilizer

There are three main processes that occur during emulsification (Walstra, 1993). First, droplets are deformed and eventually broken up followed by adsorption of surfactants onto the deformed and newly formed droplets. The droplets then encounter each other leading to possible coalescence. These processes occur simultaneously with each occurring several times during emulsion formation, indicating that a steady state may not be achieved (Walstra, 1993). Consequently, surfactants play two very significant roles in the preparation of emulsions. They lower the interfacial tension, making it easier for droplets to break up. They also prevent re-coalescence of droplets to a varying degree and improve the long-term stability of emulsions (Yang et al., 2013; Walstra, 1993).

2.7 Characterization of Emulsions

Characterization of emulsions is essential since it helps to ensure the desired droplet size and stability with time. Analytical techniques include particle size determination, zeta potential measurement, nuclear magnetic resonance, polydispersity index (PDI), surface tension determination and differential scanning calorimetry. A number of these techniques are summarized below.

2.7.1 Particle Size Analyzers

Particle size affects various properties of emulsions and is an essential indicator of quality and performance. Smaller droplet sizes and higher surface charge will typically improve suspension and emulsion stability. In the paint and pigment industries, particle size influences appearance properties such as gloss and tinctorial strength. Several analytical techniques can be used to determine the particle size of emulsions. Two of the frequently used techniques are dynamic light scattering and electron microscopy.

2.7.1.1 Dynamic Light Scattering

Dynamic light scattering (DLS), sometimes referred to as photon correlation spectroscopy or quasi-elastic light scattering, is one of the most popular and useful techniques used for determining the size of particles in the sub-micron region, usually dispersed in a liquid. DLS is composed of a light source, a spectrometer consisting of an optical system for defining the scattering angle, a signal analyzer, a detector, and a computer for processing and presenting the data. It is quite simple to operate with short experiment time, and may not require extensive experience for routine measurement. DLS uses a laser beam to illuminate a suspension of particles or molecules undergoing Brownian motion (Sartor, 2003; Pecora, 1985). It is based on the intensity of the light scattered from dispersions of particles and macromolecules. It is understood that the intensity fluctuates with time and happens very fast. The time-dependent fluctuations in the intensity of the scattered light that occur are analyzed by an autocorrelator which determines the autocorrelation of the signal. Thus, DLS measures the diffusion coefficient of the particles or molecules undergoing Brownian motion and relates this to the particles size. Instrument such as Malvern Zetasizer Nano ZS (Worcestershire, UK) is used for measurement of particle size.

2.7.2 Polydispersity Index

Another technique used for the characterization of emulsions is the polydispersity index (PDI). Dynamic light scattering can be used to determine the polydispersity index of an emulsion. PDI is significant since it characterizes the particle size distribution of emulsions and suspensions. Usually, a narrow size distribution is required, since larger polydispersity indices results in particle aggregation , and indicate a broad size distribution.

2.7.3 Zeta Potential

During the movement of a colloidal particle in a dispersion medium, a layer of the bulk dispersant remains attached to the particle. The boundary of this layer is referred to as shear plane (slipping plane)**.** The value of the electric potential at the shear plane represents the Zeta potential. It is considered as a physical property exhibited by a particle in suspension and emulsion. Understanding zeta potential could help to control and optimize the formulations of emulsions and suspensions. It helps in determining the long-term stability of emulsions and suspensions. In an emulsion, if all the particles have a large negative or positive zeta potential they will repel each other leading to dispersion stability. In contrast, if the particles have low zeta potential values then there is no force to prevent the particles sticking together and this may lead to dispersion instability. Unstable emulsions and suspensions occur between +30 or -30 mV and particles outside these limits are usually considered stable (Larsson et al., 2012).

2.8 Emulsion Components

In making medium-chain-length (mcl) poly(3-hydroxyalkanoate) (O/W) emulsion, the dispersed phase consists of mcl-PHA dissolved in an organic solvent, while the continuous phase is aqueous surfactant.

2.9 Poly(3-hydroxyalkanoates) (PHAs)

Poly(3-hydroxyalkanoates) (PHAs) are polyesters that are synthesized by a wide range of bacteria and produced from renewable resources, making it a promising alternative material for various applications in industry. Properties such as biodegradability and non-toxicity make PHAs more advantageous compared to conventional petroleum-based plastics (Wu et al., 2009).

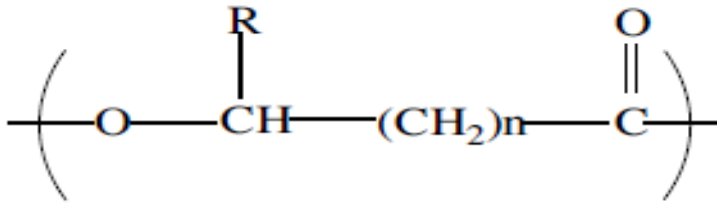


Fig. 2-4 General structure of poly(3-hydroxyalkanoates) (PHAs) (Anjum et al., 2016)

n = 1	R = hydrogen	poly (3-hydroxypropionate)
	= methyl	poly (3-hydroxybutyrate)
	= ethyl	poly (3-hydroxyvalerate)
	= propyl	poly (3-hydroxyhexanoate)
	= pentyl	poly (3-hydroxyoctanoate)
	= nonyl	poly (3-hydroxydodecanoate)
n = 2	R = hydrogen	poly (4-hydroxybutyrate)
	= methyl	poly (4-hydroxyvalerate)

n = 3	R = hydrogen	poly (5-hydroxyvalerate)
	= methyl	poly (5-hydroxyhexanoate)
n = 4	R = hexyl	poly (6-hydroxydodecanoate)

Depending on the number of carbon in the R groups, PHAs may be classified as short-chain-length (scl) containing up to five carbon subunits or medium-chain-length (mcl) containing six to fourteen carbon subunits. Scl-PHAs consisting of mcl monomers are referred to as scl-mcl-PHA.

2.10 Medium-chain-length Poly(3-hydroxyalkanoates) (mcl-PHAs)

The elastomeric properties of mcl-PHAs make them suitable for numerous applications, contrary to its stiff or brittle scl counterpart. Not only are mcl-PHAs fully compostable and entirely derived from renewable resources, they also have a low or no crystallinity and a low melting temperature (40°C to 60°C). The glass transition temperatures (T_g) ranges between -50°C to -25°C (Witholt and Kessler, 1999). The literature has discussed many prospective applications of PHAs including drug delivery (Lu et al., 2011; Kilicay et al., 2011), films and coatings (Marchessault et al., 1995; Dufresne and Samain, 1998) and tissue engineering (Wu et al., 2009).

2.11 Poly(3-hydroxyalkanoate) Suspensions

There have been few studies on either scl or mcl-PHA suspensions. The first PHA colloidal suspension was reported by Horowitz and Sanders (1994). Various authors have since

studied PHA suspensions for diverse applications including paper coating (Bourbonnais and Marchessault, 2010), paint formulation (Van der Walle et al., 2001), toners (Sacripante et al., 2012; Fukui et al., 2009; McAneney-Lannen et al., 2012), and drug delivery (Lee et al., 2011; Lu et al., 2011; Shrivastev et al., 2013; Xiong et al., 2010; Bayram et al., 2008; Zhang et al., 2010; Kassab et al., 1997; Kilicay et al., 2011).

Preparing PHA nanoparticle suspensions may require the use of either high energy methods with mechanical devices such as ultrasonicators (Marchessault et al., 1995; Heathman et al., 2014) or high energy homogenizers with the aid of surfactants and co-surfactants or low energy methods (Poletto et al., 2008). PHA suspensions made of scl (Horowitz and Sanders, 1994), scl-mcl (Zhang et al., 2010; Xiong et al., 2010; Kilicay et al., 2011), and mcl (Gaucher et al., 2010) require a surfactant to retain stability. However, stable mcl-PHA nanoparticle suspensions can be fabricated with no surfactant, if the final solids content is lower than 0.4 % (w/v) (Ramsay et al., 1994; Marchessault et al., 1995; Beauregard et al., 2016). This is because the relatively longer side chain of mcl-PHA monomers provide steric stabilization, in contrast to poly-3-hydroxybutyrate (PHB) containing methyl group (Marchessault et al., 1995). Without surfactant mcl-PHA nanoparticles tend to aggregate if the solids content is beyond 0.4 % (w/v) (Beauregard et al., 2016). A much higher mcl-PHA solids content in the range of 25-30 % is required for most industrial applications such as xerographic toners with particle sizes less than 300 nm (Sacripante et al., 2012). Preparation of concentrated (30 %) stable mcl-PHA suspensions has not been reported in the literature.

2.12 Mcl-PHA Containing Functionalized Side Chains

Mcl-PHAs can have a relatively long non-polar side chains. As a result, these polymers tend to be more hydrophobic. However, depending on the production and post-production modification, mcl-PHAs can contain various functional groups (Figure 2-5) at the terminal position in their long pendant side chains (Kai and Loh, 2014). These functional groups include olefins (Fritzche et al., 1990; Ulmer et al., 1994; Kim et al., 1995), halogen (Arkin et al., 2000; Doi and Abe, 1990; Kim et al., 1996), ester (Hany et al., 2004), aromatic (Hazer et al., 1996; Curley et al., 1996), hydroxyl (Lee et al., 2000), epoxide (Bear et al., 1997; Park et al., 1998), carboxyl (Kurth et al., 2002; Lee and Park, 2000; Stigers and Tew, 2003), and amide (Hany et al., 2004).

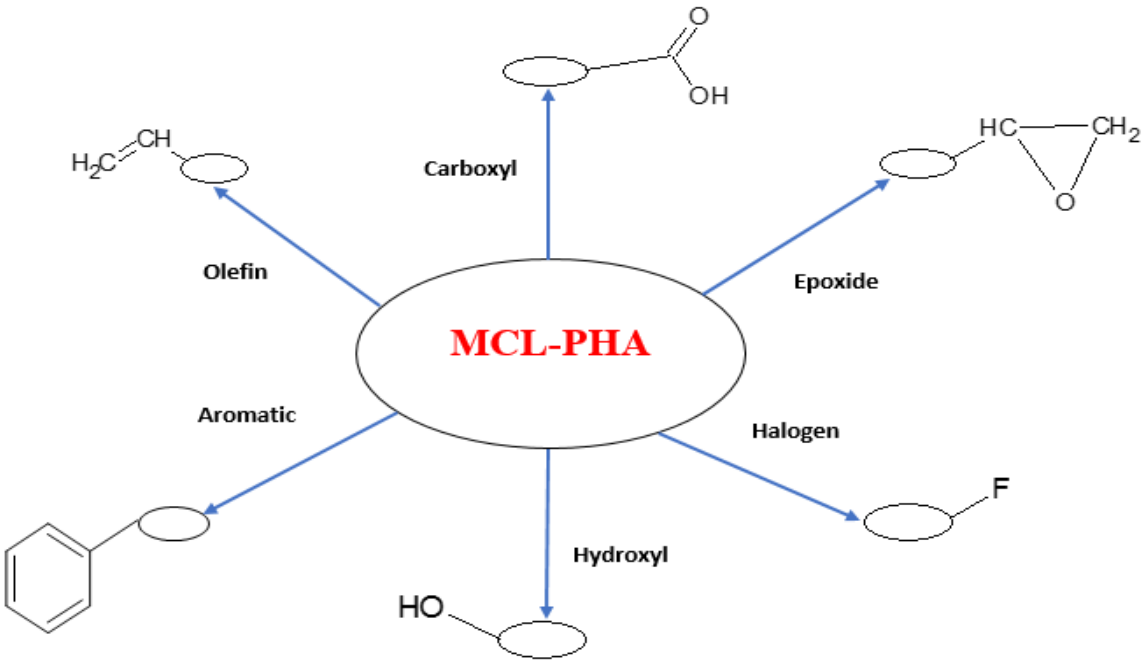


Fig. 2-5 Illustration of the various functional groups that mcl-PHA can contain via chemical modification (modified from Kai and Loh, 2014)

Incorporating functional groups in mcl-PHAs can influence their physical properties and present site for chemical modicfication. PHA properties such as mechanical strength, surface features, and hydrophilicity can be altered to suit the requirements of particular applications via chemical modification, giving it more value and increasing the use of the material (Lee et al., 2000; Kai and Loh, 2014). For example, mcl-PHAs containing pendant hydroxyl groups (Fig. 2-6) were synthesized via chemical modification of the side chain olefins, using potassium permanganate ($KMnO_4$), to enhance the hydrophilicity of the material (Lee at al., 2000).

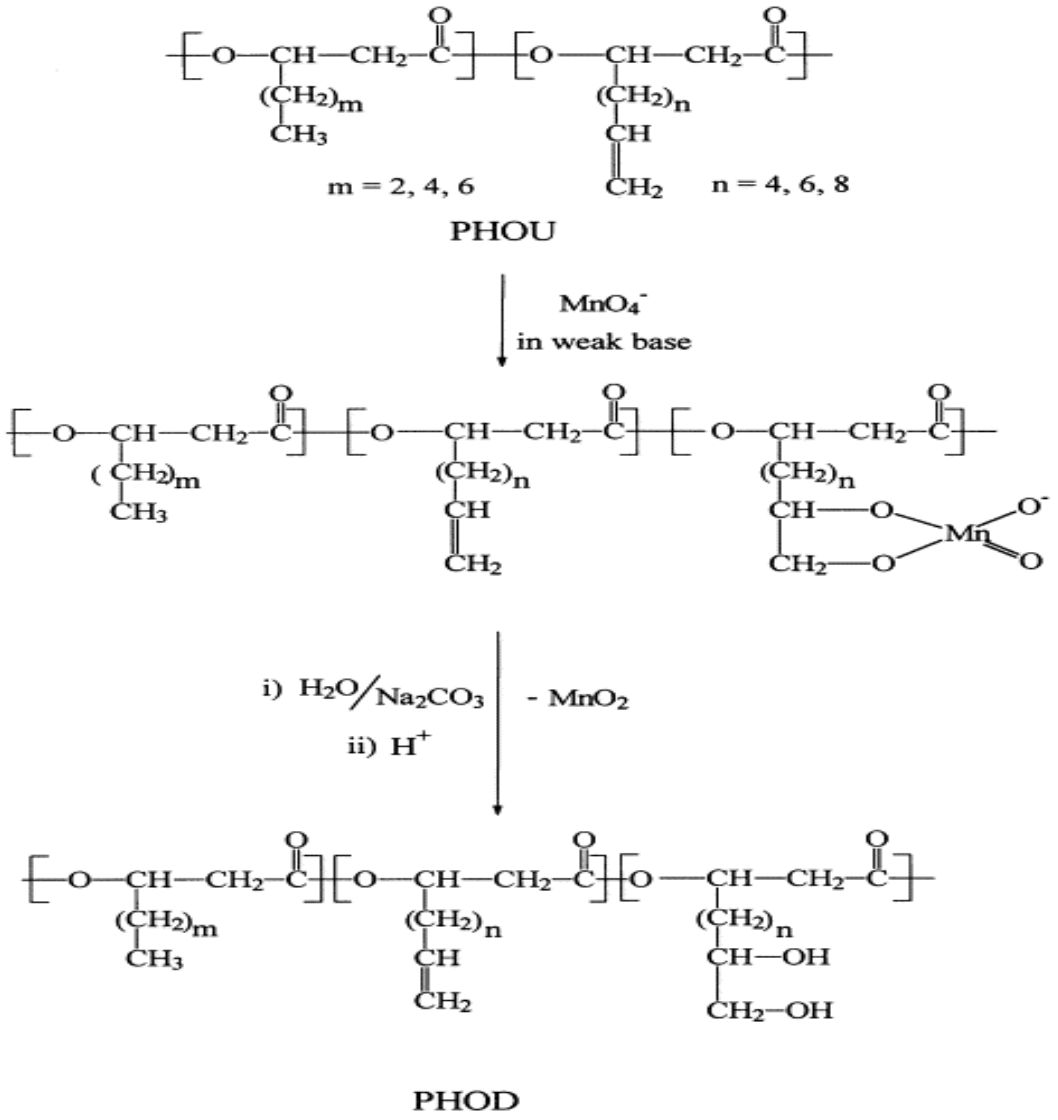


Fig. 2-6 Reaction scheme for the synthesis of hydroxyl mcl-PHAs by chemical modification (Lee et al., 2000). PHOD is the hydroxylated product of poly(3-hydroxyoctanoate-co-3-hydroxyundec-10-enoate) (PHOU)

2.12.1 Incorporating of Side Chain Carboxyl Groups

The synthesis of carboxylated mcl-PHAs has been reported by various authors (Bear et al., 2001; Hany et al., 2004, Kurth et al., 2002; Timbart et al., 2007; Lee and Park, 2000; Stigers and Tew, 2003; Zhang et al., 2000). Mcl-PHAs can also contain a carboxylic group at one end and a hydroxyl group at the other end (Timbart et al., 2007). The production of carboxylated mcl-PHA has been accomplished by chemical modification of mcl-PHAs containing unsaturated side chains (Kurth et al., 2002; Lee and Park, 2000; Stigers and Tew, 2003; Hany et al., 2004). The presence of carboxyl groups was identified using proton nuclear magnetic resonance (^{1}H-NMR) and infrared (IR) spectroscopy. The reactions reported by Lee and Park (2000) and Kurth et al. (2002) used potassium permanganate ($KMnO_4$), as an oxidizing agent. A significant loss of the polymer molecular weight (MW) was reported when 50 % of mcl-PHA containing olefin side chains was converted to carboxylic acid (Lee and Park, 2000). Kurth et al. (2002) also obtained an undesirable loss of the polymer molecular weight upon synthesizing the carboxylic mcl-PHAs by this method. Stigers and Tew (2003) used osmium tetraoxide (OsO4) and oxone to oxidize the unsaturated side chains, with minimal polymer molecular weight loss. Higher conversions were observed, with no molecular weight loss when 11-mercaptoundecanoic acid was used to convert the side chain olefins to carboxylic acid (Figure 2-7) via radical addition reaction (Hany et al., 2004).

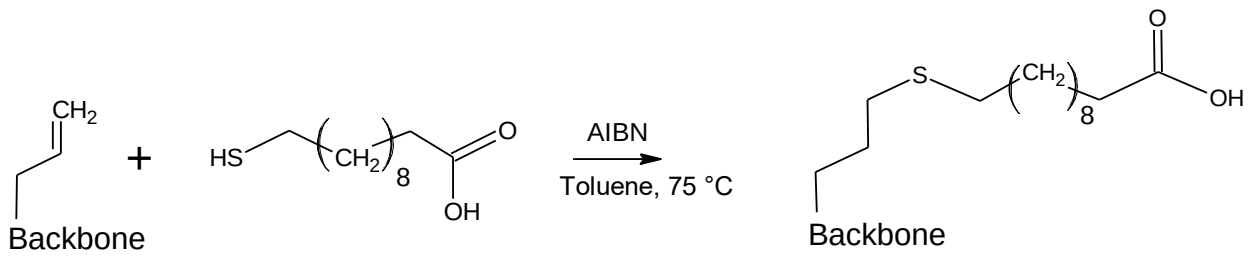


Fig. 2-7 Conversion of unsaturated mcl-PHA to carboxylated mcl-PHA with no molecular weight loss (Hany et al., 2004)

Carboxyl functional groups are useful because they increase the hydrophilicity of mcl-PHA (Stigers and Tew, 2003; Kurth et al., 2002). They are of great use to bind bioactive molecules, hydrophilic components, or targeting proteins (Kai and Loh, 2014; Kurth et al., 2002). Carboxyl groups also enhance reactivity for possible subsequent chemical reactions. For example, mcl-PHA containing terminal carboxyl group can be transformed to either an amide or an ester group using tridecylamine and octadecanol, respectively (Hany et al., 2004).

Carboxylated mcl-PHA nanoparticle suspensions have been prepared by Kurth et al. (2002) and Beauregard et al. (2016). No signficant decreased in nanoparticle size was observed with increasing carboxylic acid content (Kurth et al., 2002). Beauregard et al. (2016) reported a significant decreased in particle size when suspensions were prepared by carboxylated mcl-PHA compared to that of unsaturated and aliphatic mcl-PHAs. Both studies were made at a low final solids content of 0.2 and 0.4 % (w/v), respectively. There has been no work done on the preparation of concentrated carboxylated PHA suspensions. In addition, introducing carboxylic groups could potentially reduce particle size and stabilizer requirement needed to stabilize high solids mcl-PHA suspensions. Evaluating the surfactant concentration required to stabilize

concentrated (10 %) carboxylated mcl-PHA nanoparticle suspensions has not been reported in the literature.

2.13 References

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Chapter 3

Dense Stable Nanoparticle Suspensions of Medium-chain-length Poly(3-hydroxyalkanoate)

Submitted to European Polymer Journal

3.1 Abstract

This is the first study to examine the formulation (selection of ionic and nonionic surfactants and their concentrations) and processing conditions (ultrasonication time and amplitude, and selection of solvent) to make dense suspensions (10 and 30 % (w/v)) of medium-chain-length poly-3-hydroxyalkanoate (mcl-PHA) with particles less than 300 nm. A two-stage emulsification-solvent evaporation process was used. Previous studies have made suspensions at much lower solids content (up to 0.4 % (w/v)). The dispersed phase was mcl-PHA initially dissolved in methylene chloride, while the continuous phase was water containing one or more surfactant. When ionic surfactants were evaluated to stabilize the oil-in-water emulsion, the anionic, sodium dodecyl sulfate (SDS), and the cationic, dodecyltrimethylammonium bromide, produced the smallest particle sizes (both ~100 nm). Nanoparticles were more stable when an ionic surfactant, SDS, was combined with any of the non-ionic surfactants tested. The zeta potential of nanoparticles stabilized with SDS and polyoxyethylene octyl phenyl ether (Triton X-100) or polyoxyethylene (20) sorbitan monooleate (Tween 80) increased slightly over 30 days, indicating that they may be more effective than the other non-ionic surfactants stabilizers where a decrease was observed. Using the same surfactant formulation, similar size mcl-PHA stable nanoparticle suspensions were produced using either an ultrasonic probe or a more scalable high-shear microfluidic device.

3.2 Introduction

Poly-3-hydroxyalkanoates (PHAs) are aliphatic polyesters, produced as intracellular granules from renewable resources by many bacterial species (Park et al., 2012). PHAs are classified as short-chain-length (scl) containing 3 to 5 carbon subunits or medium-chain-length (mcl) containing 6 to 14 carbon subunits. Scl-PHAs which also contain some mcl monomers are referred to as scl-mcl-PHA. Mcl-PHAs are elastomeric or tacky materials with low or no crystallinity and low or no melting point, whereas scl-PHAs are typically highly crystalline and stiff. PHAs are non-toxic, and biodegradable in most biotic environments. They have been intensely studied in the last several decades (Zinn et al., 2001; Hazer and Steinbüchel, 2007; Anderson and Dawes, 1990). Mcl-PHAs attract increasing attention as they find applications in toners (Fukui et al., 2009; Sacripante et al., 2012; McAneney-Lannen et al., 2012), drug delivery (Shrivastav et al., 2013; Lu et al., 2011; Xiong et al., 2010), coatings and paint formulations (Van der Walle et al., 2001). For example, mcl-PHAs can replace polyester toners incorporating bisphenol A (Sacripante et al., 2012) which has been banned in certain applications in Canada and Europe because it disrupts hormonal functions and is carcinogenic (Vogel, 2009). Many of these applications require mcl-PHA as a dense suspension of nanoparticles.

Nanoparticles are defined as solid, colloidal particles that are 10–500 nm. They have better stability against particle aggregation and gravitational separation than larger particles (McClements, 2012; Tadros et al., 2004). Small particle size reduces creaming and sedimentation, allows a more uniform deposition on substrates, and coalescence can be prevented using surfactants that minimize disruption of the liquid film between particles (Tadros et al., 2004). Polymer nanoparticles can be made by (i) high energy methods using mechanical devices such as ultrasonicators or high pressure homogenizers which disrupt interfaces to form small particles with the help of surfactants and co-surfactants or (ii) low energy methods in

which small particles form spontaneously due to altered conditions such as phase inversion (McClements, 2012). High energy methods require much less surfactant to achieve small particles than low energy methods. For example, formation of droplets smaller than 200 nm via microfluidization required a surfactant to oil ratio (SOR) < 0.1 but when spontaneous emulsification was used a SOR > 1 was necessary (Yang et al., 2012).

Few studies have used low energy methods to make PHA colloidal suspensions (Poletto et al., 2008) but high energy methods, specifically emulsification followed by solvent evaporation, have been commonly used to make both scl- and scl-mcl-PHA suspensions (Xiong et al., 2010; Bayram et al., 2008; Horowitz and Sanders, 1994; Ramsay et al., 1994; Kassab et al., 1997; Kiliçay et al., 2011; Lee et al., 2011). This approach is popular because of its simplicity and suitability for scale-up (Reis et al., 2006). Particle size and distribution dictate specific applications and also determine significant properties such as viscosity, surface area, and packing density (Rao and Geckeler, 2011).

In the emulsification-solvent evaporation method, PHA is solubilized in an organic solvent, added to water with vigorous mixing to make an oil-in-water emulsion before the solvent is removed by evaporation to leave the mcl-PHA as a suspension. A surfactant must be added to the aqueous phase to make a stable latex of scl-PHAs (Horowitz and Sanders, 1994). Although a surfactant or emulsifier is routinely added to stabilize latexes of scl-mcl-PHAs (Xiong et al., 2010; Kiliçay et al., 2011; Zhang et al., 2010) and mcl-PHAs (Gaucher et al., 2010), it is not needed if the final solids content is less than 0.4 % (w/v) (Ramsay et al., 1994; Marchessault et al., 1995; Beauregard et al., 2016). The stability of mcl-PHA suspensions without a surfactant was attributed to steric stabilization due to the relatively longer side chains of mcl-PHA monomers compared to the methyl group in poly-3-hydroxybutyrate (PHB)

(Marchessault et al., 1995). However, without a surfactant, mcl-PHA nanoparticles agglomerate above 0.4 % solids (Beauregard et al., 2016). The thermoelastomeric scl-PHAs, poly-4-hydroxybutyrate and poly-4-hydroxybutyrate-co-3-hydroxybutyrate, have not been found to form stable nanoparticles even with a surfactant (Marchessault et al., 1995). Studies with scl-mcl PHAs for drug delivery (Xiong et al., 2010; Kiliçay et al., 2011; Zhang et al., 2010) or mcl-PHAs for paper coating (Bourbonnais and Marchessault, 2010) were made at a low final solids content of 0.01-0.25 % (w/v). Certain applications such as xerographic toners require much higher final solids content of 25-30 % (w/v) and particles sizes of 25-300 nm (Sacripante et al., 2012). There are no reports on the processing conditions and surfactants needed to make stable mcl-PHA nanoparticles at such high solids content.

This study is the first to demonstrate the preparation of concentrated (i.e. 10-30 % w/v) mcl-PHA nanoparticles in suspension. The emulsification-solvent evaporation method was used. The effect of processing parameters such as the ultrasonication time and amplitude, and its formulation such as the choice of surfactants, their concentration, and the choice of the organic solvent to solubilize mcl-PHA on particle size and stability were investigated. In addition, we show that mcl-PHA nanoparticles can also be fabricated using a high-shear microfluidic device.

3.3 Material and Methods

3.3.1 Preparation of Concentrated mcl-PHA Suspension

Mcl-PHA was prepared by fed-batch fermentation of *Pseudomonas putida* KT2440 as described previously (Gao et al., 2016) and had a composition of 60 mol% 3-hydroxydecanoate, 20 mol% 3-hydroxyoctanoate, and 20 mol% 3-hydroxyhexanoate. Distilled, deionized water was used to prepare solutions. All chemicals were analytical grade.

Mcl-PHA suspensions were prepared by the emulsification-solvent evaporation method in two stages (Meleson et al., 2004; Jafari et al., 2007). In stage 1, the suspension was prepared by adding the appropriate amount of polymer solubilized in 10 mL methylene chloride (unless otherwise stated), to 9 mL of an aqueous surfactant solution at the specified concentration (Fig. 3-1). The pH was adjusted to 7.0 ± 1.6 with 2 M NaOH before forming the "premix" emulsion in an ice bath by homogenization for three min with a rotor-stator system (SDT Tissumizer, Tekmar Company, USA) equipped with an 18 mm diameter stator at 10 % of the power output of the Tekmar power controller (model TR-10). In stage 2, an oil-in-water nanoemulsion was formed by applying high shear using ultrasonication (20 KHz Ultrasonic Homogenizer 4710 Series, Cole Parmer Instrument Co., IL, USA), equipped with a microtip probe of 3.2 mm diameter in an ice bath, except when a microfluidizer (six passes at 40 psi, Microfluidizer® Processor 110S, Microfluidics Cooperation, MA, USA) was used. The solvent was removed by rotary evaporation at 40^{o} C, then transferred to a graduated centrifuge tube. Distilled, deionized water was added to obtain the final solids concentration of the mcl-PHA suspension.

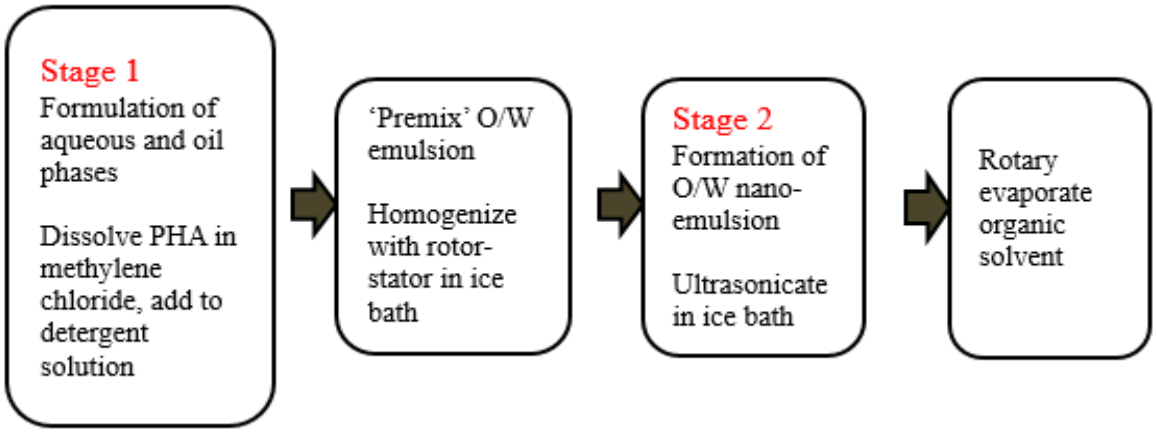

Fig. 3-1 Schematic diagram illustrating the two-stage process to make mcl-PHA nanoparticle suspensions

3.3.2 Particle Characterization

Mean particle diameter (Z average), polydispersity index (PDI) and zeta potential were measured by laser light scattering (Malvern Zetasizer Nano ZS, Worcestershire, UK) at 25ºC. Samples were diluted with distilled, deionized water to 1 g/L and measured at day 1, except if otherwise indicated. Data are expressed as the average of three measurements ± the standard deviation.

3.3.3 Ultrasonication Time and Amplitude in Stage 2

"Premix" oil-in-water emulsions of 10 % (w/v) mcl-PHA in 20 mM SDS or 30 % mcl-PHA in 50 mM SDS were made in stage 1, as described above. In stage 2, the ultrasonication time was varied from 1 to 15 min at 20 % amplitude or the percent amplitude was varied between 10 to 30 % for 10 min and the particle size was assessed at day 1.

3.3.4 SDS Concentration in Stage 1

The "premix" oil-in-water emulsion was made in stage 1 with an increasing SDS concentration from 5 to 150 mM. In stage 2, ultrasonication was performed at 25 % amplitude for 10 min for 10 % (w/v) mcl-PHA, and at 20 % amplitude for 10 mins for 30 % (w/v) mcl-PHA.

3.3.5 Selection of Ionic Surfactant

The cationic surfactants evaluated were dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB) and the anionic surfactants were alkyldiphenyloxide disulfonate (Dowfax® 2A1), sodium dodecylbenzenesulfonate (SDBS), and sodium dodecyl sulfate (SDS). All surfactants were obtained from Sigma-Aldrich Canada

(Oakville ON, Canada). Each surfactant was evaluated at only 10 % (w/v) mcl-PHA at a sonication amplitude of 25 % for 10 min at a concentration of 100 mM SDS.

3.3.6 Combination of SDS with a Non-ionic Surfactant

Nanoparticle suspensions were made as described above for 10 % (w/v) mcl-PHA at a sonication amplitude of 25 % for 10 min in 20 mM SDS with and without 40 mM of one of the following non-ionic surfactants: polyoxyethylene octyl phenyl ether (Triton X-100, OmniPur®), sorbitan monooleate (Span 80), polyoxyethylene (20) cetyl ether (Brij 58), polyoxyethylene (20) sorbitan monolaurate (Tween 20), polyoxyethylene (20) sorbitan monooleate (Tween 80), and sorbitan trioleate (Span 85), purchased from Sigma-Aldrich Canada (Oakville ON, Canada).

3.3.7 Combination of SDS ± Triton X-100 at 30 % Solids

A 30 % (w/v) mcl-PHA "premix" oil-in-water emulsion was prepared as described above except it was made in 8 mM SDS with and without Triton X-100 and ultrasonicated at 20 % amplitude for 10 min.

3.3.8 Solvent for mcl-PHA Dissolution

Mcl-PHA (30 % (w/v)) was solubilized in either chloroform, tetrahydrofuran (Fisher Scientific), 1, 2-dichloroethane (OmniSolv®), trichloroethylene (Sigma-Aldrich), dichloromethane or acetone (ACP) then added to 8 mM SDS and 24 mM Triton X-100 to form the "premix" emulsion. Stage 2 ultrasonication was done at 20 % amplitude for 10 min.

3.4 Results and Discussion

3.4.1 Choice of Solvent

The choice of the organic solvent for the dissolution of mcl-PHA is critical to the production of a suitable oil-in-water emulsion, the first step in making mcl-PHA nanoparticles. Acetone has been used successfully to solubilize the polymer for production of mcl-PHA suspensions up to 0.4 % (w/v) (Marchessault et al., 1995; Beauregard et al., 2016). However, our initial attempts with mcl-PHA solubilized in acetone to make 10 % (w/v) nanoparticles using an ultrasonication probe and the same conditions described by Marchessault et al. (1995) and Beauregard et al. (2016) were unsuccessful. Almost all polymer immediately adhered to the rotor-stator probe during the preparation of the 'premix' emulsion in stage 1 (Fig. 3-2). Use of tetrahydrofuran was also unsuccessful and produced the same gummy mass.

Fig. 3-2 Image showing mcl-PHA adhering to the rotor-stator device during formation of 'premix' emulsion when acetone was used as the solvent

Both acetone and tetrahydrofuran are water-soluble. When solvents that are poorly soluble in water such as methylene chloride, chloroform, 1, 2-dichloroethane or trichloroethylene were used, uniform nanoparticles were formed (Table 3-1). It would appear that any solvent in which the polymer dissolves may be suitable when making mcl-PHA nanoparticle suspensions at relatively low concentrations (e.g. < 0.4 % (w/v)). However, for higher density suspensions (e.g. ≥ 10 % (w/v)), only those solvents with low water solubility can produce nanoparticles. Hence all further experiments were done using methylene chloride.

Table 3-1 Particle size and zeta potential at day 1 for 30 % (w/v) mcl-PHA initially solubilized in different solvents. The values correspond to the averages of one experiment for n=3

Solvent	**Water Solubility (g/L)**	**Particle size (nm)**	**Zeta Potential (mV)**
Methylene chloride	17.50 at 25°C	240 ± 1	-42.9 ± 0.9
Chloroform	8.09 at 20°C	247 ± 1	-40.1 ± 0.6
1,2-Dichloroethane	8.70 at 20°C	241 ± 1	-42.0 ± 0.6
Trichloroethylene	1.28 at 25°C	245 ± 3	-41.1 ± 1.0
Tetrahydrofuran	Miscible	Not measurable	Not measurable
Acetone	Miscible	Not measurable	Not measurable

3.4.2 Ultrasonication Time and Amplitude in Stage 2

The “premix” emulsions and the nano-emulsions were prepared in an ice bath because a lower temperature increases the viscosity of the continuous phase thus decreasing the collision

rate between droplets, reducing the opportunity for coalescence (Marchessault et al., 1995). This should result in smaller particle sizes after the solvent is removed. Energy input disrupts droplets, producing smaller ones. The size and stability of PHA particles are affected by these processing conditions as shown by Heathman et al. (2014) although they used a different PHA (scl-mcl-PHA with 86 mol % 3HB, 14 mol % 3-hydroxyhexanoate) at a much lower maximum solids content of 0.2 % (w/v). Increased sonication time and amplitude decreased the particle size of latexes formulated with either 10 % (w/v) mcl-PHA in 20 mM SDS or 30 % (w/v) mcl-PHA in 50 mM SDS (Fig. 3-3). The initial selection of SDS was based on Horowitz and Sanders (1994) who had studied the fabrication of scl-PHA artificial granules using a wide range of surfactants and found that SDS was one among several that produced stable granules. The SDS concentration chosen was based on the results of Marchessault et al. (1995) to ensure an excess of surfactant. The role of a surfactant such as SDS is to reduce the interfacial tension at the oil- (i.e. mcl-PHA solubilized in methylene chloride) water interface. This reduces the amount of energy needed to reduce droplet size and the presence of the surfactant at the interface also helps to stabilize the droplets by reducing coalescence (Tadros et al., 2004).

With increasing sonication time, for 10 % (w/v) mcl-PHA the particle size decreased from 212 ± 0.9 nm at 1 min to 165 ± 0.5 nm, the minimum particle size, after 10 mins of sonication (Fig. 3-3a). Increasing the concentration of the mcl-PHA in the dispersed phase would have a direct influence on particle size, as it changes the polymer/solvent ratio (Kassab et al., 1997). At a higher solids content of 30 % (w/v) and the same “premix” conditions as at 10 % solids, the particle size was larger (298 ± 2.4 nm at 1 min) with the majority of the size reduction occurring after 10 min of sonication to 191 ± 2.3 nm. Further sonication had much less impact on

particle size. Hence, a sonication time of 10 min was used in subsequent experiments at both solids content.

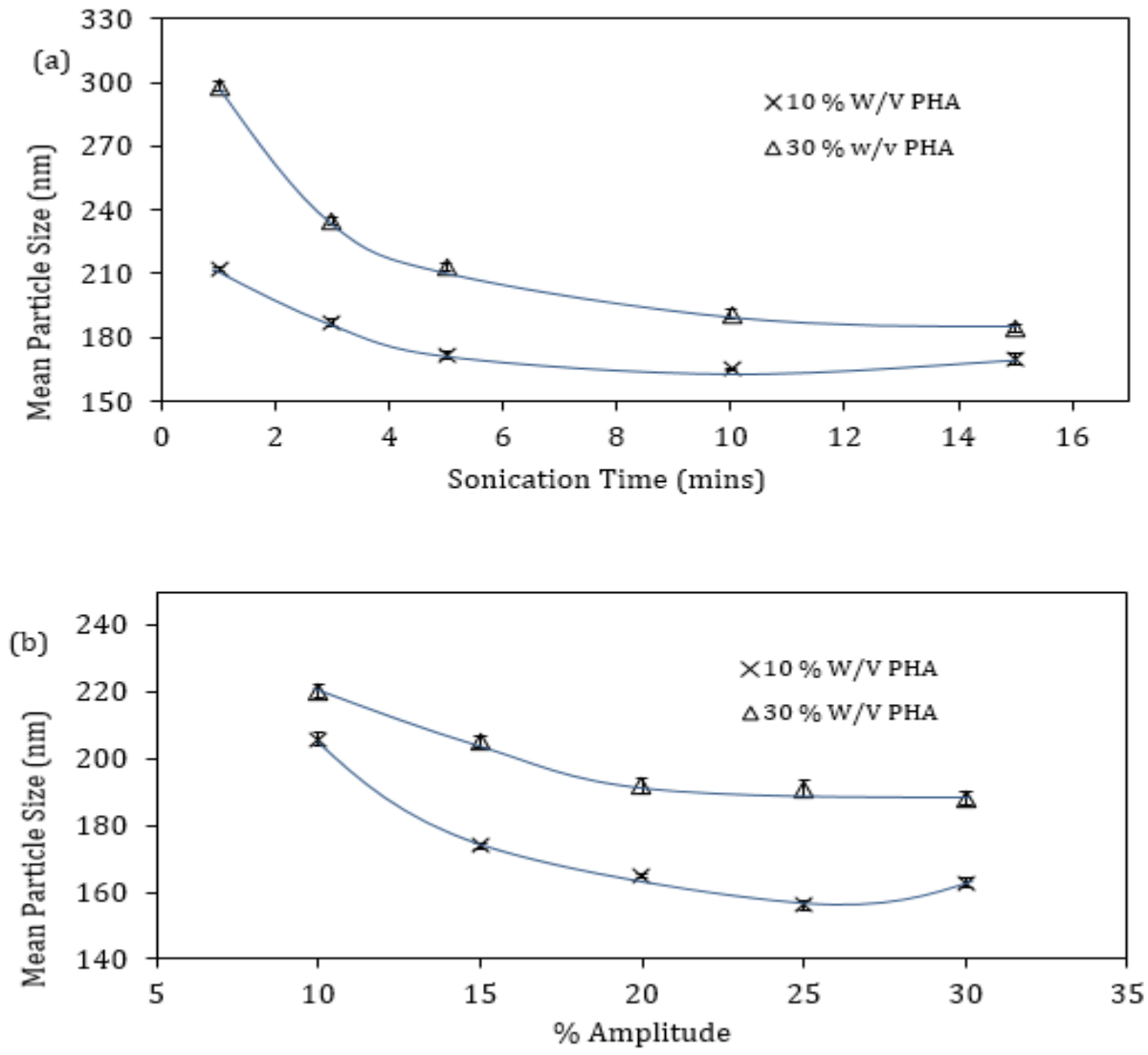

Fig. 3-3 Particle size at (a) increasing sonication time (at 20 or 25 % amplitude) or (b) increasing percent amplitude (at 10 mins ultrasonication time) on 10 % (w/v) mcl-PHA in 20 mM SDS and on 30 % (w/v) mcl-PHA in 50 mM SDS. Measurements were taken at day 1 and data are mean ± standard deviation for n = 3. Refer to Table A-1 and A-2 (appendix) for independently replicated experiment of the particle sizes in 10 % (w/v) PHD suspensions. Reproducible results obtained

Fixing the sonication time at 10 mins and increasing the sonication amplitude decreased the mcl-PHA particle size at both 10 % and 30 % (w/v) mcl-PHA. At the higher solids content, particle sizes were larger and decreased more gradually (Fig. 3-3b). As ultrasonication breaks up the mcl-PHA/solvent droplets, new surfaces are formed. If there is sufficient surfactant available to immediately coat the new surfaces, when the droplets collide, there should be sufficient repulsion between the droplets to maintain a small size. If there is insufficient surfactant, as the droplets collide they coalesce producing larger droplets (and eventually larger particles) (Meleson et al., 2004). Hence, it is likely that there was not enough surfactant to totally coat the mcl-PHA droplets at the higher solids content.

The increase in mcl-PHA particle size observed at 10 % solids at sonication times longer than 10 min at a fixed amplitude (Fig. 3-3a) or at 30 % amplitude at a fixed sonication time (Fig. 3-3b) may also be attributed to insufficient surfactant available to coat the droplets due to a larger surface area at a smaller particle size. At 10 % solids, the smallest particle size (156 ± 1.33 nm) was at 25 % amplitude while at 30 % solids, increasing the amplitude beyond 20 %, resulted in minimal improvement. Thus 25 and 20 % amplitude were selected for use at 10 and 30 % mcl-PHA respectively, in subsequent experiments.

3.4.3 Effect of SDS Concentration

At a higher concentration of mcl-PHA, the collision frequency of mcl-PHA droplets and possible coalescence increase during emulsification. The use of a surfactant with a high adsorption rate on the mcl-PHA droplets will help form the droplets and to reduce coalescence. The surfactant must be soluble in the continuous phase (i.e. the aqueous phase) and if it adsorbs too slowly on the “oil” droplet, coalescence will occur before the surfactant can create a

repulsive barrier in the form of an electrical double layer around the droplet, resulting in larger droplets and hence larger particles. Typically, ionic surfactants are used to achieve this type of stabilization (Tadros, 2009; Hellgren et al., 1999). We chose to examine the effect of ionic surfactant concentration using the common anionic surfactant, SDS. Although increased amplitude and sonication time can form smaller nanoparticles, it is important to use enough surfactant to help form and stabilize these particles. Increasing the surfactant concentration decreases particle size but excess surfactant may increase particle size (Komaiko and McClements, 2014) due to the formation of gel-like clumps that have been attributed to cubic phases (Davidov-Pardo and McClements, 2015) or a large increase in viscosity that makes emulsification more difficult (Saberi et al., 2013). Excess surfactant also adds unnecessary cost and may be undesirable in the final application. Nanoparticles were made with 10 % (w/v) mcl-PHA in 5-110 mM SDS or with 30 % (w/v) mcl-PHA in 8-150 mM SDS. Mcl-PHA particle size decreased as the SDS concentration increased at both solids contents (Fig. 3-4a). At 30 % solids increasing the SDS concentration decreased the particle size from 251 ± 2.1 nm at 8 mM to 192 ± 2.4 nm at 50 mM but higher SDS concentrations had little effect on the particle size, while at 10 % solids, the particle size steadily decreased until 100 mM SDS to achieve a particle size of 100 ± 0.36 nm. This is about 1.5 times smaller than was obtained with 20 mM SDS. Although there was one third the solids content, a higher surfactant concentration was needed to coat the larger surface area generated by the much smaller particles at 10 % solids. Since 100 mM SDS gave the smallest nanoparticles, this concentration was chosen to investigate the effect of other ionic surfactants on PHA particle size at 10 % solids.

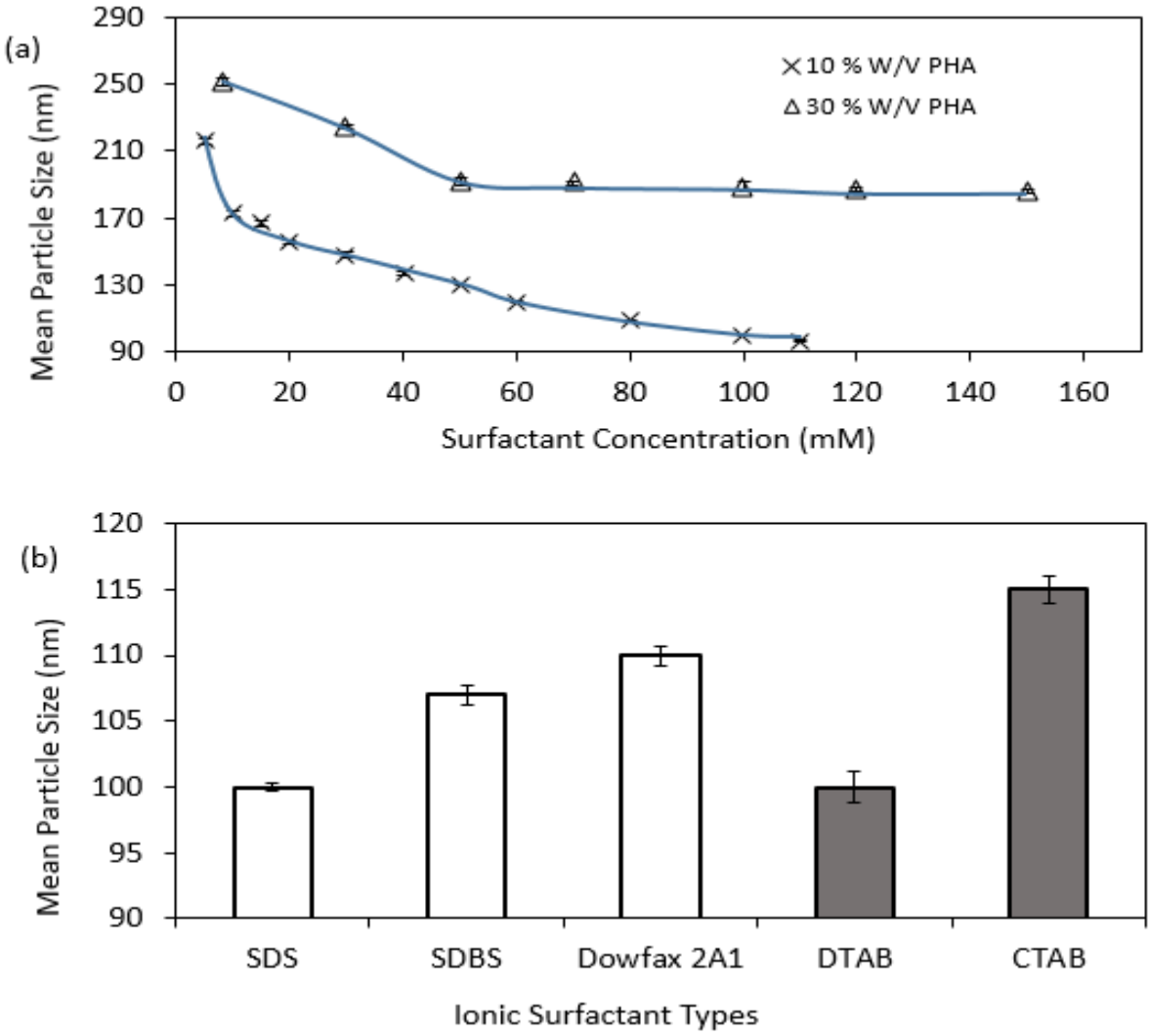

Fig. 3-4 Effect of (a) SDS concentration on 10 % (w/v) mcl-PHA at 25 % amplitude and 10 mins of sonication or on 30 % (w/v) mcl-PHA at 20 % amplitude and 10 mins of sonication and (b) different ionic surfactants (100 mM) on 10 % (w/v) mcl-PHA at 25 % amplitude and 10 mins of sonication. Measurements were done at day 1 and data are the mean ± standard deviation for n = 3. Sodium dodecyl sulfate (SDS), sodium dodecylbenzenesulfonate (SDBS) and sodium dodecyldiphenyl oxide sulfonate (Dowfax® 2A1) were the anionic surfactants and dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB) were the cationic surfactants evaluated

3.4.4 Selection of an Ionic Surfactant

While SDS was successful at creating nanoparticles, other ionic surfactants may be superior. Both cationic and anionic surfactants were evaluated at 10 % (w/v) mcl-PHA. Surfactants are also classified semi-empirically based on their hydrophilic-lipophilic (HLB) balance which indicates their relative solubility in oil and water such that an HLB of zero is very soluble in oil and a value of 40 is very water soluble. All surfactants tested were within the HLB range of 8-18 recommended to form an oil-in-water emulsion (Tadros, 2009) except for SDS, Span® 80 and Span® 85 which had HLB values of 40, 4.3, and 1.8, respectively (Table 2). Surfactants with HLB > 7 can form oil-in-water emulsions (Tadros et al., 2004).

All the ionic surfactants tested produced small nanoparticles that ranged from 100 ± 0.36 nm to 115 ± 1.07 nm. There was no apparent effect on particle size based on the charge of the surfactant as the lowest particle sizes were obtained with SDS and DTAB (Fig. 3-4b), an anionic and cationic surfactant respectively. This may be because SDS and DTAB had the lowest interfacial tension (not measured) between the nanoparticle and the aqueous phase during emulsification, leading to smaller PHA particles.

Among the surfactants tested, SDS and SDBS are the cheapest and similarly priced at US $1500-2000 per metric ton while the other ionic surfactants, including DTAB, are about 10 times more expensive at current commodity market prices (http://www.alibaba.com, accessed on May 25, 2016). Hence, SDS was selected as the ionic surfactant for subsequent experiments since it is less expensive than DTAB.

Table 3-2 Surfactant characteristics (Housaindokht and Pour, 2012; Omwoyo et al., 2015)

Surfactant	Chemical name	Chemical formula	HLB	Type
SDS	Sodium dodecyl sulfate	$C_{12}H_{25}SO_4Na$	40	Anionic
SDBS	Sodium dodecylbenzenesulfonate	$C_{18}H_{29}NaO_3S$	10.6	Anionic
Dowfax® 2A1	Sodium dodecyldiphenyl oxide sulfonate	$C_{22}H_{28}Na_2O_7S_2$	16.7	Anionic
DTAB	Dodecyltrimethylammonium bromide	$C_{15}H_{34}BrN$	12.9	Cationic
CTAB	Cetyltrimethylammonium bromide	$C_{19}H_{42}BrN$	10	Cationic
Triton X-100	Polyoxyethylene octyl phenyl ether	$C_{34}H_{62}O_{11}$	13.4	Non-ionic
Tween® 80	Polyoxyethylene (20) sorbitan monooleate	$C_{64}H_{124}O_{26}$	15	Non-ionic
Tween® 20	Polyoxyethylene (20) sorbitan monolaurate	$C_{58}H_{114}O_{26}$	16.7	Non-ionic
Brij® 58	Polyoxyethylene (20) cetyl ether	$C_{56}H_{114}O_{21}$	16	Non-ionic
Span® 80	Sorbitan monooleate	$C_{24}H_{44}O_6$	4.3	Non-ionic
Span® 85	Sorbitan trioleate	$C_{60}H_{108}O_8$	1.8	Non-ionic

3.4.5 Selection of a Non-ionic Surfactant in Combination with SDS

While an ionic surfactant like SDS is used to overcome attractive forces like van der Waals' by forming a repulsive electrical double layer, non-ionic surfactants create another type of repulsive force when they cover the surface of an "oil" droplet or particle to provide steric stabilization. Either anionic (Hellgren et al., 1999) or cationic (Chu and Hwang, 1997)

surfactants in combination with a non-ionic surfactant have been used to make polymer dispersions and the steric stabilization provided by the non-ionic surfactant improves shear stability (Hellgren et al., 1999) and is needed at high solids content (Walstra, 1993). The mixture of an anionic and a non-ionic surfactant has a synergistic effect on stabilization compared to each surfactant individually (Paria and Khilar, 2004). Longer, more hydrophobic tails of the non-ionic surfactants interact more strongly with the hydrophobic portion of the anionic surfactant to create the synergistic effect (Ćirin et al., 2012).

Horowitz and Sanders (1994) examined a wide range of surfactants (cationic, anionic, non-ionic and soaps) to individually stabilize scl-PHA particles, while studies with scl-mcl-PHAs (Xiong et al., 2010; Kiliçay et al., 2011; Zhang et al., 2010) and mcl-PHAs (Ramsay et al., 1994; Marchessault et al., 1995; Gaucher et al., 2010) used a single surfactant. This is the first published study to examine the production of mcl-PHA nanoparticles using a combination of an ionic with a non-ionic surfactant. Several non-ionic surfactants (polyoxyethylene octyl phenyl ether (Triton X-100), sorbitan monooleate (Span® 80), polyoxyethylene (20) cetyl ether (Brij® 58), polyoxyethylene (20) sorbitan monolaurate (Tween® 20), polyoxyethylene (20) sorbitan monooleate (Tween® 80), and sorbitan trioleate (Span® 85)) were studied in combination with SDS.

Suspensions of 10 % w/v mcl-PHA prepared with SDS alone resulted in 156 ± 1.33 nm particles at day 1 increasing to 190 ± 3.76 nm by day 30 (Fig. 3-5b). The addition of any of the non-ionic surfactants tested in combination with SDS resulted in a similar initial particle size except that Triton X-100 (148 ± 1.72 nm) formed a slightly smaller size and Span 85 (175 ± 3.27 nm) a slightly larger particle. The presence of a non-ionic surfactant helped to prevent coalescence of the mcl-PHA particles over time since with SDS alone, the particle size increased

by 21 % over 30 days compared to SDS with Span 85 where the particles increased by only 2.9 % while all other combinations demonstrated a small decrease in coalescence (compared to SDS alone) over the same time period.

If the repulsive forces keeping two droplets or particles apart are greater than the attractive forces then the emulsion or suspension will be stable and coalescence will not occur. According to the DLVO theory, the repulsive forces which keep two particles or droplets apart create an energy barrier which can be measured by the zeta potential. The magnitude of the zeta potential is indicative of the size of this energy barrier and hence the repulsive forces, so large positive or negative zeta potentials indicate large repulsive forces. Unstable emulsions and suspensions occur between a zeta potential of +30 to -30 mV, and particles outside these limits are usually considered stable (Larsson et al., 2012). The zeta potential of nanoparticles made with SDS alone was initially -47.6 ± 0.52 mV but decreased sharply by day 30 to -36.2 ± 3.00 mV (Fig. 3-5a). Although this value is still within the range of a stable suspension, a decrease of 24 % indicates that 20 mM SDS alone may not maintain stable nanoparticles over the long term, even though the zeta potential is still within the stable range. By day 30, the zeta potential of all combinations of SDS with a non-ionic surfactant was greater than that of SDS alone. Except for the combination of SDS with Triton X-100 or with Tween 80, the zeta potential decreased but to a lesser extent than what was observed with SDS alone, after 30 days. The slight increase in zeta potential of SDS with Triton X-100 or with Tween 80 may indicate a better stability with these two non-ionic surfactants. Of the two, Tween 80 should exhibit better synergism with SDS as it has a longer hydrophobic tail (C18) than Triton X-100 (C10) to allow a stronger interaction with the hydrophobic portion of SDS (Ćirin et al., 2012).

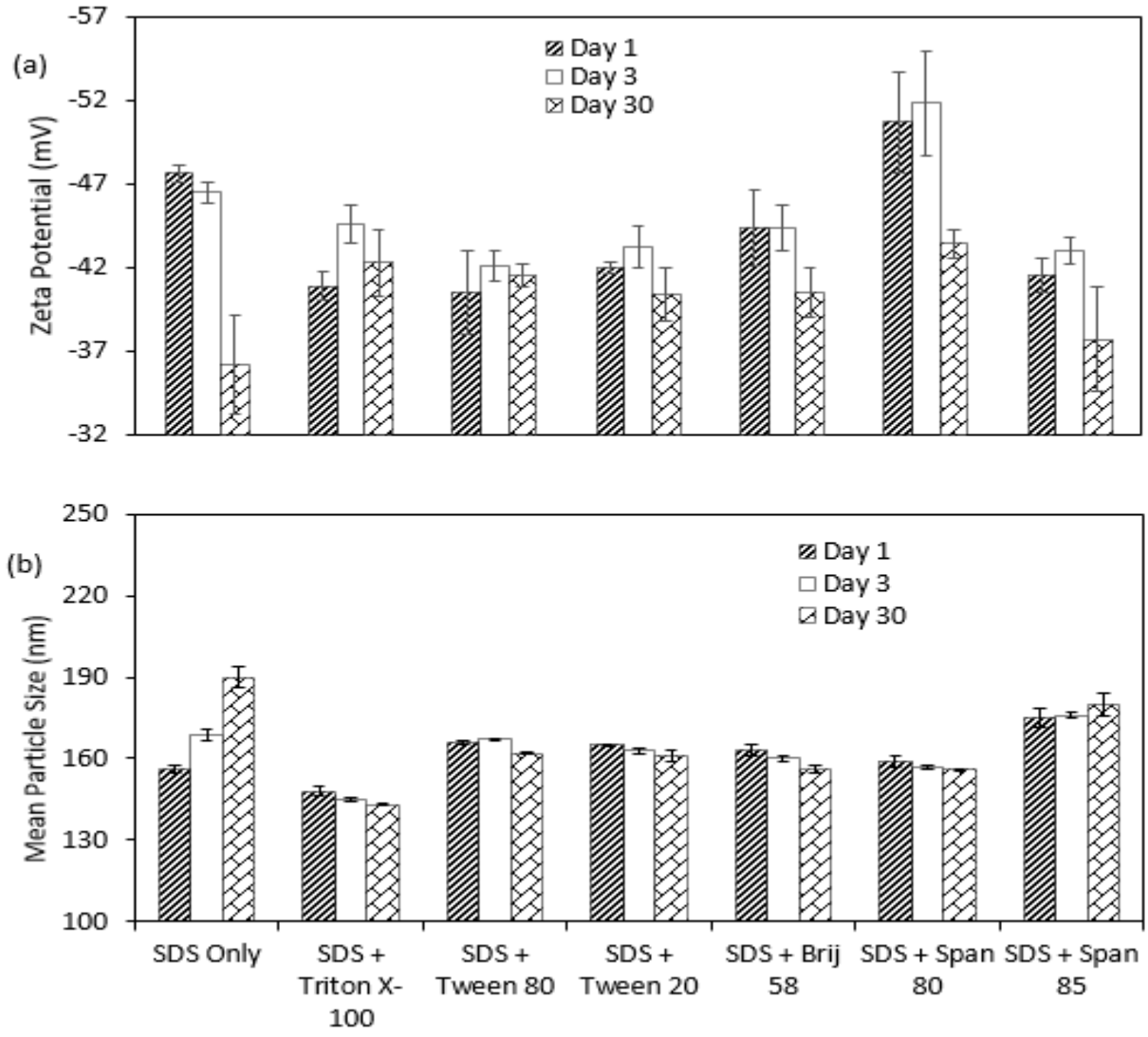

Fig. 3-5 Effect of 20 mM SDS ± 40 mM non-ionic surfactants concentrations on (a) zeta potential and (b) particle size in 10 % (w/v) mcl-PHA suspensions at 25 % amplitude and 10 mins of sonication. Zeta potential measurement showed that all suspensions were stable, but Triton X-100 gave the lowest particle size over time, therefore it was selected for subsequent experiments. Suspension prepared by SDS only resulted in an increase in particle size over time. Data are the mean ± standard deviation for n = 3. The non-ionic surfactants were polyoxyethylene octyl phenyl ether (Triton X-100, OmniPur®), sorbitan monooleate (Span® 80), polyoxyethylene (20) cetyl ether (Brij® 58), polyoxyethylene (20) sorbitan monolaurate (Tween® 20), polyoxyethylene (20) sorbitan monooleate (Tween® 80), and sorbitan trioleate (Span® 85)

However, based on the zeta potential and the smaller particle size obtained with Triton X-100, the repulsive forces in SDS-Triton X-100 are slightly greater than that of SDS-Tween 80. This may also be due to a better adsorption rate by Triton-X-100 than Tween 80, allowing it to provide a better protective shield around the droplets or particles resulting in stronger repulsion. Hence, Triton X-100 was selected as the non-ionic surfactant for further formulation studies.

3.4.6 SDS and Triton X-100 at 30% Solids

Having selected the SDS-Triton X-100 combination to stabilize mcl-PHA nanoparticles, the appropriate formulation to stabilize 30 % mcl-PHA suspension was investigated. Increasing the molar ratio of Triton X-100 in the continuous phase to SDS (8 mM) decreased the particle size (Fig. 3-6b) and increased the zeta potential (Fig. 3-6a) indicating larger repulsive forces and hence better stability. The stability of suspensions containing only SDS and the lower Triton X-100 concentrations (8 and 16 mM) exhibited less stability after 215 days as seen in the zeta potential values (Fig. 3-6a) and increasing particle sizes (Fig. 3-6b), most likely because there was insufficient surfactant. Incorporating more non-ionic surfactant ($\geq$ 24 mM Triton X-100) improved stability as seen by the higher zeta potential. The higher amount of Triton X-100 increased steric stabilization and perhaps produced a greater synergism. Based on these results, a minimum of three parts Triton X-100 to one part SDS based on molar concentration is recommended for the preparation of stable nanoparticles for 30 % (w/v) mcl-PHA.

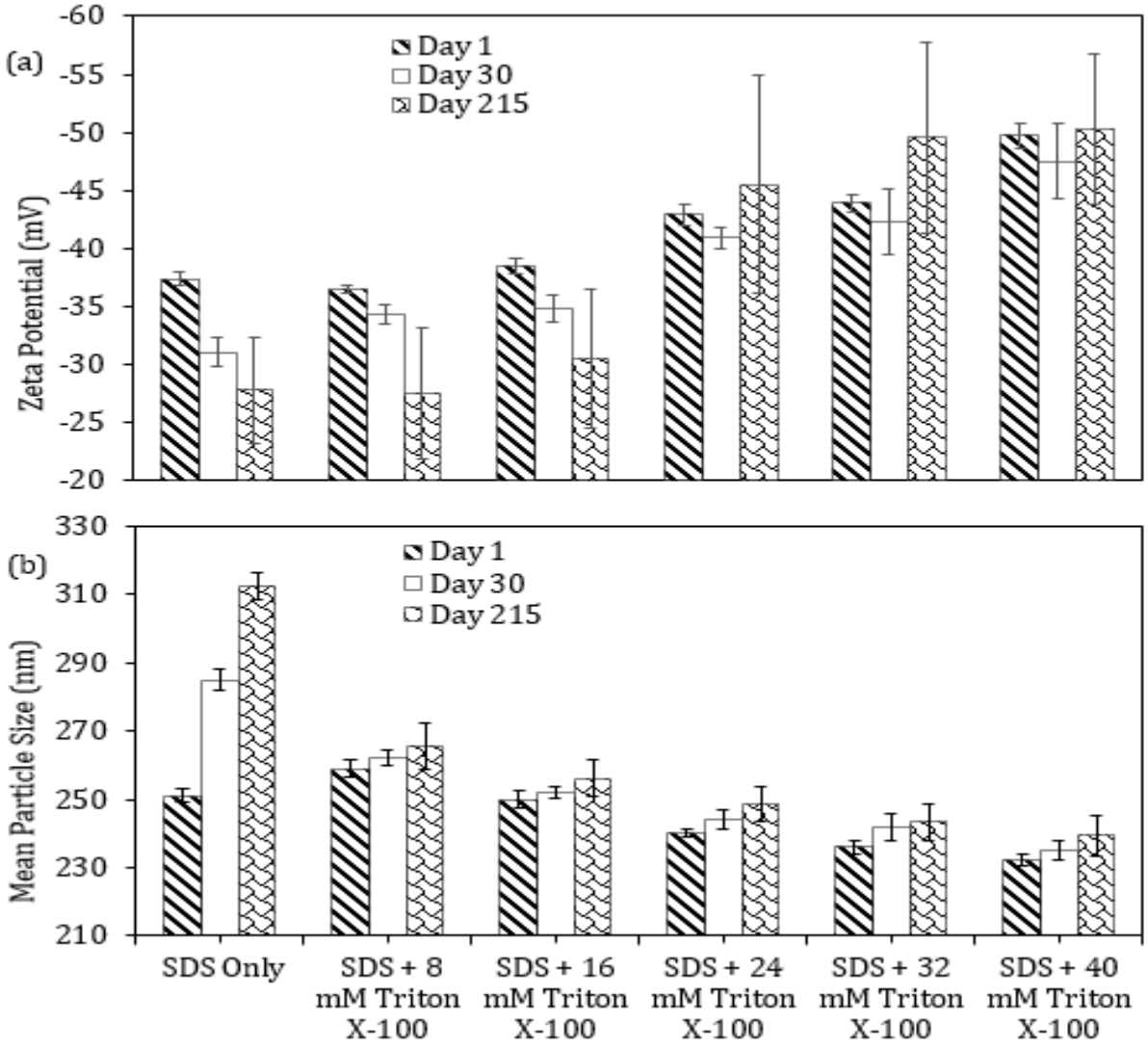

Fig. 3-6 Effect of 8 mM SDS ± Triton X-100 concentrations on (a) zeta potential and (b) particle size in 30 % (w/v) mcl-PHA suspension at 20 % amplitude and 10 mins of sonication. Data are the mean ± standard deviation for n = 3

Mcl-PHA is hydrophobic and requires a high amount of surfactant to produce stable nanoparticles. Typically, higher surfactant concentration results in a higher amount of impurities which may negatively impact application. More hydrophilic mcl-PHAs, such as those with carboxyl groups at the end of the side chain form smaller particles at low concentration in the

absence of surfactants (Beauregard et al., 2016). At higher polymer concentration less surfactant may be required. This should be investigated as it would reduce cost and may be of value in certain applications sensitive to surfactant content, such as toner formulation.

3.4.7 Microfluidization

Although the roto-stator/ultrasonic homogenization was capable of making the desired particle size and stability, high pressure homogenization can make smaller droplets with a narrower size distribution (Strawbridge et al., 1995) and may do so with more energy efficiency (Tadros et al., 2004). Suspensions were prepared as described in Fig. 3-1, except that in stage 2, a microfluidizer with no cooling was used instead of ultrasonic homogenization in an ice bath. Similar particle sizes were obtained with both devices (Fig. 3-7) and they were stable with zeta potential above ± 30 mV but a higher polydispersity index was obtained with microfluidization, possibly due to lack of cooling.

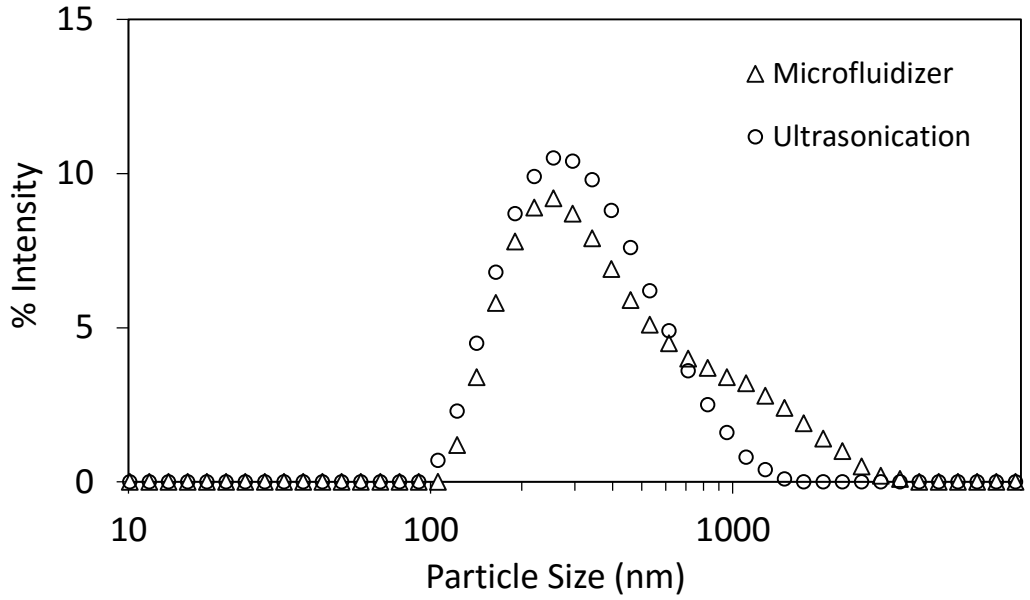

Fig. 3-7 Mcl-PHA nanoparticles made by ultrasonication or by microfluidization. Higher polydispersity index obtained with the microfluidizer

3.5 Conclusions

It has been demonstrated for the first time that stable nanoparticles of mcl-PHA latexes could be achieved at 10 and 30 % solids content using a two-stage emulsification-solvent evaporation process. Of the ionic surfactants tested, both the anionic, SDS, and the cationic, DTAB, produced the smallest particle sizes (~100 nm). Higher zeta potentials and more stable particles were obtained when the ionic surfactant, SDS, was combined with any of the non-ionic surfactant tested, with Triton X-100 and Tween 80 showing a slight increase in zeta potential over 30 days while the zeta potential with other non-ionic surfactants decreased.

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Chapter 4

Carboxylated Medium-chain-length Poly(3-hydroxyalkanoates) Suspensions

To be submitted to Journal of Polymers and the Environment

4.1 Abstract

Suspensions of mcl-PHA containing carboxyl groups (11 and 18 %) were prepared and compared to that of poly-3-hydroxynonanoate (PHN) and poly-3-hydroxydecanoate (PHD) at 10 % (w/v) solids content. The synthesis of carboxylated mcl-PHAs was done via a free radical addition reaction of 11-mercaptoundecanoic acid to the pendant double bonds of unsaturated poly-3-hydroxynonanoate (PHNU). Complete conversion of vinyl groups to carboxyl groups was achieved with a minimum of 3.5 equiv. of 11-mercaptoundecanoic acid per double bond in PHNU. Nanoparticle suspensions prepared with mcl-PHA containing 11 % carboxylation (PHNC-1) required surfactant to retain stability at 0.4 % solids under ultrasonic treatment. A variety of non-ionic surfactants reduced particle aggregation and produced similar particle sizes and particle size distribution (155.6 ± 8.4 to 163.4 ± 11.3 nm and 0.42 ± 0.03 to 0.49 ± 0.04), with polyoxyethylene octyl phenyl ether (Triton X-100) being the lowest. Smaller nanoparticles (97.1 ± 1.1 to 121.7 ± 5.7 nm) with a narrower particle size distribution (0.21 ± 0.001 to 0.25 ± 0.003) were obtained when Triton X-100 was combined with several ionic surfactants. A combination of SDS and Triton X-100 was selected to investigate other mcl-PHAs at 10 % solids. Suspensions of PHN containing 18 % of carboxyl groups (PHNC-2) produced slightly smaller nanoparticles and higher zeta potential value (143.1 ± 2.9 nm and -44.1 ± 0.5 mV) when compared to those of PHNC-1 (170.3 ± 4.6 nm and -39.6 ± 0.9 mV), PHD (181.2 ± 3.2 nm and -35.2 ± 1.3 mV), and PHN (183.5 ± 4.7 nm and -34.4 ± 2.6 mV).

4.2 Introduction

Medium-chain-length poly(3-hydroxyalkanoates) (mcl-PHAs) have attracted extensive interest due to their potential applications as biomaterials. They are made from renewable resources, are biodegradable and biocompatible thermoelastomers, usually with a low melting temperature, tensile strength, and a low or no crystallinity. These polymers contain a relatively long pendant non-polar side chain, making the material substantially more hydrophobic (Hazer et al., 2012; Levine et al., 2016). However, depending on the production process or post-production modification, mcl-PHAs can contain various functional groups at the terminal position of their pendant side chains, including olefin (Fritzche et al., 1990; Ulmer et al., 1994; Kim et al., 1995), halogen (Arkin et al., 2000; Doi and Abe, 1990; Kim et al., 1996), ester (Scholz et al., 1994; Hany et al., 2004), aromatic (Hazer et al., 1996; Curley et al., 1996), hydroxyl (Lee et al., 2000; Renard et al., 2005), epoxide (Bear et al., 1997; Park et al., 1998), carboxyl (Kurth et al., 2002; Hany et al., 2004; Zhang et al., 2000; Lee and Park, 2000; Stigers and Tew, 2003), and amide (Hany et al., 2004). Mcl-PHAs contain a carboxylic acid group at one end and a hydroxyl group at the other end of the polymer side chains (Timbart et al., 2007).

The presence of functional groups can influence properties such as mechanical strength, surface features, and hydrophilicity to suit the requirements of particular applications. In particular, introducing polar functional groups such as hydroxyl or carboxylic groups into the pendant side chains enhances hydrophilicity. For example, poly-3-hydroxyoctanoate (PHO) containing 40-60 % of hydroxyl groups in the side chains has been shown great enhancement of hydrophilicity, and is completely soluble in polar solvents such as methanol, dimethyl sulfoxide (DMSO), and 80/20 acetone-water mixture (Lee et al., 2000). Renard et al. (2005) used a borane-tetrahydrofuran (BH_3-THF) complex to synthesize PHO with 25 % hydroxylation in the pendant

side chains, with the polymer showing a considerably increase in hydrophilicity. These modified mcl-PHAs could be used to make novel graft polymers with useful properties (Kai and Loh, 2014; Renard et al., 2005).

The presence of carboxyl groups has attracted increasing attention because it could be used to bind bio-active molecules, hydrophilic components, and target proteins (Kai and Loh, 2014; Kurth et al., 2002). Improved hydrophilicity of PHO containing 25 % of carboxyl groups in the lateral chains was achieved by Kurth et al. (2002), and the modified polymer exhibited complete solubility in polar solvents such as methanol and acetone-water mixtures and pH dependent solubility in a polar mixture of acetone-water. Carboxylated PHAs have been proposed to produce nanoparticle-drug conjugates for site-specific delivery (Kurth et al., 2002). The introduction of carboxyl ions on PHA via ion implantation has been employed to enhanced wettability (Zhang et al., 2000). Carboxylated PHA has also been used as a chemically reactive site to synthesize mcl-PHA containing either an amide or an ester functional group (Hany et al., 2004).

Most studies introduced the carboxyl groups via chemical modification of the terminal carbon of the olefinic side chain, except for Zhang et al. (2000) who successfully produced carboxylated mcl-PHA via ion implantation. Of the few studies on synthesis of carboxylated mcl-PHA, Kurth et al. (2002) and Lee and Park (2000) used potassium permanganate ($KMnO_4$), as an oxidizing agent, with the former resulting in loss of the polymer's molecular weight. Stigers and Tew (2003) used osmium tetraoxide (OsO4) and oxone to oxidize the long pendant side chain olefins, achieving a minimal molecular weight loss. However, higher conversions were reported, with no loss of molecular weight when 11-mercaptoundecanoic acid was used to convert the side chain olefins to carboxylic acid via radical addition reaction (Hany et al., 2004).

Nanoparticle suspensions have been produced with mcl-PHA containing carboxylic acid functional group (Kurth et al., 2002; Beauregard et al., 2016). Kurth et al. (2002) used a surfactant stabilizer but Beauregard et al. (2016) showed that a stable latex can be fabricated without a surfactant at 0.4 % solids. Significantly smaller nanoparticles were obtained with carboxylated mcl-PHAs, in contrast to suspensions of mcl-PHAs containing either vinyl or aliphatic side chains (Beauregard et al., 2016). Increasing the carboxyl content could not significantly decrease the mcl-PHA nanoparticle size (Kurth et al., 2002). Both studies were made at a low final solids content of 0.2 % (w/v) (Kurth et al., 2002) and 0.4 % (w/v) (Beauregard et al., 2016), respectively. Most industrial applications typically require suspensions above 10 % solids and particle sizes below 300 nm (Beauregard et al., 2016), but to date no work has been reported on the making of carboxylated mcl-PHA latexes above 0.4 % solids.

A recent study has shown that 30 % solids latexes of mcl-PHA containing aliphatic side chains require high surfactant concentration (5.9 % of PHA weight) to maintain stability (chapter 3). In order to stabilize emulsions, surfactants modify the interfaces by lowering the interfacial tension, making it easier for droplets to break up. They also prevent re-coalescence of droplets to a varying degree and improve the long-term stability of emulsions (Yang et al., 2013; Walstra, 1993). High surfactant concentration may actually lead to an increase in particle size (Komaiko and McClements, 2014), increase the cost of formulation, and may be undesirable in the final product.

Carboxylated PHA latexes likely have lower particle-water interfacial tension, and hence, would probably require less surfactant to maintain suspension stability. This study investigates the stoichiometry of carboxylation of mcl-PHA with vinyl side-chains as well as the surfactant

requirements for the stabilization of 10 % (w/v) carboxylated mcl-PHA latexes. The effect of carboxylation on particle size and zeta potential is also reported.

4.3 Materials and Methods

4.3.1 Biosynthesis of PHN and PHNUs

PHAs were produced by fed-batch fermentation of *Pseudomonas putida* KT 2440 (Sun et al., 2009). Poly-3-hydroxynonanoate (PHN) containing 70 mol % 3-hydroxynonanoate and 30 mol % 3-hydroxyheptanoate, poly-3-hydroxydecanoate (PHD) having a composition of 60 mol % 3-hydroxydecanoate, 20 mol % 3-hydroxyoctanoate, and 20 mol % 3-hydroxyhexanoate, PHN with some unsaturation in the side-chains (vinyl groups) (PHNUs), PHNU-1 consisting of 61 mol % 3-hydroxynonanoate, 28 mol % 3-hydroxyheptanoate, 6 mol % C9:1 3-hydroxynonenoate, 4 mol % C11:1 3-hydroxyundecenoate, and 1 mol % C7:1 3-hydroxyheptenoate, and PHNU-2 composed of 72 mol % 3-hydroxynonanoate, 10 mol % 3-hydroxyheptanoate, 7 mol % C9:1 3-hydroxynonenoate, 9 mol % C11:1 3-hydroxyundecenoate, and 2 mol % C7:1 3-hydroxyheptenoate.

4.3.2 Carboxylation of PHNUs Containing 11 and 18 % of Unsaturated Groups

Synthesis of PHNCs (carboxylated products of PHNU) was performed as described by Hany et al. (2004). PHNU was dissolved in a mixture of toluene (1:10 weight of PHNU/V, Anachemia Canada Inc., Montreal QC, Canada), 11-mercaptoundecanoic acid (3.5 equiv. per unsaturated units in PHNU, unless otherwise stated, W & J PharmaChem Inc., MD, USA), and 2,2´-azobis(2-methylpropionitrile) (AIBN, 0.2 equiv. per unsaturated units in PHNU, Sigma-Aldrich, Oakville ON, Canada) under nitrogen. The reaction solution was heated to 75°C for 17 h, cooled to room temperature, dropped slowly into ice cold methanol (1:10 v/v, ACP, Montreal

QC, Canada), and left in the fume hood overnight. The solvent was removed, raw product dissolved in a minimal amount of methylene chloride (ACP, Montreal QC, Canada), and then dropped slowly into ice cold methanol (1:10 v/v) for further purification and precipitation of product. The solution was left in the fume hood overnight, then dried under high vacuum in a desiccator. The yields of carboxylated PHN were usually in the range of 70 %.

4.3.3 Polymer Characterization

^{1}H NMR spectra were recorded in $CDCl_3$ using a Bruker AVANCE 300 spectrometer at 300 K with samples of about 20 mg mcl-PHA in 0.8 mL of $CDCl_3$ (Sigma-Aldrich 99.8 atoms %D).

Gel permeation chromatography (GPC) analysis was performed with a Waters 2690 Gel Permeation Chromatograph equipped with a Waters 410 differential refractometer using polystyrene standards. Each sample was prepared by dissolving 40 mg of polymer in 10 mL of tetrahydrofuran (THF) (Fisher Scientific, Canada) overnight to ensure complete dissolution, then passed through a 0.2 µm nylon filter into a 2.5 mL GPC vial. THF was used as the eluent at a flow rate of 0.3 mL/min through four Waters Styragel columns (HR 0.5, 1, 3, and 4), maintained at 40 °C with an injection volume of 30 μL.

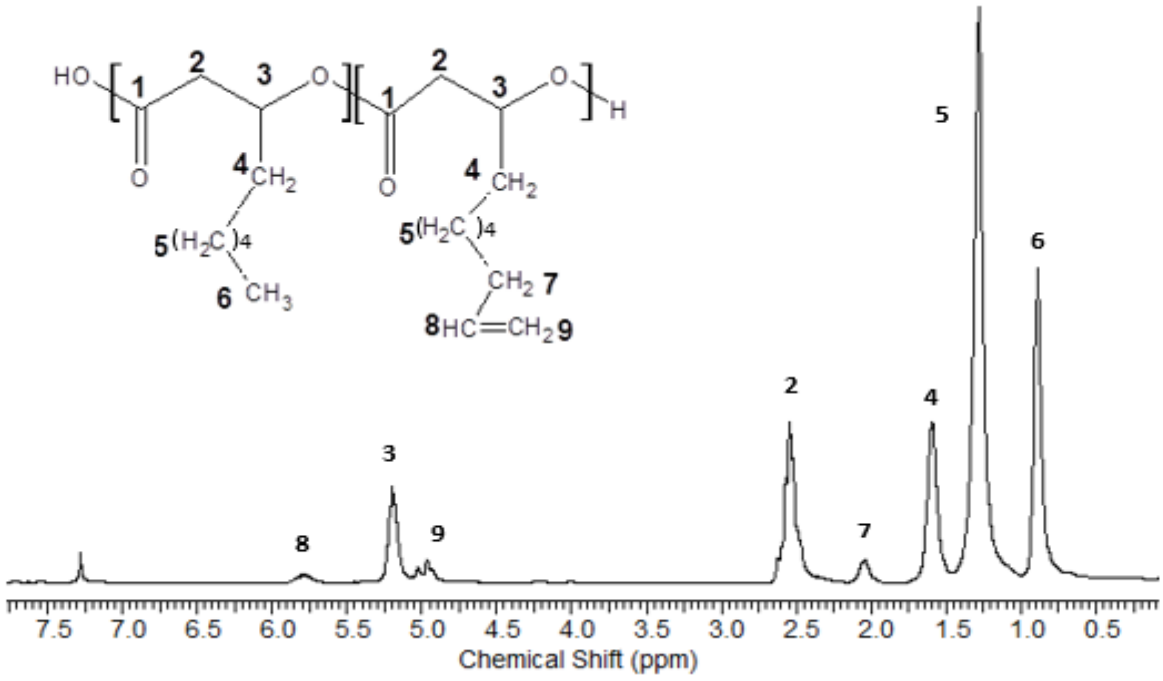

Fig. 4-1 ^{1}H NMR spectrum of PHNU-1 in $CDCl_3$

The intensity of individual peaks in the ^{1}H NMR spectrum was used to calculate the percent conversion of double bonds into carboxyl groups in accordance with equation 1 (Renard et al., 2005).

$$\%\,\text{Conversion} = 100\,\frac{[(\%C{=}C)\text{initial} - (\%C{=}C)\text{unreacted}]}{(\%C{=}C)\text{initial}} \qquad [1]$$

$$(\%C = C)\text{unreacted} = \frac{(100 \times P8)}{P3}$$

Where P3, P8 and P9 are the integration of the peaks centered at 5.18, 4.95 and 5.75 ppm, respectively, as shown in Fig. 4-1. The intensity of the methine backbone protons at 5.18 ppm was set to 1 in each spectrum.

4.3.4 Preparation of Mcl-PHA Suspensions

Mcl-PHA nanoparticle suspensions were prepared as previously described (chapter 3). PHA dissolved in methylene chloride was added to an aqueous surfactant solution, and homogenized for 3 min using a rotor-stator system (SDT Tissumizer, Tekmar Company, USA, equipped with an 18 mm diameter stator at 10 % of the power output of the Tekmar power controller (model TR-10)), to form a premix emulsion in an ice bath. The premix emulsion was ultrasonicated (20 KHz Ultrasonic Homogenizer 4710 Series, Cole Parmer Instrument Co., IL, USA, equipped with a microtip probe of 3.2 mm diameter) in an ice bath to produce a suspension in water. For example, 10 % (w/v) suspensions were prepared by dissolving 1 g of the desired PHA in 10 mL of methylene chloride before adding to 9 mL of aqueous surfactant solution. The homogenized emulsion was then subjected to ultrasonication. Methylene chloride was removed by rotary evaporation, and the suspension transferred to a graduated centrifuge tube. Distilled, deionized water was added to obtain 10 % (w/v) solids content.

4.3.5 Carboxylated Mcl-PHA at 0.4 % Solids without Surfactant

Colloidal suspensions of 0.4 % (w/v) mcl-PHA (11 % carboxylation) (PHNC-1) were made without surfactant. Suspensions were fabricated with and without the formation of the premix emulsion, and subjected to ultrasonication by varying the time from 1 to 10 min at 20 % amplitude, and particle size assessed at day 1.

4.3.6 Selection of a Non-ionic Surfactant at 0.4 % Solids

The particle size at day 1 was evaluated for four non-ionic surfactants (5 mM) at 0.4 % (w/v) PHNC-1 at a sonication amplitude of 20 % for 10 min. The non-ionic surfactants were

polyoxyethylene octyl phenyl ether (Triton X-100, OmniPur®), sorbitan monooleate (Span 80), polyoxyethylene (20) cetyl ether (Brij 58), and polyoxyethylene (20) sorbitan monooleate (Tween 80), purchased from Sigma-Aldrich Canada (Oakville ON, Canada).

4.3.7 Combination of Triton X-100 with an Ionic Surfactant at 0.4 % Solids

Suspensions containing 0.4 % (w/v) PHNC-1 were fabricated as described above at a sonication amplitude of 20 % for 10 min in 5 mM Triton X-100 (unless otherwise stated) with 2 mM of one of the following ionic surfactants: cationic surfactants include dodecyltrimethylammonium bromide (DTAB) and cetyltrimethylammonium bromide (CTAB), and the anionic surfactants were alkyldiphenyloxide disulfonate (Dowfax® 2A1) and sodium dodecyl sulfate (SDS). All surfactants were obtained from Sigma-Aldrich Canada (Oakville ON, Canada).

4.3.8 SDS Concentration at 10 % Solids

Submicron-sized particles of PHNC-1 at 10 % (w/v) were made as described above with an increasing SDS concentration from 1 to 8 mM, at ultrasonication time and amplitude of 10 min and 20 % respectively, and particle size assessed at day 1.

4.3.9 Combination of SDS ± Triton X-100 at 10 % Solids

A 10 % (w/v) PHNC-1 was fabricated as described above in 5 mM SDS with and without Triton X-100, at a sonication amplitude of 20 % for 10 min.

4.3.10 Different Mcl-PHAs at 10 % Solids

Colloidal suspensions were made as described above with different mcl-PHAs in 5 mM SDS and 20 mM Triton X-100 at 10 % solids, at an ultrasonication time and amplitude of 10 min and 20 % respectively. The mcl-PHAs evaluated include poly(3-hydroxydecanoate) (PHD), poly(3-hydroxynonanoate) (PHN), PHNC-1**,** and PHN with 18 % of carboxylation (PHNC-2).

4.4 Results and Discussion

4.4.1 Conversion of Vinyl to Carboxyl Groups

The reaction scheme and conditions studied to convert mcl-PHA vinyl groups to carboxylic acid groups are shown in Fig. 4-2. The dependence of the amount of 11-mercaptoundecanoic acid on the conversion of vinyl groups in PHNC-1 was investigated. In this work, molar ratios of 11-mercaptoundecanoic acid: unsaturated units of 0 to 4 were used.

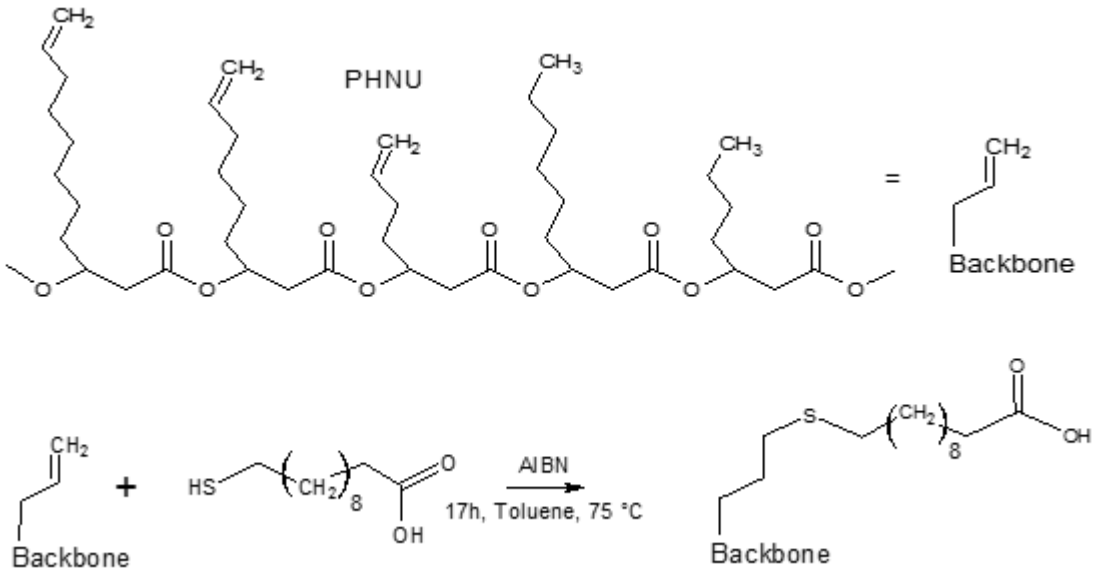

Fig. 4-2 Conversion of alkenes to carboxylic acids via a radical addition reaction with 11-mercaptoundecanoic acid in toluene (Hany et al., 2004)

Without 11-mercaptoundecanoic acid in the reaction (spectrum not shown), the double bonds were not converted to carboxyl groups. Fig. 4-3 shows the spectra obtained with increasing amounts of 11-mercaptoundecanoic acid. The solvent ($CDCl_3$) peak appeared at 7.27 ppm. The alkene signals in PHNU-1 are observed at 4.95 (-CH=C**H**$_2$) and 5.75 ppm (-C**H**=CH_2), respectively (Fig. 4-3, spectrum **f**). These peaks completely disappeared at 3.5 and 4 equivalents of 11-mercaptoundecanoic acid in spectra **d** and **e** respectively (Fig. 4-3), confirming quantitative conversion of the unsaturated groups. The signal at 2.34 ppm, designated as peak 10, represents the methylene protons adjacent to the carboxyl group. Peaks 8 and 9 centered at 5.75 and 4.95 ppm respectively for the vinyl groups are present in spectra **a**, **b**, and **c** (Fig. 4-3) suggesting incomplete carboxylation. The –COOH content increased with increasing 11-mercaptoundecanoic acid and a minimum of 3.5 equiv. of 11-mercaptoundecanoic acid was needed for complete conversion of olefin groups to carboxyl groups. Conversion data are summarized in Table 4-1. The PHNUs and PHNCs number-average molecular weight (M_n), weight-average molecular weight (M_w), and the polydispersity index (PDI = M_w/ M_n) reported in Table 4-2 show no significant molecular weight loss after the reaction.

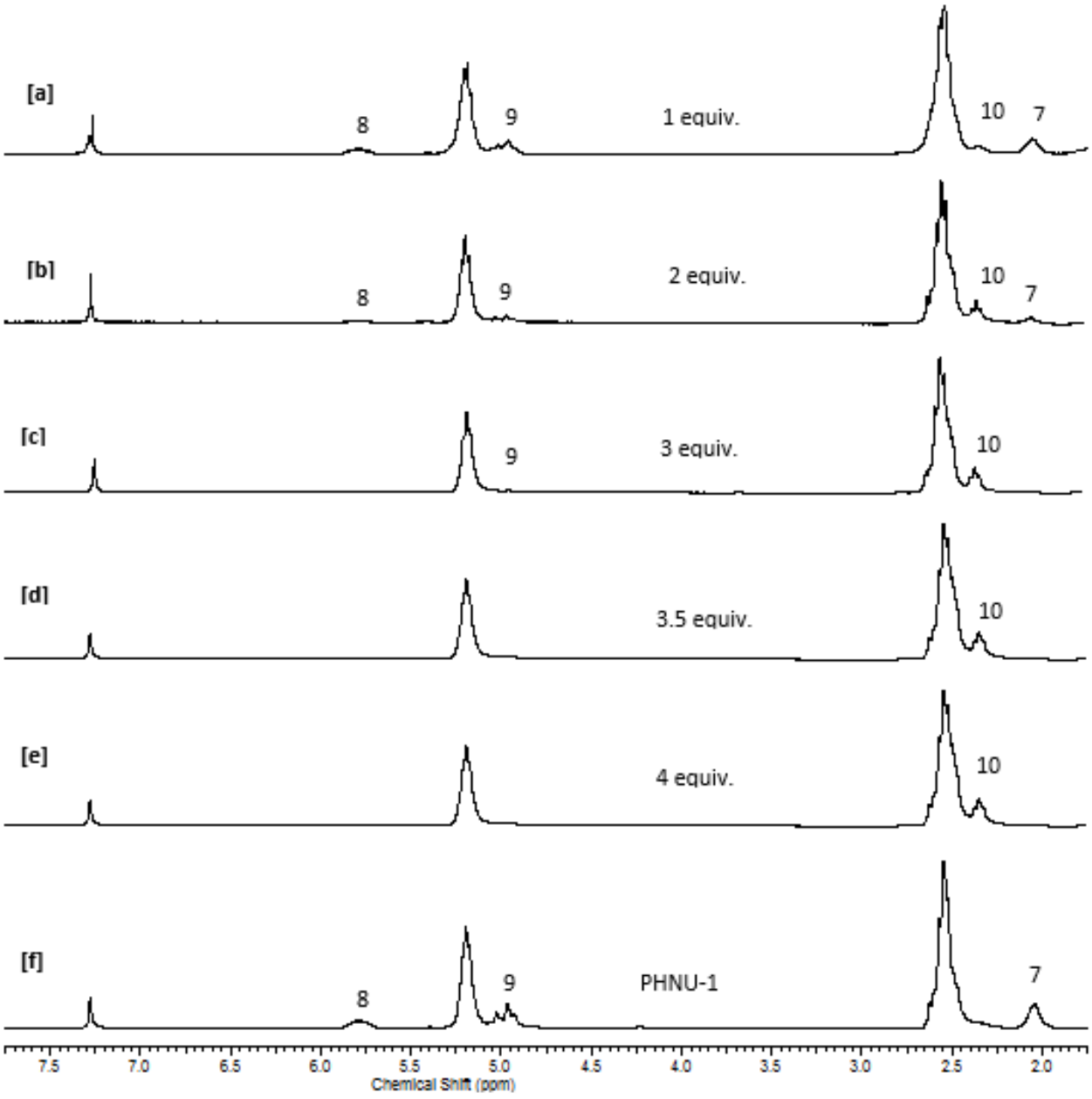

Fig. 4-3 Spectra of PHNC-1 at various stages of vinyl group conversion. A minimum 3.5 equiv. of 11-mercaptoundecanoic acid per unsaturated units in PHNU-1 was required for complete conversion of olefin groups to carboxyl groups. Only parts of the corresponding spectra with the key resonances are shown.

Table 4-1 Influence of 11-mercaptoundecanoic acid molar ratio on the carboxylation of PHNC-1

Initial molar ratio 11-mercaptoundecanoic acid: unsaturated groups	Unsaturated groups conversion (%)	Unsaturated groups (mol %)	Carboxyl groups (mol %)
0.0	0	11	0
1.0	18	9	2
2.0	64	4	7
3.0	91	1	10
3.5	100	0	11
4.0	100	0	11

Table 4-2 Mcl-PHAs molecular weight measured by GPC. M_w/ M_n is the polydispersity index

Mcl-PHA	M_n (g.mol^{-1})	M_w (g.mol^{-1})	PDI (M_w/ M_n)
PHNU-1	23377	37871	1.6
PHNC-1	30641	48413	1.6
PHNU-2	52408	94334	1.8
PHNC-2	55684	101345	1.8

4.4.2 Particle Size of PHNC-1 at 0.4 % Solids

The method used to prepare PHA nanoparticles directly affects the particle size. Stable PHO nanoparticle suspensions at 0.4 % solids have been prepared without surfactant by the titration method (Marchessault et al., 1995). With a six-blade turbine impeller and drop-wise addition of the solubilized polymer into deionized water under vigorous mixing, nanoparticle suspensions of PHN, PHN with 18 % unsaturation (PHNU), and PHN with 18 % carboxylation

(PHNC) at 0.4 % solids were stable for at least 110 days without the addition of a surfactant (Beauregard et al., 2016). In the present study, the particle size and stability of carboxylated PHNC-1 was evaluated at a solids content of 0.4 % (w/v) before increasing to 10 % solids. These suspensions were compared to those of PHD, PHN and PHNC-2. When the solubilized PHNC-1 polymer was added to deionized water and ultrasonicated at 20 % amplitude without surfactant at 0.4 % solids (no premix), the particles agglomerated with increasing ultrasonication time and particle size increased from 253.2 ± 11.8 to 295.4 ± 10.2 nm (Table 4-3) compared to the much smaller particles sizes of 76.1 ± 6.4 nm obtained by Beauregard et al. (2016) for their carboxylated PHN.

Table 4-3 Effect of premix (3 min rotor stator homogenization) and ultrasonication time on particle size at day 1 for 0.4 % (w/v) PHNC-1 without surfactant in an ice bath. Data are the mean ± standard deviation for n = 3. Polydispersity index (PDI) close to 1 indicates broad size distribution as measured by the zeta sizer

Ultrasonication time (min)	Particle size(nm)	PDI
1 (No premix)	253.2 ± 11.8	0.53 ± 0.06
3	261.1 ± 7.9	0.55 ± 0.07
5	282.3 ± 6.5	0.57 ± 0.07
10	295.4 ± 10.2	0.57 ± 0.08
10 (Premix)	320.1 ± 7.5	0.59 ± 0.08

Using the procedure described in chapter 3, forming a premix emulsion prior to ultrasonification, resulted in slightly larger polymer particles (320.1 ± 7.5 nm vs 295.4 ± 10.2 nm without premix). This may be due to higher collision rates of the particles caused by both the rotor-stator and ultrasonication. Polydispersity index (PDI) of the particle size distribution in the suspensions was about 0.5 (Table 4-3), indicating a broad size distribution as measured by the Zetasizer. In dynamic light scattering, PDI values range from 0 to 1, with values close to 0 indicating narrow size distribution. Based on this, surfactants may be required to obtain smaller, stable nanoparticles when using ultrasound treatment.

4.4.3 Evaluation of Non-ionic Surfactants on PHNC-1 Particle Size at 0.4 % Solids

Since PHNC-1 agglomerated when subjected to ultrasonication, various non-ionic surfactants were evaluated to reduce particle aggregation. Non-ionic surfactants can coat the polymer particles to provide steric stabilization, keeping the particles from approaching each other (Hellgren et al., 1999). The non-ionic surfactants examined were polyoxyethylene octyl phenyl ether (Triton X-100), sorbitan monooleate (Span® 80), polyoxyethylene (20) cetyl ether (Brij® 58), and polyoxyethylene (20) sorbitan monooleate (Tween® 80).

In the absence of surfactant, with and without formation of the premix emulsion before ultrasonication, the particle size decreased from 320.1 ± 7.5 and 295.4 ± 10.2 nm respectively (Table 4-3) to 194.2 ± 11.2 and 155.6 ± 8.4 nm respectively (Table 4-4) with addition of Triton X-100. The smaller particle size obtained with the formation of the premix emulsion with a surfactant (Table 4-4) suggests that the premix may be needed when the suspension contains a surfactant. Hence, all further suspensions were prepared with forming a premix emulsion prior to ultrasonication.

All the non-ionic surfactants evaluated produced smaller nanoparticles ranging from 155.6 ± 8.4 to 163.4 ± 11.3 nm (Table 4-4). The PDI was between 0.42 ± 0.03 and 0.49 ± 0.04, which may be indicating a broader particle distribution considering the polymer solids content (0.4 %). However, Triton X-100 gave a slightly smaller particle size and was selected for subsequent experiments.

Table 4-4 Non-ionic surfactants (5 mM) on particle size at day 1 for 0.4 % (w/v) PHNC-1 with or without formation of premix emulsion (3 min rotor stator homogenization) before ultrasonication at an amplitude of 20 % for 10 min in an ice bath. Data are mean ± standard deviation n = 3

Surfactant	Particle size (nm)	PDI
Triton X-100 (No premix)	194.2 ± 11.2	0.49 ± 0.04
Triton X-100 (Premix)	155.6 ± 8.4	0.43 ± 0.03
Tween 80	165.8 ± 10.1	0.45 ± 0.04
Brij 58	168.2 ± 9.3	0.45 ± 0.04
Span 80	163.4 ± 11.3	0.42 ± 0.03

4.4.4 Evaluation of Ionic Surfactants with Triton X-100 on PHNC-1 Particle Size at 0.4 % (w/v)

Although a non-ionic surfactant is used to keep PHA particles from aggregating by providing a protective shield around the particles, ionic surfactants are needed to reduce interfacial tension to create smaller particles during emulsification. This was the case when each ionic surfactant (dodecyltrimethylammonium bromide (DTAB), cetyltrimethylammonium bromide (CTAB), alkyldiphenyloxide disulfonate (Dowfax® 2A1), and sodium dodecyl sulfate (SDS)) was combined with Triton X-100. Smaller nanoparticles with a narrower particle size

distribution (97.1 ± 1.1 to 121.7 ± 5.7 nm and PDI of 0.21 ± 0.001 to 0.25 ± 0.003, Table 4-5) were formed than those in suspensions prepared with only a non-ionic surfactant (Table 4-4).

Among the ionic surfactants evaluated, SDS gave a slightly smaller particle size when combined with Triton X-100 demonstrating a good synergism to produce a stronger interaction to form stable smaller nanoparticles. This was also previously observed with PHD (Chapter 3).

Table 4-5 Effect of combining 2 mM ionic surfactant and 5 mM non-ionic surfactant on particle size at day 1 for 0.4 % (w/v) mcl-PHA (11 % carboxylation). Data are the mean ± standard deviation for n = 3

Surfactant	Particle size (nm)	PDI
Triton X-100		
+ SDS	97.1 ± 1.1	0.21 ± 0.001
+ CTAB	121.7 ± 5.7	0.25 ± 0.003
+ DTAB	115.6 ± 3.3	0.24 ± 0.003
+ Dowfax 2A1	120.5 ± 3.9	0.25 ± 0.001
Tween 80		
+ SDS	122.6 ± 4.4	0.31 ± 0.03
+ CTAB	136.2 ± 7.5	0.37 ± 0.03
Span 80		
+ DTAB	128.8 ± 6.7	0.32 ± 0.05

When other surfactant combinations were tested, slightly larger particles sizes (122.6 ± 4.4 to 136.2 ± 7.5 nm) were obtained (Table 4-5). Based on these results, the SDS-Triton X-100 combination was selected for subsequent experiments.

4.4.5 Evaluation of SDS Concentration on PHNC-1 Particle Size and Stability at 10 % (w/v)

The particle size of PHD was shown to decrease with increasing SDS concentration (Chapter 3). Similarly, carboxylated PHA, PHNC-1**,** nanoparticles decreased from 285.2 ± 12.3 to 198.7 ± 1.92 to nm at 10 % solids content as the SDS concentration was increased from 1 to 8 mM (Fig. 4-4b). The surfactant concentration range was selected based on the results of Chapter 3 when a 30 % (w/v) PHD nanoparticle suspension was stable over 215 days with 8 mM SDS and 24 mM Triton X-100. The surfactant concentration at the polymer-liquid interface increased upon increasing SDS concentration, and this led to a reduction of the particle-water interfacial tension, thereby resulting in smaller particles (Meleson et al., 2004). At a higher SDS concentration, the new particle surfaces formed via ultrasonication are immediately covered with SDS which helps to prevent coalescence. Not only did higher SDS concentration decrease PHNC-1 particle size, but zeta potential values increased from -18.4 ± 2.42 to -36.1 ± 0.92 mV (Fig. 4-4a). Zeta potential gives an indication of the strength of the energy barrier created by repulsive forces, which prevent particles or droplets from approaching each other. Since a suspension is considered unstable at values between +30 and -30 mV (Larsson et al., 2012), suspensions prepared with 1-4 mM SDS are unstable (-18.4 ± 2.42 to -25.7 ± 2.23 mV) and will likely agglomerate with time. This may be due to insufficient surfactant to totally coat the surface of all particles to maintain stability and prevent aggregation. An SDS concentration of 5 mM in 10 % (w/v) PHNC-1 suspension gave a stable suspension (-31.3 ± 0.36 mV) with little effect on particle size at higher concentrations. Hence, 5 mM SDS was chosen to study in combination with Triton X-100.

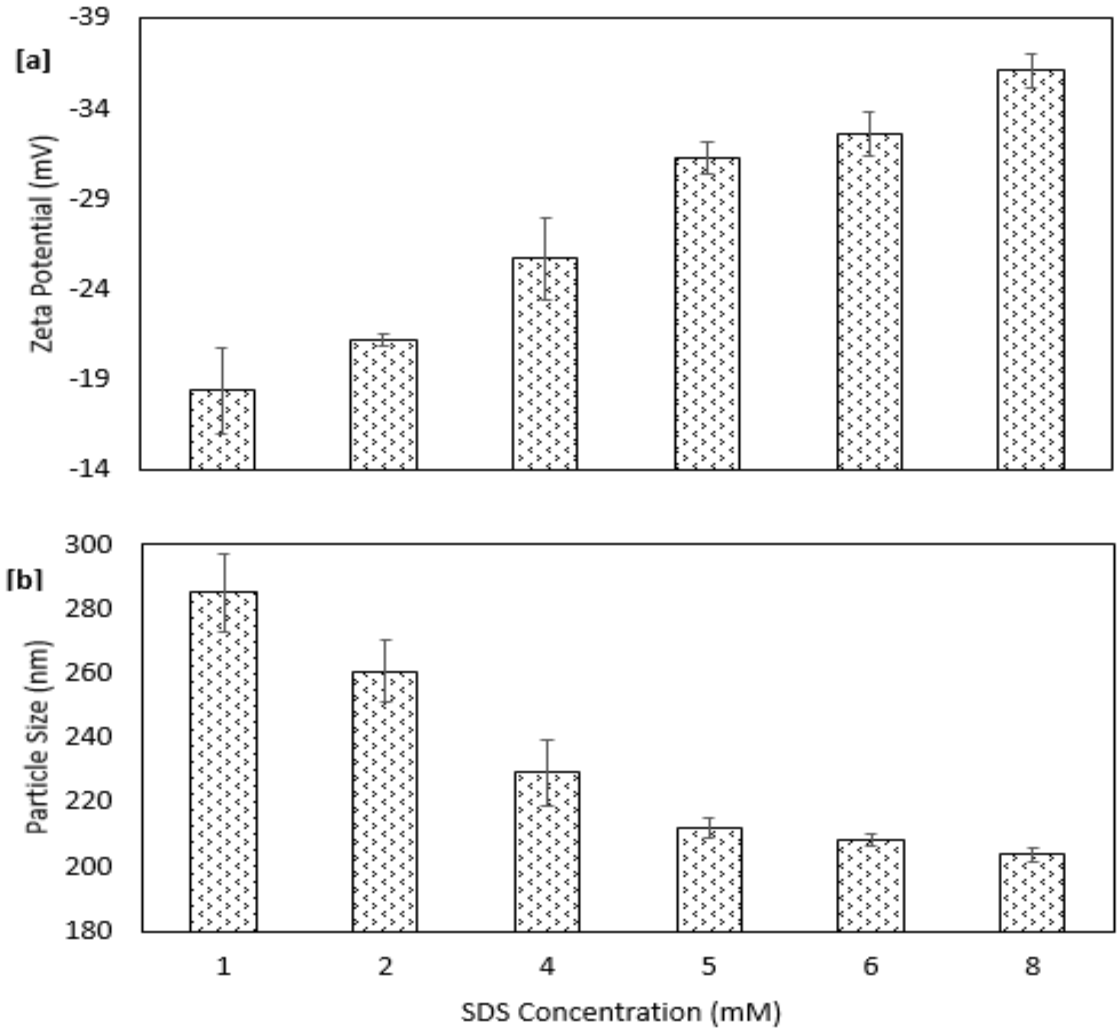

Fig. 4-4 Effect of SDS concentration on (a) zeta potential and (b) particle size in 10 % (w/v) PHNC-1 at sonication amplitude of 20 % for 10 mins. Measurements were done at day 1 and data are the mean ± standard deviation for n = 3

4.4.6 Evaluation of Triton X-100 Concentration with SDS on PHNC-1 Particle Size and Stability at 10 % (w/v)

Polymer dispersions can be prepared by combining a non-ionic surfactant with an ionic surfactant (Hellgren et al., 1999; Chu and Hwang, 1997). At high solids content, non-ionic surfactants are able to prevent particle aggregation via steric stabilization, and enhance shear stability (Hellgren et al., 1999; Walstra, 1993). Nanoparticles were made with 10 % (w/v)

PHNC-1 in 5 mM SDS and 5-25 mM Triton X-100. PHNC-1 particle size decreased as Triton X-100 concentration increased (Fig. 4-5b). Unstable suspensions were formed at 5 mM Triton X-100 (-28.5 ± 1.17 mV), while stable suspensions were formed at concentrations ≥ 10 mM with zeta potential values decreasing to -41.2 ± 0.63 mV with increasing Triton X-100 concentration (Fig. 4-5a). Since increasing Triton X-100 above 20 mM had little further effect on particle size, this concentration was selected to investigate other mcl-PHAs.

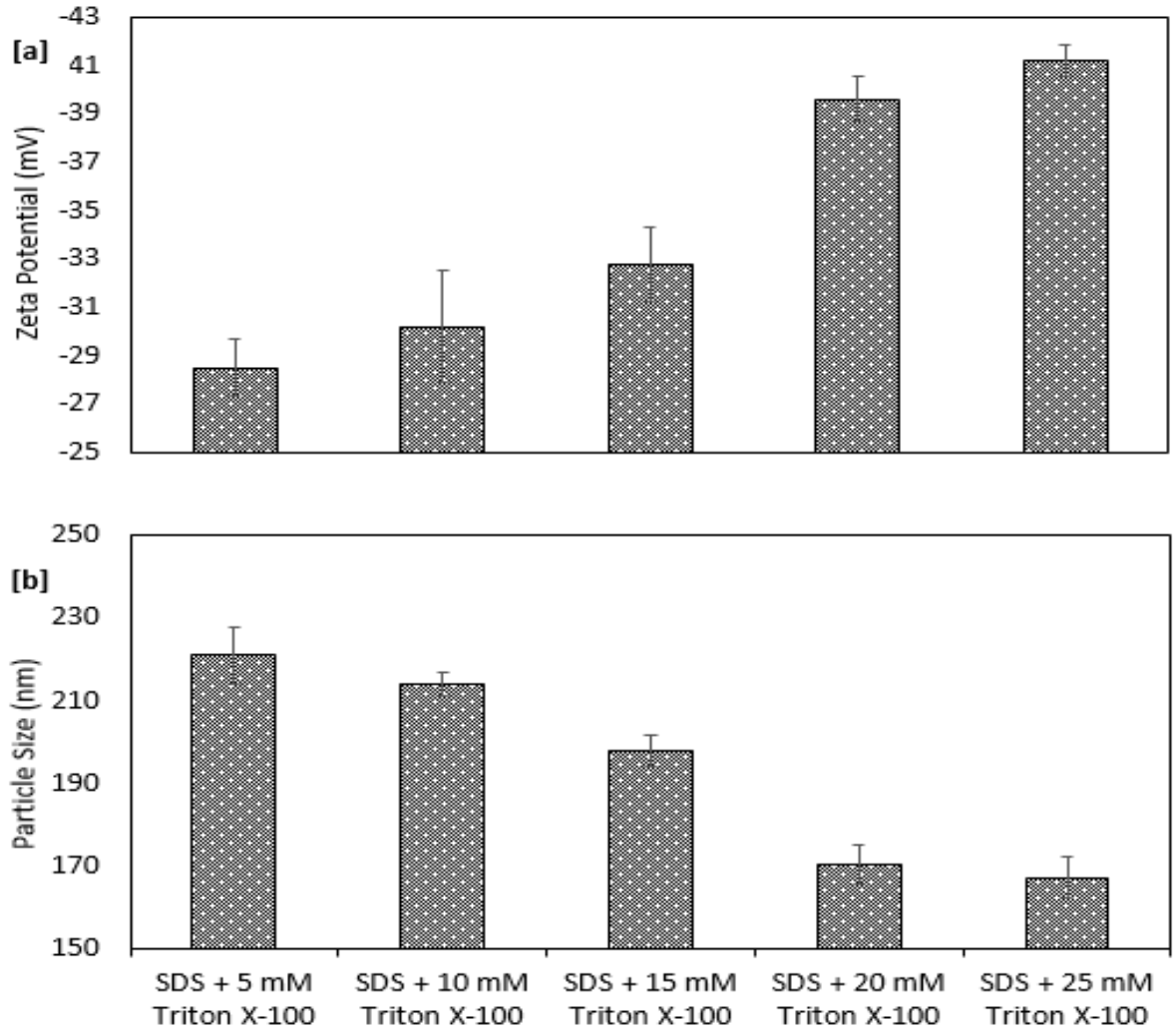

Fig. 4-5 Effect of 5 mM SDS ± Triton X-100 concentrations on (a) zeta potential and (b) particle size in 10 % (w/v) mcl-PHA suspensions at 20 % amplitude and 10 mins of sonication. Data are the mean ± standard deviation for n = 3

4.4.7 Different Mcl-PHAs at 10 % Solids

Upon selecting the appropriate SDS-Triton X-100 concentrations needed to stabilize 10 % (w/v) PHNC-1, other mcl-PHAs (PHD, PHN, and PHNC-2) were evaluated at the same solids content and surfactant concentration. Similar particle sizes and zeta potential values were obtained for both PHN (183.5 ± 4.72 nm and -34.4 ± 2.61 mV) and PHD (181.2 ± 3.19 nm and -35.2 ± 1.31 mV). Both materials have similar hydrophobic pendant side chain and may behave the same in aqueous suspension, thereby producing similar particle size and zeta potential values (Fig. 4-6). The average particle size for PHNC-1 (11 % carboxyl groups) and PHNC-2 (18 % carboxyl groups) were 170.3 ± 4.61 and 143.1 ± 2.88 nm, respectively. The PHNC-1 nanoparticles were slightly smaller than those of PHD and PHN, but the PHNC-2 were much smaller than all PHAs investigated.

Levine et al. (2016) reported a decreased in contact angle of carboxylated poly-3-hydroxybutyrate (PHBC) (74.8°) in contrast to 81.2° obtained for unsaturated PHB (PHBU). A lower contact angle indicates increased surface hydrophilicity. While PHN and PHD have aliphatic pendant side chains, PHNC-1 and PHNC-2 contain carboxyl groups (11 and 18 %, respectively) at the end of their lateral side chains. These carboxyl groups may aid in lowering of particle-water interfacial tension due to hydrophilic interaction with the aqueous phase, with the effect more pronounced as carboxyl groups increase. In addition, higher zeta potential values were obtained with higher carboxyl content, indicating that increased hydrophilic interaction in an aqueous polymer suspension may help to provide steric stabilization.

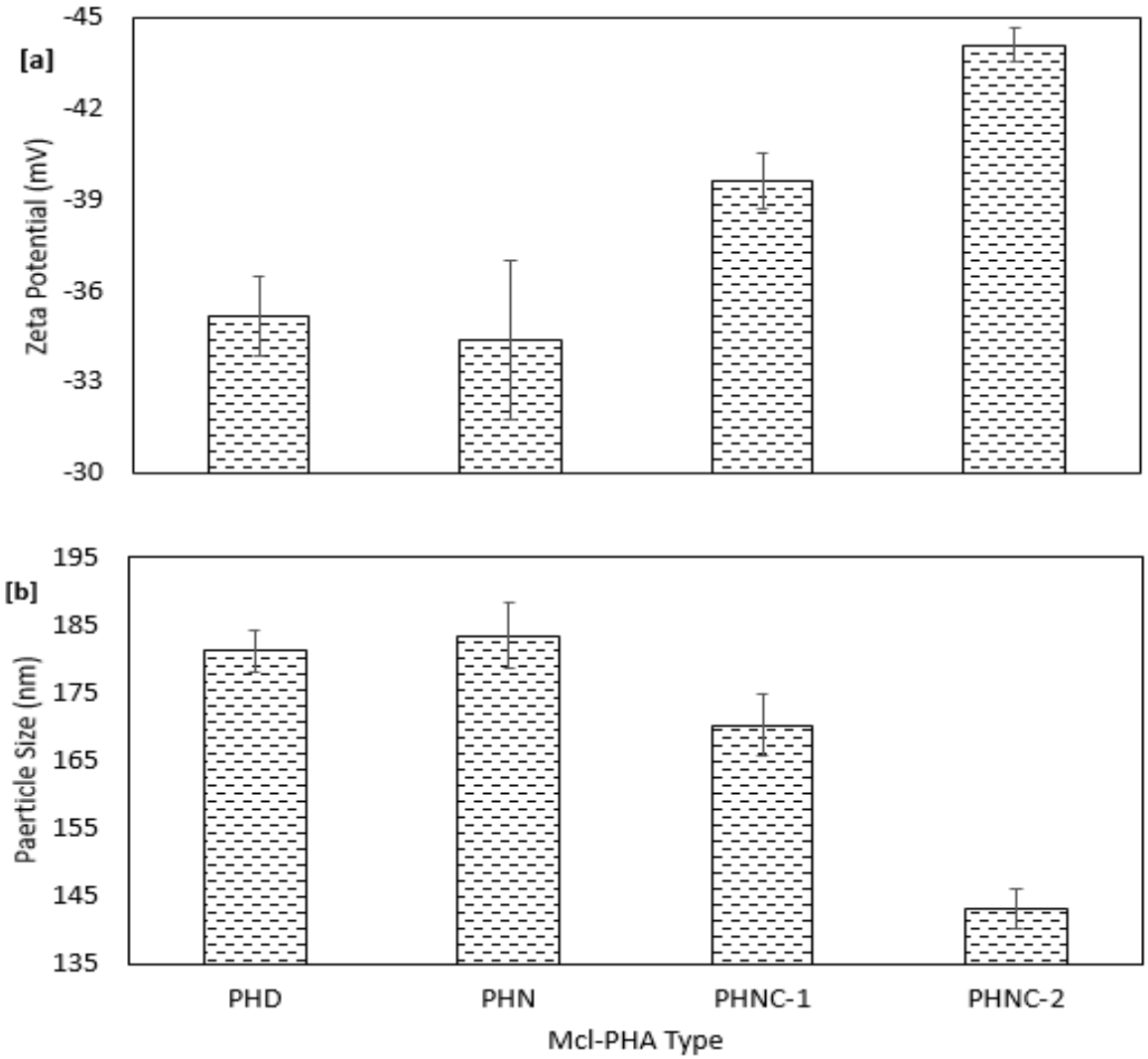

Fig. 4-6 Effect of 5 mM SDS ± 20 mM Triton X-100 concentrations on (a) zeta potential and (b) particle size in 10 % (w/v) mcl-PHA suspensions at 20 % amplitude and 10 mins of sonication. Zeta potential measurement showed that all suspensions were stable, but PHN-2 gave the lowest particle size. Data are the mean ± standard deviation for n = 3. The PHAs were poly-3-hydroxynonanoate (PHN), poly-3-hydroxydecanoate (PHD), PHN with 11 % of carboxyl groups in the pendant side-chains (PHNC-1), and PHN with 18 % of carboxyl groups in the pendant side-chains (PHNC-2)

4.5 Conclusion

A minimum of 3.5 equiv. of 11-mercaptoundecanoic acid per double bonds in PHNU (unsaturated poly-3-hydroxynonanoate) was needed for complete conversion of olefin groups to carboxyl groups. Mcl-PHA (11 % carboxylation) required surfactant to maintain stability even in 0.4 % (w/v) nanoparticle suspensions when using the emulsification-solvent evaporation method instead of the titration method. Carboxylated mcl-PHAs produced smaller nanoparticles compared to mcl-PHAs containing aliphatic pendant side chains. Much smaller particles and larger zeta potential value were obtained with higher carboxyl content.

4.6 References

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Chapter 5

Conclusions and Recommendations for Future Work

5.1 Conclusions

A two-stage emulsification-solvent evaporation process was employed to produce medium-chain-length poly-3-hydroxyalkanoate (mcl-PHA) suspensions. This reproducible preparation method was found to produce uniformly nanoscale colloidal dispersions of mcl-PHA particles in water using a rotor-stator and an ultrasonic probe. Through systematic experiments the key parameters that control the making of dense (10 and 30 % (w/v)) poly-3-hydroxydecanoate (PHD) suspensions were examined. Surfactants played a significant role in stabilizing particles during emulsification. Ionic surfactants lower the interfacial tension, thereby facilitating particle break-up whereas non-ionic surfactants provide steric stabilization by preventing particle re-coalescence. When ionic surfactants were evaluated to stabilize the suspension, the anionic, sodium dodecyl sulfate (SDS), and the cationic, dodecyltrimethylammonium bromide, produced the smallest particle sizes (both ~100 nm). Nanoparticles were more stable when an ionic surfactant, SDS, was combined with any of the non-ionic surfactants tested. However based on the zeta potential and the smaller particle size obtained, an SDS-Triton X-100 combination was selected. A minimum of three parts Triton X-100 to one part SDS based on molar concentration is recommended for the preparation of stable nanoparticles for 30 % (w/v) mcl-PHA. A similar particle size was produced when a more scalable, high-shear, microfluidic device was employed using the same surfactant formulation.

Carboxylated mcl-PHA with different carboxyl content (11 and 18 %) was synthesized via free radical addition reaction of 11-mercaptoundecanoic acid to the pendant vinyl bonds of unsaturated PHNU. A complete conversion of the pendant vinyl groups to carboxyl groups

required a minimum 3.5 equiv. of 11-mercaptoundecanoic acid per double bonds in PHNU. Surfactant was needed to maintain stability when the emulsification-solvent evaporation method was used to prepare nanoparticle suspensions of mcl-PHA containing 11 % of carboxyl groups even at 0.4 % but not when the titration method was used. Several non-ionic surfactants tested to reduce agglomeration of particles produced similar particle sizes, but Triton X-100 gave the smallest particles. When Triton X-100 was combined with the ionic surfactants tested, smaller particles were obtained. When an SDS and Triton X-100 combination was used to evaluate other mcl-PHAs at 10 % solids, the particle size obtained with carboxylated mcl-PHAs were smaller compared to those of mcl-PHA containing aliphatic lateral side chains, with mcl-PHA (18 % carboxylation) producing much smaller nanoparticles and more stable zeta potential values.

5.2 Recommendations for Future Work

Although this study has shown that 30 % (w/v) PHD uniform nanoparticle suspensions can be prepared using ultrasonic treatment, a more scalable, high-shear, microfluidic device produced similar particle size, but with slightly higher polydispersity index. Microfluidization is known to produce smaller nanoparticles with uniform size distribution compared to ultrasonication, therefore more work should focus on the formulation and processing conditions of this device to make high density monodisperse particles. Further studies are also needed on scaling-up of the two-stage emulsification-solvent evaporation process to produce large quantity of samples with smaller and uniform particle size.

Carboxylated mcl-PHAs have been shown to produce smaller particle size in contrast to mcl-PHA containing aliphatic lateral side chains, because they likely have lower particle-water interfacial tension due to hydrophilic interaction with the aqueous phase. It would be interesting

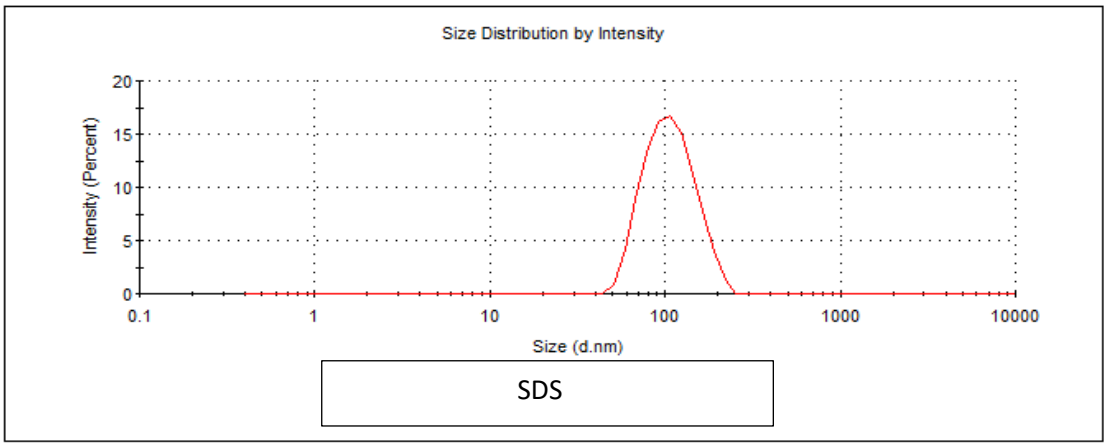
to produce nanoparticle suspensions of carboxylated mcl-PHAs having even higher carboxylation (> 18 %), as this may help to further reduce particle size. This approach may be useful to reduce the surfactant concentration required to stabilize dense (30 % (w/v)) stable mcl-PHA suspensions, and avoid problems associated with high surfactant concentration, such as increase in particle size and higher cost of formulation.

Appendix

Raw Data

[a]

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	99.70	Peak 1:	110.2	100.0	35.73
PdI:	0.096	Peak 2:	0.000	0.0	0.000
Intercept:	0.925	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



[b]

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	100.9	Peak 1:	113.4	100.0	40.46
PdI:	0.096	Peak 2:	0.000	0.0	0.000
Intercept:	0.927	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

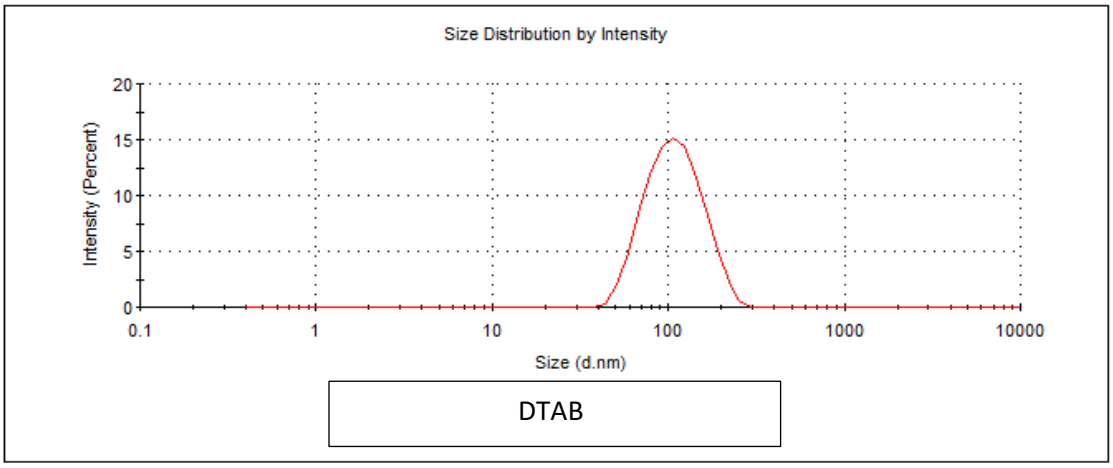
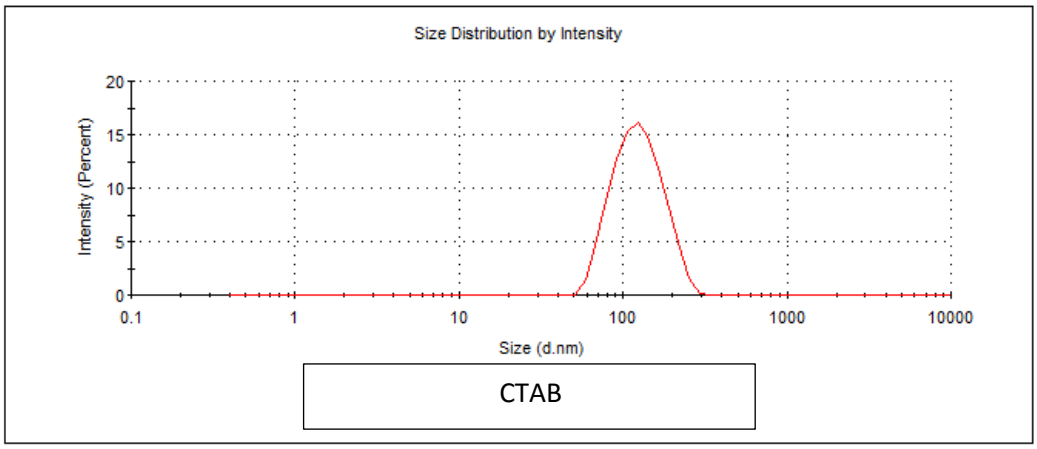


Fig. A-1 Particle size chromatogram of 100 mM (a) SDS and (b) DTAB in 10 % (w/v) PHD suspension, with measurement done at day 1

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 115.7	Peak 1:	128.7	100.0	43.37
PdI: 0.094	Peak 2:	0.000	0.0	0.000
Intercept: 0.929	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 110.5	Peak 1:	120.7	100.0	40.21
PdI: 0.140	Peak 2:	0.000	0.0	0.000
Intercept: 0.927	Peak 3:	0.000	0.0	0.000
Result quality : Good				

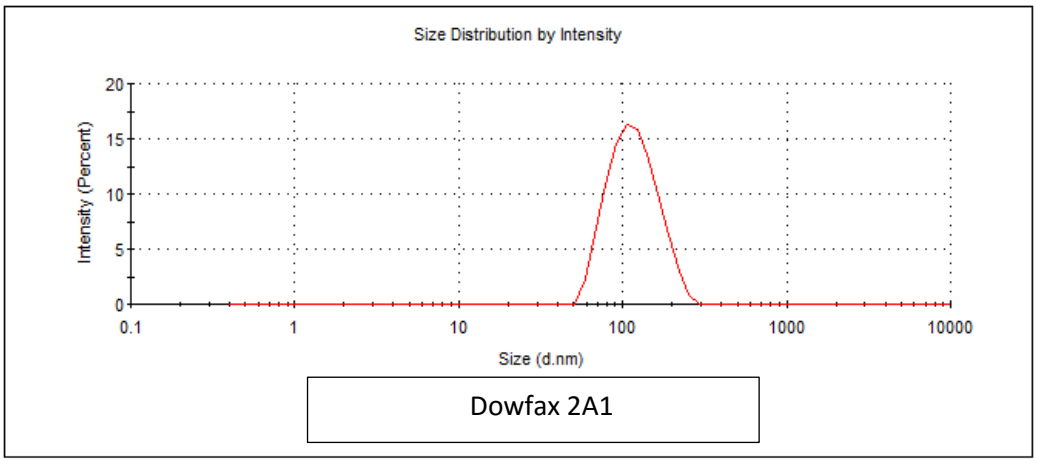
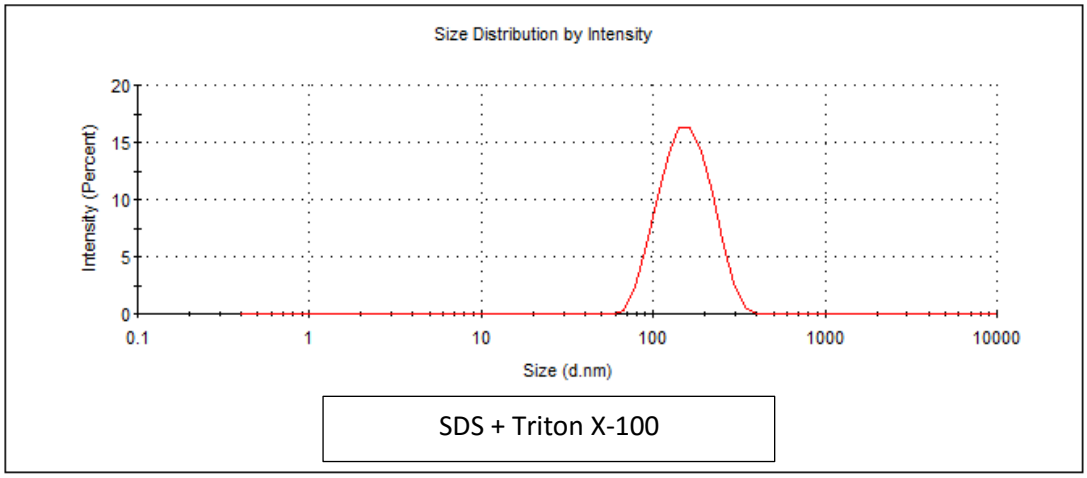


Fig. A-2 Particle size chromatogram of 100 mM (a) CTAB and (b) Dowfax 2A1 in 10 % (w/v) PHD suspension, with measurement done at day 1

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 147.0	Peak 1:	161.9	100.0	52.92
PdI: 0.075	Peak 2:	0.000	0.0	0.000
Intercept: 0.917	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 166.2	Peak 1:	183.4	100.0	59.64
PdI: 0.083	Peak 2:	0.000	0.0	0.000
Intercept: 0.918	Peak 3:	0.000	0.0	0.000
Result quality : Good				

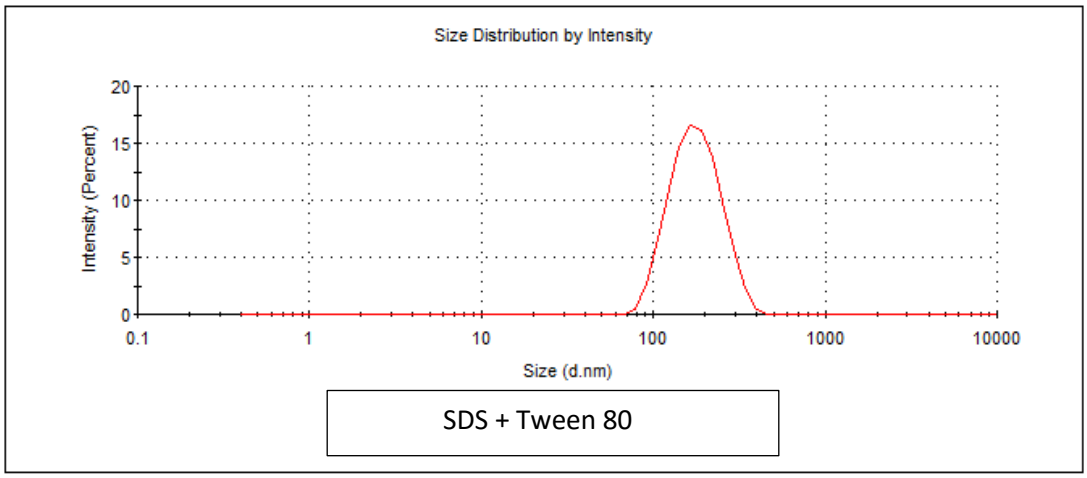
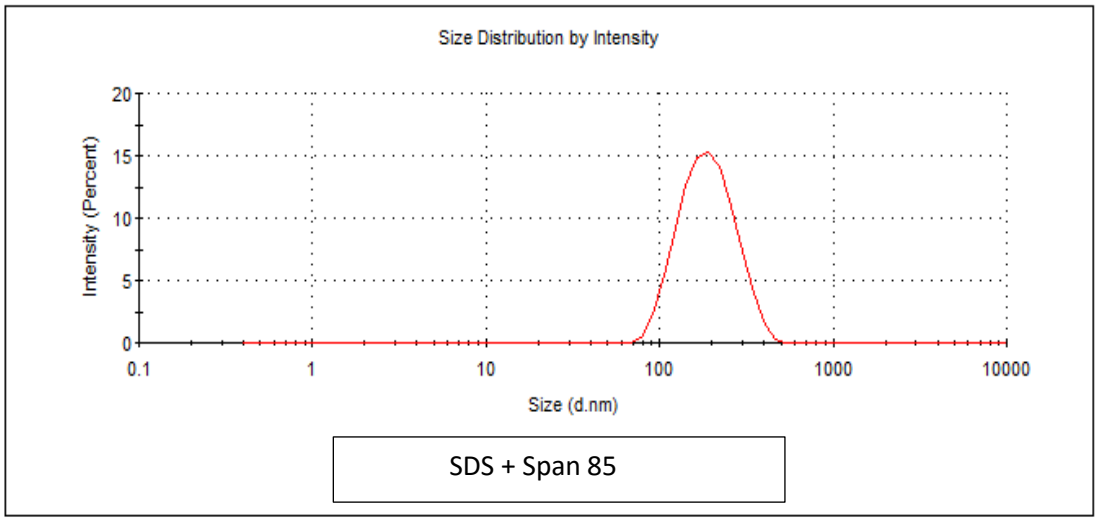


Fig. A-3 Particle size chromatogram of 20 mM SDS + 40 mM (a) Triton X-100 and (b) Tween 80 in 10 % (w/v) PHD suspension, with measurement done at day 1

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 175.0	Peak 1:	197.5	100.0	69.76
PdI: 0.107	Peak 2:	0.000	0.0	0.000
Intercept: 0.906	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 163.9	Peak 1:	180.2	100.0	57.44
PdI: 0.081	Peak 2:	0.000	0.0	0.000
Intercept: 0.917	Peak 3:	0.000	0.0	0.000
Result quality : Good				

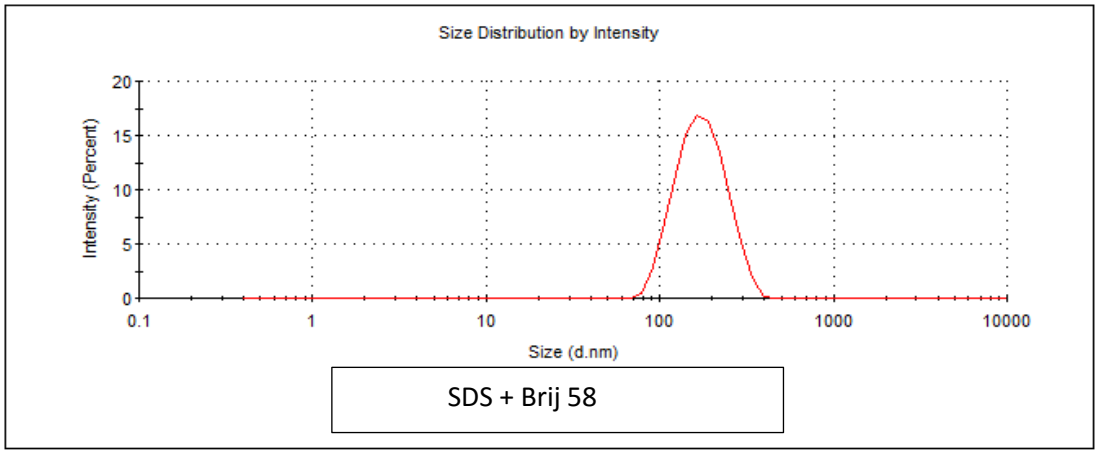
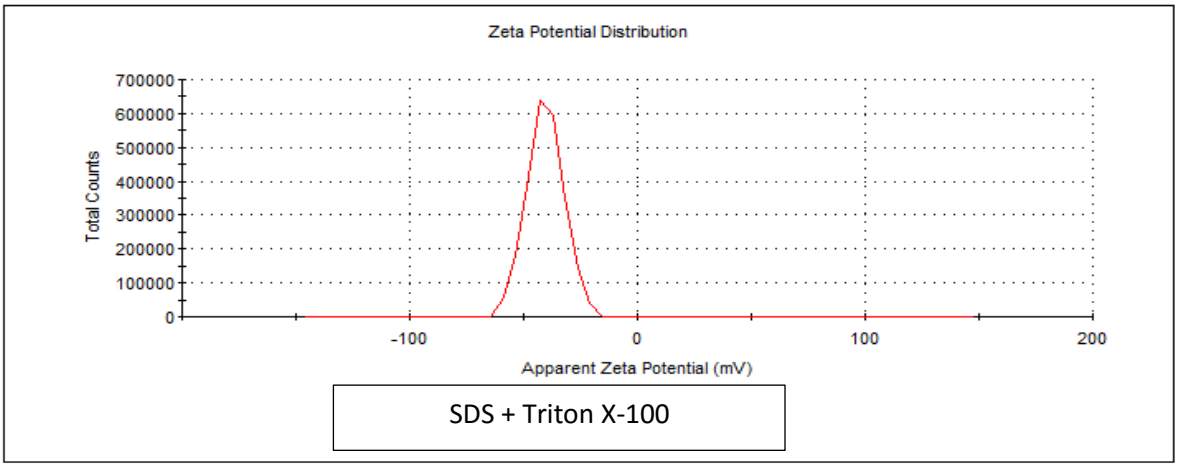


Fig. A-4 Particle size chromatogram of 20 mM SDS + 40 mM (a) Span 85 and (b) Brij 58 in 10 % (w/v) PHD suspension, with measurement done at day 1

[a]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-40.4	Peak 1: -40.4	100.0	7.95
Zeta Deviation (mV):	7.95	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	0.0148	Peak 3: 0.00	0.0	0.00

Result quality : Good



[b]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-39.7	Peak 1: -39.7	100.0	11.3
Zeta Deviation (mV):	11.3	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	0.0185	Peak 3: 0.00	0.0	0.00

Result quality : Good

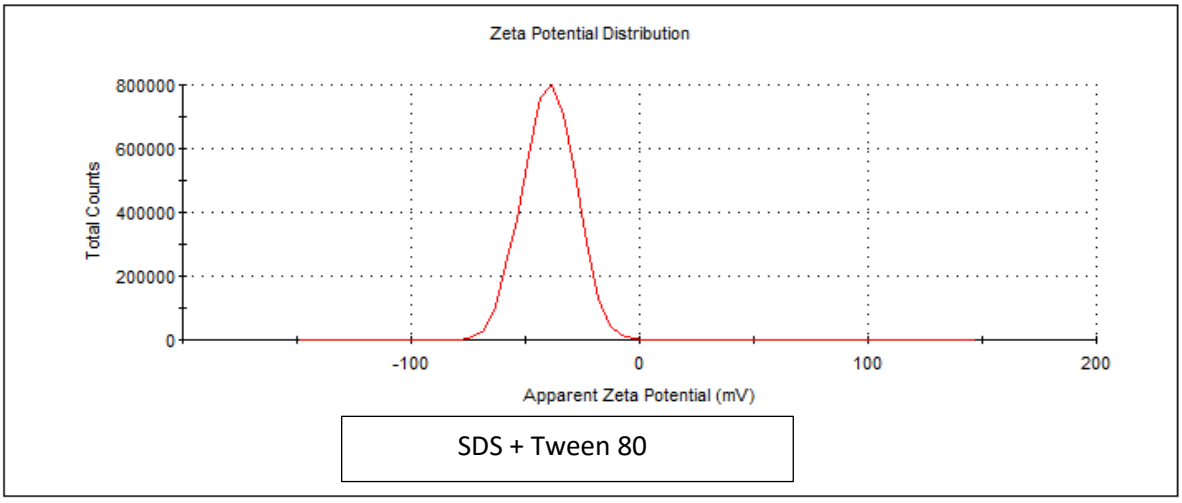
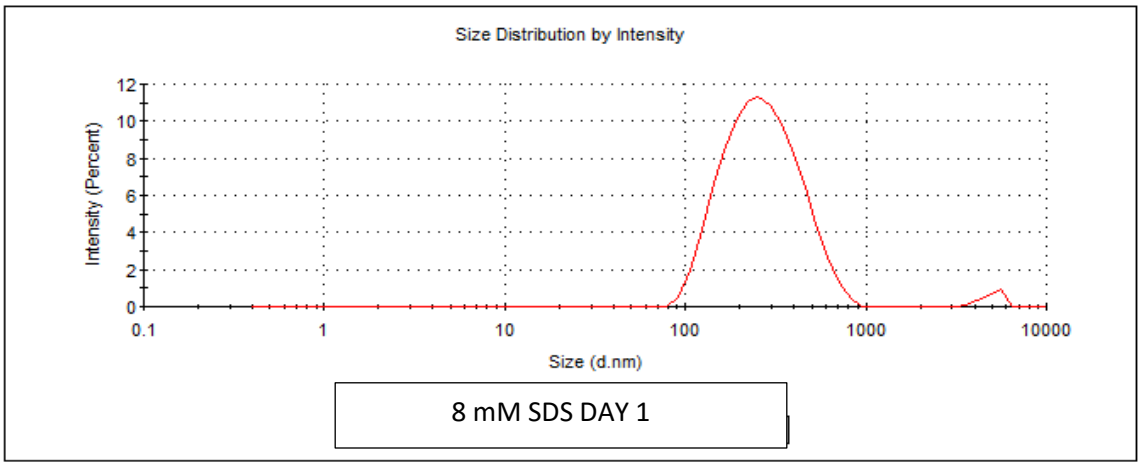


Fig. A-5 Zeta potential chromatogram of 20 mM SDS + 40 mM (a) Triton X-100 and (b) Tween 80 in 10 % (w/v) PHD suspension, with measurement done at day 1

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 250.5	Peak 1:	289.4	98.0	137.5
PdI: 0.217	Peak 2:	5015	2.0	601.6
Intercept: 0.923	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 321.2	Peak 1:	278.7	89.0	77.47
PdI: 0.343	Peak 2:	4797	11.0	724.9
Intercept: 0.883	Peak 3:	0.000	0.0	0.000
Result quality : Good				

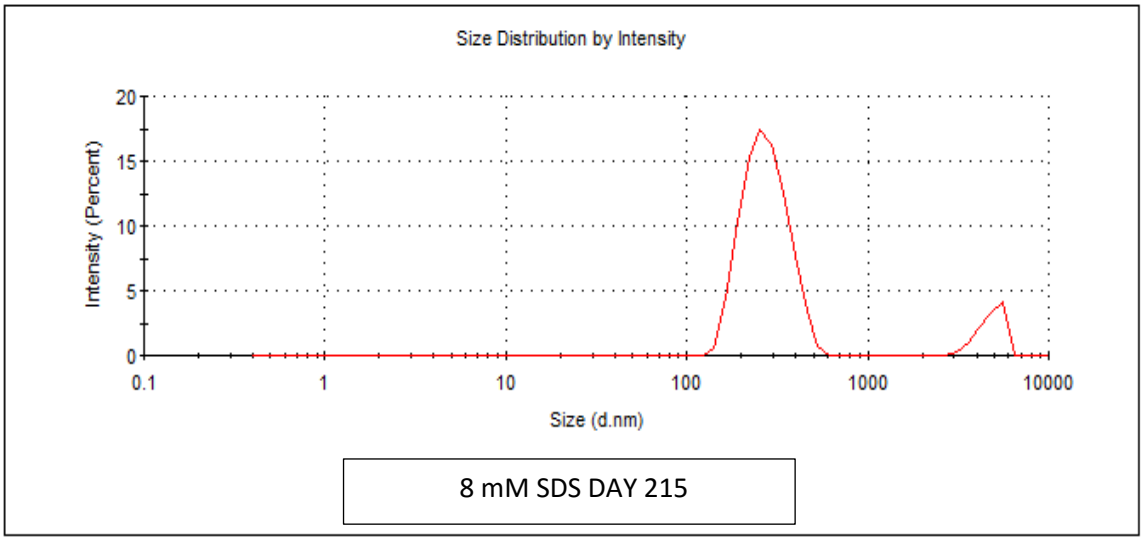
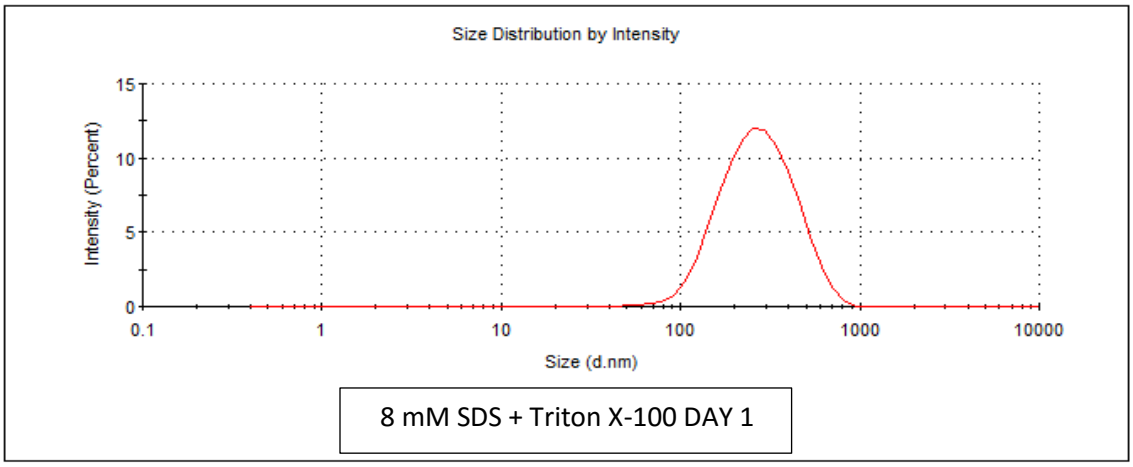


Fig. A-6 Particle size chromatogram of 8 mM SDS in 30 % (w/v) PHD suspension measured at (a) day 1 and (b) day 215. Bimodal distribution shows the particle aggregation that occurred when the suspension was stabilized by SDS only, resulting in larger particle size and higher PDI.

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 244.3	Peak 1:	293.7	100.0	134.3
PdI: 0.187	Peak 2:	0.000	0.0	0.000
Intercept: 0.930	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 247.4	Peak 1:	290.6	100.0	132.9
PdI: 0.175	Peak 2:	0.000	0.0	0.000
Intercept: 0.922	Peak 3:	0.000	0.0	0.000
Result quality : Good				

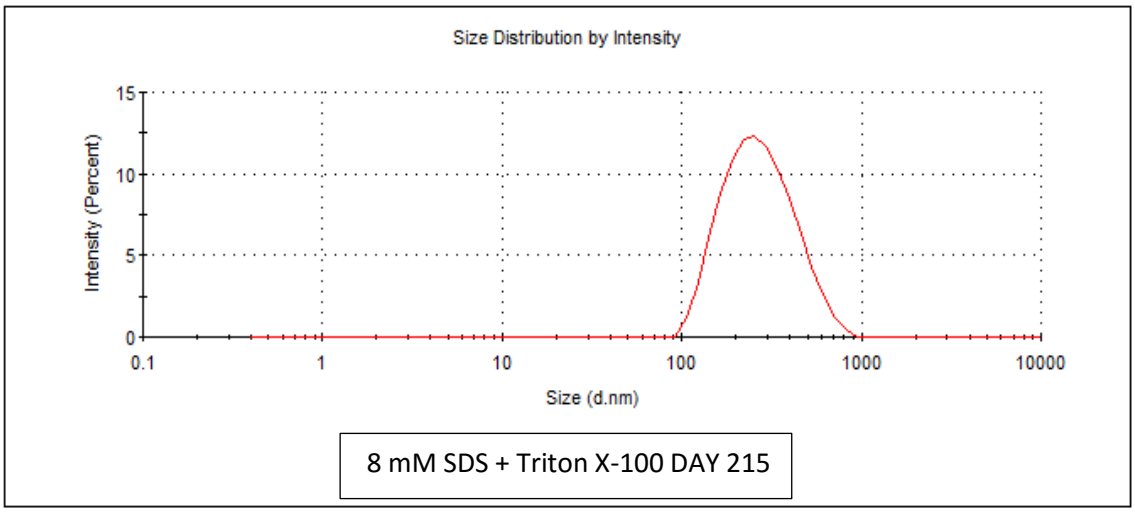
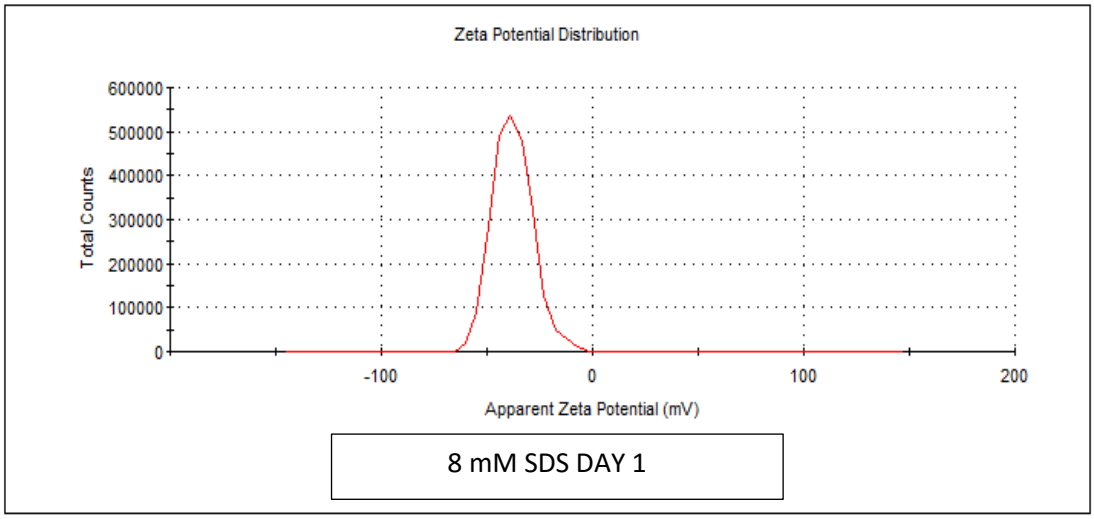


Fig. A-7 Particle size chromatogram of 8 mM SDS + 24 mM Triton X-100 in 30 % (w/v) PHD suspension measured at (a) day 1 and (b) day 215

[a]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -37.9	Peak 1:	-37.9	100.0	9.33
Zeta Deviation (mV): 9.33	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0154	Peak 3:	0.00	0.0	0.00

Result quality : Good



[b]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -27.7	Peak 1:	-27.7	100.0	7.26
Zeta Deviation (mV): 7.26	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0104	Peak 3:	0.00	0.0	0.00

Result quality : Good

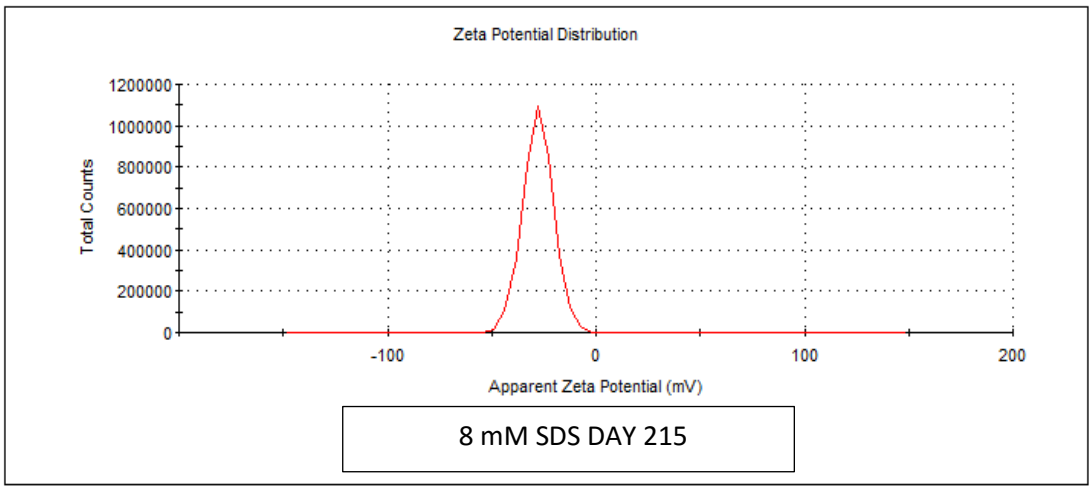
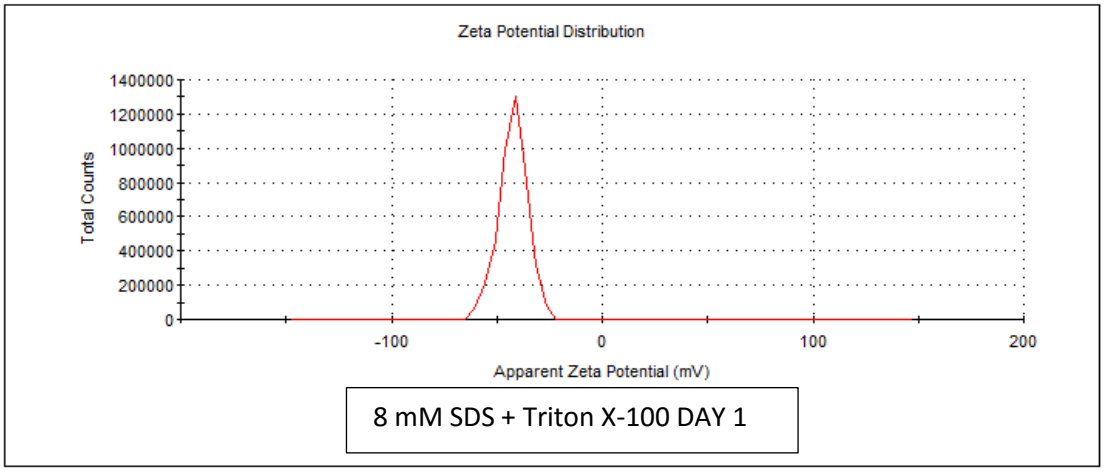


Fig. A-8 Zeta potential chromatogram of 8 mM SDS in 30 % (w/v) PHD suspension measured at (a) day 1 and (b) day 215

[a]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -42.3	Peak 1:	-42.3	100.0	6.72
Zeta Deviation (mV): 6.72	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0159	Peak 3:	0.00	0.0	0.00

Result quality : Good



[b]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -45.7	Peak 1:	-45.7	100.0	6.64
Zeta Deviation (mV): 6.64	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0186	Peak 3:	0.00	0.0	0.00

Result quality : Good

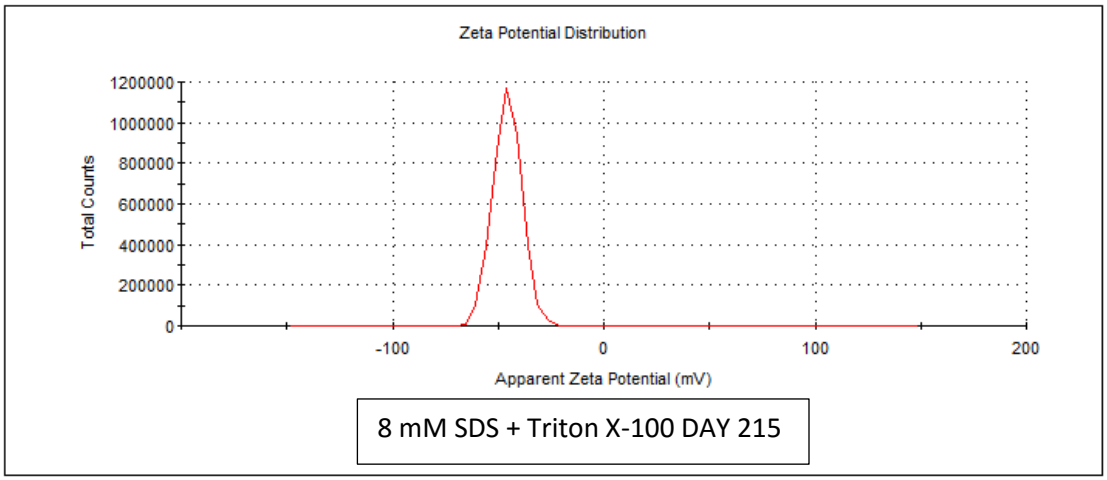


Fig. A-9 Zeta potential chromatogram of 8 mM SDS + 24 mM Triton X-100 in 30 % (w/v) PHD suspension measured at (a) day 1 and (b) day 215

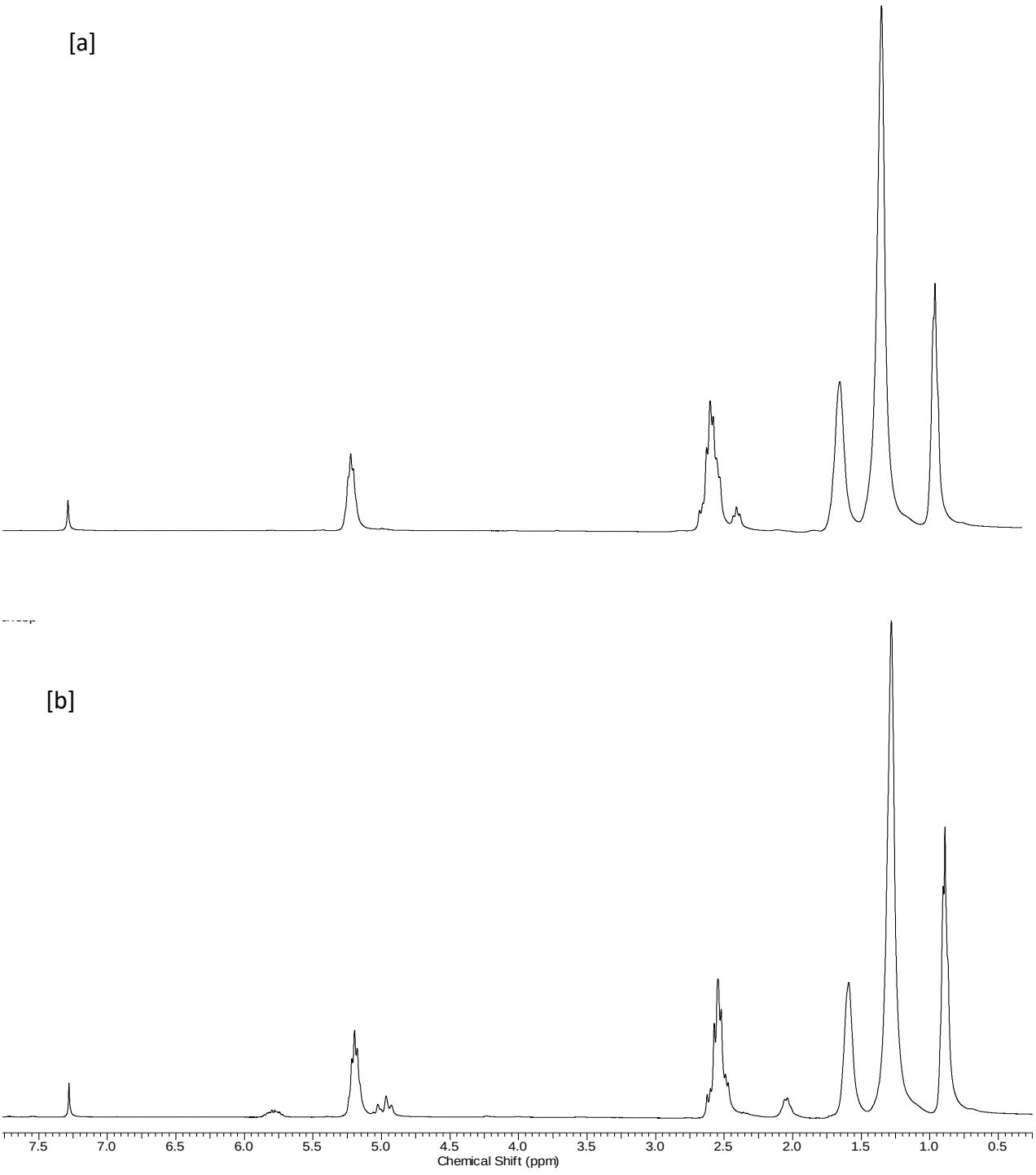


Fig. A-10 Complete spectra of (a) PHNC-1 and (b) PHNU-1 in $CDCl_3$

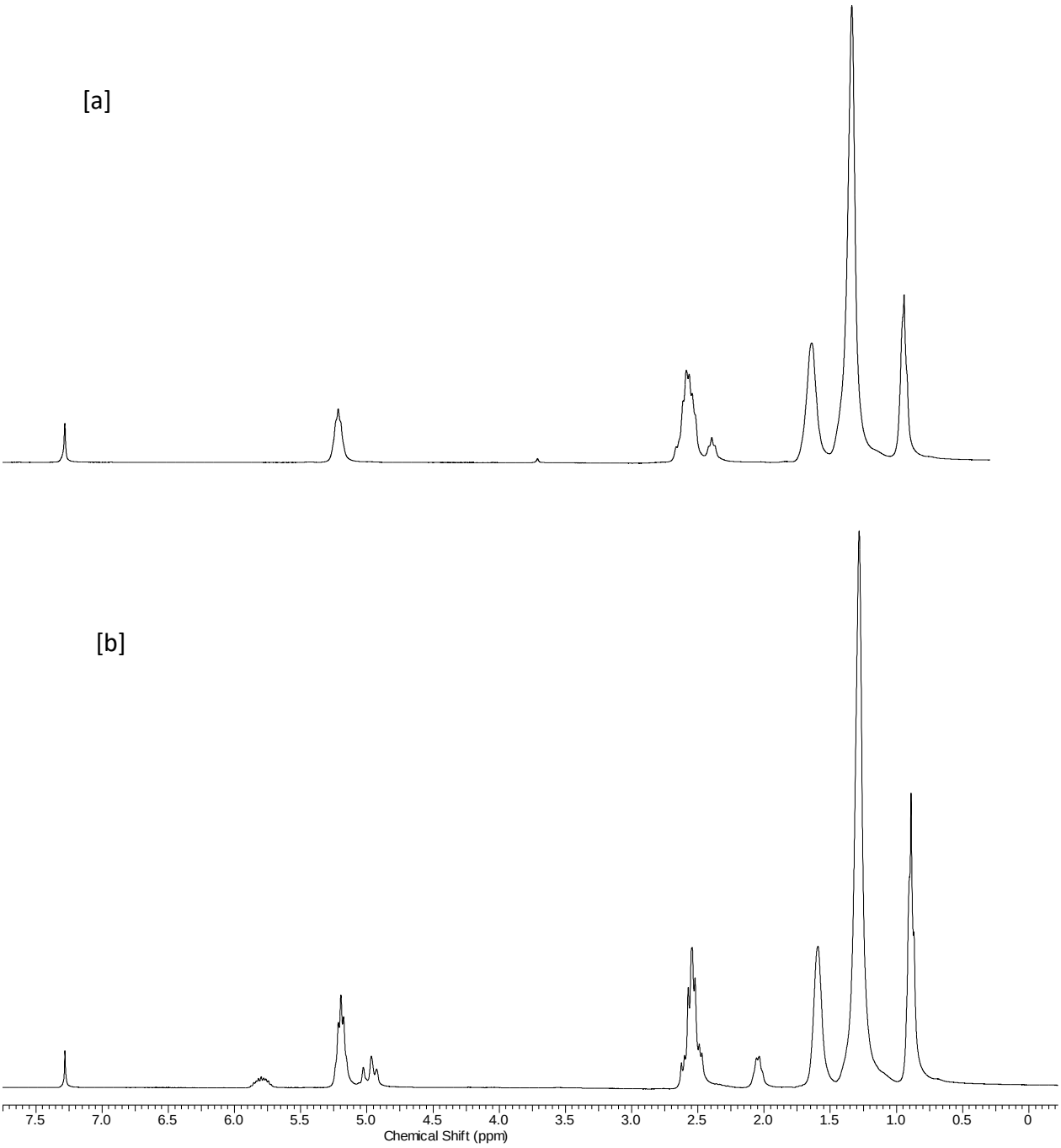
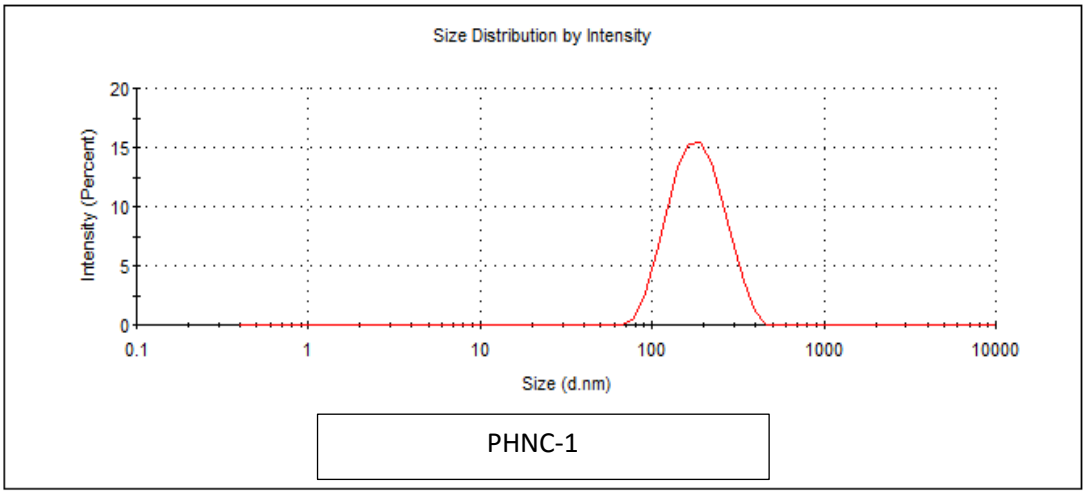


Fig. A-11 Spectra of (a) PHNC-2 and (b) PHNU-2 in $CDCl_3$

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 170.5	Peak 1:	191.4	100.0	66.30
PdI: 0.104	Peak 2:	0.000	0.0	0.000
Intercept: 0.917	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 146.9	Peak 1:	159.2	100.0	45.79
PdI: 0.077	Peak 2:	0.000	0.0	0.000
Intercept: 0.918	Peak 3:	0.000	0.0	0.000
Result quality : Good				

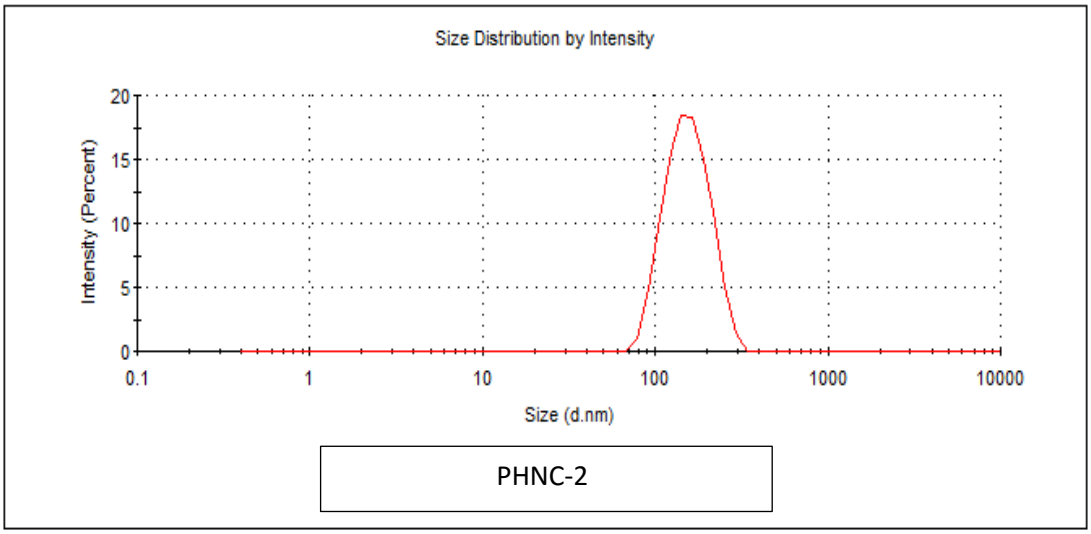
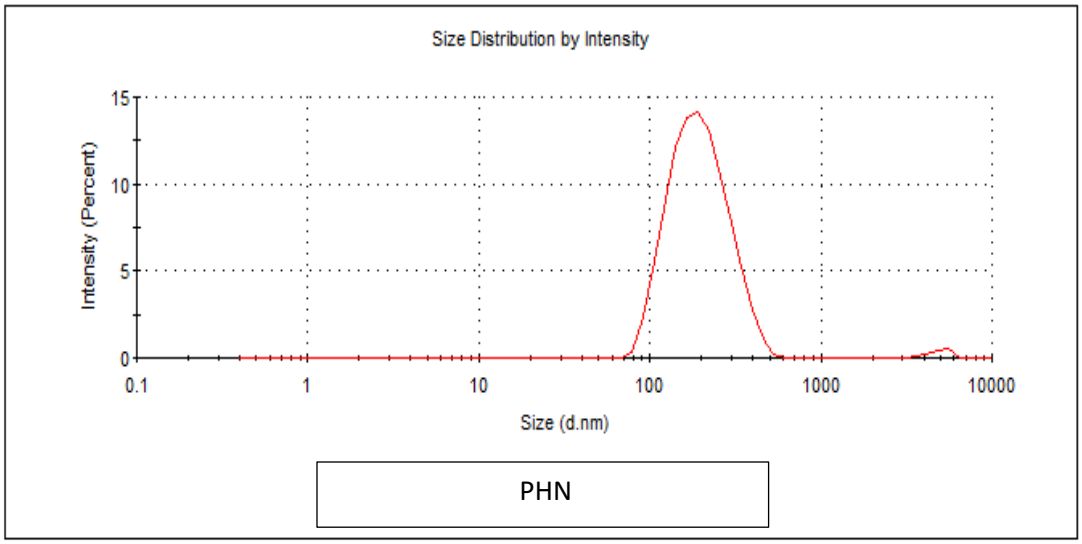


Fig. A-12 Particle size chromatogram of (a) PHNC-1 and (b) PHNC-2 with 5 mM SDS + 20 mM Triton X-100 in 10 % (w/v) suspension, and measurement done at day 1

[a]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 183.9	Peak 1:	204.5	98.8	79.35
PdI: 0.163	Peak 2:	4994	1.2	610.9
Intercept: 0.919	Peak 3:	0.000	0.0	0.000
Result quality : Good				



[b]

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 179.6	Peak 1:	193.4	98.6	66.57
PdI: 0.160	Peak 2:	5031	1.4	590.7
Intercept: 0.924	Peak 3:	0.000	0.0	0.000
Result quality : Good				

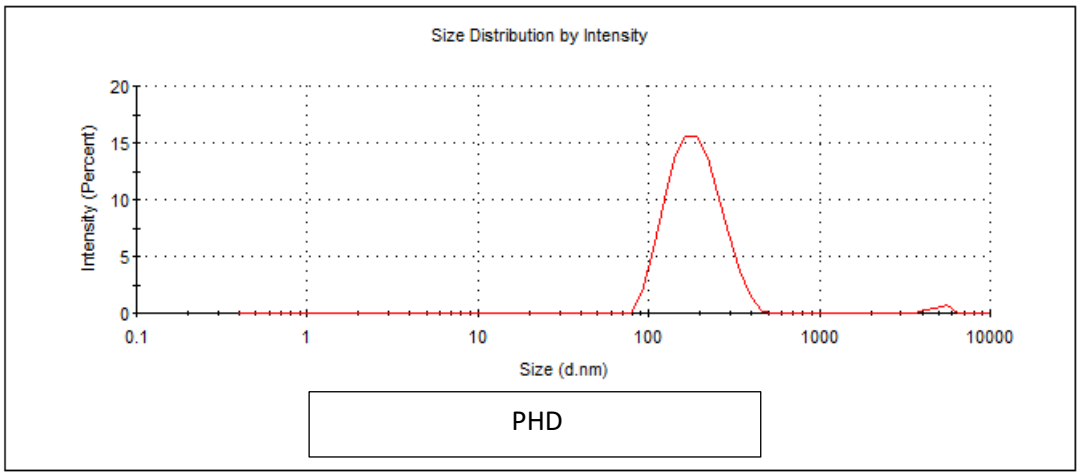
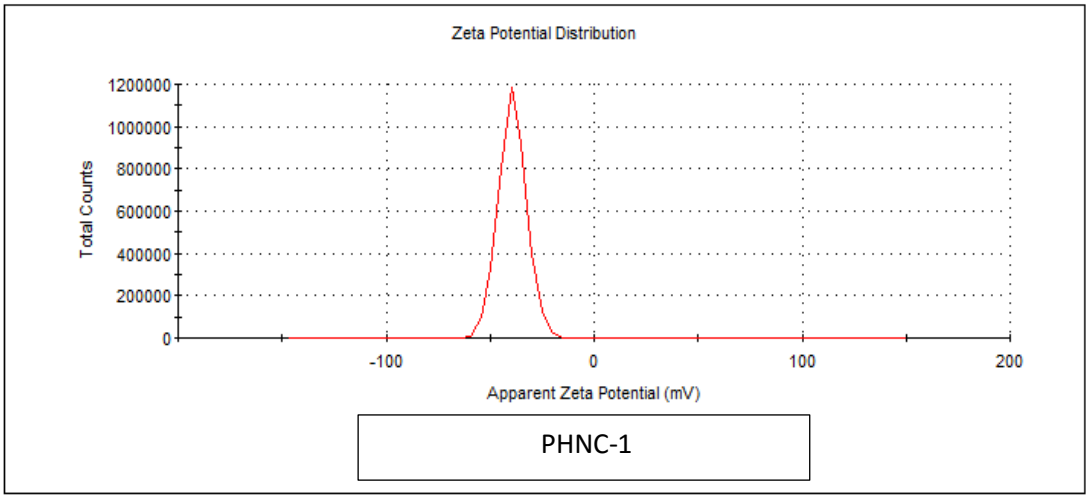


Fig. A-13 Particle size chromatogram of (a) PHN and (b) PHD with 5 mM SDS + 20 mM Triton X-100 in 10 % (w/v) suspension, and measurement done at day 1

[a]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -39.2	Peak 1:	-39.2	100.0	6.61
Zeta Deviation (mV): 6.61	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0146	Peak 3:	0.00	0.0	0.00

Result quality : Good



[b]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -44.1	Peak 1:	-44.1	100.0	7.63
Zeta Deviation (mV): 7.63	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0168	Peak 3:	0.00	0.0	0.00

Result quality : Good

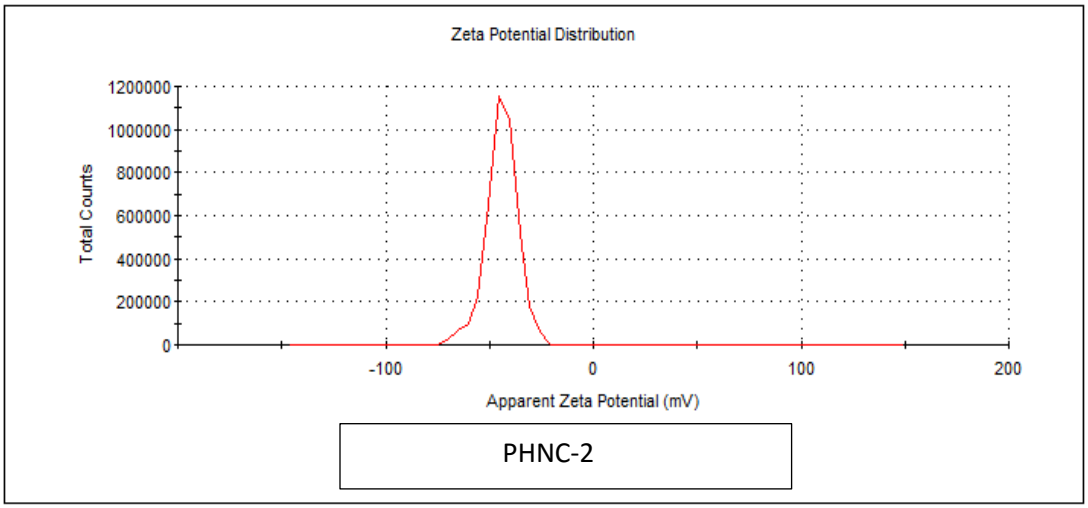
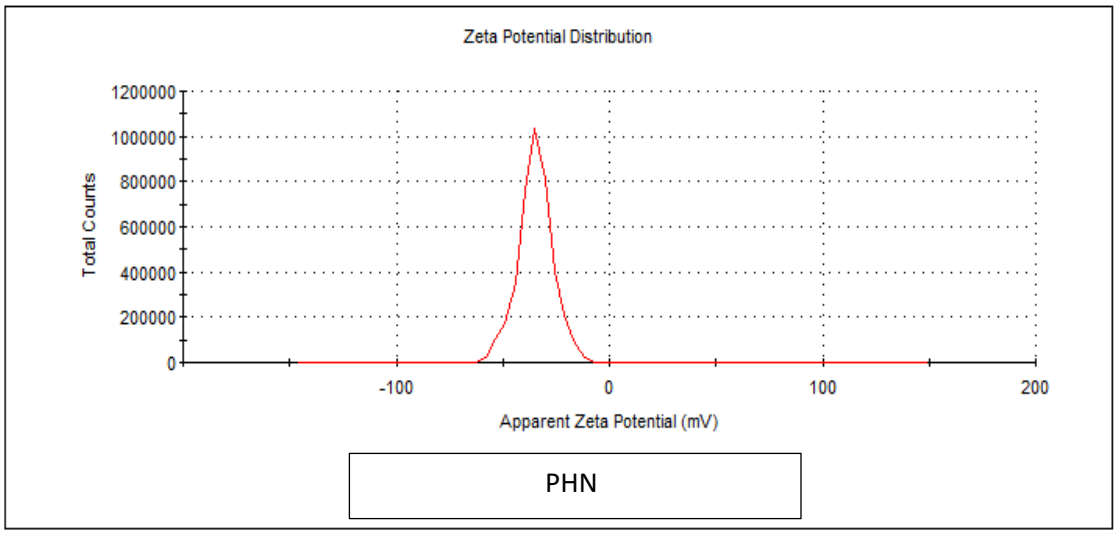


Fig. A-14 Zeta potential chromatogram of (a) PHNC-1 and (b) PHNC-2 with 5 mM SDS + 20 mM Triton X-100 in 10 % (w/v) suspension, and measurement done at day 1

[a]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -34.7	Peak 1:	-34.7	100.0	8.12
Zeta Deviation (mV): 8.12	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0141	Peak 3:	0.00	0.0	0.00

Result quality : Good



[b]

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -35.6	Peak 1:	-35.6	100.0	7.87
Zeta Deviation (mV): 7.87	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0100	Peak 3:	0.00	0.0	0.00

Result quality : Good

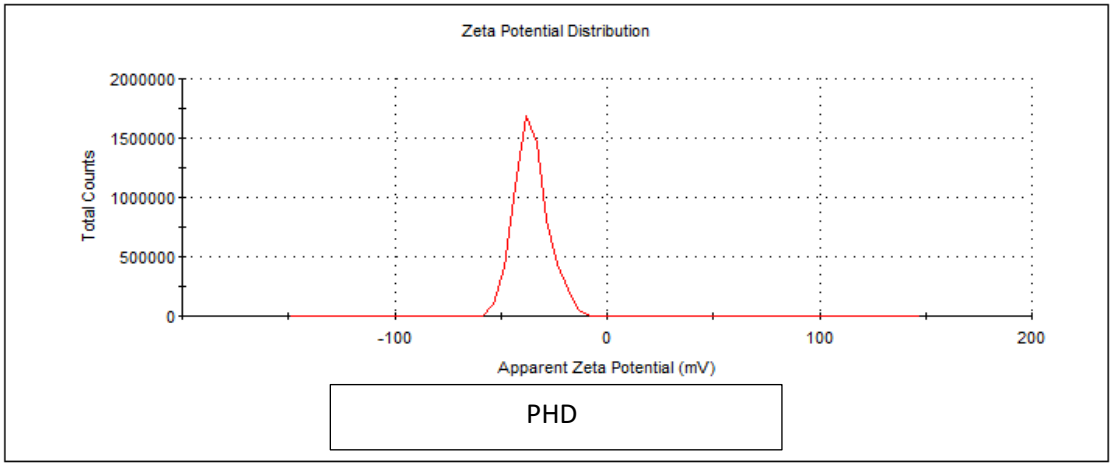


Fig. A-15 Zeta potential chromatogram of (a) PHN and (b) PHD with 5 mM SDS + 20 mM Triton X-100 in 10 % (w/v) suspension, and measurement done at day 1

Table A-1 Particle size at increasing sonication time and 20 % amplitude on 10 % (w/v) PHD in 20 mM SDS. Measurements were taken at day 1 and data are mean ± standard deviation for n = 3 for each experiment. Reproducible results obtained

Time (min)	Experiment 1	Experiment 2
1	212 ± 0.9	210 ± 1.3
3	187 ± 1.6	188 ± 0.7
5	172 ± 1.9	171 ± 0.6
10	165 ± 0.5	163 ± 1.6
15	170 ± 2.7	169 ± 3.1

Table A-2 Particle size at increasing amplitude and 10 mins of sonication time on 10 % (w/v) PHD in 20 mM SDS. Measurements were taken at day 1 and data are mean ± standard deviation for n = 3 for each experiment. Reproducible results obtained

Amplitude (%)	Experiment 1	Experiment 2
1	206 ± 1.9	209 ± 2.1
3	174 ± 0.9	175 ± 1.2
5	165 ± 0.5	164 ± 0.9
10	156 ± 1.3	157 ± 0.8
15	163 ± 1.3	162 ± 1.6