Small Angle Neutron Scattering Studies of Native and Chemically Modified Phytoglycogen Nanoparticles

by

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ABSTRACT

SMALL ANGLE NEUTRON SCATTERING STUDIES OF NATIVE AND CHEMICALLY MODIFIED PHYTOGLYCOGEN NANOPARTICLES

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Phytoglycogen is a highly branched polymer of glucose produced as soft, compact nanoparticles by sweet corn. Properties such as softness, porosity, and mechanical integrity, combined with nontoxicity and biodegradability, make phytoglycogen nanoparticles ideal for applications involving the human body. We used small angle neutron scattering (SANS) to study the structure, hydration and interaction of these nanoparticles. SANS measurements on dilute dispersions of highly purified, native phytoglycogen nanoparticles revealed that the particles are well described by the core-chain model in which the core of radius 21.0 nm is decorated by linear, “hairy” chains. SANS measurements of concentrated dispersions of native particles were consistent with a hard sphere model using the Percus-Yevick approximation, allowing the determination of the concentration dependence of the interparticle spacing. Hydrophobic modification of the particles with octenyl succinic anhydride (OSA) using a high degree of substitution resulted in the observation of a distinctive high-q scattering feature that we associated with the hydrophobic collapse of the hairy chains to form “seeds” on the outer surface of the particles within the context of the raspberry particle geometry. This SANS measurement of OSA-modified particles allowed the determination of the average length and packing density of the hairy chains on the native particles. SANS measurements of concentrated dispersions of OSA-modified particles revealed complex interparticle interactions due to the soft, sticky and
charged nature of the OSA-modified particles. Hydrolysis of the particles using either hot acid or the amyloglucosidase enzyme resulted in smaller particles, increased polydispersity and increased hydration water, with interparticle interactions similar to those for the native particles. Collectively, the SANS results presented in this thesis provide new, unique, fundamental information on the structure, hydration and interactions of native and chemically modified phytoglycogen nanoparticles, establishing a quantitative basis for the development of new applications of this promising sustainable nanotechnology.
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# TABLE OF CONTENTS

Abstract ................................................................................................................................. ii

Acknowledgements .............................................................................................................. iv

Table of Contents ............................................................................................................... v

List of Tables ..................................................................................................................... x

List of Figures ................................................................................................................... xi

Chapter 1 : Introduction and Background .......................................................................... 1

1.1 Dendrimers ...................................................................................................................... 1

1.1.1 Dendrimer Structure ................................................................................................. 1

1.1.2 Radial Density Profile of Dendrimers (Back folding) ............................................... 2

1.1.3 Applications of Dendrimers ..................................................................................... 4

1.2 Phytoglycogen ............................................................................................................... 6

1.2.1 The Dendrimeric Structure of Phytoglycogen .......................................................... 8

1.2.2 Small Angle Neutron Scattering Measurements Phytoglycogen ............................ 10

1.2.3 Quasielastic Neutron Scattering Measurements of Phytoglycogen ....................... 13

1.2.4 Rheology of Concentrated Phytoglycogen Dispersions .......................................... 15

1.2.5 Morphology Revealed by Atomic Force Microscopy Force Spectroscopy ............ 16

1.2.6 Water Structure in Phytoglycogen Nanoparticles .................................................. 18

1.2.7 Osmotic Pressure Experiments on Phytoglycogen Nanoparticles ......................... 19
1.2.8 Computer Simulations on Phytoglycogen .......................................................... 24
1.2.9 Coarse Grained Simulations ........................................................................... 25
1.2.10 Atomistic Simulations .................................................................................... 26
1.2.11 Applications of Phytoglycogen ...................................................................... 27
1.3 Chemical Modifications of Phytoglycogen and Other Polysaccharides ............. 28
1.4 Scope of Thesis ...................................................................................................... 31
1.5 Summary of Research Results ............................................................................. 32

Chapter 2 - Materials, Methods and Analysis ............................................................. 33

2.1 Material Production ............................................................................................. 33
2.1.1 Extraction and Purification of Phytoglycogen .................................................. 33
2.1.2 Preparation of Very Concentrated Phytoglycogen Samples ............................ 33
2.1.3 Acid Hydrolysis ............................................................................................... 35
2.1.4 Enzymatic Hydrolysis ..................................................................................... 36
2.1.5 Octenyl Succinic Anhydride Modification of Phytoglycogen ......................... 37
2.1.6 Determination of the Degree of Substitution .................................................. 40
2.1.7 Preparation of Aqueous Dispersions of Phytoglycogen of Native and Chemically Modified Phytoglycogen ................................................................. 40
2.2 Deuterated OSA Synthesis .................................................................................. 41
2.3 Small Angle Neutron Scattering ......................................................................... 44
2.3.1 Porod/Scattering Invariant and Contrast Matching ......................................... 45
2.3.2 Kratky Plots ........................................................................................................................................ 46
2.3.3 SANS Modeling of Polymers ............................................................................................................. 47
2.3.4 Different Models of the Form Factor P(q) ........................................................................................ 49
2.3.5 Polydispersity in SANS Models ......................................................................................................... 56
2.3.6 The Structure Factor S(q) ................................................................................................................ 57
2.4 Dynamic Light Scattering (DLS) ........................................................................................................... 63
2.5 Small Angle X-ray Scattering (SAXS) .................................................................................................. 65
2.6 Infrared Spectroscopy (IR) ................................................................................................................... 66
2.7 Preparation of Phytoglycogen Dispersions for SANS Measurements ................................................. 68

Chapter 3 - SANS Measurements of Aqueous Dispersions of Native Phytoglycogen Nanoparticles

3.1 Refinement of the Native Particle Form Factor ..................................................................................... 69
3.2 Kratky Plot ........................................................................................................................................... 71
3.3 Contrast Matching and Hydration ......................................................................................................... 72
3.4 Concentrated Native Dispersions ......................................................................................................... 77
3.5 SANS from Very Concentrated Phytoglycogen Samples ...................................................................... 79

Chapter 4 - SANS Measurements of Aqueous Dispersions of Octenyl Succinic Anhydride Modified Phytoglycogen Nanoparticles ............................................................................................... 81
4.1 Determination of the Match Point of pOSA ........................................................................................ 81
4.2 OSA Modified Phytoglycogen in Dilute Limit ...................................................................................... 82
4.3 Concentrated dispersions of OSA-modified phytoglycogen ......................................................... 89

4.4 Degree of Substitution .................................................................................................................... 92

Chapter 5 - Characterization of Phytoglycogen Nanoparticles Modified by Hydrolysis .......... 94

5.1 Dynamic Light Scattering on Hydrolyzed Phytoglycogen Nanoparticles ............................... 94

5.2 Rheology of Hydrolyzed Phytoglycogen ..................................................................................... 97

5.3 Contrast Matching of Hydrolyzed Phytoglycogen ..................................................................... 99

5.4 Fits to the SANS Curves for Dilute Dispersions of Hydrolyzed Phytoglycogen
Nanoparticles Using the Core-Chain Model ................................................................................. 102

5.5 Fits of the SANS Curves for Concentrated Dispersions of Hydrolyzed Phytoglycogen
Nanoparticles ...................................................................................................................................... 105

Chapter 6 - Preliminary Characterization of Cationically modified Phytoglycogen ............. 108

6.0 Cationic Phytoglycogen (GTAC-PG) ........................................................................................ 108

Chapter 7 - Summary and Future Work .......................................................................................... 113

7.1 Summary of Results ..................................................................................................................... 113

7.2 Future Work ................................................................................................................................. 114

7.2.1 RheoSANS .................................................................................................................................. 114

7.2.2 Tuning the Electrostatic Interaction Between OSA-Modified Phytoglycogen
Nanoparticles ...................................................................................................................................... 115

7.2.3 Succinic Anhydride Modification – No hydrophobic modification ..................................... 115

7.2.4 Concentrated Diffusion Measurements .................................................................................. 115
7.2.5 Additional Cationically Modified Phytoglycogen SANS Measurements ............... 116

References ...................................................................................................................................................... 117
LIST OF TABLES

TABLE 1: SUMMARY OF DLS RESULTS FOR HYDROLYZED STUDY ................................................................. 96

TABLE 2: SUMMARY THE SANS RESULTS FOR HYDROLYZED STUDY ............................................................. 104
LIST OF FIGURES

FIGURE 1-1: IDEALIZED DENDRIMER AS PREDICTED BY DE GENNES AND HERVERT ................................................. 2
FIGURE 1-2: SIMULATED IMAGES OF A NINTH-GENERATION DENDRIMER ................................................................. 4
FIGURE 1-3: DENDRIMER STRUCTURE OF PHYTOGLYCOGEN NANOPARTICLES ......................................................... 7
FIGURE 1-4: CHAIN LENGTH DISTRIBUTIONS OF PHYTOGLYCOGEN AND ACID TREATED PHYTOGLYCOGEN ........ 9
FIGURE 1-5: SANS DATA FOR PHYTOGLYCOGEN DISPERSIONS, ........................................................................... 11
FIGURE 1-6: NEUTRON CONTRAST SERIES DATA OF PHYTOGLYCOGEN ............................................................... 13
FIGURE 1-7: FITS TO THE QENS DATA, .................................................................................................................... 14
FIGURE 1-8: RELATIVE VISCOSITY OF PHYTOGLYCOGEN ......................................................................................... 16
FIGURE 1-9: AFM HEIGHT IMAGE OF A SINGLE PHYTOGLYCOGEN NANOPARTICLE ............................................. 17
FIGURE 1-10: DIFFERENCE BETWEEN ATR-IR ABSORPTION .................................................................................... 18
FIGURE 1-11: ATR-IR SPECTRA TACKING THE TIME EVOLUTION OF THE DEUTERIUM EXCHANGE ......................... 19
FIGURE 1-12 MASTER LOG-LINEAR PLOT OF OSMOTIC PRESSURE Π VERSUS CONCENTRATION C ............................ 21
FIGURE 1-14: BULK MODULUS Kp (SOLID SYMBOLS) AND INVERSE OF THE HYDRATION FORCE DECAY LENGTH λ .......... 24
FIGURE 1-15: CROSS SECTION OF A PHYTOGLYCOGEN NANOPARTICLE ............................................................. 26
FIGURE 1-16: RADIUS OF GYRATION Rg VERSUS THE NUMBER OF MONOMERS N IN AN AMYLOSE CHAIN .......... 27
FIGURE 1-17: SCHEMATIC DIAGRAMS OF THE MOLECULES USED TO MODIFY PHYTOGLYCOGEN ................... 29
FIGURE 2-1 PHOTOGRAPH OF THE PHYTOGLYCOGEN PELLET AFTER ULTRA-CENTRIFUGATION .......................... 34
FIGURE 2-2: THE MECHANISM OF ACID HYDROLYSIS .......................................................................................... 35
FIGURE 2-3: HYDRODYNAMIC RADIUS OF PHYTOGLYCOGEN AS MEASURED BY DYNAMIC LIGHT SCATTERING ...... 36
FIGURE 2-4: THE AMYLOGLUCOSIDASE MOLECULE .......................................................................................... 37
FIGURE 2-5: SCHEMATIC DIAGRAM OF THE ESTER LINKAGE ........................................................................ 38
FIGURE 2-6: SET UP FOR THE OSA MODIFICATION .......................................................................................... 39
FIGURE 2-7: REACTION EQUATIONS FOR DOSA (2-OCTENYLSUCCINIC ANHYDRIDE-D13) ..................................... 42
Chapter 1: Introduction and Background

1.1 Dendrimers

1.1.1 Dendrimer Structure

Dendrimers are highly branched molecules that have rich three-dimensional structures. These spherical molecules are typically symmetrical about their core which serves as the ‘root’ for linear polymeric chains to grow and in turn branch into several more chains, which in turn branch into more chains, suggesting in a structure that is shown schematically in Figure 1-1a and is alluded to by sharing etymology with the Greek work ‘dendron’ which means tree. The structure becomes easier to visualize if we consider how dendrimers are synthesized in the lab.

One method of dendrimer synthesis, known as divergent synthesis, highlights the iterative process that results in the dendrimer’s highly branched structure. Starting with a trifunctional core, known as generation zero (G0), the unit that makes up the next generation (chain or monomer) are added to each of the functional sites, forming the first generation (G1). Each chain in G1 has at least two functional sites that serve as branching points for linear chains that correspond to the second generation (G2) of the dendrimer.\(^1\)\(^-\)\(^3\) This growth process continues until it is artificially terminated or until the density of the chains is large enough that the addition of more chains is sterically blocked; there is no accessible volume for new monomers to occupy. This method of growth leads naturally to a maximum and uniform particle size and is referred to as self-limited growth.\(^3\)\(^,\)\(^4\)
1.1.2 Radial Density Profile of Dendrimers (Back folding)

Given the exponential growth of the number of monomers with generation, it was originally thought that the density of monomers increased exponentially with radial distance within the molecule, leading to a “hollow core – dense shell” picture (Figure 1-1a). This picture was demonstrated mathematically by de Gennes and Hervert and brought great excitement to the field of dendrimer research, because of the possibility of using the “hollow core” particles as a vehicle to deliver drugs. However, because they assumed \textit{a priori} that subsequent generations occurred at larger radial distances, this necessarily produced a hollow core radial profile. Subsequent computer simulations and calculations showed that this was not necessarily true because of the flexibility of the chains within the molecule. Computer simulations, refinements of self-consistent field theory calculations, together with small angle neutron...
scattering (SANS) experiments\textsuperscript{11,12} on high generation dendrimer molecules, have shown that
growth at higher generations involves the folding back of the flexible chains into the center of
the molecule, resulting in high molecular weight, monodisperse molecules that have a highly-
branched outer surface and a dense core (Figure 1-1b).

Back folding of the chains into the centre of the molecule at high generations was
observed in Monte Carlo simulations performed by Goddard\textsuperscript{13} and Likos & I.O Götze\textsuperscript{14} in which
they simulated polyamidoamine (PAMAM) dendrimers with generations 1 to 11 and tracked the
location of monomers in each generation. Likos\textsuperscript{1} has provided a colour-coded pictorial
representation of the distribution of monomers of different generations (Figure 1-2). This shows
clearly that the monomers of the highest generation are uniformly distributed throughout the
molecule.
Figure 1-2: Simulated images of a ninth-generation dendrimer. A) Cross-section though the centre of the dendrimer, revealing that higher order generation monomers are distributed uniformly throughout the volume of the dendrimer. B) Terminal groups (blue) decorate the surface of the dendrimer. Adapted from Likos1.

1.1.3 Applications of Dendrimers

The synthesis of dendrimers is more involved and complicated than the synthesis of linear or branched polymers, but the resulting architecture offers many advantageous properties. These properties include monodispersity, high symmetry, high molecular weight, control of the chemical and physical conditions of each generation, and high density of functional groups on the surface. Monodispersity is advantageous for sample purification, since it is easier to separate larger monodisperse particles from the smaller unreacted subunits. Monodispersity of the
molecules controls the functional groups per particle and improves the reproducibility of experimental results. There is some controversy about how polydispersity of commercially sold dendrimers is presented, choosing to present the polydispersity index of the dendrimer as it was before performing further chemistry and modifications. The ability to control the functional core G0, the intermediate generations, and the terminal generation of a dendrimer during synthesis is a powerful way to determine the structure of the dendrimer. Controlling the functionality of the core G0 influences the resulting structure, which can be either spherical or elliptical. Careful choice of the building blocks used in the interior generations govern the global properties of the dendrimer and the choice of the final or surface generation sets the functional groups that interact with the external environment or can be used for further chemical modification. Controlling the number of generations also has a profound effect on the mechanical properties. In the case of PAMAM dendrimers it was found that dendrimers of G4 or lower resulted in a soft particle with many voids, resembling what is known as a ‘starburst’ polymer, whereas dendrimers of generations 5 or greater were found to be more rigid and globular in nature.

The architecture of dendrimers serves as the basis for many of their applications. It was observed that, because the functional groups are close together, binding affinity of dendrimers for small molecules is greater than their monomeric constituents. One study has shown that the binding affinity of a sugar binding protein (concanavalin A) to dendrimeric mannose was 240x larger than that for monomeric mannose. The large size and molecular weight of dendrimers are also advantageous during chemical modification; moderate functionalization of dendrimers will not have a significant effect on the chemical properties of the dendrimers. This is particularly interesting for drug carrying applications where hydrophobic particles can be carried on hydrophilic dendrimers.
Most chemotherapeutic drugs are hydrophobic, which makes delivery via the bloodstream difficult. In addition, the kidneys would quickly remove these compounds. Covalent attachment of drugs to high molecular weight, hydrophilic dendrimers will result in a composite system that will be water soluble, if the degree of modification is not extreme. Drug carrying dendrimers are substantially larger than drug molecules and cannot pass the 5 nm particle size threshold that would lead to their filtering by the kidneys. Another consequence of the attachment of drug molecules to a large macromolecule is that tumorous cells have an increased uptake of large macromolecules, known as the enhanced permeation and retention (EPR) effect. The EPR effect reduces the severity of the side effects of chemotherapeutic drugs since they would normally act non-specifically on all cells.

It is possible to chemically modify the dendrimer so that the modified particle itself acts as a drug. Cationic agents are toxic to microbials which is of great interest with the rise of development of drug resistant bacteria. It was observed that polypropylenimine (PPI) dendrimers cationically modified with quaternary ammonium groups were able to kill microbes, with higher generations and higher degrees of modification being more effective. However, it was found that cationic dendrimers were also toxic to eukaryotic (human) cells. Improvements in the selectivity for killing microbial cells are necessary.

1.2 Phytoglycogen

Phytoglycogen is a naturally occurring glucose-based polymer produced as a highly branched, compact nanoparticle (44 nm in diameter) in certain plants such as sweet corn, due to a deficiency of the debranching enzyme isoamylase. The inhibition of debranching during the synthesis of amyllopectin results in a dense polymeric structure similar to glycogen, and this
similarity is reflected in the naming convention, with “phyto-“ meaning from plants, indicating that phytoglycogen is glycogen from plants. Glycogen and phytoglycogen consist of glucose monomers that form chains via $\alpha$-(1→4) glycosidic linkages and are regularly branched via $\alpha$-(1→6) linkages\textsuperscript{20–22} (Figure 1-3). Glucose monomers participating in the $\alpha$-(1→4) linkage are known as anhydroglucose units (AGUs) because the repeating monomeric unit is lacking a water molecule upon polymerization ($\text{C}_6\text{H}_{10}\text{O}_5$ versus $\text{C}_6\text{H}_{12}\text{O}_6$). This repeating cycle of chain growth and branching is indistinguishable from that in synthetic dendrimers.

**Figure 1-3:** Dendrimer structure of phytoglycogen nanoparticles composed of linear chains of anhydroglucose units (AGUs) bonded through $\alpha$-1,4 linkages with regular branching through $\alpha$-1,6 linkages. The chains are flexible enough to fold back towards the center of the particle, uniformly filling the volume of the entire particle.\textsuperscript{1} The inset shows the three liable hydrogens (-OH group bonded to C2, C3, and C6) that are available for deuterium exchange, as discussed in Figure 1-11.
Glycogen has been extensively studied and its basic structure\textsuperscript{4,23–26}, branching and debranching enzymes\textsuperscript{27–29}, and evolutionary significance are well understood\textsuperscript{30}. The body of knowledge of the physical properties of phytoglycogen is rapidly expanding, with recent studies on the structure, hydration, mechanical and flow properties,\textsuperscript{15–18} as well as enzymatic and chemical modification.\textsuperscript{31}

1.2.1 The Dendrimeric Structure of Phytoglycogen

The dense branching of chains in phytoglycogen is a dendrimeric architecture (Figure 1-3). As discussed in Section 1.1.1 Dendrimer Structure, there is a maximum generation number of a dendrimer before growth is terminated by steric restriction, corresponding to self limiting growth. To evaluate the maximum generation number of native phytoglycogen particles, it is necessary to determine the total number of AGUs in one particle and the average length of each linear chain. From small angle neutron scattering (Section 3.1 Refinement of the Native Particle Form Factor it was determined that there are $6.66 \times 10^4$ glucose monomers in a phytoglycogen particle.\textsuperscript{15,32} Chromatography measurements on native phytoglycogen and acid hydrolyzed phytoglycogen (Section 5.4 Fits to the SANS Curves for Dilute Dispersions of Hydrolyzed Phytoglycogen Nanoparticles Using the Core-Chain Model) allowed the measurement of the chain length distributions.\textsuperscript{33} Chain length distributions with peaks around 12 (similar to glycogen) and 18 were measured in Figure 1-4.
Figure 1-4: Chain length distributions of phytoglycogen and acid hydrolyzed phytoglycogen as measured by the Martinez lab using a Dionex and HPSEC-dRI instruments. Through careful hydrolysis they were able to disrupt the $\alpha$-1,6 linkages without cleaving the $\alpha$-1,4 linkages, thus providing a measure of the degree of polymerization (DP) of the chains. In both chromatography techniques, two DP centered on 12 and 18 AGU per chain were measured.

To estimate which generation phytoglycogen is we can use the following equation which calculates the number of monomers $N(g)$ in a dendrimers of generation $g$ with a functionality branch point $z$:\textsuperscript{9}

$$N(g) = 1 + \frac{z((z - 1)^g - 1)}{z - 2}$$

(1)

To adapt Equation 1 for phytoglycogen, $z = 3$, and we need to adjust our total number of AGUs to AGUs per unit added, $N(g) \rightarrow \frac{N(g)}{DP}$, because the equation was developed for adding one monomer per generation and we are considering adding a linear chain of several monomers. Thus, rearranging Equation 1 for $g$ for phytoglycogen becomes:
A DP of 12 and 18 both predict a generation number of around 10.8 and 10.3 respectively. This could suggest we have a 10th generation with excess chains on the surface as discussed in Section 3.1 Refinement of the Native Particle Form Factor. A 10th generation dendrimer is very large compared to the typical synthetic dendrimers discussed in Section 1.1.1 Dendrimer Structure.

1.2.2 Small Angle Neutron Scattering Measurements Phytoglycogen

In this section, we summarize our first small angle neutron scattering (SANS) study on phytoglycogen. Subsequent improvements in the methods that we use to purify phytoglycogen have led to refinements in the morphology of the particles discussed in Section 3.1 Refinement of the Native Particle Form Factor, but it is worthwhile to discuss our initial results here.

SANS measurements were performed on the Extended Q-range Small Angle Neutron Spectrometer (EQ-SANS) beamline at the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory (ORNL). The SANS curves collected on a dilute dispersion of phytoglycogen in D2O was consistent with uniform density spherical particles of radius 17.4 nm (Figure 1-5a). By increasing the concentration of the phytoglycogen dispersions, SANS measurements allowed us to probe the interparticle interactions and revealed that phytoglycogen nanoparticles behave as hard spheres. Furthermore, the onset of jamming was observed for dispersion concentrations \( C \gtrsim 20 \% \text{w/w} \) (Figure 1-5c).
Figure 1-5: SANS data for phytoglycogen dispersions. a) Scattered intensity versus scattering wavevector \( q \) as a function of phytoglycogen concentration. Intensity data were normalized by the concentration, \( C \). The data for the lowest concentration were fitted to obtain the radius and NSLD of the phytoglycogen nanoparticles (error reported is \( \sigma^2 \)). b) The emergence of a structure factor from interparticle scattering was used to determine the interparticle distance. c) The interparticle distance approached the nanoparticle diameter at high phytoglycogen concentrations. d) A schematic showing the particle diameter and interparticle spacing shown in c. Adapted from Nickels, 2016.
We also used contrast variation to vary the contrast between the solvent and the particles by varying the ratio of D\textsubscript{2}O to H\textsubscript{2}O. The effect of varying the D\textsubscript{2}O:H\textsubscript{2}O ratio on the SANS curves is shown in Figure 1-6a, in which the overall scattering intensity shows a minimum near D\textsubscript{2}O:H\textsubscript{2}O = 50\%. The contrast match point, for which the neutron scattering length densities of the particles and solvent are equal, is determined by calculating the Porod Invariant \(Q^*\):

\[
Q^* = \int_0^\infty q^2 I(q) dq
\]  (3)

and fitting the data to a quadratic curve to determine the D\textsubscript{2}O:H\textsubscript{2}O ratio corresponding to the minimum (Figure 1-6c). By determining the contrast match point, we were able to calculate the AGU monomer volume to be 166.8 Å\(^3\). This in turn allows us to compare the contrast match point with 100\% D\textsubscript{2}O condition to determine that the number of water molecules associated with each AGU in phytoglycogen (hydration number \(n_H\)) to be 22.5 ± 2.5.
Figure 1-6: Neutron contrast series data used to determine the NSLD of phytoglycogen. SANS data for two phytoglycogen concentrations: a) 12.9% w/w and b) 22.4% w/w, for different % D2O. (c) The total scattering, as indicated by the total scattering invariant $Q^*$, shows a clear minimum that is independent of concentration. (d) Contrast matching used to predict the NSLD of phytoglycogen as a function of the H$_2$O/D$_2$O ratio Adapted from Nickels, 2016.\(^{15}\)

1.2.3 Quasielastic Neutron Scattering Measurements of Phytoglycogen

Quasielastic neutron scattering allows the determination of the energy transfer ($\Delta E$) between incident neutrons and the sample, i.e., the energy gain or loss in the scattered neutrons.
The resulting dynamic structure factor \( S(q, E) \) can be used to quantify the dynamics and relaxations of different molecules within the sample. For dispersions of biopolymers in water, one could expect to see relaxations from the biopolymer chains and bulk water, with the water contribution due to both water strongly associated with the biopolymer chains (bound water) and surrounding bulk water. QENS measurements allow the quantification of these relaxations through the fitting of Lorentz curves to the data for different concentrations and temperatures of phytoglycogen dispersions (Figure 1-7 bc).

**Figure 1-7**: Fits to the QENS data. (a) A representative fit is shown for 24.4% w/w phytoglycogen in D_2O at 280 K for \( q = 1.3 \) Å\(^{-1}\). The fit required three Lorentzian functions, in addition to the instrumental resolution function, to account for the elastic scattering. The fwhm for each Lorentzian component is shown for data collected at (b) 280 K and (c) 295 K. The q-range is truncated for the phytoglycogen process, due to it leaving the dynamic window of the instrument (errors reported are 2\( \sigma \)). The data in panels b and c are shown for different phytoglycogen concentrations: 10.5% (top), 19.7% (middle) and 24.4% (bottom). In each plot, data is shown for bulk water (black), hydration water (red), and phytoglycogen (blue). In panels b and c, the dashed line corresponds to the best fit of the bulk water to a \( q^2 \) dependence. Adapted from Nickels, 2016.
The $S(q,E)$ curves in Figure 1-7 show that the bulk water has a $q^2$-dependent relaxation, as one would expect for freely diffusing molecules (black lines), a very slow diffusion from the phytoglycogen chains (blue), and an intermediate relaxation corresponding to bound water with reduced mobility (red). The bound water had a $q^{2.6}$-dependent relaxation, which reflects the sub-diffuse nature of the bound water. By computing the ratio of the integrated areas of the Lorentzian curves for bound water and total water in combination with the molar ratio of phytoglycogen to water allowed us to determine the total number of water molecules within each phytoglycogen nanoparticle. This provided an independent measure of the hydration number of phytoglycogen, $25.8 \pm 4.6$ water molecules per AGU, which agreed quite closely with the hydration number determined using SANS: $22.5 \pm 2.5$ water molecules per AGU.

1.2.4 Rheology of Concentrated Phytoglycogen Dispersions

Phytoglycogen nanoparticles are highly soluble in water. Rheology experiments performed in the Dutcher Lab showed that very large concentrations are required before significant increases in viscosity are observed (Figure 1-8).\textsuperscript{18} Dispersions with concentration $C$ up to 32% w/w were prepared, corresponding effective volume fraction $\phi_{\text{eff}}$ up to 1.13, which is notably larger than the maximum volume fraction of $\phi_{\text{HS}} = 0.58$ that can be achieved for hard spheres. This is a direct indication of the soft and compressible nature of phytoglycogen nanoparticles.
Figure 1-8: Relative viscosity of phytoglycogen (zero shear viscosity relative to that of water) as a function of phytoglycogen concentration C and effective volume fraction $\phi_{eff}$. The solid line was calculated using the best-fit parameters obtained by fitting the data points to the Vogel-Fulcher-Tannmann model of the glass transition. The dashed line corresponds to dispersions of hard spheres, and the deviation of the data from the dashed line is an indication of the soft nature of phytoglycogen nanoparticles. Adapted from Shamana, 2018. 18

1.2.5 Morphology Revealed by Atomic Force Microscopy Force Spectroscopy

Atomic force microscopy (AFM) is a powerful technique to measure the morphology and mechanical properties of nanoparticles. A protocol developed in the Dutcher Lab to covalently bond phytoglycogen nanoparticles to atomically flat gold substrates using an intermediary self-assembled monolayer of 4-mercaptophenylboronic acid (4-MPBA). This allowed AFM force spectroscopy to be used to collect dense arrays of force-distance curves. These data allowed the visualization of the phytoglycogen nanoparticle height for different applied forces, as well as the
determination and visualization of the Young’s modulus within each particle. In Figure 1-9a, we show an AFM image of an isolated phytoglycogen nanoparticle with a zero applied force (initial contact between the tip and the particle). In Figure 1-9b we show an AFM image of the same particle subjected to an applied force of 2 nN. Clearly, the application of this modest force results in a dramatic change to the morphology of the particle, revealing an inner branched structure. By accounting of the close proximity of the AFM tip to the underlying gold surface, the Young’s modulus $E$ of the hydrated phytoglycogen nanoparticles was determined, identifying a larger value for the inner region of the particles ($E = 596 \pm 16$ kPa) from a softer outer region of the particles ($E = 273 \pm 11$ kPa), with an average value of the Young’s modulus $\bar{E} = 690 \pm 20$ kPa.

**Figure 1-9:** a) AFM height image of a single phytoglycogen nanoparticle measured in Milli-Q water corresponding to the contact point between the AFM tip. The maximum height of the nanoparticle is 43.4 nm. (B) AFM height image of the nanoparticle in (A) measured in Milli-Q water corresponding to a force of 2 nN on the AFM cantilever. The maximum height of the nanoparticle is 7.4 nm. Adapted from Baylis, 2021 (in press).
1.2.6 Water Structure in Phytoglycogen Nanoparticles

Attenuated Total Reflection Infrared (ATR-IR) spectroscopy (IR) measurements of ultrathin films of phytoglycogen nanoparticles at different relative humidities allow the study of degree of ordering of the water within the particles. Measurements of the O-H stretching band revealed that the water within phytoglycogen nanoparticles was much more highly ordered than other polysaccharides (Figure 1-10).17

![Figure 1-10: Difference between ATR-IR absorption ΔA within the wavenumber range corresponding to the OH-stretching mode of water measured for phytoglycogen films at different relative humidities (RH) relative to that measured for the lowest value of RH for phytoglycogen (left), hyaluronic acid (middle) and chitosan (right). The large peak at 3200 cm⁻¹ for phytoglycogen corresponds to highly ordered water within the particles. Adapted from Grossutti, 2016.17](image)

The ratio of the absorbance at 3250 cm⁻¹ and 3400 cm⁻¹ can be used to define a parameter

\[ R_{\text{network}} = \frac{A_{3250}}{A_{3400}} \]

that quantified the degree of ordering of the water molecules within the phytoglycogen with larger values of \( R_{\text{network}} \) corresponding to a more ordered hydrogen bonded network.
ATR-IR was also used to determine the time scale for the exchange of H\textsubscript{2}O and D\textsubscript{2}O within the particles. Phytoglycogen films hydrated with H\textsubscript{2}O were exposed to D\textsubscript{2}O vapour and the spectra were collected as a function of exposure time Figure 1-11. It was observed that within 5 minutes most of the hydroxyl protons were exchanged, with complete exchange occurring after 25 hours. This demonstrates that the entire interior of phytoglycogen nanoparticle is accessible to the solvent.\textsuperscript{16}

### 1.2.7 Osmotic Pressure Experiments on Phytoglycogen Nanoparticles

**Dialysis**

An osmotic pressure $\Pi$ can be imposed on a dispersion of phytoglycogen nanoparticles by placing the dispersion into a sealed bag made of dialysis membrane. The sealed bag is placed
in a solution of osmotically stressing molecules such as polyethylene glycol (PEG) or dextran for which the concentration of the osmotically stressing molecules determines the magnitude of the osmotic pressure imposed on the dispersion within the sealed bag. The membrane pores are sufficiently small that the phytoglycogen nanoparticles and the osmotically stressing molecules cannot pass through - only water can pass through the membrane and it will flow until the chemical potential of the water is the same on both sides of the membrane. In this way, the externally imposed osmotic pressure $\Pi$ results in a change in the concentration $C$ of the phytoglycogen dispersion. This method was used in the Dutcher Lab to measure the dependence of the phytoglycogen dispersion concentration $C$ on osmotic pressure $\Pi$ for concentrations ranging from very dilute to $C \approx 100\%$ w/w. The results are the solid circles in Figure 1-12.

Ellipsometry

Phytoglycogen nanoparticles spincoated into ultrathin films experience an osmotic pressure or disjoining pressure $\Pi$ that acts across the film thickness. For these films that contain little water, phytoglycogen concentrations $C$ that range from 80% w/w up to 100% w/w can be achieved by varying the relative humidity (RH) above the film surface. A positive disjoining pressure $\Pi$ causes the film to swell and a negative disjoining pressure $\Pi$ causes a decrease in film thickness. $\Pi$ is determined by the RH:

$$\Pi = \frac{\mu}{V_m} = - \left( \frac{RT}{V_m} \right) \ln \left( \frac{p}{p_0} \right) = - \left( \frac{RT}{V_m} \right) \ln \left( \frac{RH}{100} \right)$$

(4)
where $R$ is the gas constant, $T$ is the absolute temperature, $\mu$ is the chemical potential, $V_m$ is the molar volume of water, $p$ is the partial pressure of water vapour, and $p_0$ is the equilibrium vapour pressure. These results are the open circles in Figure 1-12.

**Combined Osmotic Pressure Data**

The dialysis data was combined with the ellipsometry data, together with additional gravimetric analysis data, to characterize the dependence of $\Pi$ on concentration $C$ over the complete range of concentrations $0 < C < 100\% \text{ w/w}$ (Figure 1-12).[^34]

![Figure 1-12 Master log-linear plot of osmotic pressure $\Pi$ versus concentration $C$ for phytoglycogen nanoparticles (red symbols) collected using dialysis (solid circles), ellipsometry (open circles) and gravimetric analysis (half solid circles). The top axis shows the corresponding effective volume fraction $\phi_{eff}$ values. Data for dextran are also shown as black symbols, collected using dialysis (solid squares) and ellipsometry (open squares). Adapted from Grossutti, 2021.][^34]
Using the particle volume data obtained using SANS,\textsuperscript{15,32} these data were recast as $\Pi$ versus the volume $V_p$ of the particles, which allowed the calculation of the bulk modulus $K_p$ using the thermodynamic derivative

$$K_p = -V_p \frac{\partial \Pi}{\partial V_p} = -\Pi \frac{\partial \ln \Pi}{\partial \ln V_p}$$

(5)

and the plotting of $K_p$ versus the hydration water content of the phytoglycogen nanoparticles

(Figure 1-13).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure13.png}
\caption{Log-linear plot of the bulk modulus $K_p$ versus the hydration water content of the phytoglycogen nanoparticles, relative to that of fully hydrated particles. The solid curve was calculated based on the dependence of the osmotic pressure on particle volume; the dashed curve is the extrapolation of the solid curve to the fully hydrated particle. Adapted from Grossutti, 2021.\textsuperscript{34}}
\end{figure}
The curve shown in Figure 1-13 quantifies the relationship between the mechanical properties and the water content of the particles. For fully hydrated particles, we can compare the value of the bulk modulus $K_p = 200$ kPa determined from the osmotic pressure measurements with the value of the average Young’s modulus $\bar{E} = 690$ kPa determined from the AFM force spectroscopy measurements. Because the particles can be described by a zero Poisson’s ratio, the relationship between $K_p$ and $\bar{E}$ is particularly simple: $K_p = \bar{E}/3$. We can see that this relationship is quite closely satisfied by the results of the osmotic pressure and AFM experiments.

**Relationship Between Water Structure and Mechanical Modulus**

Recently, the Dutcher Lab has correlated infrared spectroscopy measurements of water structure with ellipsometry measurements of mechanical modulus. Increases in the order of the hydrogen bond network of the hydration water, as specified by the ATR-IR parameter $R_{\text{network}}$, led to linear increases in $K_p$ (Figure 1-14). In addition to the data for native phytoglycogen, data is also shown for additional materials: hyaluronic acid (HA), dextran and two modified versions of phytoglycogen (positively charged GTAC-PG and negatively charged CM-PG). It is interesting to note that the addition of charged polar groups, both cationic and anionic, to phytoglycogen significantly decreased its mechanical modulus, highlighting opportunities to tailor its properties for different applications through chemical modification.
Figure 1-14: Bulk modulus $K_p$ (solid symbols) and inverse of the hydration force decay length $\lambda$ (hollow symbols) versus $R_{\text{network}}$. The different symbols correspond to PG (blue); dextran (green); GTAC-PG, DS = 0.9 (violet); GTAC-PG, DS = 1.5 (black); CM-PG, DS = 0.9 (gold); and HA (red). The solid lines correspond to the best linear fits to each data set, with $r^2 > 0.995$.

1.2.8 Computer Simulations on Phytoglycogen

Hendrick de Haan’s group in the CNAB Lab at the Ontario Technology University have performed computer simulations of the growth, structure, and dynamics of phytoglycogen nanoparticles. Their work involves both coarse grained simulations of the growth of phytoglycogen nanoparticles and atomistic simulations of amylose chains and small portions of phytoglycogen nanoparticles.
1.2.9 Coarse Grained Simulations

The SANS results presented later in this thesis show that a phytoglycogen nanoparticle consists of 66 600 AGUs. This large number of AGUs, together with approximately 1.5 million associated water molecules, corresponds to approximately 5.8 million atoms within each phytoglycogen nanoparticle. This is very expensive to simulate and would take a large amount of time. Coarse graining is an effective way to reduce the simulation time as the 21 atoms for an AGU (C₆H₁₀O₅) and the 3 atoms for a water (H₂O) molecule are each represented as small spheres. In these simulations, the phytoglycogen particle is grown from a central seed, with linear chains allowed to grow by adding the small spheres until they reach a specified chain length and branching occurs. This process is repeated until the number of small spheres within the particles is the same as the number of AGUs measured in the small angle neutron scattering experiments (Figure 1-15). The magnitude of an attractive interaction parameter between the linear chains was adjusted so that the amount of bound water and the overall diameter of the particle was consistent with that measured using SANS (person communication, Nicole Drossis). A cross section of a simulated particle is shown in Figure 1-15, corresponding to an attractive interaction parameter of $\epsilon = 0.45 \ k_BT$. 


Figure 1-15: Cross section of a phytoglycogen nanoparticle obtained by performing a coarse-grained computer simulation. Simulations from Nicole Drossis and Hendrick de Haan (personal communication).

1.2.10 Atomistic Simulations

Atomistic simulations of single amylose chains and collections of amylose chains provide valuable information concerning chain structure, dynamics and interactions. Hendrick de Haan’s group at the Ontario Technology University have simulated amylose chains consisting of different number of monomers in a water solvent and this has allowed them to determine the radius of gyration $R_g$ of the chains (Figure 1-16). $R_g$ can be related to the Flory parameter as

$$R_g = R_0 N^\nu$$

By fitting the simulation results to Equation 6, a Flory exponent value of $\nu = 0.6239$ was obtained, which is consistent a polymer chain in a good solvent ($\nu = 3/5$). This value of the Flory parameter is used in the present study in the core-chain model discussed in Section 3.1 Refinement of the Native Particle Form Factor.
Figure 1-16: Radius of gyration $R_g$ versus the number of monomers $N$ in an amylose chain calculated from atomistic simulations. The red line was calculated using the best-fit power law exponent (Flory parameter) $\nu = 0.62$ obtained by fitting the data points to the relation $R_g \sim N^\nu$. The best-fit value of $\nu$ is consistent with the theoretical result for a good solvent ($\nu = 3/5$).

1.2.11 Applications of Phytoglycogen

The soft, porous nature of phytoglycogen nanoparticles, together with their biodegradability and non-toxicity, make them desirable for a broad range of personal care, nutraceutical and biomedical applications. Applications in personal care are being commercialized by Mirexus Biotechnologies Inc. (Mirexus has supplied the phytoglycogen nanoparticles used in the present study). For example, the slow release of sorbed water from phytoglycogen nanoparticles makes them attractive as a long-term moisturizing agent in cosmetics formulations. There are many promising applications of phytoglycogen in nutrition and biomedicine that can be unlocked through the knowledge gained in studying the morphology.
of the native and chemically modified phytoglycogen nanoparticles, as presented in this thesis. These applications include solubilizing hydrophobic molecules, e.g., bioactive molecules, masking undesirable flavours, and delivering drugs. To better exploit these potential applications and identify new applications, carbohydrate chemistry techniques can be used to chemically modify the particles, both by covalently attaching molecules to the surface of the particles and by hydrolyzing the particles using acids and enzymes.21,27,38,39

1.3 Chemical Modifications of Phytoglycogen and Other Polysaccharides

The performance of nanoparticles depend directly on their size, shape, and surface charge.40 This is especially true in applications related to drug delivery.41 Size is very important, with smaller nanoparticles being able to pass the blood brain barrier to and having an improved rate of cellular uptake. Thus, it is of interest to reduce the size and characterize the physical properties of the size-reduced phytoglycogen nanoparticles. In this thesis, acid hydrolysis and enzymatic degradation were used to achieve a reduction in the size of the particles.

We have also covalently attached small molecules to the surface of phytoglycogen nanoparticles that alters their functionality. Glucose-based polymers that have β-linkages, such as native cellulose, have limited functionality and applications due to their insolubility in water. Chemical modification of the hydroxyl rich repeating units can be performed to enhance the solubility and applications of these otherwise insoluble materials.40,42 In these modifications, the degree of substitution (DS), which corresponds to the fraction of the available chemical groups that have been modified, can be controlled. In contrast, phytoglycogen is readily soluble in water
and has the same number of hydroxyl groups per repeating unit as starch, which means that we can use the modifications that have been developed to functionalize starch in various ways to increase the range of applications of phytoglycogen. In this thesis, the structure, hydration, and interactions of phytoglycogen nanoparticles that have undergone esterification with octenyl succinic anhydride (OSA) and etherification with glycidyltrimethylammonium chloride (GTAC) are studied and discussed.

![Schematic diagrams of the molecules used to modify phytoglycogen.](image)

**Figure 1-17:** Schematic diagrams of the molecules used to modify phytoglycogen. a) Octenyl succinic anhydride (OSA), b) glycidyltrimethylammonium chloride (GTAC). c) The esterification of OSA with a glycogen repeat unit, conferring a negative charge and hydrophobicity to the phytoglycogen, and d) cationic etherification of GTAC with the glycogen repeat unit, conferring a positive charge.

The preparation of octenyl succinic anhydride (OSA) modified starches (so-called OS-starches) dates back to 1953 when a patent was filed using OS-starches to create emulsifiers using a procedure that is recognized as safe for food use (up to 3% addition). These OS-starches demonstrate many different physical properties because of their amphiphilic
properties. Gelation of 3% OS-starches (waxy maize) occurred at lower temperatures (63.9 °C vs 69.2 °C) with a smaller enthalpy of gelation (4.2 J/g less) because of a weakened hydrogen bonding network. OS-starches also have notably different pasting properties, forming higher viscosity pastes at lower temperatures compared to those formed using native starches. The swelling of OS-starches when exposed to water is also improved and is attributed to the bulky OS groups causing a weakening of the intramolecular hydrogen bonding, allowing more water to interact with the hydroxyl groups of the starch. This increased swelling and absorption of water results in OS-starch pastes that are more optically clear, which enhances consumer appeal. OS-starches take longer to digest than their native counter parts, with the OSA groups either sterically hindering enzymatic activity or creating a hydrophobic microenvironment, with the rate of digestion controlled by the DS value.

The tunable physical properties that are conferred through the addition of OSA to starches allow them to be used in a wide variety of food, pharmaceutical, and industrial products. The ability to emulsify mixtures and stabilize emulsions is important for dressings and creams. The amphipathic nature of OS-starches is critical for its role as an encapsulating agent for hydrophobic bioactive molecules within water-based dispersions. OS-starches have been used successful as an encapsulating agent for coenzyme Q10, conjugated linoleic acid, and salt encapsulation for enhanced saltiness. They show promise for use in functional foods, resisting digestion and replacing added fats that are used to emulsify the formulations, resulting in healthier products with lower fat content that can help to combat obesity and heart disease. OS-starches have also been used in paints, coatings, adhesives and biodegradable plastics.

Cationic modification of starch is also commonly used to increase the range of paper, textile, oil, and biomedical applications. Cationic starches demonstrate desirable
flocculating and absorption properties in applications such as harvesting biomass flocculants and waste water treatment to remove organic and inorganic materials. Several papers and US patents also show that the use of cationic modified starches results in increased mechanical strength of paper without the need for other fillers, which reduces the overall costs of raw fibers. In addition, cationically modified cellulose nanocrystals have been shown to enhance the binding-affinity to viruses.

1.4 Scope of Thesis

The goal of this thesis is to present a detailed characterization of the structure, hydration, and interactions of native phytoglycogen nanoparticles and chemically modified phytoglycogen. The primary tool that we have used is small angle neutron scattering (SANS). The results of this study have been crucial in providing structural and hydration data that is necessary to interpret physical property data collected using other techniques such as rheology, dialysis, ellipsometry, and atomic force microscopy.

In Chapter 2, I describe the extraction and purification of native phytoglycogen particles, chemical functionalization of the particles, and the theoretical background for the experimental techniques that are used in the present study. In Chapter 3, I discuss the results of SANS measurements on native phytoglycogen nanoparticles. In Chapter 4, I present the results of SANS measurements on phytoglycogen nanoparticles modified with octenyl succinic anhydride (OSA). In Chapter 5, I present the results of SANS measurements on phytoglycogen nanoparticles that have been hydrolyzed using acid or enzyme treatments. In Chapter 6, I present preliminary results of SANS measurements of cationic modified phytoglycogen nanoparticles.
Finally, in Chapter 7, I present a summary of the results and I provide suggestions for future experiments.

1.5 Summary of Research Results

The present study has allowed the unambiguous determination of the structure and morphology of the phytoglycogen nanoparticles as hairy nanoparticles with a core radius of 22 nm that is decorated by linear chains that are, on average, 20 AGUs long. The detailed understanding of the nature of the hairiness of the particles was possible through the study of OSA-modified particles. The hydrophobic collapse of highly-modified surface chains produced a distinctive high-\(q\) scattering feature in the SANS curve that we interpreted as being due to small, uniform “seeds” on the outer surface of the particles within the context of the raspberry model.

We have characterized the changes to the radius and hydration of phytoglycogen nanoparticles that have been subjected to acidic or enzymatic hydrolysis. We find that the radius is reduced for both hydrolysis methods at the expense of increasing the polydispersity, with the degree of hairiness enhanced for the acidic treatment.

A preliminary analysis of cationically modified phytoglycogen is also presented. The added charge can be adequately described by the screened Coulomb interaction between particles, but a complicated form factor needs to be developed in order to fully describe the SANS curves.
Chapter 2 - Materials, Methods and Analysis

2.1 Material Production

2.1.1 Extraction and Purification of Phytoglycogen

Frozen kernels of sweet corn were slowly thawed and then ground into a mush. The liquid component was collected by removing the solid components using a cheese cloth. The collected juice was subjected to physical separation techniques (centrifugation and ultrafiltration) to remove the remaining solid components, proteins, and lipids, yielding a water-based slurry containing only phytoglycogen nanoparticles. The particles were then precipitated in ethanol and dried overnight in a vacuum oven to yield dried “R&D grade” particles that were subsequently dispersed in either Milli-Q water (H$_2$O), D$_2$O, or mixtures of H$_2$O and D$_2$O to produce the dispersions used in the present study. Further details on the extraction and purification procedure are provided in Nickels et al.$^{15}$

2.1.2 Preparation of Very Concentrated Phytoglycogen Samples

To study the limit of very concentrated phytoglycogen dispersions, we used centrifugation to produce a phytoglycogen gel and glass. A 4.5% w/w dispersion of phytoglycogen was ultracentrifuged with a relative centrifugal force of 154071.6 x g for 1 hour, this resulted in a pellet of concentrated phytoglycogen pellet with a water supernatant (Figure 2-1a). The supernatant water was removed using a pipette and the pellet was carefully removed from the centrifuge tube using a scoopula, and the pellet was placed onto a quartz window of an aluminium SANS cell (Figure 2-1b). A second quartz window was placed on the cell to provide an airtight seal for the sample. A second concentrated phytoglycogen sample was lyophilized
after centrifugation and before mounting and sealing it in a second aluminium SANS cell to achieve the highest possible concentration. The samples were shipped to Oak Ridge National Laboratory and the SANS measurements were performed.

Figure 2-1 a) Photograph of the phytoglycogen pellet after ultra-centrifugation. A clear separation of the pellet (opalescent material on the lower left of the centrifuge tube) and the supernatant water can be seen. 
b) A picture of the centrifuged sample being loaded into the vessel, left shows the entire vessel before the second quartz windows is applied and right image shows the clear gel between the two quartz windows.
2.1.3 Acid Hydrolysis

![Chemical structure diagram]

**Figure 2-2:** The mechanism of acid hydrolysis. The excess hydronium from the low pH environment destabilizes the oxygen participating in the linkage (depicted here as the $\alpha$-1,4). A water molecule will then cleave this unstable charged intermediate, disrupting the linkage.

Dispersions of native phytoglycogen nanoparticles (20% w/w) were prepared by dispersing dried particles in Milli-Q water and the pH was adjusted to pH 2 by adding sulfuric acid (Figure 2-2). The solution was then heated to 90 °C and mixed for 2.5 hours with a magnetic stirring hot plate. At elevated temperature, the excess hydronium disrupts the oxygen participating in the glycosidic bonds, cleaving the $\alpha$-1,4-linkages and $\alpha$-1,6-linkages. The hydrolysis was terminated by cooling the dispersion to room temperature and neutralizing with 1M NaOH. The final product was then purified through dialysis against a 12-14 kDa MWCO filter and lyophilized. Aliquots were removed at regular time intervals during the reaction and the particle radius was measured using dynamic light scattering (DLS) to track the particle radius as a function of hydrolysis time (Figure 2-3).
Figure 2-3: Hydrodynamic radius of phytoglycogen as measured by dynamic light scattering as a function of time when exposed to pH 2 sulfuric acid at an elevated temperature of 90 °C.

2.1.4 Enzymatic Hydrolysis

Amyloglucosidase (AMG) was used to enzymatically hydrolyze the native phytoglycogen. AMG hydrolyzes the terminal α-1,4 glycosidic bonds and will hydrolyze α-1,6 bonds once it becomes a terminal AGU. 25 U (U ≡ enzyme unit with units μmol/min) per gram of PG was added to 20% w/w NPG dispersed in a 50 mM sodium acetate buffer at room temperature for 30 minutes until terminated by adding 1 M NaOH. The final product was then dialyzed against a 12-14 kDa MWCO filter and lyophilized.
Figure 2-4: a) The amyloglucosidase molecule with the active site highlighted in yellow (PDB ID: 1DOG) b) Schematic of the action of amyloglucosidase that works in a stepwise manner removing the terminal unit and releasing glucose into the solvent.

2.1.5 Octenyl Succinic Anhydride Modification of Phytoglycogen

Protiated 2-octenyl succinic anhydride (pOSA) was purchased from Vertellus (material # 104850, molecular weight of 210.1 g/mol). Deuterated OSA (dOSA) was synthesized using the procedure described in Section: 2.2 Deuterated OSA Synthesis (resulting in the structure shown in Figure 2-5b). The succinic anhydride headgroup forms a monoester with one of the three hydroxyl groups on one of the AGUs in phytoglycogen. The succinic anhydride will also react with water; to maximize the reaction efficiency, the succinic anhydride should not be previously dispersed in water. 56
To perform OSA-modification of phytoglycogen, 8 g of phytoglycogen was dispersed in 32 mL of water. The dispersion was homogenized using a T18 ULTRA-TURRAX homogenizer (IKA) equipped with a S18N-10G dispersing element at 11,000 rpm for the entire process (~2.5 hr) and held at 35°C. Either pOSA or dOSA was added (4000 μL, 240 μL, 800 μL, 247 μL for pOSA_HDS, pOSA_LDS, dOSA_MDS and dOSA_LDS, respectively, where DS is the degree of substitution, and HDS, MDS and LDS refer to high, medium and low degrees of substitution) at a controlled rate using a syringe pump (33 μL/min for pOSA_HDS, 10 μL for dOSA_MDS, and 3 μL/min for both pOSA_LDS and dOSA_LDS). The pH was maintained between 8-8.5 through the addition of 4 M NaOH. After adding the OSA, the reaction continued until the pH remained within the accepted range for at least half an hour without the addition of base to ensure completion of the reaction. The pH of the mixture was then adjusted to 5 by adding 1 M HCl, dialysed against a filter with a
MWCO of 12-14 kDa for three days with a total of 10 water changes, and then lyophilized. A photograph of the reaction set up is shown in Figure 2-6.

Figure 2-6: Set up for the OSA modification. The reaction mixture was continually homogenized to ensure thorough mixing of the water solvent and the water-insoluble octenyl succinic anhydride; without homogenization the two phases separated out. The water bath maintained the elevated temperature of the reaction mixture. OSA was added at a controlled rate using a syringe pump and NaOH was added by hand using pipettes to control the pH. With a larger setup it would be possible to use a burette. The reaction takes at least 2.5 hours.
2.1.6 Determination of the Degree of Substitution

The DS values of the resulting products, defined as the average number of OSA groups per anhydroglucose unit (AGU) within the phytoglycogen particles, were determined by combining SANS and infrared spectroscopy (IR) data using the following procedure. The value of the degree of substitution (DS) for the highly modified pOSA<sub>HDS</sub> sample was determined by the best fit to the SANS data. This determination was possible because of the observation of a distinctive, high-\(q\) scattering peak in the SANS data measured for this sample (Figure 4-2). The DS measured in this way for pOSA<sub>HDS</sub> was 0.1 OSA particles per AGU. Because a high-\(q\) scattering peak was not observed for samples with smaller DS values, we instead used infrared spectroscopy (IR) to determine the DS values relative to that for the pOSA<sub>HDS</sub> sample. At high pH, corresponding to fully deprotonating the carboxyl group on OSA, IR spectroscopy provided a measure of the relative amount of ester linkages that are formed with binding of OSA to phytoglycogen (Figure 4-6), and therefore the relative DS value. The DS values for the different samples were: 0.10 for pOSA<sub>HDS</sub>, 0.027 for dOSA<sub>MDS</sub>, and 0.010 for pOSA<sub>LDS</sub> and dOSA<sub>LDS</sub>. This analysis is discussed in Section 4.4 Degree of Substitution.

2.1.7 Preparation of Aqueous Dispersions of Phytoglycogen of Native and Chemically Modified Phytoglycogen

Phytoglycogen, OSA-modified phytoglycogen, and hydrolyzed phytoglycogen were dispersed in H<sub>2</sub>O/D<sub>2</sub>O mixtures at concentrations \(C\) ranging from 1% to 30% w/w using either a stir plate and stir bar for several hours until fully dissolved for lower concentrations (\(C < 15%\) w/w), or using a T18 digital ULTRA-TURRAX homogenizer (IKA) equipped with a S18N-10G...
dispersing element at 11,000 rpm for more concentrated samples \((C > 15\% \text{ w/w})\) for several minutes, repeating as necessary until homogenous. Samples were allowed to equilibrate for at least 1 h before the SANS experiments were performed. Sample concentrations are reported as a weight/weight percentage \((\% \text{ w/w})\), defined as the phytoglycogen mass divided by the total mass of phytoglycogen and solvent. Samples used for SANS studies, specified as a function of \% D\textsubscript{2}O, were formulated at the same concentrations in weight per volume for H\textsubscript{2}O and D\textsubscript{2}O and are reported as their equivalent \% w/w at 100\% D\textsubscript{2}O.

### 2.2 Deuterated OSA Synthesis

**Preparation of dOSA (2-octenylosuccinic anhydride-\text{d\textsubscript{13}})**

dOSA was prepared using deuterated components and the standard protocol for the preparation of pOSA.\textsuperscript{57} We used a highly deuterated 1-octene, which was prepared by the reaction of commercially available reagents vinyl magnesium bromide (Sigma-Aldrich) and 1-bromohexane-\text{d\textsubscript{13}} (CDN Isotopes). 1-Octene-\text{d\textsubscript{13}} was synthesized according to Figure 2-7 and the experimental protocol outlined below. The octane is an immediate synthetic precursor to dOSA, with deuteria occupying each alkyl carbon. 1-Octene-\text{d\textsubscript{13}} and maleic anhydride were heated together to promote the ene reaction which gives directly dOSA as a mixture of cis and trans (major) isomers. The deuteration sites are indicated below and are assigned based on the known concerted mechanism of the ene reaction\textsuperscript{58} and with support from the characterization data. Particularly, the high-resolution mass spectrum (TOF MS EI) reveals an accurate mass consistent with the C\textsubscript{12}H\textsubscript{5}D\textsubscript{13}O\textsubscript{3} molecular formula. The \textsuperscript{1}H NMR spectrum reveals resonances with chemical shifts suitable for H\textsuperscript{1}, H\textsuperscript{2}, H\textsuperscript{3}/H\textsuperscript{4} and vinylic resonance H\textsuperscript{5} (see below). The \textsuperscript{2}H NMR spectrum
offers resonances fully assignable to the molecule, including a lone vinylic resonance at 5.55 ppm and a 2.67 ppm resonance assigned to the deuterium on the carbon beside a carbonyl.

![Chemical reaction diagram](image)

**Figure 2-7:** Reaction equations outlining the synthetic preparation of dOSA (2-octenylsuccinic anhydride-d_{13}).

**Synthesis of 1-octene-d_{13}**

![Schematic diagram](image)

**Figure 2-8:** Schematic diagram of 1-octene-d13.

1-bromohexane-d_{13} (1.0 equiv) was added to a flame dried round bottom flask and the flask was then purged with argon. A 0.1 M solution of freshly prepared Li_{2}CuCl_{4} (0.04 equiv)^{59} in dry THF and dry Et_{2}O (0.5 M substrate concentration) were added to the flask and it was cooled to 0 °C in an ice-water bath. Vinylimagnesium bromide (1.2 equiv, 1.0 M in THF) was then added to the mixture. The reaction was monitored via GC and the reaction was quenched after an hour with H_{2}O. The mixture was extracted with ether (3 × 20 mL), and the combined organic layers were washed with brine (1 × 20 mL). The organic layer was dried using MgSO_{4} and filtered. The mixture was first distilled under vacuum at 50 Torr to remove solvent and product from the distillation pot. A second distillation was performed with heating at
atmospheric pressure to remove THF and Et₂O from 1-octene-d₁₃ (b.p. 121 °C at 760 Torr) (Figure 2-8). ¹H NMR (400 MHz, CDCl₃) δ 5.81 (dd, J = 10.2, 17.2 Hz, 1H), 4.99 (dd, J = 2.2, 17.1 Hz, 1H), 4.93 (dd, J = 2.2, 10.2 Hz, 1H).

**Synthesis of 2-octenylsuccinic anhydride-d₁₃ (dOSA)**

1-octene-d₁₃ (1.69 g, 13.5 mmol) and maleic anhydride (2.65 g, 27.0 mmol) were added to a pressure vessel. The mixture was stirred at 200 °C in a heated sand bath for 24 hours. The addition of 1:1 hexane:DCM precipitated a solid that was filtered off. The resulting organic filtrate was evaporated under reduced pressure and purified via Kuglerrohr short path distillation (b.p. 150 °C at 0.1 Torr) to afford dOSA as a colourless oil (1.09 g, 36% yield) (Figure 2-9). Cis and trans isomers:

¹H NMR (600 MHz, CDCl₃) δ 5.30 (t, J = 7.1 Hz, 1H), 2.90-2.87 (m, 1H), 2.54-2.50 (m, 1H), 2.46-2.42 (m, 1H), 2.25-2.22 (m, 1H). Cis and trans isomers: ²H NMR (92 MHz, CDCl₃) δ 5.55 (s, 1D), 2.67 (s, 1D), 1.95 (s, 2D), 1.26 (m, 6D), 0.83 (s, 3D). Trans isomer: ¹³C {¹H} NMR (101 MHz, CDCl₃) δ 180.74, 178.51, 124.93, 41.08, 34.50, 28.98. Cis isomer: ¹³C {¹H} NMR (101 MHz, CDCl₃) δ 180.80, 178.46, 124.33, 41.14, 34.70, 28.98. Trans isomer: ¹³C {¹H,²H} NMR (151 MHz, CDCl₃) δ 181.03, 178.82, 134.54, 124.91, 41.14, 34.51, 31.32, 29.92, 28.97, 27.74, 21.15, 12.84. Cis isomer: ¹³C {¹H,²H} NMR (151 MHz, CDCl₃) δ 181.08, 178.75, 133.40, 124.30, 41.20, 34.76, 31.32, 30.06, 27.93, 26.18, 21.20, 13.14. FTIR (cm⁻¹): 2924, 2856, 1861, 1784, 1710, 1439, 1419, 1294, 1235, 1051, 948, 920, 7210. HRMS (TOF MSEI): Calculated for C₁₂H₅D₁₃O₃ [M⁺] 223.2072. Found: 223.2079. MS (TOF MS EI), m/z: 223.21 (100, M⁺), 222.20 (50), 173.11 (65), 121.16 (25), 98.06 (30).
A sample of pOSA prepared in the same manner provided the following mass spectrometric data. HRMS (TOF MS EI): Calculated for C_{12}H_{18}O_3 [M]^+ 210.1256. Found: 210.1263. MS (TOF MS EI), m/z: 210.13 (100, M^+), 167.07 (25), 110.10 (35), 85.94 (30), 83.94 (45).

### 2.3 Small Angle Neutron Scattering

Neutron scattering is an ideal technique to study hydrated biopolymers. This is because of the isotopic sensitivity of the scattering of neutrons from hydrogen and its heavier isotope deuterium. Neutrons have isotopic sensitivity because the incident radiation is scattered from nuclear interactions. This is contrasted with X-rays and light which scatter from interactions with the electron cloud density, which is very insensitive to different isotopes of the same element. The nucleus’ cross section is significantly smaller than the electron cloud that envelops it, resulting in a weak scattering efficiency. In addition, flux of neutron sources is significantly smaller than X-ray sources, which results in very long collection times. This low scattering efficiency can be a boon in that it dramatically reduces the amount of secondary scattering that would otherwise complicate the analysis and allows the study of highly concentrated dispersions.

Small angle neutron scattering is a powerful tool to characterize the size and shape of particles in dispersions, as well as the interparticle interactions at higher concentrations. Furthermore, it is possible to determine the hydration of the glucose monomers in a phytoglycogen nanoparticle, which is difficult using other techniques.
2.3.1 Porod/Scattering Invariant and Contrast Matching

The Porod Invariant $Q^*$ is a model-independent quantity that was introduced in Section 1.2.2 Small Angle Neutron Scattering Measurements Phytoglycogen and can be calculated using Equation 3.

The Porod Invariant method is used to determine the relationship between the volume fraction and contrast of an incompressible system.\(^\text{62}\) We can also use $Q^*$ as a measure of the total scattering for different contrast conditions to determine the contrast match point, which is the D$_2$O:H$_2$O composition of the solvent for which the scattering between the particle and solvent is minimized. Because $Q^*$, Equation 3, is proportional to the scattering intensity $I(q)$ which then proportional to the square of the contrast (difference in scattering length density squared), the contrast match point corresponds to the minimum of $Q^*$ vs the solvent scattering length density. By calculating the scattering length $b_{AGU}$ from the chemical formula for anhydroglucose at the match point and measuring the scattering length density $\rho_{\text{match}}$ corresponding to the match point, the volume of the monomer $V_{AGU}$ can be determined:

$$V_{AGU} = \frac{b_{AGU}}{\rho_{\text{match}}} \quad (7)$$

It should be noted that typically extrapolations to $q = 0$ and extremely high-$q$ are required for solving Equation 3. In this work we did not extrapolate to these extreme regimes but instead limited the bounds of our integration. We did this because the Porod invariant in a volume integration of the data, represented as the area under the Kratky plot (2.3.2 Kratky Plots).
Our Kratky plot in Figure 3-2 shows a distinct bell that approaches zero in the limits of our data and thus are justified with the limits of our integral.

### 2.3.2 Kratky Plots

A Kratky plot represents the data as $q^2 * I(q)$ vs $q$ is useful for assessing the compactness of a system without modeling. This is directly related to the large-$q$ trend for compact spheres which exhibit $q^{-4}$ decrease versus a random chain which exhibits $q^{-2}$. The Kratky plot representation will show a bell-shaped curve for the compact sphere and a plateau for random chains. Normalization of the intensity by the zero angle scattering $I(q = 0)$ and the $q$ by the radius of gyration $R_g$ makes possible to assess partial unfolding and allow for the comparison between samples. Figure 2-10 shows the normalized Kratky plots for a globular protein (PolX, blue), less compact structures that consists of subunits linked together (P47, green and P67, red), random coil (XPC, grey), and a fully disordered protein (IB5, purple). The distinct shapes of the curves in Figure 2-10 provides a qualitative means to assess the particle’s shape to inform modeling choices.
Figure 2.3.3 SANS Modeling of Polymers

The SANS scattering intensity, $I(q)$, depends on the shape and interactions of particles dispersed in a solvent. Information about the particle shape and structure is described by the form factor amplitude $F(q)$, where the square of the absolute value is defined as the form factor $P(q)$:
\[ P(q) = |F(q)|^2 \]  

(8)

The scattering length density of the system, \( \rho(r) \), of the system contributes to form factor amplitude, as discussed in greater detail later in Section 2.3.4 Different Models of the Form Factor \( P(q) \). The scattering events that occur between two or more different particles are described by the structure factor \( S(q) \). For dispersions in liquid that do not have long order structure, which is the typical for crystals, the structure factor is related to the interparticle potential between particles, as discussed in Section 2.3.6. The scattering intensity \( I(q) \) can be written as:

\[ I(q) = |F(q)|^2 \ast S(q) + bkd \]  

(9)

where \( bkd \) is the incoherent (q-independent) background.

Using models to describe \( I(q) \) is an iterative process. One chooses a model to describe the system of interest and initializes the model parameters with \( a \ priori \) information. \( I(q) \) is calculated using these parameters which are evolved using the DREAM algorithm and the quality of the fit is assessed by computing the reduced chi-squared value.\(^6\) The DREAM algorithm is a sophisticated population-based differential routine included in the SASview package that is able to compute the parameter uncertainties.\(^6\)
2.3.4 Different Models of the Form Factor $P(q)$

The form factor $P(q)$ contains information about the particle size and shape. The form factor amplitude $F(q)$ is expressed generally as:\(^{61,67}\)

$$F(q) = \int \rho(r)e^{i(q \cdot r)} d^3r$$  \hfill (10)

where $\rho(r)$ is the radial density profile of the particle. Different models correspond to different choices of $\rho(r)$.

Uniform Density Sphere

A uniform sphere of radius $R$ has a volume $V = \frac{4}{3}\pi R^3$ and a uniform radial scattering length density profile such that:

$$\rho(r) = \begin{cases} 
\rho_{sphere} & \text{for } r \leq R \\
\rho_{solv} & \text{for } r > R 
\end{cases}$$  \hfill (11)

with the contrast defined as $\Delta \rho \equiv |\rho_{sphere} - \rho_{solv}|$. Starting from Equation 10 we limit the integral from all space to the volume $V$ of the particle:

$$F_{sphere}(q) = \Delta \rho \int_V e^{i(q \cdot r)} d^3r$$

where $\Delta \rho$ is the contrast between the sphere and the solvent, and $V$ is the volume of the particle.
\[ F_{\text{sphere}}(q) = \Delta \rho \int_{\phi=0}^{2\pi} \int_{\theta=0}^{\pi} \int_{r=0}^{R} e^{i(qr)} r^2 dr \sin \theta \, d\theta \, d\phi \]

which can be integrated to yield

\[ F_{\text{sphere}}(q) = 3 \Delta \rho V_{\text{sphere}} \frac{\sin(qR) - qR \cos(qR)}{(qR)^3} \]  

Thus, the form factor of a uniform sphere can be expressed as:

\[ P(q) = \frac{\phi}{V} \left[ 3V(\Delta \rho) \frac{\sin(qr) - qr \cos(qr)^2}{(qr)^3} \right]^2 \]  

Core-Chain Model

The core-chain model was developed to describe neutron scattering from uniform density, spherical particles with polymer chains grafted to the particle surface (Figure 2-11), and we use it to model the scattering from phytoglycogen nanoparticles with hairy chains on the particle surface. The scattering intensity \( I(q) \) for dilute dispersions (for which the structure factor \( S(q) \approx 1 \)) can be written as the sum of four contributions: (i) scattering from the particle core; (ii) scattering from the grafted polymer chains; (iii) correlations between the particle core and the grafted polymers; and (iv) correlations between the grafted polymers.\(^{69}\)
\[ I(q) = \phi \left[ \Delta \rho_c^2 F_c^2(q) + \Delta \rho_p^2 V_p^2 N_p P_p(q) + 2N_p \Delta \rho_c \Delta \rho_p F_c(q) E(q) F_p(q) + N_p(N_p - 1) \Delta \rho_p^2 F_p^2(q) E^2(q) \right] \]  

where \( \phi \) is the particle volume fraction, \( \Delta \rho_x \) is the difference between the scattering length densities of component \( x \) (core \( c \) or polymer \( p \)) and the solvent (\( \Delta \rho_x = |\rho_x - \rho_{solv}| \)), \( F_x(q) \) is the form factor amplitude of component \( x \), \( P_x(q) \) is the form factor of component \( x \), \( E(q) = j_0(qR) \) is a spherical Bessel function, \( V_p \) is the pervaded volume of the polymer, and \( N_p \) is the number of grafted polymers per particle.

The form factor amplitude \( F_c(q) \) of the spherical core is given by Equation 12. The form factor amplitude \( F_p(q) \) of the polymers is given by:

\[ F_p(q) = \frac{V_p}{2U^{2v}} \gamma \left( \frac{1}{2v}, U \right), \]  

and the corresponding form factor \( P_p(q) \) is given by:

\[ P_p(q) = \frac{1}{U^{2v}} \gamma \left( \frac{1}{2v}, U \right) - \frac{1}{U^{2v}} \gamma \left( \frac{1}{v}, U \right); \]  

where
\[ U = q^2 \frac{R_g^2(2\nu + 1)(2\nu + 2)}{6} \]  

and \( \gamma(x,y) \) is the lower incomplete gamma function. The polymer radius of gyration \( R_g \) is specified by

\[ R_g^2 = \frac{N^{2\nu}a^2}{(2\nu + 1)(2\nu + 2)} , \]

where \( a \) is the length of a monomer, \( N \) is the number of monomers per polymer and \( \nu \) is the Flory exponent.
Figure 2-11: Schematic for the Core-Chain (CC) model, highlighting the contributions of the four terms. The first term being correlations within the core, the second from correlations between the chain, the third resulting from correlations from the core and the chains, and the final interaction from correlations between chains. Adapted from Pokorski & Hore, 2019.69

The core-chain model was not included in the SasView library but was implemented through Sasview using a python script.

Raspberry Model

The raspberry model was developed to describe the neutron scattering intensity for a uniformly dense, spherical particle for which the particle surface is decorated with many smaller spherical structures or “seeds”, resulting in a structure that resembles a raspberry.70
The corresponding form-factor is the summation of four contributions: (i) the self-correlation of the core ($\Psi_c$), (ii) the self-correlation of the seed ($\Psi_s$), (iii) correlations between the core and the seeds ($S_{CS}$), and (iv) the correlations between the seeds ($S_{SS}$). The different contributions to the form factor are given by: \[ \Psi_c = \frac{3[\sin(qR_c) - qR_c \cos(qR_c)]}{(qR_c)^2} \] \[ \Psi_s = \frac{3[\sin(qR_s) - qR_s \cos(qR_s)]}{(qR_s)^2} \] \[ S_{CS} = \Psi_c \Psi_s \frac{\sin(q(R_c + R_s))}{q(R_c + R_s)} \] \[ S_{SS} = \Psi_s^2 \left[ \frac{\sin(q(R_L + R_s))}{q(R_L + R_s)} \right]^2 \] The contributions to the form factor in Equations 19-22 are shown in Figure 2-12.
Figure 2-12: Schematic depicting the different length scales in the raspberry model, the radius of the core and the radius of the seeds. There are four terms in this model, i) the spherical form factor of the core, ii) the spherical form factor of the seeds, iii) structure factor cross terms between the core and the seeds, and iv) the structure factor cross term between seeds. The seeds are on the surface.

We write the excess scattering length density between the core and the solvent as $\Delta \rho_C = |\rho_C - \rho_{Solw}|$, that between seeds and the solvent as $\Delta \rho_S = |\rho_S - \rho_{Solw}|$, and the total amount of scattering as $M = \Delta \rho_C V_C + N_S \Delta \rho_S V_S$, where $V_C$ is the volume of the core and $V_S$ is the volume of the seeds, so that we can write the form factor $P(q)$ as:

$$P(q) = \frac{1}{M^2} \left[ (\Delta \rho_C)^2 V_C^2 \Psi_C^2 + N_S (\Delta \rho_S)^2 V_S^2 \Psi_S^2 + N_S (1 - N_S) (\Delta \rho_S)^2 V_S^2 S_{SS} + 2 N_S \Delta \rho_C \Delta \rho_S V_C V_S S_{CS} \right] (23)$$
The number $N_s$ of seeds in the raspberry model can be calculated using the equation $N_s = \phi_S R_S^3 / \phi_C R_C^3$, where $\phi_S$ and $\phi_C$ are the volume fractions of the seeds and core, respectively, and $R_S$ and $R_C$ are the radius of the seed and core, respectively.

2.3.5 Polydispersity in SANS Models

Polydispersity was handed by the SasView software by assuming a particle size distribution $f(x, \bar{x}, \sigma)$ with a mean value $\bar{x}$ and width $\sigma$ and using it to weigh the form factor amplitude $F^2(q, x)$ in Equation 24.

$$P(q) = \frac{\text{scale}}{V} \int f(x; \bar{x}, \sigma)F^2(q, x)dx + bkrd$$ (24)

We used a Schultz distribution in our fitting routine, which is similar skewed towards larger values, like a lognormal distribution, but offers computational advantages. The Schultz can be described as:

$$f(x) = \frac{1}{\text{Norm}}(z + 1)^{z+1} \left(\frac{\bar{x}}{x}\right) e^{-\left(\frac{z+1}{\bar{x}}\right)x}$$ (25)

where
\[ z = 1 - \left( \frac{\sigma}{\bar{x}} \right)^2 \] (26)

which can then yield the final polydispersity PD as

\[ PD = \frac{\sigma}{\bar{x}} \] (27)

### 2.3.6 The Structure Factor \( S(q) \)

In concentrated dispersions, the effect of interparticle interactions can be observed in the measured SANS curves and this is modeled as the structure factor \( S(q) \) (Equation 9). In a similar fashion to the form factor in Equation 10, \( S(q) \) can be described by:

\[ S(q) = 1 + \int e^{iqr} g(r) dr \] (28)

where \( g(r) \) is the radial distribution function which describes the variation of the density varies in the vicinity of a particle, which indicates the probability of finding a particle some distance \( r \) away. In crystalline samples, sharp peaks in \( S(q) \) are observed, corresponding to long-range correlations from the periodic structure within the crystalline lattice. In contrast, for liquids, there are no long-range correlations, and therefore no sharp peaks.
The shape of $g(r)$ is very sensitive to the potential around the particle, which models the interparticle interactions. To determine $S(q)$, an expression for the radial distribution function $g(r)$ is required. The Ornstein-Zernike equation is a fluid dynamic equation that relates the pair correlation function $g(r)$ to the forces a molecule experiences from another molecule’s potential. The total correlation function (associated two-particle Ursell function) $h(r)$ is defined by:

$$h(r) = g(r) - 1$$  \hspace{1cm} (29)$$

Ornstein and Zernike then proposed that the total correlation $h(r)$ has contributions directly between particles, where some particle 1 acts on particle 2, and indirect contributions, where some particle 1 acts on particle 3 which influences particle 2. These contributions are denoted as $c(r_{12})$ and $c(r_{13})$ respectively. The indirect contribution is weighted by the number density of possible positions for the third particle. Thus, the total correlation function $h(r)$ can be written in the following form, known as the Ornstein-Zernike equation: 

$$h(r_{12}) = c(r_{12}) + \rho \int c(r_{13}) h(r_{32}) \, dr_3$$  \hspace{1cm} (30)$$

where the last term results from a convolution with the third particle. Defining $\hat{C}(k)$ and $\hat{H}(k)$ to be the Fourier transforms of $c(r)$ and $h(r)$ respectively, convolution theorem yields:
\[ \hat{c}(k) = \frac{\hat{H}(k)}{1 + \rho \hat{H}(k)} \]  

(31)

\[ \hat{H}(k) = \frac{\hat{c}(k)}{1 + \rho \hat{c}(k)} \]  

(32)

which can only be solved with a closure relation. Closure relations are independent equations that relate the total correlation \( h(r) \) and direct correlation \( c(r) \) such that the set equations are “complete” and unique solutions for \( h(r) \) and \( c(r) \), resulting in “closure”. We describe two structure factors and their closures that are relevant to the present study: the hard sphere structure factor and the Hayter-Penfold rescaled mean spherical approximation for charged particles.

Hard Sphere Structure Factor

For hard spheres dispersed in a solvent, there are no interactions between the particles except when they are in contact and the particles are not allowed to overlap. This can be represented mathematically by the potential:

\[ U(r) = \begin{cases} \infty & \text{for } r < 2R \\ 0 & \text{for } r \geq 2R \end{cases} \]

(33)

where \( r \) is the center-to-center distance between particles and \( R \) is the radius of each spherical particle. This potential results in an analytical solution to the Ornstein-Zernike equations.
(Equation 30) when used with the Percus-Yevick closure to determine the structure factor for hard sphere. The Percus-Yevick closure states that the radial distribution function \( g(r) \) is the sum of the direct \( c(r) \) and indirect radial distributions functions \( g_{\text{indirect}}(r) \):

\[
c(r) = g(r) - g_{\text{indirect}}(r)
\]  

(34)

It is then assumed that

\[
g(r) = e^{-\beta w(r)}
\]

\[
g_{\text{indirect}}(r) = e^{-\beta [w(r)-U(r)]}
\]  

(35)

where \( \beta = 1/k_B T \), \( w(r) \) is the potential of mean force and \( U(r) \) are the direct interactions between pairs of spheres. Thus, combining Equation 33 and Equation 34:

\[
c(r) = e^{-\beta w(r)} - e^{-\beta [w(r)-U(r)]}
\]  

(36)

which can be used in Equation 29. The resulting equation can be solved analytically as done by Stell.\(^2\) The resulting form for the structure factor is: \(^3\)
\[ S_{HS}(q) = \frac{1}{1 - \bar{N}C(q)} \]  

where

\[ \bar{N}C(q) =  
\begin{align*}
-24\phi & \left\{ \lambda_1 \left[ \frac{\sin(2Rq) - (2Rq)\cos(2Rq)}{(2Rq)^3} \right] \\
-6\phi\lambda_2 & \left[ \frac{(2Rq)^2\cos(2Rq) - (2Rq)\sin(2Rq) - 2\cos(2Rq) + 2}{(2Rq)^4} \right] \\
-\phi & \frac{\lambda_1}{2} \left[ \frac{(2Rq)^4\cos(2Rq) - 4(2Rq)^3\sin(2Rq) - 12(2Rq)^2\cos(2Rq) + 24(2Rq)\sin(2Rq) + 24\cos(2Rq) - 24}{(2Rq)^6} \right] 
\end{align*} \]

\[ \phi \] is the volume fraction, \( \lambda_1 = \frac{(1+2\phi)^2}{(1-\phi)^4} \) and \( \lambda_2 = \frac{-(1+\phi)^2}{(1-\phi)^4} \).

Hayter-Penfold Rescaled Mean Spherical Approximation

Including charges on the particles adds complexity to the system as the charge of the particles, the counter ions in the solvent, and the dielectric properties of the solvent must be accounted for using a screened Coulomb interaction. The screened Coulomb interaction, also known as the Yukawa potential, can be written as
\[ U(r) = \pi \epsilon_0 \epsilon (2R)^2 \psi_0^2 \frac{\exp \left(-\kappa (r - 2R)\right)}{r} \text{ for } r > 2R \]  \hspace{1cm} (39)

where \( \epsilon_0 \) is the permittivity of free space, \( \epsilon \) is the dielectric of the solvent, \( 2R \) is the particle diameter, \( \kappa^{-1} \) is the Debye length, and \( \psi_0 \) is the surface potential for a macroion of charge \( z \):

\[ \psi_0 = \frac{z}{\pi \epsilon_0 \epsilon (2 + \kappa 2R)} \]  \hspace{1cm} (40)

Typically, one defines dimensionless parameters: \( x = \frac{r}{D} \), \( k = \kappa 2R \), and \( K = q 2R \). Instead of using the Percus-Yevick closure (Equation 34), the Rescaled Mean Spherical Approximation closure is used in the Ornstein-Zernike equation (Equation 30) with the conditions:

\[ c(r) = \beta U(r) \text{ for } r > 2R \]

\[ h(r) = -1 \text{ for } r < 2R \]

The solution has the same form as Equation 3 with:
\[
\frac{\bar{N}\mathcal{C}(q)}{24\phi} = \frac{A(sin(K) - Kcos(K))}{K^3} + \frac{B\left(\frac{2}{K^2} - 1\right)Kcos(K) + 2 \sin(K) - \frac{2}{K}}{K^3} \\
+ \frac{\phi A \left(\frac{24}{K^3} + 4 \left(1 - \frac{6}{K^2}\right)sin(K) - \left(1 - \frac{12}{K^2} + \frac{24}{K^4}\right)Kcos(K)\right)}{2K^3} \\
+ \frac{C(k \cosh(k) \sin(K) - K \sinh(k) \cos(K))}{K(K^2 + k^2)} + \frac{F(k \sinh(k) \sin(K) - K(\cosh(k) \cos(K) - 1))}{K(K^2 + k^2)} \\
+ \frac{F(cos(K) - 1)}{K^2} - \frac{\beta \pi \varepsilon_0 \varepsilon \Psi_0(k \sin(K) + K \cos(K))}{K(K^2 + k^2)}
\]  

(41)

The constants A, B, C and F are polynomial expressions that are provided in the original publication from Hayter-Penfold.\textsuperscript{73,74}

2.4 Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) is a powerful tool to measure the hydrodynamic radius \(r_H\) of a particle dispersed in a liquid. Light is scattered from particles that have an index of refraction that differs from that of the liquid. A particle that diffuses through the scattering volume causes fluctuations in the measured intensity that occur on a time scale for which particles move a distance \(\Delta x\) comparable to the wavelength \(\lambda\) of the light. For Brownian motion of the particles, \((\Delta x)^2 = Dt\), where \(D\) is the diffusion coefficient. The scattering intensity at \(t = 0\) can be compared with that at later times by calculating an autocorrelation function (ACF). The ACF can be thought of how similar an observation is to itself after some time has elapsed, which for the Brownian motion of the particles decays with a constant rate \(\Gamma\). The decay rate \(\Gamma\) of the ACF is directly related to the diffusion coefficient \(D\) via
\[ \Gamma = q^2 D \tag{42} \]

such that large particles that diffuse slower will decay at a slower rate as more energy is required to move them.

The hydrodynamic radius \( R_H \) of a monomodal distributions of particles can be determined from the diffusion coefficient \( D \) of the particles. For spherical particles, this is determined by the Stokes-Einstein relation\(^75\):

\[ R_H = \frac{k_B T}{6\pi \eta D} \tag{43} \]

where \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature, and \( \eta \) is the solvent viscosity.

Experimentally, one measures \( \Gamma \) from the ACF, then calculates \( D \) from Equation 42, and then calculates \( R_H \) using Equation 43.

The intensity \( I \) of the light scattered from small particles scales as the particle radius to the sixth power \( (I \propto r^6)\).\(^76\) Although the intensity-weighted particle radius distributions are most reliably determined from DLS experiments,\(^76\) polydispersity of the particle radius results in the skewing of the intensity-weighted particle radius distribution to larger particle radius values. It is also possible to convert the measured intensity-weighted particle radius distribution to a number-average particle radius distribution, which can be compared to particle radius values measured
directly using SANS or microscopy techniques such as atomic force microscopy and
transmission electron microscopy.

DLS measurements were performed on two different instruments: a Malvern Zetasizer
Nano ZSP spectrometer and a Brookhaven BI-200SM spectrometer. The ZSP spectrometer uses
a large static angle ($\theta = 173^\circ$) to measure the sample in polystyrene cuvettes, whereas the BI-
200SM allows variable scattering angles (and therefore different scattering wavevectors $q$) to
measure the sample in glass scintillation vials in a decahydronaphthalene bath.

### 2.5 Small Angle X-ray Scattering (SAXS)

X-rays are scattered from the electron cloud density of an atom instead of the nucleus as
in neutron scattering, such that the two techniques provide complementary information. X-rays
have more energy than neutrons, which can damage biological samples. During SAXS
experiments, it is important to monitor for any degradation of the material by tracking any
changes in the intensity spectra as a function of time. We used the in-house Anton Parr SAXSess
mc² SAXS instrument at ORNL to perform SAXS measurements on some of the samples that
were measured using SANS. The limited scattering wavevector ($q$-range) (0.01-0.68 Å) was
designed for the study of small proteins and was not ideal for the larger phytoglycogen
nanoparticles. For comparison, SANS measurements extended another order of magnitude in the
low-$q$ range. This prevented a detailed analysis of the SAXS spectra but it provided some useful
complementary information.

The larger $q$ values probed in the SAXS experiments provided additional insight into the
smaller length scale features observed in the SANS curves for chemically modified
phytoglycogen nanoparticles. However, low-\(q\) values, comparable to that measured using SANS, could not be accessed in the SAXS experiments, so that a detailed interpretation of the SAXS results was not possible and fitting of the SAXS was not performed. We did not observe degradation of the phytoglycogen samples due to the X-ray beam during the SAXS experiments.

2.6 Infrared Spectroscopy (IR)

The bonds in a molecule oscillate, twist, and bend at infrared frequencies. Infrared (IR) spectroscopy is used to measure the absorption of the IR radiation by molecular bonds to infer information about the functional groups that are present and their interactions with the solvent. IR is well-suited to the characterization of water structure in polysaccharides. The O-H stretching mode of water is sensitive to hydrogen bonding and therefore the environment of hydrated biopolymers. For the case of two water molecules, hydrogen bonding between the molecules decreases the strength of the covalent O-H bonding within each molecule, depending on the angle of the hydrogen bond that is formed. Because of this, the O-H stretching mode can be used to quantify the ordering of the water network: an increase (decrease) in the number, linearity, or strength of the of the hydrogen bonding network results in a decrease (increase) in the frequency of the O-H IR absorption band. The effect of the hydrogen bond network on the O-H stretching region of an IR spectrum is shown schematically in Figure 2-13 for an attenuated total reflection infrared (ATR-IR) spectrum of the O-H stretching region.
Figure 2-13: IR spectrum of O-H stretching for bulk water at ~25 °C with arrows indicating network and multimer water subpopulations.

In ATR-IR spectra of polysaccharide films, O-H modes are observed within the wavenumber range 3600-3000 cm\(^{-1}\) and C-H stretching modes occur within the range 3000-2800 cm\(^{-1}\).\(^{17}\) In the case of chemically modified materials, such as OSA, an ester linkage is formed and this contributes a carbonyl signal at ~1720 cm\(^{-1}\). When OSA is complexed to phytoglycogen, there is a carbonyl group exposed to the solvent that is pH dependent. When the solvent exposed carbonyl group is protonated (low pH), it contributes to the 1720 cm\(^{-1}\) band, whereas when it is deprotonated (high pH), it contributes to the ~1575 cm\(^{-1}\) band.
Succinic acids have two carbonyl groups with dissociation constants at $pK_{a,1} \approx 4.2$ & $pK_{a,2} \approx 5.5$.\textsuperscript{77-79} The mixed protonation states of the succinic acid allow one to determine the degree of substitution (DS) of the modified materials, as discussed in Section 4.4 Degree of Substitution.

### 2.7 Preparation of Phytoglycogen Dispersions for SANS Measurements

SANS measurements were performed on phytoglycogen dispersions with different concentrations and H$_2$O/D$_2$O ratios at the Extended Q-Range Small Angle Neutron Spectrometer (EQ-SANS)\textsuperscript{80}, which is located at the Spallation Neutron Source (SNS) [Oak Ridge National Laboratory (ORNL)]. Samples were loaded in 2 cm diameter, 1 mm thick circular quartz cells (Hellma), which were then placed in a temperature regulated 15-position sample holder. Data was collected using three instrumental configurations: 1.3 m sample-to-detector distance, using 5.5-9 Å wavelength neutrons; 4.0 m sample-to-detector distance using 10-13.4 Å wavelength neutrons; and 8.0 m sample-to-detector distance using 12.5-15 Å wavelength neutrons. These three configurations yielded a total scattering wavevector range (q-range) of $0.002 < q < 0.30$ Å\textsuperscript{-1}. The scattered neutrons that were captured by the detectors resulted in two-dimensional scattering intensity data that was reduced using the Mantid software environment and normalized to a Porasil standard to establish an absolute scale \textsuperscript{81}. Data was corrected for pixel sensitivity, dark current, and sample transmission. For the resulting one-dimensional scattered intensity curves of I(q) vs q, the background was subtracted based on the empty can and solvent conditions.
Chapter 3 - SANS Measurements of Aqueous Dispersions of Native Phytoglycogen Nanoparticles

3.1 Refinement of the Native Particle Form Factor

The SANS data measured for a dilute (1% w/w) dispersion of high purity, native phytoglycogen particles in D$_2$O is shown in Figure 3-1. The data has a small but distinctive dip at an intermediate $q$-value ($\sim 0.015$ Å$^{-1}$; indicated by blue arrow in Figure 3-1a), which corresponds to the first fringe expected for scattering from monodisperse particles.$^{62}$ This feature allowed us to accurately determine the particle radius $r$. By fitting the SANS data to a uniform sphere model, shown as the grey curve in Figure 3-1a, we obtained a best-fit value of $r = 220$ Å with an associated Schultz polydispersity index PDI = 0.26. The best-fit to the data using a uniform sphere model fits the data quite well, but it does not explain the presence of enhanced scattering at high-$q$ values. We obtained a significant improvement to the fit to the native particle SANS scattering data at large $q$-values ($0.05 - 0.09$ Å$^{-1}$) by allowing for the presence of short, hairy chains on the surface of the particles (core-chain model described in Section 2.3.4 Different Models of the Form Factor P(q)).$^{68}$ This result is consistent with rheological measurements of concentrated phytoglycogen dispersions that showed an additional relaxation process that was attributed to the presence of hairy chains,$^{18}$ as was also observed for star polymers.$^{82}$ The best-fit to the SANS data (red curve in Figure 3-1b) was obtained using a core particle radius of $r = 210$ Å and assuming the same value of PDI = 0.26 as used for the fit to the uniform sphere model.

For the hairy chains on the surface of the phytoglycogen particles, we initially assumed that they were the same average length as between branch points within the hyperbranched particles, corresponding to $N_c = 12$ AGUs, which yielded a best-fit packing density of the hairy chains of
\( \sigma = 0.0020 \pm 0.0001 \text{ chains/Å}^2 \). We note that the best-fit value of \( \sigma \) was highly correlated with the choice of \( N_c \), with similar excellent fits to the SANS curve obtained for different combinations of \( N_c \) and \( \sigma \), i.e. longer (shorter) chains separated by larger (smaller) distances gave equivalent fits. Despite the strong correlation between the values of \( N_c \) and \( \sigma \), the ratio \( \beta \) of the total number of AGUs in the hairy chains to that in the particle core was insensitive to the choice of \( N_c \): \( \beta = 0.26 \pm 0.01 \) for \( 12 < N_c < 24 \). We thus refined our choice of \( N_c \) through our analysis of the SANS results for OSA-modified particles presented in 4.2 OSA Modified Phytoglycogen in Dilute Limit. This allowed us to obtain a reliable estimate of \( N_c = 20 \), corresponding to \( \sigma = 0.00125 \pm 0.00008 \text{ chains/Å}^2 \), with values of the radius of gyration \( R_g = 15 \text{ Å} \) and a Flory exponent of \( \nu = 0.5 \) that were extrapolated from the results of previous computer simulations of amylose chains in water.\(^{83-85}\) The resulting best-fit curve to the SANS data is shown in Figure 3-1b.

![Figure 3-1](image_url)

**Figure 3-1:** SANS scattered intensity \( I \) versus scattering wavevector \( q \) for 1% w/w native phytoglycogen dispersed in D\(_2\)O. (a) The grey curve corresponds to the best-fit to the data using the uniform sphere model with the following best-fit values: nanoparticle radius \( r = 223 \text{ Å} \), polydispersity index \( PDI = 0.26 \), and NSLD = 0.586 fm/Å\(^3\). (b) The red curve corresponds to the best-fit to the data using a core-chain model\(^{68}\) with the following best-fit values: nanoparticle radius \( r = 212 \text{ Å} \), polydispersity index \( PDI = 0.26 \), and
hairy chains with $N_c = 20$ AGUs and a packing density of 0.00125 chains/Å$^2$. In both (a) and (b), the straight blue lines and blue arrows are used to highlight the small dip in the data at intermediate $q$ values.

The best-fit value of the phytoglycogen nanoparticle core radius of $r = 210$ Å is considerably larger than that determined in our previous SANS study.$^{15}$ We note that the presence of the small dip in the SANS data in the present study, indicated by the blue arrow in Figure 3-1, allowed us to eliminate ambiguity in the joint determination of the particle radius and PDI values and obtain accurate values for each parameter. We note that the best-fit value of $r$ for the core-chain model is slightly less than that obtained by fitting the data to the uniform sphere model, which is consistent with the presence of short chains on the surface of the particles.

3.2 Kratky Plot

Kratky analysis is a model independent analysis used to qualitatively probe the shape and flexibility of the sample. Kratky plots are common in protein studies to evaluate the degree of folding of a protein; a well-folded protein is globular/spherical and has a bell-shaped Kratky plot, whereas an unfolded protein has a Kratky plot that monotonically increases with increasing $qR_g$, a mix of these two confirmations will have a superposition of these shapes (see Figure 3-2b). The shape of the Kratky plots in Figure 3-2a, in which $(qR_g)^2 I(q)/I(q = 0)$ versus $qR_g$ indicate that native phytoglycogen nanoparticles and phytoglycogen nanoparticles modified with OSA are compact and spherical in shape, which is consistent with our choice of the core-chain and raspberry model to fit the measured SANS curves.$^{69}$
Figure 3-2: Kratky plot for a) Native and pOSA_{HDS} samples and b) a representative schematic of different shapes. The bell-shape of the plot exhibited in (a) indicates that native phytoglycogen and pOSA_{HDS} are spherical in nature, supporting the form factor chosen for fitting (core-chain and raspberry). Similar to the Kratky plot for 7th generation dendrimer. c) Fifth generation (G5) polyamidoamine (PAMAM) dendrimers, triangles are experimentally measured highest contrast and squares are calculated with the maximum contrast. The dashed line is the calculations for a homogenous sphere. NPG is more like the uniform sphere than the G5 PAMAM, this reflects the higher number of generations resulting in a more defined surface.

3.3 Contrast Matching and Hydration

We also determined the hydration of the phytoglycogen nanoparticles from SANS experiments, as in our previous study. Because hydrogen isotopes scatter differently in neutron
scattering experiments, we performed SANS experiments at different H$_2$O/D$_2$O ratios, commonly referred to as a contrast series (Figure 3-3). The minimum of the curve shown in Figure 3-3b occurs at 51.2% D$_2$O ($\rho = 0.296$ fm/Å$^3$, scattering length of 47.5 fm), corresponding to equal neutron scattering lengths for the solvent and phytoglycogen (contrast match point). This result is consistent with the results of our previous measurements,$^{15}$ for which the contrast match point was 48.8% D$_2$O. We determined the number of water molecules $n_H$ associated with each AGU in the phytoglycogen particle (hydration number). Briefly, we assumed three exchangeable hydrogens per AGU$^{87}$ and used the D$_2$O concentration at the contrast match point to calculate the corresponding stoichiometry of the AGU, C$_6$H$_{8.46}$D$_{1.54}$O$_5$, and its volume of 160.5 Å$^3$. We then calculated the value of $n_H$ needed to increase the NSLD value from that measured at the contrast match point at 51.2% D$_2$O (0.296 fm/Å$^3$) to that measured for 100% D$_2$O (0.586 fm/Å$^3$). The NSLD of the phytoglycogen particle in pure D$_2$O (Figure 3-3c) can be written as:

$$\text{NSLD}_{100\%D_2O} = \frac{b_{\text{phytoglycogen}} + b_{D_2O}n_H}{v_{\text{phytoglycogen}} + v_{D_2O}n_H}$$

(44)

where the scattering length of D$_2$O is $b_{D_2O} = 19.15$ fm, the volume of D$_2$O is $v_{D_2O} = 29.96$ Å$^3$, and the scattering length of glucose is $b_{\text{phytoglycogen}} = 62.7$ fm, corresponding to three exchangeable protons on each AGU. Rearranging Equation 4 to solve for $n_H$, we obtain $n_H = 20 \pm 2$ water molecules per AGU, which is slightly less than the value of 22.5 determined previously.$^{15}$ This value of $n_H$ corresponds to an effective volume fraction $\phi_{\text{eff}} = 0.032$ for the 1% w/w
dispersion that is consistent with the results of rheology measurements on dilute dispersions of phytoglycogen nanoparticles.\textsuperscript{32}

As in our previous study,\textsuperscript{15} the SANS data can also be used to estimate the molecular weight of the phytoglycogen nanoparticles. We calculated the number $N$ of AGUs in the core of a nanoparticle by dividing the nanoparticle volume $V_p = 3.99 \times 10^{-23}$ m$^3$ (calculated using the best-fit particle radius of $r = 210$ Å) by the average volume per AGU (including the associated water) $v_m = 7.60 \times 10^{-28}$ m$^3$, which yields a value of $N = 5.25 \times 10^4$ AGUs within the core of the nanoparticle. Using the values of $n_H$ and $N$, we calculate the total number of water molecules within the core of a phytoglycogen nanoparticle as $N_H = 1.05 \times 10^6$. By considering the $1.41 \times 10^4$ AGUs in the hairy chains, calculated from the surface density of the chains and the surface area of the core, there are a total of $6.66 \times 10^4$ AGUs in one phytoglycogen nanoparticle. We note that the hairy chains on the surface of the nanoparticles contain a significant percentage (21.2\%) of the total number of AGUs in the nanoparticles. Using the molecular weight for anhydroglucose (162 g/mol), a “dry” molecular weight of $1.08 \times 10^7$ g/mol is obtained. By including the mass of the hydration water within each nanoparticle and assuming that the hairy chains on the outside of the particles have the same amount of associated water as the chains within the core of the particles, we obtain a molecular weight of $3.48 \times 10^7$ g/mol for fully hydrated phytoglycogen nanoparticles, with each nanoparticle containing 220\% of its mass in water. We note that we used a number of assumptions in this analysis, such as assuming that the volume of hydration water is unchanged from that of bulk water, that water properties do not change substantially with deuteration, and that all of the water inside the nanoparticles is hydration water.

Our new, more precise determination of the phytoglycogen nanoparticle radius and morphology, with a core radius of 212 Å and short hairy chains on the surface of the particle,
together with the best-fit values of the NSLD and number $n_H$ of hydration water molecules, results in dry and hydrated molecular weight values for the phytoglycogen nanoparticles that are ~ 2.5 times those quoted in our previous study.\textsuperscript{15} We reiterate that the reduced polydispersity of the phytoglycogen nanoparticles in the present study reduced the ambiguity in the determination of the phytoglycogen particle radius $r$ and polydispersity index PDI, resulting in a more accurate determination of $r$. 
Figure 3-3: SANS contrast series data for native phytoglycogen particles. (a) Scattered intensity $I$ versus scattering vector $q$ for dispersions of 12.9% w/w phytoglycogen in solvents with different % D$_2$O. (b) The total scattering invariant $Q^*$, calculated using the SANS data shown in (a), which has a minimum value for NSLD = 0.296 fm/Å$^3$ at the contrast match point of 51.2% D$_2$O. (c) NSLD versus % D$_2$O for water (black) and phytoglycogen (red). The red data points correspond to the measured NSLD values for phytoglycogen at 51.2% and 100% D$_2$O.
3.4 Concentrated Native Dispersions

SANS experiments were also performed on concentrated dispersions (up to 30% w/w) of native phytoglycogen (Figure 3-4). The concentrated dispersions show a prominent peak at intermediate \( q \), which corresponds to interparticle correlations that can be described by the structure factor \( S(q) \). To calculate \( S(q) \) for each sample (Figure 3-4b), we divided each SANS data set, normalized by its concentration, by the data set measured for the dilute sample. In Figure 3-4b, we also show solid curves that were calculated using the best-fit parameters obtained by fitting the \( S(q) \) data to a hard sphere model using the Percus-Yevick approximation.\(^{71}\) In Figure 3-4c, we show the average interparticle spacings (average center-to-center distances) corresponding to the primary peak in the \( S(q) \) data in Figure 3-4b. The interparticle spacing decreases with increasing concentration, approaching the average diameter of the phytoglycogen nanoparticles at the largest concentrations, indicating the onset of solidification of the dispersions. The large error bars are in part from the large data spacing in the q-values in this regime.
Figure 3-4: SANS data for concentrated dispersions of phytoglycogen. (a) Scattered intensity versus scattering vector \( q \) for different concentrations of phytoglycogen in \( \text{D}_2\text{O} \), normalized by the concentration \( C \). (b) The structure factors \( S(q) \) were calculated by dividing each SANS data set shown in (a) by the data set measured for the dilute sample. The inset shows each of the \( S(q) \) data sets on a linear scale, which are offset vertically to allow comparison of the different data sets. The curves were calculated using the best-fit parameters from the hard sphere model using the Percus-Yevick approximation.\(^{22}\) (c) Average interparticle spacings (average center-to-center distances), corresponding to the primary peak in the \( S(q) \) data, versus concentration \( C \). (d) the surface separation as predicted using the Kuwabara cell model from Hao & Riman (2004)\(^ {38}\) using concentration to volume fraction approximations outlined in the supplemental information of Grossutti and Dutcher (2021).\(^ {34}\)
3.5 SANS from Very Concentrated Phytoglycogen Samples

![SANS spectra](image)

**Figure 3-5:** a) Scattering intensity for the centrifuged phytoglycogen sample (violet) and the centrifuged and lyophilized phytoglycogen sample (grey-blue). The scattering peak observed for the centrifuged sample corresponds to a length scale of 37 nm, which is slightly less than the particle diameter of 42 nm, indicating that the particles are compressed at very large concentrations. The lack of a scattering feature in the SANS curve for the centrifuged and lyophilized phytoglycogen sample indicates that the sample is very homogenous with no distinction between their neighbouring particles. b) SANS spectra of the centrifuged sample and centrifuged and lyophilized sample plotted with the concentrated dispersions of the refined phytoglycogen material seen in Figure 3-4a. Decreasing low-q values correspond to increasing concentrations.

The scattering curves measured for the highly concentrated phytoglycogen sample are intriguing. The SANS curves for the centrifuged phytoglycogen samples contains a peak at $q = 0.0177 \, \text{Å}^{-1}$, which corresponds to an interparticle spacing of ~37 nm. This value is slightly less than the diameter of the core of a fully hydrated phytoglycogen nanoparticles ($2R = 42$ nm), indicating that the particles are compressed and/or interpenetrating for this large value of phytoglycogen concentration. For the centrifuged and lyophilized phytoglycogen sample, no
scattering peak was observed and the scattering intensity decreased as $q^{-4}$, which is consistent with Porod’s Law.\textsuperscript{90} Apparently, the phytoglycogen nanoparticles are so compressed/interpenetrated that the sample is a continuous material with no distinguishable interfaces between the individual particles on the length scales probed. It is possible that extending to lower $q$ could show grain sizes if there are any.
Chapter 4 - SANS Measurements of Aqueous Dispersions of Octenyl Succinic Anhydride Modified Phytoglycogen Nanoparticles

4.1 Determination of the Match Point of pOSA

The contrast match point for pOSA was determined from SANS data collected on 28.5 mM OSA in solvents with different ratios of D$_2$O: H2O (Figure 4-1a). These data were used to calculate the total scattering invariant $Q^*$, using Equation 3 and shown in Figure 4-1b, which had a minimum value for NSLD = 0.101 fm/Å$^3$ at the contrast match point of 22.9% D$_2$O. This value corresponds to a volume of an OSA molecule of 291.5 Å$^3$, and it is useful as input to the form factor models discussed below when evaluating possible particle geometries as well as stoichiometric calculations. I would like to take a moment to reiterate that these are the pOSA molecules and not hydrophobically modified phytoglycogen. The q-range is limited as only the middle-q range configuration measured as high resolution low-q and high-q data was not required for contrast fitting.
Figure 4-1: (a) Scattered intensity $I$ versus scattering vector $q$ for dispersions of 28.5 mM pOSA in solvents with different ratios of D$_2$O: H$_2$O. (b) The total scattering invariant $Q^*$, calculated using the SANS data shown in (a), which has a minimum value for NSLD = 0.101 fm/Å$^3$ at the contrast match point of 22.9% D$_2$O.

4.2 OSA Modified Phytoglycogen in Dilute Limit

In Figure 4-2, we show the SANS data measured for dilute (1% w/w) dispersions of native, pOSA$_{LDS}$ and pOSA$_{HDS}$ phytoglycogen nanoparticles in D$_2$O, together with a solid curve corresponding to the best-fit of the pOSA$_{HDS}$ data to the raspberry model. The SANS data for the pOSA$_{LDS}$ sample is only slightly different from that measured for the native particles. In contrast, for the pOSA$_{HDS}$ sample, we observed an additional peak centered at $q \sim 0.15$ Å$^{-1}$, indicating the presence of an additional, well defined small length scale in this highly modified material. The additional scattering peak cannot be described by the core-chain model that was used to fit the SANS data for native phytoglycogen nanoparticles (Figure 4-2b). Instead, we found that the measured data was well described by the raspberry model$^{70}$ (red curve in Figure 4-2b), in which small spherical seeds decorate the surface of uniform spherical particles.
We fit the pOSA\textsubscript{HDS} data to the raspberry model using a highly iterative fitting procedure. Using a core radius of 21.2 nm with a scattering length density of 0.586 fm/Å\textsuperscript{3}, as determined from the core-chain model of native phytoglycogen, we used high-q peak in the 1\% pOSA\textsubscript{HDS} SANS data in Figure 4-2a to determine the seed radius of 1.4-1.6 nm, which resulted in the correct curvature. The intensity of the high-q feature required a scattering length density of the seeds to be 0.470 fm/Å\textsuperscript{3}, which is consistent with the seeds having an effective DS of 0.45 OSA molecules per AGU in the chains and hydration of 12 waters per AGU. An effective DS of 0.45 is equivalent to a total DS of 0.1 when considering the core AGUs.\textsuperscript{32}

**Figure 4-2:** (a) SANS data for dilute (1\% w/w) dispersions of native phytoglycogen (blue data points), pOSA\textsubscript{LDS} (green data points) and pOSA\textsubscript{HDS} (black data points), together with a red curve calculated using the best-fit of the pOSA\textsubscript{HDS} data to the raspberry model with the number of AGUs in the hairy chains $N_c = 20$ (schematic diagram shown, not to scale). (b) SANS data of the pOSA\textsubscript{HDS} with the red curve corresponding to the best fit parameters for the core-chain model which fails to describe the high-q feature.

We can better quantify the core-chain model from these raspberry results. We first evaluated the results corresponding to an average number of $N_c = 12$ AGUs in each hairy chain
(corresponding to a best-fit packing density of $\sigma = 0.0020 \pm 0.0001$ chains/Å$^2$). Using the particle core radius of 212 Å determined from the best-fit of the native particle data to the core-chain model (Figure 3-1a), we obtained an excellent fit to the SANS curve. Using the best-fit parameter values, together with our choice of $N_c$ and $\sigma$, we calculated the average number of hairy chains in each seed $N_s$. For the case of $N_c = 12$ and $\sigma = 0.0020 \pm 0.0001$ chains/Å$^2$, $N_s = 1.57$ chains/seed. This result implies that neighbouring hairy chains are required to aggregate to form each small seed on the surface of the particles.

If we consider larger values of $N_c$, i.e., longer chains, the number of chains per seed $N_s$ decreases and, for sufficiently long chains, it falls below 1 chain per seed, which is not physically reasonable. The case of $N_s = 1$ corresponds to the upper limit for the average number of AGUs in the hairy chains $N_c = 20$, for which single chains collapse to form individual seeds. Not only does $N_c = 20$ provide an upper limit on the hairy chain length, it also provides a reasonable estimate of the most likely average chain length, given the hydrophobic nature of OSA-modified chains that would discourage association of neighbouring chains and make the collapse of individual chains to form the small seeds more likely, resulting in the well-defined scattering feature observed in the pOSAHDS SANS curve (Figure 4-2a). For this reason, we chose $N_c = 20$, with the corresponding best-fit value of $\sigma = 0.00125 \pm 0.00008$ chains/Å$^2$, as the best description of the hairy chains that decorate the surface of the phytoglycogen nanoparticles. The best fit to the SANS curve for $N_c = 20$ shown in Figure 4-2a corresponds to a seed radius of 14.6 Å, and an average center-to-center distance between seeds of 30.0 Å, values that indicate closely packed seeds on the particle surface (the ideal close packing center-to-center distance for the seeds is 28.2 Å).
It is remarkable that the SANS results obtained for the pOSA\textsubscript{HDS} sample allowed us to determine the properties of the hairy chains on the surface of the native phytoglycogen nanoparticles – a result that is very useful for understanding the chemical modification of phytoglycogen nanoparticles by a wide variety of chemical species. We also note that there is a well-defined ratio $\beta = 0.269$ for the total number of AGUs in the hairy chains relative to that in the particle core that is insensitive to the choice of $N_c$ ($\beta = 0.269 \pm 0.005$ for $12 < N_c < 24$). Because of the robust nature of the ratio $\beta$, the value of DS = 0.10 for the pOSA\textsubscript{HDS} sample is also well-defined.

In comparing the best-fit curve and the SANS data for the pOSA\textsubscript{HDS} sample (Figure 4-2a), we note that the measured intensities at the smallest $q$ values are slightly larger than the calculated values. Because the scattering intensity values are very large, these small differences are significant and indicate a measurable interaction between particles, i.e. a structure factor $S(Q)$ that is larger than 1, even for the very dilute 1% w/w concentration.

To further test the validity of the raspberry model in describing the pOSA\textsubscript{HDS} SANS data, we used the best-fit parameter values determined for the 100% D\textsubscript{2}O solvent condition to calculate SANS curves corresponding to our measurements of the same sample in two other solvent conditions with different D\textsubscript{2}O:H\textsubscript{2}O ratios, accounting for changes in the deuteration of the labile hydroxyl groups of the AGUs with changing D\textsubscript{2}O:H\textsubscript{2}O (Figure 4-3a): 50% D\textsubscript{2}O, for which the solvent is matched to phytoglycogen; and 23% D\textsubscript{2}O, for which the solvent is matched to pOSA (Figure 4-1). We obtained reasonable agreement between the data sets and the curves calculated using the set of raspberry model best-fit parameters determined from the 100% D\textsubscript{2}O data, validating our interpretation of the data. For the pOSA- and dOSA-modified samples with lower DS values, there was no additional scattering peak in the SANS data that could be
attributed to seeds in the raspberry model. For these samples, we found that the data was well described by using the core-chain model with the DS values determined from IR spectroscopy (Section 4.4 Degree of Substitution). The SANS data for these samples are shown for different solvent conditions in Figure 4-3b, c and d, corresponding to maximum contrast, contrast matching of the OSA, and contrast matching of the phytoglycogen, respectively, as illustrated in Figure 4-3e. In addition, we show curves calculated using the best-fit parameters obtained by fitting the native phytoglycogen SANS data to the core-chain model (Figure 3-1b), using the best-fit DS values of 0.010, 0.027 and 0.010 for the pOSALDS, dOSAMDS and dOSALDS samples, respectively. Clearly, the agreement between the data and calculated curves is excellent in all cases, further validating our interpretation of the data.

The physical picture that emerges from our analysis is that the hydrophobic modification of phytoglycogen nanoparticles with pOSA and dOSA can be accommodated on the hairy chains that decorate the surface of the particles. For low to medium DS values, the modified chains are sufficiently far apart that they remain isolated from one another and the SANS data can be interpreted in terms of the core-chain model with hairy chains that are, on average, slightly modified. For the largest DS value for pOSA – for which we observed an additional scattering peak in the SANS data – we can interpret the SANS data in terms of the raspberry model, in which the hydrophobically modified hairy chains collapse to minimize their contacts with the surrounding water solvent, forming small, well-defined seeds on the surface of the phytoglycogen nanoparticles. The seeds consist of a mixture of glucose, OSA and a reduced amount of associated water due to the hydrophobic character of the seeds. The progression from native particles (core-chain model of uniform sphere decorated by hairy chains) to particles with low to medium DS values (core-chain model of uniform sphere decorated by hairy chains with
well separated modified chains) to particles with the largest DS value (raspberry model with uniform sphere decorated by small seeds) is shown schematically in Figure 4-4. The deviations that can be seen between the data and the calculated curve for 1% \( \text{pOSA}_{\text{HDS}} \) in 50% \( \text{D}_2\text{O} \) (Figure 4-3a) for which we only observe scattering from the seeds on the surface of the particles, can possibly be attributed to the roughness of the particle surface.

**Figure 4-3:** (a) SANS data for dilute dispersions of \( \text{pOSA}_{\text{HDS}} \) for different percentages of \( \text{D}_2\text{O} \) in the solvent: 100% \( \text{D}_2\text{O}; 50% \text{D}_2\text{O}, \) for which the solvent is matched to phytoglycogen; and 23% \( \text{D}_2\text{O} \), for which the solvent is matched to \( \text{pOSA} \). The curves were calculated using the parameters determined by fitting the \( \text{pOSA}_{\text{HDS}} \) data measured in 100% \( \text{D}_2\text{O} \) to the raspberry model, accounting for changes in the deuteration of the labile hydroxyl groups of the AGUs with changing \( \text{D}_2\text{O}: \text{H}_2\text{O} \). (b) SANS data for dilute dispersions of...
pOSA\textsubscript{LDS} under the same solvent conditions as (a). The curves were calculated using the best-fit parameters obtained by fitting the native phytoglycogen SANS data to the core-chain model (Figure 4-2b), using the best-fit DS value of 0.010. (c) SANS data for dilute dispersions of dOSA\textsubscript{MDS} for different percentages of D\textsubscript{2}O in the solvent: 100% D\textsubscript{2}O; 90.8% D\textsubscript{2}O, for which the solvent is matched to dOSA; and 50% D\textsubscript{2}O, for which the solvent is matched to phytoglycogen. The curves were calculated using the best-fit parameters obtained by fitting the native phytoglycogen SANS data to the core-chain model (Figure 4-2b), using the best-fit DS value of 0.027 and accounting for changes in the deuteration of the labile hydroxyl groups of the AGUs with changing D\textsubscript{2}O:H\textsubscript{2}O. (d) SANS data for dilute dispersions of dOSA\textsubscript{LDS} under the same solvent conditions as (c). The curves were calculated using the best-fit parameters obtained by fitting the native phytoglycogen SANS data to the core-chain model (Figure 4-2b), using the best-fit DS value of 0.010. (e) Schematic diagrams of scattering contrast for (left to right, not to scale): 100% D\textsubscript{2}O, contrast match of solvent to either pOSA (23% D\textsubscript{2}O) or dOSA (90.8% D\textsubscript{2}O), and contrast match of solvent to phytoglycogen (50% D\textsubscript{2}O).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{schematic}
\caption{Schematic diagrams of the phytoglycogen nanoparticle core-chain geometry with increasing OSA degree of substitution (left to right, not to scale): native particles, with chains on the surface of the particles; low DS modified particles, with a small number of modified chains (shown in orange); and high DS modified particles, with a large number of modified chains that collapse to form close-packed seeds (orange circles) on the surface of the particles.}
\end{figure}
4.3 Concentrated dispersions of OSA-modified phytoglycogen

The interactions between native phytoglycogen nanoparticles in high concentration dispersions is dominated by the presence of hairy chains on the surface of the particles, as is observed in rheology and osmotic pressure measurements.\textsuperscript{18,34} Chemical modification of the particles with OSA introduces hydrophobicity, due to the presence of oily chains on the OSA molecules. When dispersed in water at high concentrations, the hydrophobic character of the OSA-modified phytoglycogen nanoparticles leads to a net attraction between the particles. In addition, there is the possibility of electrostatic repulsion due to the presence of the carboxylate group (Figure 2-5) that can be either protonated (uncharged) or deprotonated (negatively charged) – the pKa of the carboxylate group is ~ 4.2.\textsuperscript{78,79} At neutral pH, as for the present measurements, the particles have a considerable negative charge. The interplay between particle hairiness, hydrophobicity and electrostatics produces rich possibilities for particle-particle interactions in high concentration dispersions of OSA-modified phytoglycogen nanoparticles.

We have used SANS to study the structure of concentrated dispersions of OSA-modified particles, a technique that is sensitive to interparticle correlations at high concentrations through the structure factor $S(q)$ (Equation 9).

In Figure 4-5, for both pOSAHDS and pOSALDS, we compare the SANS results measured on dilute (1% w/w) dispersions with those measured on concentrated dispersions in 100% D$_2$O, except for the 12.9% OSAHDS sample, for which the buffer was 23% D$_2$O with a correction applied to account for the incoherent scattering. For both DS values, we observed that, with increasing concentration $C$, the interparticle correlation peak occurred at larger values of $q$, corresponding to smaller length scales, \textit{i.e.} smaller interparticle spacing. For these samples, we calculated $S(q)$ by dividing the high concentration data, normalized by their concentration, by
the best fit of the raspberry form factor to the dilute (1% w/w) dispersion data (Figure 4-5c and d). We chose to divide by the best-fit raspberry form factor curve, instead of the dilute dispersion data (as was done for the native particles (Figure 3-4b)), because of the enhanced scattering observed at low-\( q \) for the dilute dispersion of OSA-modified particles, which indicated a small but measurable interaction between the particles in the dilute limit. This approach assumes that there is no change in the nanoparticle size as a function of concentration, which seems to be a reasonable assumption based on the results obtained in contrast series experiments on native phytoglycogen samples for concentrations less than 22.4% w/w,\(^{15} \) in which the contrast match point was unaffected by the phytoglycogen concentration. In Figure 4-5c and d, we also show solid curves that were calculated using the best-fit parameters obtained by fitting the structure factor to a hard sphere model using the Percus-Yevick approximation (Equation 37).\(^{71} \) In Figure 4-5e, we show the average interparticle spacings (average center-to-center distances) corresponding to the primary peak in the \( S(q) \) data in Figure 4-5c and d, as well as the native particle data from Figure 3-4c. In each case, the interparticle spacing decreases with increasing concentration, approaching the average diameter of the phytoglycogen nanoparticles at the largest concentrations, indicating the onset of solidification of the dispersions.

We note that, although the \( q \)-value of the primary peak in \( S(q) \) is well described by the best-fit curves to the hard sphere model in Figure 4-5c and d, there are significant deviations between the measured and calculated curves. These deviations are not surprising, given the soft, sticky and charged nature of the OSA-modified particles. For example, it has been shown recently that electrostatic repulsion of negatively charged nanoparticles can lead to solidification of particle dispersions at lower concentrations due to overlap of the Debye screening lengths for
the particles.\textsuperscript{91–95} Clearly, more complex models of the interactions between OSA-modified particles are required to accurately describe the $S(q)$ data for concentrated dispersions in detail.

**Figure 4-5:** SANS data for concentrated dispersions of pOSA-modified phytoglycogen nanoparticles. (a)-(b) Scattered intensity $I$ versus scattering vector $q$ for different concentrations of OSA-modified particles in 100% D$_2$O, normalized by the concentration $C$ for (a) pOSA$_{HDS}$ and (b) pOSA$_{LDS}$. (c)-(d) Structure factors $S(q)$ calculated by dividing each SANS data set shown in (a) by the data set measured for the dilute sample for (c) pOSA$_{HDS}$ and (d) pOSA$_{LDS}$. The solid curves correspond to the best fits of the $S(q)$ data to a hard sphere model using the Percus-Yevick approximation. (e) Average interparticle spacings (average center-to-center distances) for pOSA$_{HDS}$ (green), pOSA$_{LDS}$ (red) and native phytoglycogen particles (black),
corresponding to the primary peak in the $S(q)$ data, versus concentration $C$. The red horizontal dashed line corresponds to the core diameter measured for native particles. The interparticle spacing values calculated for the dilute (1% w/w) dispersions are much larger: 1130 Å ± 250 Å (pOSA$_{HDS}$) and 1460 Å ± 300 Å (pOSA$_{LDS}$) (not shown), corresponding to the low-$q$ features that can be seen in (c) and (d).

4.4 Degree of Substitution

Attenuated total reflection infrared (ATR-IR) absorption measurements of (p/d)OSA-PG films were measured to directly observe the ester linkage in OSA modified phytoglycogen. Figure 4-6 shows the spectra from 1800-1500 cm$^{-1}$. This range contains a few peaks at ~1720, 1640, and 1575 cm$^{-1}$ which are from carbonyl groups, sorbed water, and carboxylate groups respectively. The ester linkage between PG and OSA contributes to the carbonyl signal (~1720 cm$^{-1}$). The carboxyl group on the OSA is pH dependent and can either contribute to the 1720 cm$^{-1}$ band when protonated (low pH) or contribute to the ~1575 cm$^{-1}$ band when deprotonated. The pKa of this group is ~4.2 and measurements in that environment will have a mixed protonated state in the population.

To determine the relative degree of substitution, we created full protonated and deprotonated films by adjusting the initial solution to pH 2 and 10 respectively. While deprotonated (pH 10) the carboxyl group will contribute to the 1575 cm$^{-1}$ peak instead of the 1720 cm$^{-1}$ peak, thus the intensity in the 1720 cm$^{-1}$ peak is only from the ester linkage between the PG and OSA. The absorbance in the 1720 cm$^{-1}$ peak is then proportional to the number of ester linkages in each sample. Taking the ratio of the intensity of these peaks allows the determination of the relative degree of substitution of the lower DS samples.
Figure 4-6: (a) IR absorption spectra for the pOSA_{HDS} (red), dOSA_{MDS} (blue), dOSA_{LDS} (black), pOSA_{LDS} (green) samples at pH 10. The band centered at 1725 cm\(^{-1}\) can be attributed to ester linkages between phytoglycogen and the OSA molecules. By comparing the integrated intensities of the bands for dOSA_{MDS}, dOSA_{LDS} and pOSA_{LDS} to that for pOSA_{HDS}, the relative values of DS can be determined. The relative ratios for dOSA_{MDS} and dOSA_{LDS} are consistent to those inferred from a comparison of the integrated intensities for the C-D stretching band centered at 2100 cm\(^{-1}\) (not shown). (b) IR absorption spectra for the pOSA_{HDS} (red), dOSA_{MDS} (blue), dOSA_{LDS} (black), pOSA_{LDS} (green) samples at pH 2. The protonation of the carboxyl group shifts the band at 1575 cm\(^{-1}\) to \(\sim 1720\) cm\(^{-1}\).
Chapter 5 - Characterization of Phytoglycogen Nanoparticles Modified by Hydrolysis

As discussed in Section 1.3 Chemical Modifications of Phytoglycogen, phytoglycogen nanoparticles can be modified through hydrolysis, which breaks bonds between the AGUs within the particles. This can be achieved either using dilute acids at elevated temperature, or by using enzymes that break specific bonds within the particles. In this chapter, we explore the changes to the physical properties of phytoglycogen nanoparticles produced by both methods of hydrolysis.

5.1 Dynamic Light Scattering on Hydrolyzed Phytoglycogen Nanoparticles

Dynamic light scattering (DLS) was used to quantify the reduction in particle radius and polydispersity produced by acid and enzymatic hydrolysis. As discussed in Section 2.4 Dynamic Light Scattering (DLS), for a symmetric, monomodal particle radius distribution measured in DLS, the intensity weighted particle radius distribution can be determined directly from the autocorrelation function (Equation 42), which allows the determination of the hydrodynamic radius $R_H$ as the radius value corresponding to the maximum value in the distribution. For polydisperse particles, the dependence of the scattering intensity on the sixth power of the particle radius results in a skewing of the intensity-weighted distribution to larger radius values. Alternatively, the number-weighted particle radius distribution can also be determined. In Figure 5-1, we provide the intensity weighted and number weighted particle radius distributions for native phytoglycogen nanoparticles (PG), acid hydrolyzed particles (Acid-PG), particles
hydrolyzed by amyloglucosidase (AMG-PG), as well as enzymatically synthesized glycogen (ESG). The corresponding values of the hydrodynamic radius $R_H$, the number average radius $R_N$, and the polydispersity index PDI are listed in Table 1. We note that the value of $R_N$ for native PG is very close to the particle radius measured using SANS ($r = 21$ nm).

![Figure 5-1: DLS distributions of particle radius as measured by DLS for 0.2% w/w for dispersions of native phytoglycogen (PG, black), acid hydrolyzed PG (Acid-PG, green), PG hydrolyzed by amyloglucosdase (AMG-PG, red), and enzymatically synthesized glycogen (ESG, blue). a) Intensity weight distributions for each material showing the hydrodynamic radius $R_H$. b) Number weighted distributions show the number weighted radius $R_N$. Through controlled hydrolysis the native material the particle size can be reduced and can be made to be comparable to ESG but at the expense of a greater polydispersity. The smaller materials have larger polydispersity indices than the native material. These results are summarized in Table 1.](image-url)
Table 1: Hydrodynamic radius $R_h$, number average radius $R_N$ and polydispersity index PDI for native PG, acid hydrolyzed PG (Acid-PG), and PG hydrolyzed by amylglucosidase (AMG-PG). Measured values are also provided for enzymatically synthesized glycogen (ESG).

<table>
<thead>
<tr>
<th></th>
<th>$R_h$ (nm)</th>
<th>$R_N$ (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>69.1</td>
<td>21.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Acid-PG</td>
<td>37.8</td>
<td>10.5</td>
<td>0.16</td>
</tr>
<tr>
<td>AMG-PG</td>
<td>67.6</td>
<td>18.9</td>
<td>0.12</td>
</tr>
<tr>
<td>ESG</td>
<td>33.7</td>
<td>12.0</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Acid hydrolysis (Section 2.1.3 Acid Hydrolysis) had a significant impact on the PG particles, resulting in a significant reduction in the particle radius by a factor of ~1.9, and a large increase in the PDI by 60%. AMG hydrolysis (Section 2.1.4 Enzymatic Hydrolysis) resulted in a smaller decrease in the particle radius by a factor of ~1.07 and a smaller increase in the PDI by 20%. The DLS results for ESG show a significantly smaller particle radius than the native PG with a larger associated PDI. In Figure 5-2, we show SANS spectra collected on dilute dispersions of the four different types of particles. The relative particle radii measured using DLS ranks in the same order as the low-$q$ intensities in the SANS curves. This is consistent with Equation 12 which in the limit of $q \to 0$ becomes $F(q = 0) = \Delta \rho V_{sphere}$, where the volume of a sphere is $4\pi R^3/3$. 
Figure 5-2: SANS intensity curves for dilute dispersions of the phytoglycogen and glycogen samples listed in Table 1. The progression of decreasing scattering intensity in the low-q limit of PG to AMG-PG to ESG to Acid-PG is consistent with the progression of decreasing particle radii measured using DLS. Core-chain fits are presented in Figure 5-5.

5.2 Rheology of Hydrolyzed Phytoglycogen

The work in this section is attributed by Hurmiz Shamana. To properly determine the hydration and NSLD of the modified particles, the volume fraction values of the nanoparticle dispersions must be determined. We used to rheology in the dilute regime which can be described by the Einstein equation: $^{18,96}$

$$\eta_r = 1 + 2.5\phi_{eff}$$ (45)
where $\eta_r$ is the zero-shear viscosity relative to that of water and $\phi_{\text{eff}}$ is the effective volume fraction. In the dilute limit, the effective volume fraction is proportional to the concentration $C$, so that we can express the volume fraction as $\phi_{\text{eff}} = kC$. Therefore Equation 4 can be written as:

$$
\eta_r = 1 + 2.5kC
$$

(46)

The best-fit values of $k$ were used to calculate the effective volume fraction values. We note that ESG can contain a significant amount (up to 20% w/w) of impurities, such as resistant starch (up to 20% w/w), which would significantly affect the determination of the $k$ values.

In calculating the particle form factors, e.g., Equation 13, the volume fraction $\phi$ is typically calculated from the solute mass fraction or by relating the Porod Invariant (Equation 3) to the volume fraction through the relationship:

$$
Q^* = 2(\pi \Delta \rho)^2 \phi (1 - \phi)
$$

(47)

We chose to use the effective volume fraction $\phi_{\text{eff}}$ as measured by rheology because we account for the hydration water (water inside the particles) in the neutron scattering length density (Equation 4). The hydration water is not taken into account when using the glucose mass fraction and using the Porod Invariant has been shown to be extremely sensitive to background subtractions: changing the background by 0.0001 (second significant digit) changes the predicted volume fraction by a factor of 2 and is thus unreliable. Our confidence in the use of $\phi_{\text{eff}}$ in the
particle form factors is reinforced by the SANS curves shown in Figure 5-2. The progression of decreasing scattering intensity in the low-q limit from PG to AMG-PG to ESG to Acid-PG is consistent with a decreasing particle radius that is described by $\phi_{eff}$ and measured directly using DLS.

![Graph](image)

**Figure 5-3:** Zero shear viscosity relative to that of water for PG (black), Acid-PG (green), AMG-PG (red), and ESG (blue). The solid line fits yield the best-fit values of $k$, which determines the volume fraction values used in the fitting of SANS curves. (Data collected and analyzed by Hurmiz Shamana)

### 5.3 Contrast Matching of Hydrolyzed Phytoglycogen

Contrast matching experiments were performed to compare the contrast match point and the hydration of the hydrolyzed particles with those values determined for native PG. The values of the scattering invariant $Q^*$ described in Section 2.3.1 Porod/Scattering Invariant and Contrast
Matching for the hydrolyzed particles yielded similar contrast match points of approximately 50% D$_2$O (Figure 5-4). This indicates that the AGUs in each particle are not fundamentally altered by the hydrolysis; each AGU volume was consistent with 160 ± 2 Å$^3$. By determining the contrast match point and the neutron scattering length density (NSLD) at 100% D$_2$O, we were able to determine the hydration $n_H$ (the number of water molecules associated with each AGU).  

Acid-PG and AMG-PG had larger hydration numbers ($n_H$ values of 23.2 and 30.6 water molecules per AGU respectively) than for native PG ($n_H$ value of 20 ± 2). These increases in $n_H$ for the hydrolyzed particles are likely due to the removal of AGUs within the PG particles during the hydrolysis process, which would result in more water within the particles. We also found that the hydration number of ESG ($n_H$ value of 14.2) which is likely due to denser branching (and therefore less water) within the ESG particles.
Figure 5-4: Contrast matching of the a) native material, b) AMG-phytoglycogen, c) acid hydrolyzed phytoglycogen, and d) synthetic glycogen. Each sample has a contrast match point about 50% and because all materials share a match point, they all share the common anhydroglucose. By measuring the scattering length density of material at 100% and comparing it to the fully exchanged monomer we can determine the hydration number by calculating how many water molecules are needed to create the measured density using the method discussed in 15. The results are summarized in Table 2. It was
observed that both methods of hydrolysis on PG increases the amount of water molecules per monomer. This is consistent with Copeland’s work which saw that acid hydrolysis on starches increases the swelling power. ESG sorbs significantly less water than the other materials.

5.4 Fits to the SANS Curves for Dilute Dispersions of Hydrolyzed Phytoglycogen Nanoparticles Using the Core-Chain Model

For native PG, we found that the uniform sphere model did not adequately describe the measured SANS curve (Figure 5-5, grey lines); a significant improvement in the fit to the data was obtained using the core-chain model (Figure 5-5, red lines). The best fit of the core-chain model to the native PG data corresponded to a core radius of 212 Å (PD 0.26) and showed that 21.2% of the AGUs in the native PG particles resided in the hairy chains that decorated the outer surface of the particles. The native radius is consistent with the number weighted DLS measurement of 212 Å, PDI 0.1. By using the core-chain model to fit the measured SANS curves for Acid-PG and AMG-PG, we found that the core radius was reduced to 85.4 Å (PD 0.40) and 159 Å (PD 0.311), respectively. Similar to the number weighted DLS results of 105 Å (PDI 0.16) and 189 Å (PDI 0.12) for Acid-PG and AMG-PG respectively. For comparison, ESG had a core radius 97.3 Å (PD 0.35), 120 Å (PDI 0.12) by number weighted DLS. The values of the core radii and the Shultz polydispersity are provided in Table 2.
Figure 5-5: SANS scattered intensity $I$ versus scattering wavevector $q$ for 1% w/w dispersions of a) native phytoglycogen, b) Acid-PG, c) AMG-PG, and d) enzymatically synthesized glycogen dispersions in 100% D$_2$O. The grey curve corresponds to the best-fit of the data to the uniform sphere model and the red curve corresponds to the best fit to the core-chain model. The best-fit parameter values from the fit to
the core-chain model are summarized in Table 2.e) The dependence of the best-fit $\beta$ values (Equation 4) on the corresponding core radius values. f) The values of $\beta r$ normalized to the value of $\beta r$ for native PG, where values greater than one indicate that the particle is hairier than native PG.

<table>
<thead>
<tr>
<th>Material</th>
<th>Core Radius ( r ) (nm)</th>
<th>Shultz PD</th>
<th>NSLD in 100% D(_2)O (fm/(\text{Å}^3))</th>
<th>$\phi$ (C=1)</th>
<th>( n_H )</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>21.2</td>
<td>0.26</td>
<td>0.586</td>
<td>0.032</td>
<td>20</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>Acid-PG</td>
<td>8.54</td>
<td>0.4</td>
<td>0.5922</td>
<td>0.033</td>
<td>23.2</td>
<td>0.90±0.04</td>
</tr>
<tr>
<td>AMG-PG</td>
<td>15.9</td>
<td>0.311</td>
<td>0.6006</td>
<td>0.04</td>
<td>30.6</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>ESG</td>
<td>9.73</td>
<td>0.35</td>
<td>0.574</td>
<td>0.02</td>
<td>14.2</td>
<td>0.68±0.12</td>
</tr>
</tbody>
</table>

**Table 2:** Summary the best-fit parameter values for native PG, acid hydrolyzed PG, AMG hydrolyzed PG and ESG. The core radius \( r \), Shultz PD, \( n_H \) and $\beta$ values correspond to the best fit of the SANS curves to the core-chain model. The NSLD in 100% D\(_2\)O was obtained from the contrast variation measurements, and the volume fraction values were obtained from rheology measurements. Note that the $\phi(C = 1)$, the volume fraction at 1% w/w, is from Figure 5-3.

For native PG, we determined using SANS that the particles consisted of dense cores that are decorated with short linear chains of AGUs that are, on average, 20 AGUs long. Hydrolysis of the PG nanoparticles changes the particle morphology, making the particles smaller and also altering the chains at the surface of the particles since these chains are very accessible. For native PG, we defined $\beta$ as the number of AGUs in the outer surface chains relative to the number of AGUs in the particle core, i.e.
\[ \beta = \frac{N_c \sigma (4\pi r^2)}{\left( \frac{4}{3} \pi r^3 \right) V_{AGU}} = \frac{3N_c \sigma V_{AGU}}{r} \]  

(48)

where \( N_c \) is the number of AGUs per outer surface chain, \( \sigma \) is the grafting density of outer surface chains, and \( V_{AGU} \) is the volume of one AGU. For native PG, we found that \( \beta = 0.26 \pm 0.01 \). However, if we compare particles of different radii \( r \), \( \beta \) is not the best choice since \( \beta \sim 1/r \) (Equation 4). Instead, we can compare the values of \( \beta r \) that should be independent of \( r \) for particles with the same degree of hairiness. By doing so, we are comparing the values of the product of \( N_c \sigma \) for the different particles, and both \( N_c \) and \( \sigma \) could change with hydrolysis. In part (a) of Figure 5-5e, we plot the best-fit values of \( \beta \) versus the best-fit values of the particle radius \( r \) for the different particles and in part (b) we plot the corresponding values of \( \beta r \) versus \( r \). If \( N_c \sigma \) was the same for each particle, then \( \beta r \) should be independent of \( r \). Deviations from the value of \( \beta r \) for native PG indicate changes in \( N_c \sigma \) produced by hydrolysis. It was found that hydrolysis of PG through acidic or enzymatic hydrolysis increased the degree of hairiness of the particle by \(~40\%\).

5.5 Fits of the SANS Curves for Concentrated Dispersions of Hydrolyzed Phytoglycogen Nanoparticles

SANS experiments were performed on concentrated dispersions of Acid-PG, for concentrations \( C \) as large as 18% w/w, and we compare these results to those obtained for native PG. We also show the results of the concentrated dispersions of ESG up to concentrations \( C = \)
30%, however, AMG-PG was not measured in these experiments since there was insufficient material generated in the enzymatic process. The SANS curves for the concentrated dispersions show a prominent peak at intermediate q, which corresponds to interparticle correlations that can be described by the structure $S(q)$ (Figure 5-6a and b). To calculate the S(q) values for each sample, we divided each SANS curve, normalized by its concentration, by the SANS curve measured for 1% w/w dilute dispersions. In Figure 5-6c and d, we show solid curves calculated using best-fit parameters obtained by fitting the S(q) data to the hard sphere model using the Percus-Yevick approximation.\textsuperscript{71} In Figure 5-6e we show the average particle separation (distance between particle surfaces) corresponding to the primary peak in the $S(q)$ curves, which is an indication of the onset of jamming at high concentrations.
Figure 5-6: SANS data for concentrated dispersions of ESG and APG nanoparticles. (a)-(b) Scattered intensity $I$ versus scattering vector $q$ for different concentrations of ESG and APG in 100% D$_2$O, normalized by the concentration $C$ for (a) ESG and (b) APG. (c)-(d) Structure factors $S(q)$ calculated by dividing each SANS data set shown in (a) by the data set measured for the dilute sample for (c) ESG and (d) APG. The solid curves correspond to the best fits of the $S(q)$ data to a hard sphere model using the Percus-Yevick approximation. (e) Average interparticle spacings (average center-to-center distances) for APG (green), ESG (red) and native phytoglycogen particles (black), corresponding to the primary peak in the $S(q)$ data, versus concentration.
Chapter 6 - Preliminary Characterization of Cationically modified Phytoglycogen

6.0 Cationic Phytoglycogen (GTAC-PG)

Phytoglycogen that has been chemically modified with glycidyltrimethylammonium chloride (GTAC) (DS = 1.46, as determined using NMR) was supplied by Mirexus Biotechnologies. The starting PG material for GTAC-PG was not the refined material discussed in this thesis. The GTAC modification confers a positive charge to PG, introducing a long-range electrostatic repulsion between particles without the addition of a hydrophobic group. In Figure 6-1a, we show small angle x-ray scattering (SAXS) spectra I measured on dilute dispersions (1% w/w) of GTAC-PG. The long-range electrostatic interaction between particles is screened by the addition of 10 mM salt (Figure 6-1a, blue points). Unfortunately, the low-q limit of the SAXS system was limited to 0.01 Å⁻¹, which did not allow the determination of the structure factor $S(q)$. We also measured a more concentrated (2% w/w) dispersion using SAXS (Figure 6-1bc). By normalizing the scattering intensity by the dispersion concentration, the high-q feature at $q \sim 0.2$ Å⁻¹ overlaps in the two SAXS spectra, suggesting that its presence is part of the form factor $P(q)$ with the addition of GTAC leading to the formation of small length scale feature that is similar to that observed for highly-modified OSA-PG (pOSA_HDS). The feature in the lower q regime are from the structure factor emerging at these low concentrations, suggesting that the GTAC system has strong interparticle interactions.
**Figure 6-1:** a) SAXS data of 1% GTAC-PG with varying amounts of added salts. A high-q feature can be seen and is reminiscent of the high-q scattering feature seen in the SANS data for pOSA_HDS. In the low-q regime, a feature can be seen due to interparticle interactions even in the dilute limit, and the interaction is quenched by adding salt. b) Increasing concentration of the GTAC-PG without added salt. The feature at low-q is determined by the electrostatic interaction. c) The same curve as in b but normalized by concentration. The overlap at high-q suggests that the feature is due to the addition of GTAC, and the deviations at lower-q are likely due to electrostatic effects.

We also collected SANS spectra for a 1% w/w dispersion of GTAC-PG (Figure 6-2). The data contains a high-q feature that is similar to that observed in the pOSA_HDS SANS curves (Figure 4-2a), which corresponds to the presence of an additional small length scale due to the GTAC modification. In the low-q regime, the electrostatic interaction between particles in GTAC-PG has significantly more detail than in the SAXS curves (Figure 6-1) with a resolved peak at \( q \sim 0.006 \text{ Å}^{-1} \), indicating that the structure factor has a significant contribution to the scattering curves even in the limit of dilute dispersions. An initial fit to the SANS data was performed using the uniform sphere form factor with the Hayter mean spherical approximation (MSA) (screened Coulomb potential) structure factor and the results are shown in Figure 6-2b. For this demonstration we fixed the parameters that describe the PG particles (neutron scattering length density, radius, polydispersity, see Figure 3-1) and added charges to the system to capture the structure factor. At this point we assume the form factor is unaltered by the modification. Figure 6-2b shows that the screened coulomb potential is able to describe the low-q feature but high-q feature still needs to be addressed.

Nuclear magnetic resonance (NMR) measurements determined the DS of GTAC-PG to be 1.46. This value is reaffirmed by IR which estimate a similar order of magnitude modification. This is a substantial degree of modification as nearly half of the mass added is
GTAC. If we consider the number of available (labile) binding sites to be 3 per AGU the maximum value of the DS for full modification of the chains decorating the out surface of the particle is 0.63. This suggests that GTAC penetrates the volume of phytoglycogen. A core-shell geometry form factor is unable to generate the high-q feature observed. Therefore GTAC-PG must have a complicated form factor that captures a reduced virgin PG core, a shell that describes the penetrated GTAC, and some other small length scale feature that captures the high-q feature. To resolve this, more information about the GTAC molecule is required to determine its scattering length density and monomer volume to perform careful stoichiometry to inform choices in modeling the form factor.
Figure 6-2: a) Comparison of dilute 1% w/w dispersions of native PG (red) and GTAC-PG (black) as measured by SANS. A structure factor can be seen in the low-q for the GTAC-PG and some additional small length scale feature can be seen in the high-q regime, reminiscent of the pOSA_HDS. B) Preliminary fit to GTAC-PG using the uniform form factor and a Hayter (charged) sphere structure factor. The screened coulomb interaction can describe the low-q feature. The high-q feature is complicated to describe, the DS value is prohibitive to using the raspberry model, there is a substantial amount of GTAC penetrating the volume. A more complicated form factor is required to be able to describe the GTAC modified PG.
Chapter 7 - Summary and Future Work

7.1 Summary of Results

Small angle neutron scattering is a powerful way to investigate the properties of biological nanomaterials such as phytoglycogen (PG) and chemically modified PG. In the present study, we demonstrated the refinement of the isolation and purification of native PG nanoparticles resulted in an improved understanding of the nanoparticle morphology: a dense core with radius of 21.2 nm ± 0.1 nm and a Shultz polydispersity of 0.26, with short (hairy) chains decorating the outer surface of the particles. This corresponded to a total number of 52,500 AGUs in the dense core, and a total number of 14,100 AGUs in the chains, i.e., 21.2% of the AGUs are in the hairy chains. Contrast matching experiments revealed that native PG can sorb 220% of their dry weight in water.

We modified PG with octenyl succinic anhydride (OSA), a food grade modification of polysaccharides. For the largest degree of substitution (DS=0.10) of protonated OSA (pOSA), an additional high-q feature, corresponding to an additional small length scale, was observed in a SANS curve collected on a dilute dispersion of the particles. Excellent fits were obtained to the pOSA$_{HDS}$ SANS curve using the raspberry model, assuming that pOSA was bound to all of the labile binding sites on the hairy chains, resulting in the collapse of the hydrophobic hairy chains to form “seeds” that decorated the outer surface of the particles. Excellent fits to the SANS curves collected for lower degrees of modifications (pOSA$_{LDS}$, dOSA$_{MDS}$, and dOSA$_{LDS}$) and different contrast conditions were obtained using the core-chain model. Concentrated dispersions showed rich interparticle interactions due to the interplay of electrostatic and hydrophobic interactions.
Controlled hydrolysis of native PG particles using either dilute sulfuric acid at elevated temperature or the amyloglucosidase (AMG) enzyme produced modified PG with reduced radii as measured using SANS: 8.54 nm for Acid-PG and 15.9 nm for AMG-PG. Hydrolysis also resulted in increased polydispersity and increased water sorption (Acid-PG: polydispersity index PDI = 0.4, hydration number \( n_H = 23.2 \); AMG-PG: PD = 0.31, \( n_H = 30.6 \)). As for the native PG particles, the hydrolyzed particles are well described by the core-chain model, i.e., the hydrolyzed particles also have hairy chains that decorate the outer surface of the particles. The best fits to the SANS curves allowed us to compare the number of AGUs in the hairy chains for each type of modified particles using the parameter \( \beta r \). We found that hydrolysis of PG nanoparticles results in smaller nanoparticles that are 40% hairier. Concentrated dispersions of the modified particles were described by a hard-sphere particle interaction as for the native PG.

7.2 Future Work

7.2.1 RheoSANS

Concentrated dispersions of native PG, OSA-modified PG and Acid-PG show complex rheological behaviour. The NGB30m SANS beamline at the National Institute of Standards and Technology (NIST) offers the possibility of performing SANS and rheology at the same time (Rheo-SANS). The Dutcher Lab has been awarded beamtime to perform a series of Rheo-SANS experiments on OSA-modified PG, and these experiments will provide important insights into the stimuli-responsive structure-flow properties of OSA-modified PG dispersions.
7.2.2 Tuning the Electrostatic Interaction Between OSA-Modified Phytoglycogen Nanoparticles

The OSA molecule has a carboxylate group (see Figure 2-5) for which the pKa is ≈4.2.\textsuperscript{78,79} By varying the pH and salt content, the magnitude of the repulsive electrostatic interaction and the attractive hydrophobic can be tuned. This will affect the balance between the repulsive electrostatic interaction and the attractive hydrophobic interaction and provide insight into the complex interactions for high concentration dispersions.

7.2.3 Succinic Anhydride Modification – No hydrophobic modification

Because OSA contributes both repulsive electrostatic interactions and an attractive hydrophobic interaction between OSA-modified PG nanoparticles, the interparticle interactions are complex. It would be interesting to modify the PG nanoparticles with OSA molecule with the octenyl chain removed since this would eliminate the attractive hydrophobic interaction.

7.2.4 Concentrated Diffusion Measurements

DLS can be used to measure the diffusion of PG nanoparticles but only in the dilute limit in which there is no secondary scattering. This limitation does not allow the study of concentrated dispersions for which interparticle interactions dominate the sample behaviour. Alternatively, pulse field gradient NMR can be used to measure single particle diffusion in concentrated dispersions. This provides a powerful way to bridge SANS and rheological data and single particle diffusion in concentrated dispersions.
7.2.5 Additional Cationically Modified Phytoglycogen SANS Measurements

The SANS curve for GTAC-PG presented in Figure 6-2 is very rich and should be explored in greater depth. Contrast variation of GTAC in solution would be valuable to determine the neutron scattering length density and monomer volume for GTAC monomers to allow for careful stoichiometric calculations. Pushing into lower-q will grant access to the Guinier region \((R_g q < 1)\), in this region we could look at the model independent \(R_g\) as a function of D\(_2\)O solvent fraction to determine the penetration depth of the GTAC. Furthermore, the interparticle interactions are very strong, thus being able to perform measurements under different ionic solvent conditions, by changing the salt content, a more careful analysis can be performed. This in addition to reducing the DS of GTAC-PG, and therefore the total charge, would be useful in characterizing these interactions.
REFERENCES


