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Steric Stabilising Properties of Hydrophobically Modified Starch: Amylose vs. Amylopectin

Rammile Ettelaie[a,*], Melvin Holmes[a], Jianshe Chen[b], Amin Farshchi[c]

[a]Food Colloids Group, School of Food Science and Nutrition, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK.

[b]School of Food Science and Bioengineering, Zhejiang Gongshang University, Hangzhou, Zhejiang 310018, China

[c]Institute of Particle Science and Engineering, School of Chemical and Process Engineering, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK.

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* Corresponding author. Tel: + 44 113 343 2981, Fax: +44 113 343 2982

E-mail: r.ettelaie@leeds.ac.uk (R.Ettelaie).

ABSTRACT

Self-Consistent Field Theory of Scheutjens and Fleer is extended to deal with highly branched polymer chains. Using the method, the surface adsorption and the steric stabilising properties of hydrophobically modified amylose and amylopectin were compared. For unmodified starch, both biopolymers induce attractive depletion interactions between emulsion droplets. However, for chains of comparable molecular weight, the forces are stronger and longer ranged for amylose. Upon hydrophobic modification, amylopectin has a higher level of surface adsorption, but forms thinner and denser interfacial layers. While both starch molecules provide a sufficient degree of steric repulsion to keep fine emulsions stable, those induced by amylose layers are longer ranged and decay more slowly with inter-droplet separations. This desirable property is partially offset by the greater propensity of linear chains to cause bridging. It is found that mixed layers of amylose and amylopectin lead to superior steric stabilising properties, as compared to either component alone.

1. Introduction

Most naturally occurring polysaccharides are hydrophilic macromolecules that exhibit no significant level of surface activity at hydrophobic-hydrophilic interfaces (Dickinson, 2003; Nishinari & Doi, 1993). Their tendency to remain in the aqueous solutions, combined with their relatively large size, allows for the formation of entangled networks of these biopolymers at very low polysaccharide concentrations. The presence of such a network in a solution has a dramatic impact on the viscosity (Lapasin & Pricl, 1999; Ren, Ellis, Ross-Murphy, Wang, & Wood, 2003; Stephen & Phillips, 2006), making polysaccharides the functional ingredient of choice for use as rheology modifiers and thickeners in food colloid formulations. The macromolecular networks are further enhanced by the ability of the chains to weakly cross-link through inter-molecular hydrogen bonds (Ross-Murphy, 1987; Rossmurphy & Shatwell, 1993). This also gives the polysaccharides their other desirable property, namely their excellent water holding capability (Spiller, 2001; Stephen, et al., 2006).

Although most natural polysaccharides display no affinity for adsorption onto a hydrophobic surface, there are a few rather known notable exceptions. The most commonly encountered commercial example of this class of surface active polysaccharides is gum Arabic (Chanamai & McClements, 2002; Williams & Phillips, 2009). This is frequently used as an emulsifier and emulsion stabiliser in manufacturing of citrus soft drink products (Paraskevopoulou, Boskou, & Kiosseoglou, 2005; Qian, Decker, Xiao, & McClements, 2011; Ray, Bird, Iacobucci, & Clark, 1995). Other examples include pectin (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002), polysaccharide extracted from okra (O'Toole, 1999), and more recently almond gum (Mahfoudhi, et al., 2014) and cashew tree gum (Porto & Cristianini, 2014), but to name a few. It is now well established that the surface affinity of these molecules invariably arises from the complexation of the polysaccharide with protein. It is the presence of the hydrophobic amino acids amongst the residues of the protein part that entices the conjugated chains to adsorb at hydrophobic-hydrophilic interfaces (Dickinson, 2003). It turns out that in all known cases, the proteinaceous fraction responsible for the surface activity of the polysaccharide is a rather small component of the total polysaccharide, perhaps as low as 12% (Randall, Phillips, & Williams, 1989), with only around 1-2% actually ending up adsorbed on the surface of the droplets (Randall, Phillips, & Williams, 1988). It is this

small portion that provides the desired steric repulsion between the droplets and contributes to their colloidal stability, as a genuine emulsifier.

The presence of a small amount of free polysaccharide in a colloidal dispersion is often detrimental to stability of the particles. This is mainly due to the ability of free polymer in solution to induce depletion flocculation between the emulsion droplets (Dickinson, 1992; McClements, 1998). Although aggregates thus formed are normally rather weak and easily redispersed under gentle shear, their presence increases the rate of creaming and speeds up any possible coalescence and breakup of the emulsion. This consideration, combined with only having a low fraction of proteinaceous chains in the natural surface active polysaccharides, has prompted many researches to attempt to deliberately synthesis these types of conjugate biopolymers. This is achieved by the covalent bonding of a suitable protein with a polysaccharide, facilitated by Maillard reactions between the two.(Dickinson, 2009) Conjugate biopolymers involving ovalbumin-dextran (Kato, Sasaki, Furuta, & Kobayashi, 1990), β-casein-dextran (Dickinson & Semenova, 1992; Semenova, Belyakova, Polikarpov, Antipova, & Dickinson, 2009), bovine serum albumin-dextran (Dickinson, et al., 1992), β–lactoglobulin-propylene glycol alginate (Dickinson & Galazka, 1991), β–lactoglobulin-dextran (Akhtar & Dickinson, 2003), α-actalbumin-acacia gum (de Oliveira, et al., 2015), peanut protein isolate-dextran (Li, Xue, Chen, Ding, & Wang, 2014) and whey protein-maltodextrin (Akhtar & Dickinson, 2007) have all been successfully produced through this route. In most of these studies, it has been found that the resulting protein-polysaccharide complexes do indeed exhibit a significantly superior colloidal stabilising property compared to protein alone. In particular, it has been reported that at lower pH values close to the pI for many proteins, where protein stabilised emulsions have a tendency to break up, the emulsion droplets stabilised by the conjugates show no sign of any appreciable change in their size, even after a relatively long period of storage (Akhtar, et al., 2007; Dickinson, et al., 1992).

Despite their superiority, conjugate protein-polysaccharides are still not widely used as commercial emulsifiers in large scale production of food emulsions. This may be due to variability in the quality of the resulting complexes, arising from the difficulty of controlling the reaction conditions. The Maillard reactions also only occur efficiently at certain optimum value for water activity. Achieving this optimum value requires a significant level of drying that is normally carried out in a batch process. This adds to the costs of large scale

manufacturing of the conjugates for commercial use. Furthermore, the presence of small amounts of impurity, in the form of low molecular sugar moieties, greatly diminishes the reaction efficiency. Under such circumstances, the much larger polysaccharides have to compete with these undesirable sugar molecules for the limited reaction sites on the protein. The number density, even for a small amount of impurity, greatly favours the sugar moieties, depriving polysaccharide chains from the potential protein residues through which covalent bonds are to be formed. These issues have led some researchers to explore an alternative method to modify polysaccharides, turning them from purely hydrophilic molecules to more surface active amphiphilic ones. This route involves the attachment of small hydrophobic groups, often randomly distributed, throughout the backbone of the polysaccharide molecule. By adjusting the number of such sites, the amphiphilic nature of the hydrophobically modified polysaccharide can be fine-tuned. The technique has most widely been applied to cellulose and its derivatives (Charpentier, et al., 1997; Kawakami, Ihara, Nishioka, Kitsuki, & Suzuki, 2006; Rosilio, Albrecht, Baszkin, & Merle, 2000; Taubner, et al., 2013), dextran (Carrier, Covis, Marie, & Durand, 2011; Rotureau, Leonard, Dellacherie, & Durand, 2004; Rouzes, Durand, Leonard, & Dellacherie, 2002), chitosan (Calejo, Kjoniksen, Maleki, Nystrom, & Sande, 2014; Dowling, et al., 2011; Shedge & Badiger, 2014; Sjoholm, Cooney, & Minteer, 2009) and starch (Nilsson & Bergenstahl, 2006, 2007; Yusoff & Murray, 2011), not surprisingly given that these are the most abundant polysaccharides. The actual modification can take a number of different forms, but often involves the attachment of short alkane side chains to the polysaccharide. Chemical modification of starch for example can be obtained by esterification of acid anhydrids, such as octenyl succinic anhydride (OSA), dodecenyl succinic anhydride (DDSA) fatty acids and inclusion of fatty acid chlorides with hydroxyl groups in starch molecules (Liu, et al., 2008; Nilsson & Bergenstahl, 2006; Nilsson, et al., 2007; Yusoff, et al., 2011), though of these only octenyl succinic anhydride (OSA) is currently a permitted food-grade reagent for the modification of starch (Liu, et al., 2008). Hydrophobically modified starch, produced by altering waxy corn starch, is now available as a commercial product and has begun to appear in some food formulations. Other modified starch include those derived from barley and potato (Nilsson, et al., 2007).

In this study we will solely be concerned with interfacial behaviour of molecularly adsorbed films of modified starch. This must be distinguished from an alternative way in which hydrophobic starch can become attached to the surface. Due to their hydrophobic groups, starch molecules can associate and remain as small granular particles dispersed in the

solution. It has been shown that these starch particles have a suitable surface chemistry and the required contact angle values in an appropriate range that allows them to adsorb at air-water or oil-water interfaces (Marefati, Rayner, Timgren, Dejmek, & Sjoo, 2013; Yusoff, et al., 2011). This in turn enables them to stabilise the emulsion droplets through the so called "Pickering mechanism" (Binks, 2002; Dickinson, 2010; Murray, 2007). Much like other types of Pickering emulsions, the stability of droplets covered by such starch granules is exceptionally good. However, the droplets do tend to be rather coarse (Marefati, et al., 2013; Sjoo, Emek, Hall, Rayner, & Wahlgren, 2015; Yusoff, et al., 2011), often having average sizes of several tens of microns. In contrast, in this study we shall focus on the adsorption and formation of macromolecular starch layers, which are reported to produce significantly finer emulsions < 1 μm (Chanamai & McClements, 2001; Chanamai, et al., 2002; Tesch, Gerhards, & Schubert, 2002). We note that to achieve this, it is often useful that the hydrophobic modification is carried out on starch hydrolysates, as for example those resulting from the action of α-amylase. Modification of enzymatically treated waxy corn starch has been shown to produce significantly enhanced emulsifying properties when compared with traditional modified waxy corn starch (Liu, et al., 2008). Presumably, enzymatic treatment prior to modification allows for a more efficient reaction that helps with the substitution of the hydrophobic groups. Also the lower molecular size of the produced chains accounts for their more rapid adsorption dynamics to the newly created interfaces, thus producing smaller emulsions. Furthermore, the intact modified starch molecules can be rather big with radii of gyration that can easily be as much as ~ 50 nm or so. This may not be desirable when one is producing sub-micron emulsions. As well as enzymatic reduction in the size of the starch chains, the application of high levels of shear has also been shown to be capable of causing a considerable degradation of the chains (Nilsson, Leeman, Wahlund, & Bergenstahl, 2006).

It is well known that starch derived from different plants, while all consisting of a mixture of amylose and amylopectin, have their widely contrasting properties with respect to the ratio of their branch to linear chains, additions such as phosphorylation of amylopectin, the degree of branching of the latter and of course the overall molecular weight distribution of the starch molecules. Even for the same species, starch obtained from crops grown under different environmental conditions can show large variations with regards to these mentioned parameters (Alvani, Qi, Tester, & Snape, 2011; Genkina, Wasserman, Noda, Tester, & Yuryev, 2004). This then poses several interesting questions regarding the hydrophobic

modification of the starch. For example, is the degree of hydrophobic substitution (DS) the same or does it vary with the molecular weight of the chains in the distribution, as well as with their degree of branching. Even if one supposes that the values of DS and the molecular weight of chains are identical, how different would the surface behaviour of the highly branched amylopectin fraction be, when they are compared to the more linear amylose? More specifically, one may ask which of these two types of starch, or perhaps a particular mix ratio, will produce the modified starch with the most optimum streric stabilising ability, for a given value of DS. In the current study we attempt to partially answer some of these questions, by performing theoretical calculations to obtain the magnitude of the repulsion forces that result from the overlap of different modified starch layers, adsorbed on the surface of emulsion droplets. Our method for performing these calculations is the technique originally proposed by Scheutjens and Fleer (1979, 1985), based on the well-known Self Consistent Field (SCF) theory in polymer science (Dolan & Edwards, 1975; Fredrickson, Ganesan, & Drolet, 2002; Grosberg & Khokhlov, 1994). It was originally introduced to the study of food colloids by Leermakers et al. (1996) and Dickinson et al. (1997; 1997) to study and compare the adsorption behaviour of two disordered milk proteins, α_{s1}-casein and β–casein, with each other. Since then, it has been successfully extended and applied by others and us to the study of a number of different mixed biopolymer layers (Ettelaie, Akinshina, & Dickinson, 2008; Parkinson, Ettelaie, & Dickinson, 2005), surface behaviour of protein-polysaccharide conjugates (Akinshina, Ettelaie, Dickinson, & Smyth, 2008), competitive displacement of protein and surfactant in mixed systems (Ettelaie, Dickinson, & Pugnaloni, 2014) and the influence of fragmentation of proteins (Ettelaie, Zengin, & Lee, 2014) such as that of κ-casein during renneting process (Ettelaie, Khandelwal, & Wilkinson, 2014; Mellema, Leermakers, & de Kruif, 1999) on the stability of the colloidal particle (e.g. casein micelles). Other slightly different SCF schemes have also proved useful in prediction of the onset of liquid crystalline phases in food related systems (Mezzenga, Lee, & Fredrickson, 2006).

In the next section we shall provide a brief sketch of our SCF calculations, since the scheme has been described in great detail as part of many studies reported in the literature. However, while several authors have applied such a method to chains containing a small number of branch points, to our knowledge highly branched polymers, such as amylopectin, have not previously been studied using the Scheutjens-Fleer SCF theory. Achieving this requires a

none-trivial extension to the scheme. Therefore, we give a more detailed account of the differences and the way that highly branched polymers were incorporated in the method, separately in appendix I. In section 3, we present our model and indicate how various parameters that specify the modified amylase and amylopectin in our calculations were chosen. In section 4, we provide the results of our SCF calculations and discuss and contrast the behaviour of hydrophobically modified branch and linear starch with each other. We also consider systems that contain a mixture of both of these two types of starch in order to investigate how the surface behaviour of each kind of starch alters, when the other component is also simultaneously present at the interface.

2. SCF calculation methodology

The purpose of the SCF calculations here is to evaluate the variation of the free energy of the system during the approach of two surfaces, immersed in a solution of appropriate macromolecules. The interaction potential between the two surfaces at a separation r is then given as the difference between the free energy of the system when the surfaces are separated by a distance r and when they are very far apart, i.e. $F(r) - F(\infty)$. To achieve this, first a statistical mechanical averaging is carried out over the position of all monomers comprising the chains and the solvent molecules. This procedure is rather elaborate and mathematically complex, but well established. The details can be found in a number of excellent review articles and books on the theory of polymers such as that of Doi and Edwards (1986) Lifshitz et al. (1978), Grosberg and Khokhlov (1994), Fredrickson et al. (2002) and using an alternative method based on polymer lattice models by Fleer, Cohen Stuart, Scheutjens, Cosgrove, & Vincent (1993). The outcome of this averaging process is a coarse-grained functional, that provides the free energy in terms of the variation of the concentration of different types of monomers across the gap between the plates. In principle any concentration profile can appear with a probability ~ $\exp[-F(\{\phi_\alpha\})/k_BT]$, where $F(\{\phi_\alpha\})$ is the free energy for that given set of density profile variation, T the temperature and k_B the Boltzmann constant. Thermodynamic quantities of interest can then be evaluated by averaging their values over all the possible density profiles, each appropriately weighted in accordance to their probability of occurrence. It turns out that carrying out this last part of the calculation is prohibitively difficult and therefore a suitable approximation is required. In SCF theory, this is achieved by assuming that the set of density profiles that minimises

$F(\{\phi_\alpha\})$ is also the one that overwhelmingly dominates the averaging. That is the say that all thermodynamic quantities of interest are essentially those obtained for this concentration profile that has the highest probability of occurrence. As such, the SCF theory shares the common feature of all mean field theories in that it ignores the fluctuations around this most probable profile. It has been argued that for problems involving dense adsorbed polymer interfacial layers, fluctuations in the density profile are generally small and the approximation a valid one (Fleer, et al., 1993).

Any monomer residue, residing at a position z in the gap between the two approaching interfaces, feels an effective field $\psi(z)$ which arises from the short ranged interactions that this residue experiences from its neighbouring monomers. If additionally the monomer also carries electric charge, then it will further interact with other charged monomers and ions in the solution, through the longer ranged Coulombic forces. In this study we assume that saccharide monomers that make up the starch molecules are not charged. In this case it can be shown that the field experienced by a monomer of type α, situated at position z, is given by

$$\psi_\alpha(z) = \psi_h(z) + \sum_\beta \chi_{\alpha\beta}\left(< \phi^\beta(z) > - \Phi^\beta\right) + \left[\delta(z) + \delta(z - a_o L)\right]\chi_{\alpha s} \qquad (1)$$

for the specific density profile that minimises the free energy (Ettelaie, Murray, & James, 2003; Evers, Scheutjens, & Fleer, 1990; Fleer, et al., 1993). In the above equation, $< \phi^\beta(z) >$ and Φ^β represent the average concentration of monomers of type β in the vicinity of point z and that in the bulk solution far from the gap, respectively. We take the range of these interactions to be of the order of the size of the monomers, denoted by a_0 from now on. Consequently, it is also useful to divide the distance between the two surfaces into layers of thickness a_0 parallel to surfaces. Thus in doing so, we represent the smooth spatially varying concentration profile in the gap between the two interfaces by a set of discrete values, specified inside each layer. We label these layers as l=1,2,… L, where $z=La_0$ is the position of the second interface relative to the first one. The strength of the short ranged interaction for two residues of different kind α and β is specified by the Flory-Huggins parameter between the two, $\chi_{\alpha\beta}$. Eq. (1) also involves a field component $\psi_h(z)$. This component, which acts equally on all monomers and solvent molecules irrespective of their type, ensures the incompressibility of the fluid and the requirement that $\sum_\alpha \phi^\alpha(l)$ remains a constant equal to

$\sum_{\alpha}\Phi^{\alpha}$, for every layer l in the gap. For residues in layers 1 and L there are also the added interactions with the interface to consider. Again the strength and nature of these are specified by the sign and magnitude of parameters $\chi_{\alpha s}$, appearing in the last term of Eq. (1). Positive values for a given species indicate the tendency of the monomers of this type to avoid the interface, while negative values show their affinity for adsorption.

For any set of fields, $\{\psi_a(l)\}$, specified for every type of monomer everywhere in the gap, it is a relatively straight forward task to calculate the resulting density profile, $\{\phi^{\alpha}(l)\}$. This is accomplished with the aid of the segment density functions $G_i^{(f)}(l;s)$ and $G_i^{(b)}(l;s)$, defined for every polymeric species i in the solution. The quantity $G_i^{(f)}(l;s)$ is the probability (to within a fixed normalisation coefficient) of finding a segment of the polymer, consisting of the first s monomers of the chain of type i, such that this segment ends in the layer l, i.e. has its s^{th} monomer residing in layer l. The s monomers can be chosen from either end of a chain. To distinguish these two possibilities for polymers that are not symmetrical, the segment density functions are labelled with subscripts (f) and (b). Note that the monomer sequence numbers for the forward (f) and backward (b) segment density functions are labelled in reverse order to each other. The great simplification in the calculation of the segment density functions occurs due to the connectivity of the chain, namely that if the monomer s is in the layer l, then the previous monomer, s–1, must have been in the same layer or one of the two adjacent layers l-1 or l+1. This simple observation leads to the following useful recurrence relation for G:

$$G_i^{x}(l;s)=\exp(-\psi_{t_i^{x}(s)}(l))\left\{\lambda_{-1}G_i^{x}(l-1;s-1)+\lambda_0 G_i^{x}(l;s-1)+\lambda_1 G_i^{x}(l+1;s-1)\right\} \quad , \qquad (2)$$

where the suffix x represents either (b) or (f). As in our previous work (Ettelaie & Akinshina, 2014; Ettelaie, Khandelwal, et al., 2014) we use the function $t_i^x(s)$ which evaluates to the type number for the monomer s. As will be discussed in the next section, in the current model of modified starch we have two kinds of monomers; a hydrophilic glycose group, which we denote as kind $\alpha = 2$, or a substituted hydrophobic ones where $\alpha = 1$. Additionally, we take the solvent molecules as being of type $\alpha = 0$. Note also that in Eq. (2), and throughout the rest of the paper, we express all energies and fields in units of k_BT. Similarly, we shall take

the unit of length as a_0, unless otherwise stated. The values of the coefficients λ_{-1}, λ_0 and λ_1 depend on the number of neighbours that a monomer has within its own layer and the two adjacent layers. Based on a cubic lattice representation, these values are chosen as $\lambda_0 = 4/6$ and $\lambda_{-1} = \lambda_1 = 1/6$.

Starting from either ends of a chain, for a segment of size one, we have $G_i^{(f)}(1,1) = \exp[-\psi_{t_i^f(1)}(1)]$ and $G_i^{(b)}(1,1) = \exp[-\psi_{t_i^b(1)}(1)]$. This allows us to begin the recursion procedure. Using Eq. (2) one can efficiently calculate all the segment density functions, up to and including those for the entire chain of type i. Finally, the determination of concentration of each type of monomer follows directly from the knowledge of the segment density functions in combination with the well-known composition law: (Evers, et al., 1990; Fleer, et al., 1993; Leermakers, et al., 1996)

$$\phi^{\alpha}(1) = \sum_i \frac{\Phi_i}{N_i} \sum_{s=1}^{N_i} \frac{G_i^{(f)}(1;s) G_i^{(b)}(1;N_i - s + 1)\delta_{\alpha,t_i^f(s)}}{\exp[-\psi_{t_i^f(s)}(1)]} \quad . \qquad (3)$$

In Eq. (3), Φ_i is the concentration of the biopolymers of type i in the bulk solution, N_i their size (number of monomer units) and the Kronecker delta function $\delta_{i,j}$ has its usual definition, evaluating to 1 if i=j and zero otherwise.

It is seen that Eq. (1) provides the values of the fields $\{\psi_a(1)\}$ from the corresponding concentrations $\{\phi^{\alpha}(1)\}$, while Eqs. (2) and (3) allow us to do the reverse. This is the basis for an iterative process in which one begins from an initial rough estimate of the values of the fields. The concentration profiles are calculated from (2) and (3) and are substituted back in Eq. (1) to yield a new set of "improved" fields. The process is repeated until no substantial changes in either $\{\psi_a(1)\}$ or $\{\phi^{\alpha}(1)\}$ are detected with further iterations. Thus the convergence produces a "self-consistent" solution, which is the density profile that minimises the free energy of the system for the desired gap size. The procedure described above applies to linear chains. For highly branched polymers, Eqs. (2) and (3) take on somewhat different forms. The modifications necessary are described in the appendix for completeness, but these differences apart, the overall procedure remains largely the same. Once the concentration profiles and the corresponding fields are determined at a given plate separation distance, these are simply substituted in the free energy expression $F(\{\phi_\alpha\})$ to yield its

required value at that gap size (Ettelaie, Dickinson, et al., 2014; Ettelaie, Khandelwal, et al., 2014; Evers, et al., 1990; Fleer, et al., 1993).

3. Models for hydrophobically modified amylose and amylopectin

Food grade starch consists of a mixture of a highly branched amylopectin fraction and an almost linear amylose component. The starch derived from different sources can vary widely with regards to the relative abundance of each type of chains. In typical corn starch, often used in the commercial manufacturing of hydrophobically modified starch, the relative ratio of amylose is around 20%, while in waxy corn starch this can be much smaller (Sweedman, Tizzotti, Schaefer, & Gilbert, 2013). The molecular weight of the amylose fraction is typically ~ 1×10^2 to 3×10^2 kDa (Mua & Jackson, 1997b; Neelam, Vijay, & Lalit, 2012), thus giving the number of glycoside residues for these chains from around 500 up to a few thousand. In contrast, highly branched amylopectin has a molecular weight that can be many tens of MDa (Mua & Jackson, 1997a). Nonetheless, it is known that this high molecular weight fraction is often subjected to enzymatic degradation prior to hydrophobic modification to improve the efficiency of the reaction (Tizzotti, Sweedman, Schaefer, & Gilbert, 2013). Furthermore, application of shear during the homogenisation and emulsification has been shown to reduce the molecular weight significantly (Modig, Nilsson, Bergenstahl, & Wahlund, 2006; Nilsson, Leeman, et al., 2006), down to values even lower than 1 MDa. It is the aim of this work to compare and contrast the adsorption behaviour and the steric stabilising properties of highly branched molecules with those of linear form. To this end, and in order to ensure that differences in molecular weights or other such parameters do not influence the results, we have chosen the number of saccharide monomers in both amylose and amylopectin models to be 1890. This value was chosen so as to be realistic for both groups of biopolymers, representing chains at the upper end of the weight distribution for amylose, while also encompassing the lower end tail for the degraded amylopectin. The architecture of our model amylose and amylopectin-like molecules is presented in Fig. 1. From experimental data, the degree of polymerisation between two branch points for amylopectin, obtained from a variety of different sources, tends to lie in the range of 5 to 35 monomers (Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008). In this study we shall take this to be 30 units, thus giving a total of 31 branch points for our model amylopectin. Out of the 1890 saccharides comprising each chain, 63 of these are hydrophobically

modified, setting the degree of substitution to DS = 3.3 %. For food grade modified starch, the substitution is carried out through attachment of short chains facilitated by the esterification of starch and octenyl succinic anhydride (OSA) under alkaline conditions (Tesch, et al., 2002). This is limited to no more than around 3%. The particular choice of DS made here means that there is exactly one modified residue for each strand between any two successive branch points. The similarity of all strands greatly simplifies our calculations, as is explained in appendix. We have chosen the substituted units to be relatively close to the branch points. Though this need not be the case in our model, we opted to do so influenced by recent experimental data which imply that the distribution of reacted saccharides favours those which are closer to the branch units (Bai, Kaufman, Wilson, & Shi, 2014). Our model-starch chains are flexible, random and disordered polymers. At sufficiently low temperatures, some polysaccharides do form secondary structures such as double helices. This makes the application of SCF theory to such molecules invalid. However, this is less of an issue with starch, particularly at higher temperatures and when it has also been hydrophobically modified.

The reaction of a sugar residue on the starch backbone with OSA, results in the attachment of a small hydrophobic side chain to the starch. Strictly speaking in our model one has to also represent this as so, with the side chain made up of 8 to 10 individual monomers. However, this greatly adds to the complexity of an already highly branched structure (Fig. 1.) making the calculations too slow and difficult to implement. Instead, in this study, we shall represent each hydrophobic side chain by a single monomer. However, we ensure that the degree of hydrophobicity (i.e. the interactions of this hydrophobic monomer with the solvent molecules) is of appropriate strength so as to represent the value attributed to the whole of the side chain. The strength of hydrophobic interactions is estimated to increase between $1.0k_BT$ to $1.5k_BT$ for each additional CH_2 group on an alkane chain (Hunter, 2000). Therefore the Flory-Huggins χ parameter for the interaction of our hydrophobic monomers with the solvent molecules is set to $12k_BT$. Similarly, the energy change associated with the adsorption of these groups onto a hydrophobic surface has a negative favourable value of $-12k_BT$ in the model. The same adsorption parameter is zero for the rest of the monomers forming the starch molecules, including those that are branch points. This indicates the hydrophilic nature and lack of any affinity for adsorption for these unmodified units. We also take water to be a good (athermal) solvent for the sugar moieties, again by setting the solvent-saccharide interaction parameter to $0\ k_BT$.

Finally, in SCF calculations it is customary to adopt a single size for all residues involved in the calculations. While this requirement is not strict, the overheads associated with relaxing it are usually quite large and yet found not to yield qualitatively different conclusions. Saccharide units have a size of ~ 0.9 nm, whereas the solvent water molecules are smaller ~ 0.3nm. As a compromise, we choose our nominal monomer unit to be a_0=0.5 nm. This will also be our unit of length in the calculations which follow, unless it is stated otherwise.

4. Results and discussion

4.1. Unmodified amylose and amylopectin.

It is instructive to begin by considering the interfacial behaviour of starch molecules, prior to their hydrophobic modification. Purely hydrophilic chains like unmodified starch, are expected to avoid the hydrophobic interfaces. This is mainly due to the conformational entropy losses suffered by these macromolecules, when they are in the proximity of such surfaces. The SCF calculated results for the variation of the polymer density profiles of both "amylose-like" and "amylopectin-like" chains, close to a hydrophobic interface, are displayed in Fig. 2. The bulk volume fraction of starch in each solution was 0.1 %. Both the branched and the linear starch molecules show a strong tendency to deplete from the interfacial region close to the surface. Further away from the interface the polymer concentrations return to the bulk value of 0.001. However, it is quite clear that the depleted region is considerably more extended for amylose, stretching for up to 20 nm away from the surface. This is despite the fact that both sets of chains comprise of exactly the same number of monomers. It is well known that a linear polymer exhibits a larger degree of swelling in a good solvent compered to its branched counterpart. The highly branched polymer is restricted in this respect by the constraints that are imposed on it by the presence of its cross-link points. On the other hand, this makes the behaviour of the more compact amylopectin somewhat similar to that for hard sphere like objects. For hard spheres, the structural ordering close to the interface leads to oscillations in the density profile, extending to distances of several particle diameters away from the surface (Dickinson, 1992; Hunter, 2000). While such oscillation also exist for polymers (Cates, 1998), the more flexible nature of the latter combined with the low polysaccharide volume fractions used in practice, greatly diminishes these. Nonetheless, even at the relatively low polymer bulk concentration used here, a maximum, arising from the

382 oscillations in the density profile, is quite noticeable at a distance of ~ 10 nm for the
383 amylopectin based system (Fig. 2).

384 The resulting depletion interactions for both systems are shown in Fig. 3, obtained for
385 emulsion droplets of size 1 μm. These interactions are essentially attractive, but as expected
386 are quite weak. At the droplet separation range considered here, the van der Waals forces
387 between the emulsion drops are much stronger than the biopolymer induced ones. For this
388 reason we have chosen to exclude these from the graphs of Fig. 3, thus allowing the energy
389 potential component solely arising from the presence of starch in solution to be more clearly
390 displayed. The more extended depletion zone for the amylose based system (Fig. 2) leads to
391 a longer ranged, and consequently also a more attractive depletion potential in comparison to
392 amylopectin. The oscillatory nature of the interactions is once again evident in the more
393 detailed graphs, shown in the insets of Fig. 3. For practical purposes, the energy barriers
394 arising in Fig. 3 (inset) have no real consequences, being all but too small to lead to any kind
395 of depletion related stabilisation mechanism (Hunter, 2000). This is even more evident from
396 the full interaction potentials, which also includes the van der Waals forces (not shown here).
397 Nevertheless, it is interesting to note that the energy barrier produced by amylopectin is
398 slightly larger than that for amylose, thus mirroring a similar trend as the density profile
399 variation seen in Fig. 2.

400 *4.2. Hydrophobically modified amylose and amylopectin at interfaces.*

401 The attachment of small hydrophobic side chains to some of the monomer residues of starch
402 turns it into an amphiphilic macromolecule. In our model this is achieved by simply
403 changing the interaction χ parameters for the substituted residues, along the lines described in
404 section 3. Altogether 63 monomers, out of a total of 1890, are altered to give a degree of
405 substitution DS = 0.033. A distinctive characteristic of many large amphiphilic
406 macromolecules is their extremely high degree of surface affinity and their strong tendency
407 for adsorption onto hydrophobic-hydrophilic interfaces, as fro example is readily
408 demonstrated by the adsorption behaviour of proteins (Dickinson, 1992; McClements, 1998).
409 This feature turns out to also be shared by modified starch. In Fig. 4 we present the predicted
410 adsorption isotherms, obtained from our SCF calculations over five decades of starch
411 concentration, ranging from a bulk volume fraction of $1\text{x}10^{-8}$ up to $1\text{x}10^{-3}$. The adsorbed
412 amount is obtained as the excess number of chains per unit area (a_0^2) in the gap between the
413 two surfaces, calculated at a sufficiently large L:

$$\theta_i^{ex} = \frac{1}{2}\sum_{l=1}^{L}\left(\phi_i(l) - \Phi_i\right) \quad . \tag{4}$$

The large distance between the two interfaces ensures that these are isolated from each other and do not perturb the adsorption on the opposite surface. The factor of half in Eq. (4) is there to to compensate for the presence of two interfaces in our setup. As the data in Fig. 4 shows, the adsorbed amount for both the branched and linear chains barely alters over the concentration range accessible to our numerical calculations, having already reached the saturation at $1x10^{-8}$ v/v. The surface excess amounts are 0.00109 chains per unit area of a_0^2 for the hydrophobically modified amylopectin and 0.00082 for amylose, at equilibrium with a starch volume fraction of $1x10^{-8}$ in the solution. At a much higher bulk concentration of $1x10^{-3}$ v/v, the corresponding values are hardly different at 0.00111 and 0.00085 chains per surface area a_0^2, respectively. The equilibrium between the almost saturated surfaces in contact with a minute amount of starch in the solution is indicative of the very high degree of surface affinity of both these hydrophobically modified biopolymers. Based on the results of Fig. 4 and with our chains each consisting of 1890 saccharide monomers, where 63 of these have been modified by attachment of octenyl succinic anhydride (Bhosale & Singhal, 2006; Sweedman, et al., 2013), we have estimated the saturated adsorbed amounts to be 2.3 mg/m^2 for the hydrophobically modified amylopectin and 1.8 mg/m^2 for the modified amylose. These values are somewhat larger than those we previously obtained for proteins (~ 1 mg/m^2) (Ettelaie & Akinshina, 2014; Ettelaie, Akinshina, & Maurer, 2012), but in line with experimental data by Nilsson and Bergenståhl, where values ranging from 1.5 to 16 mg/m^2 have been reported for modified starch (Nilsson & Bergenstahl, 2006). It must be pointed out that the molecular weight of our amylopectin-like molecules happens to be at the very low end of the size distribution for such biopolymers, and then in the partially hydrolysed or degraded samples (Nilsson & Bergenstahl, 2006; Nilsson, Leeman, et al., 2006). As shown later, increasing the size of the chains, even without altering the number of substituted residues (therefore an effective decrease in DS), can have a significant impact on elevating the amount of adsorbed starch.

For macromolecules with high adsorption energies, the saturation coverage is limited by the extent of excluded volume interactions that arise due to the overlap of chains deposited on the surface. Results from the previous section suggest that the overlap will occur at a lower surface coverage for amylose, relative to amylopectin of the same molecular weight, given

445 the more extended nature of the former. This then explains the greater level of saturation
446 coverage for the branched chains (Fig. 4) being up to ~ 30% higher than the branched starch.
447 It can also be inferred that if all else is kept the same, during the competitive adsorption
448 between these two biopolymers in mixed systems, it is the hydrophobically modified
449 amylopectin which is expected to dominate the surface. This point is discussed further in
450 section 4.4.

451 The thickness of the adsorbed interfacial layers formed by our modified starch molecules, and
452 the variation of the polymer density profile within them, is presented in Fig. 5 for each of the
453 two starch solutions at bulk concentrations of 0.001 %. The graphs show the biopolymer
454 volume fractions plotted as a function of distance away from the surface. The higher amount
455 of deposited amylopectin (solid line in Fig. 5) is once again evident at distances close to the
456 interface. But the more interesting feature of these graphs occurs at larger distances, from
457 around 8nm to 18 nm. To better highlight this feature, the volume fraction variation of the
458 chains over this range of distances is magnified by plotting the results on a finer scale, as
459 presented in the inset graphs of Fig. 5. The situation is now the reverse of what we had closer
460 to the interface, with amylose volume fraction now being higher than amylopectin at these
461 distances further from the surface. We also note that the polymer volume fractions are still
462 relatively high (~ 0.002 v/v) compared to their bulk values ($1x10^{-5}$). These results suggest
463 that while the interfacial layer consisting of amylopectin has a rather sharp and well defined
464 boundary with the surrounding solution (occurring here at ~ 8 - 9 nm), the outer part of the
465 hydrophobically modified amylose film in contrast is more extended and diffuse. This outer
466 section of the amylose layer is likely to consist of dangling ends of the linear chains that can
467 protrude further away from the surface.

468 To test the above idea, it is useful to consider how far from the interface each of the
469 monomers on the backbone of our adsorbed starch molecules are actually situated. In
470 calculating such data, we first note that it suffices to obtain the average distance away from
471 the interface for only a subset of monomers, located on a certain segment of the amylopectin.
472 This part of the molecule is highlighted in the schematic diagram of Fig. 6. Each monomer,
473 not included in the marked section, has an equivalent counterpart in the highlighted segment.
474 So for example, monomer (b) will have the same properties as monomer (a) in the marked
475 section. Similarly, residues labelled (d) and (f) will reside at the same average distance away
476 from the surface as monomers (c) and (e), respectively (Fig. 6). In Fig. 7, we present the
477 results of our calculations, showing the average position of monomers of amylopectin relative

to the surface (solid lines). The monomers are numbered sequentially from 1 to M_i along the marked segment of amylopectin, starting from monomer (a) in Fig. 6. We will denote the number of monomers in the highlighted part of amylopectin by M_i, so as to distinguish this from the overall size of the amylopectin molecules which was represented by N_i. For our "amylopectin-like" model here, $M_i = 151$. Also in Fig. 7 are included for comparison the same data for 151 consecutively chosen monomers of the hydrophobically modified amylose (dashed lines). The residues are chosen from one end of the molecule in Fig. 7a (1^{th} to 151^{th}) and from the middle section of the chain in Fig. 7b (870^{th} to 1010^{th}). The average distance is expressed in units of monomer nominal size, a_0. The graphs show that all the hydrophobically substituted monomers of the adsorbed amylopectin chains are in close contact with the interface, resting within a distance $1a_0$ of the surface. The same is also true for the hydrophobic residues of modified amylose, chosen from the middle sections of the adsorbed chains (dashed line in Fig. 7b). The hydrophilic parts of the biopolymer, between any two consecutive hydrophobes, are then seen to protrude in a loop like fashion for short distances away from the interface. Small kinks observed in the graphs for amylopectin (solid lines in Fig. 7a and 7b) arise from the fact that these are the positions of branch points in our model. As mentioned previously the modified sites were chosen to be close to such branch points. The location of one such branch point is highlighted in Fig. 7a by an arrow. While the same trends are seen in both Fig. 7a and 7b, it is quite noticeable that those hydrophobic monomers of amylose, situated close to either ends of the linear starch molecule, are no longer all completely resting on the surface. This can be deduced from the fact that the average distance for such substituted monomers is no longer $1a_0$, but several monomer size units away from the interface. As one moves towards the centre of the linear chain, the average position of these hydrophobic groups decreases (Fig. 7a) from a value ~ $5a_0$ down to $1a_0$. The presence of a small, but nonetheless significant amount of non-attached hydrophobic residues in the interfacial film manifests itself in the type of colloidal interactions that are induced by the overlap of such amylose layers. These interactions are studied next.

4.3. Interaction potentials.

The overlap of layers adsorbed on to two approaching surfaces gives rise to effective colloidal forces between the two interfaces. These interactions can be evaluated from the free energy data provided by SCF calculations, as was discussed in section 2. Furthermore, with the aid of the so called Derjaguin approximation (Hunter, 2000; Russel, Saville, &

Schowalter, 1992), it is possible to convert the potential between two flat surfaces to that mediated between two spherical particles. Applicable to cases where the radius of the particles, R, is much bigger than both the particle separation and the range of the expected interactions, Derjaguin approximation states that the force between two particles, $f_{pr}(r)$, is related to the interaction potential (per unit area) between two flat plates, $V_{pl}(r)$, according to equation $f_{pr}(r) = \pi R V_{pl}(r)$. Integrating the force then, one obtains the required energy potential between our two spherical particles:

$$V_{pr}(r) = -\pi R \int_{\infty}^{r} V_{pl}(z)\, dz \quad . \tag{5}$$

The colloidal interactions induced by adsorbed layers of hydrophobically modified amylopectin and their amylose counterparts are displayed in Fig. 8. The results were obtained for emulsion droplets of size 1 μm, immersed in 0.001% (v/v) solutions of each biopolymer. We have also included the direct attractive van der Waals interactions, given by $-AR/(12r)$ (McClements, 1998; Russel, et al., 1992), added to that mediated by the adsorbed modified starch in order to obtain the total pair potential for the particles. The value of the composite Hamaker constant, A, was taken as $1k_BT$, which is typical of edible food oils in water (Dickinson, 1992). For both the branched and the linear modified starch, a stable colloidal emulsion is predicted from the graphs of Fig. 8. Both graphs do show the presence of a shallow energy well, occurring at separations of 15 and 24 nm, for amylopectin and amylose. The depths of the corresponding minima are $2.6k_BT$ and $1.6k_BT$, respectively. These energy minima are easily overcome by a gentle degree of shear or even just the Brownian motion of the droplets themselves. However, the situation will be more problematic at larger droplet sizes, say 10 μm. The strength of the interactions tends to scale linearly with the size of the droplets (Hunter, 2000; Russel, et al., 1992) and therefore the predicted energy minima will be 26 and 16 k_BT, in these coarser emulsions. These are now quite deep enough to produce possible aggregation of the droplets, leading to faster creaming and possible coalescence. In same context it is also interesting to note that the energy minimum associated with amylopectin is substantially larger. This can be related back to the nature of the adsorbed layers discussed in the previous section. The amylopectin layers were found to be compact with well-defined outer edges (Fig. 5). Large steric repulsion arises as soon as two such interfacial films begin to overlap. The repulsive force is so strong that it simply dominates all the other possible colloidal interactions for this and at closer separation

distances. But the sharp boundary of the adsorbed film also means that at separation distances fractionally larger than the overlap, there is essentially no steric repulsion. The steric forces decays very rapidly once the particles are further apart than approximately twice the layer thickness, i.e. a distance ~ 16 nm in our model. At these distances, any van der Waals attraction as might exist between the particles can then manifest itself and lead to the minimum in the interaction potential for the amylopectin based system. This occurs at a particle separation of 15 nm, roughly twice the film thickness, as can be seen in Fig. 8. For modified amylose, the more diffuse but extended nature of the adsorbed films produces a longer ranged repulsion, albeit one that increases more gradually with decreasing distance than the one seen for amylopectin. Nonetheless, the repulsion is still sufficiently strong to overcome the van der Waals forces, thus resulting in a shallower minimum and one that occurs at larger droplet separations (24 nm).

The above discussion at first seems to suggest some merit in using hydrophobically modified starch that consists of a larger fraction of amylose. However, the graph of the interaction potential for amylose in Fig. 8 also shows the existence of a further energy minimum at a closer distance of 6 nm. Of course, there is a relatively large energy barrier (~ $15k_BT$) that a particle/droplet pair has to be overcome before they fall into this minimum. Therefore, any aggregation rate arising from the presence of this energy minimum is predicted to be slow, though still noticeable over long periods of storage. We believe that this potential well occurs as a result of the free unattached hydrophobically substituted residues, which as seen in Fig. 7, tend to be the ones residing at the extreme ends of the linear chains. The results of Fig. 7 also showed that such free residues were in contrast not present for the adsorbed amylopectin. The unattached hydrophobic groups can lead to bridging attraction (McClements, 1998) between the droplets, as the same chain can become adsorbed simultaneously to two approaching surfaces at the same time. Such interaction potentials have also been predicted by us (Ettelaie, et al., 2003) and others (Wijmans, Leermakers, & Fleer, 1994) in relation to linear hydrophobic-hydrophilic multi-block polymers. These types of polymers also suffer from the same type of tendency for bridging as the one expected here for the modified amylose.

The above results suggest several possibilities for improving the steric interaction potentials mediated by hydrophobically modified starch. Fortunately it turns out that these features are already largely present in modified starch systems used in practice. We shall briefly examine these in the final part of this section.

4.4. Effect of molecular size and mixing of amylose and amylopectin

Starch obtained from most sources is a mixture of amylose and amylopectin. Therefore, one may ask as to how hydrophobically modified starch containing a mixture of both linear and branched starch molecules will compare to either of these components alone, so far as the steric stabilising properties are considered. Furthermore, to make the comparison of the two types of starch molecules meaningful, we had used amylopectin with molecular weights that were at the very bottom end of the size distribution for these branched biopolymers. The larger size of amylopectin in real systems should in principle provide some improvements to both the range and the strength of steric force mediated by these macromolecules. Both of these issues are considered briefly in this section.

In Fig. 9, we display the calculated interaction potential curves between a pair of emulsion droplets of size 1 μm, resulting from the overlap of adsorbed amylopectin layers. In all cases the volume fraction of starch remaining in the solution was 10^{-5} v/v. As well as the original size (1890 monomers) used in the previous sections (solid line), two more curves for hydrophobically modified amylopectin are included. The dashed line represents the results for molecules roughly twice the original size, at 3810 monomers, while the dashed line is for chains almost half the size, at 946 monomers. In all cases the hydrophobic attachments were kept the same at 63. The adsorbed amount at a single isolated surface for the three different sized amylopectin chains were found to be largest for the small chains at 0.0028 chains per monomer unit area. The corresponding values for chains of size 1890 and 3810 monomers were 0.0011 and 0.0005 chains per a_0^2, respectively. The higher number of adsorbed chains predicted for the smallest chains is perhaps not surprising given the more compact nature of these, coupled with their higher level of hydrophobic substitution (DS=6.6 % for the smallest as oppose to 1.7% for our largest chains). With this information on the number of adsorbed chains, and using the molecular weight of saccharide moieties and OSA attachments, we estimate the adsorbed amounts to be 3.03, 2.33 and 2.1 mg/m^2 for each of the three sizes studied here, beginning with the smallest one. This then suggests that, provided the number of hydrophobic attachments is kept the same, the total amount adsorbed biopolymer is a weakly decreasing function of chain size, for these hydrophobically modified branched molecules. Despite the lower level of adsorption, it is found that the largest of our three starch molecules extends further and forms a thicker, and consequently less dense, surface

film. A visual inspection of the density profile variation, plotted as a function of distance away from the interface (similar to Fig. 5) for each of the three amylopectin cases, indicates the thickness of the layers to be ~ 6.5 nm, 8 nm and ~ 11 nm, for the 946, 1890 and 3810 sized chains. All layers do show a rapid drop of monomer concentration and a thus a relatively well defined edge, on their outer side. Despite its lower average monomer density throughout the film, the slightly greater extension of the adsorbed interfacial layer for the 3810 sized chains provides for a longer ranged steric force, as can be ascertain from Fig. 9. The graphs include the direct van der Waals potentials, added to the interactions mediated by the biopolymers. The increase in the thickness of the layer for larger amylopectin, results in a small reduction in the depth of the energy well from $2.6k_BT$ down to $2.0k_BT$ for 1 μm emulsions, or alternatively from $26k_BT$ to $20k_BT$ for 10 μm ones. To obtain fully stable emulsions for 10 μm droplets much larger chains are required, however, the molecular weight distribution for amylopectin can extend to tens of MDa. Therefore, it is quite conceivable that such large amylopectin are readily available in any hydrophobically modified starch. Furthermore, in the present calculation it is the total number of modified sites that we have chosen to keep constant. If instead one kept the same value of DS, it is possible that the predicted improvement in the steric stabilising behaviour of larger chains would have been even more pronounced. However, our early preliminary results do not seem to support this view. It seems that once a chain has a sufficiently large number of hydrophobic attachments to saturate the surface, further increases in the level of substitution does not produce a much larger level of adsorption. Our results once again confirm that the behaviour of highly branched modified amylopectin is more similar to hard sphere particles. There for hard spheres too, once the adsorption is high and a closed packed coverage of surface is achieved, no further increase in the number of adsorbed particles results by increasing their adsorption energy. We shall defer a more detailed investigation of the size dependence of adsorbed amount of modified starch, as well as the possible relevance of the locations of hydrophobic substitutions to a future publication.

The results of Fig. 8, show some draw backs for the steric stabilising properties of each type of hydrophobically modified starch, when used on their own. For amylopectin, the compact nature of the adsorbed layers leads to abrupt and rapid drop in the steric repulsion as inter-particle separation increases beyond the overlap distance of the two layers. In the absence of any electrostatic forces, to achieve good stability, particularly for larger emulsions (> 10 μm), one requires the presence of very high molecular weight starch molecules in the system.

Although such large amylopectin molecules are present in starch obtained from many different sources, it is beneficial to use smaller starch molecules where possible. Small chains have faster adsorption kinetics and thus likely to lead to finer emulsions. Secondly, if smaller starch molecules can be used, then one does not need to be duly concerned about degradation of the starch chains, as may occur for example under application of shear during homogenisation or during their hydrophobic modification.

Modified Amylose, compared to similar sized amylopectin, provides a longer ranged interaction, but in addition, also has the propensity for inducing bridging attraction too. This is quite similar to linear disordered protein molecules at pH values close to their pI (i.e. without any electrostatic repulsion component being present) (Akinshina, et al., 2008). Indeed the analogy between disordered proteins and hydrophobically modified amylose can be taken further. There are cases reported in the literature where the presence of a globular protein forming a compact film at the interface, in combination with a disordered protein, seems to greatly enhance the stabilising properties of the latter. One particularly interesting system of this kind is the mixture of β-lactoglobulin and sodium caseinate (Parkinson & Dickinson, 2004, 2007), where addition of a small amount of caseinate to adsorbed why protein layers, provides a degree of emulsion stability, otherwise not achieved by either component in isolation at these levels of surface coverage. This phenomenon has been attributed to the much extended conformation adopted by casein, when a compact thin layer of β-lactoglobulin is also present on the surface, in what was labelled as the "over grown" garden model by Parkinson et al. (2005). Theoretical calculations also point to a greater level of protrusion away from the surface in comparison to that at interfaces covered solely by the same amount of casein (Parkinson, et al., 2005). It is natural then to ask whether a combination of modified amylose and amylopectin may provide better stabilisation than possible with either one, based on a very similar mechanism. The Results of our calculations on mixed systems do indeed confirm this expectation. Fig. 10, displays the calculated induced interaction potential between two emulsion droplets of size 1μm, in a system with bulk starch volume fraction of 10^{-5} v/v in solution. In every other respect the system is identical to the ones that were presented in Fig. 8, apart from the fact that now 80% of the starch in solution is modified amylopectin and 20% of it amylose. To produce a mixed starch layer, it was necessary to slightly reduce the degree of hydrophobic substitution for amylopectin, from 63 down to 58, while that of amylose was increased to 66. Thus this left the average DS more or less unchanged. This was found necessary as it was observed that at

same level of modification, and with the same molecular weight chains, Amylopectin was overwhelmingly dominant on the surface, displacing all the amylose from the interface through the competitive adsorption process. With these minor changes the surface coverage of amylopectin was 0.0009 and that of amylose 0.00035 chains per monomer unit area. Comparison of the graph in Fig. 10, with those of Fig. 8, shows a clear improvement in the provision of a suitable steric repulsion. The minimum energy well is now only $0.55k_BT$ for 1 μm droplets, and even for 10 μm emulsions not particularly problematic at 5.5 k_BT. With slightly larger chains such as the one in Fig. 9, or more careful optimisation of the level of each starch component on the surface, the depth of the well could be reduced even further. However, it is important to note that now both of the desirable properties of having a longer ranged steric interaction, attributed to amylose, and lack of any bridging, associated with the amylopectin system, are present in the interaction potential of Fig. 10. It seems then that the presence of both modified amylose and amylopectin may be a useful and significant aspect of the emulsion stabilising ability of hydrophobically modified starch.

5. Conclusion

Hydrophobically modified starch provide one of the most promising routes in achieving food grade steric stabilisers that can provide emulsion stability under a wide range of pH, salt concentration and other environmental conditions. Nevertheless, very little work has been done in assessing how the architecture of starch molecules, in terms of the ratio of the linear amylose to branched amylopectin may influence the surface properties of such modified starch. In the current work we have systematically extended the Self consistent Field Theory (SCF) scheme of Scheutjens and Fleer (Scheutjens, et al., 1979, 1985) to deal with highly branched polymer structures. Using this we have conducted a theoretical study to compare the surface adsorption behaviour, and thus the steric stabilising ability of amylose and amylopectin. For unmodified starch both sets of starch molecules lead to depletion, as expected and demonstrated experimentally (Chanamai, et al., 2001). But more interestingly, it is found that for chains of equal molecular weight, the depletion induced by amylose is both stronger and longer ranged. This is attributed to the more swollen nature of the linear chains compared to their branched counterparts. Upon a sufficient degree of hydrophobic substitution (DS), both amylose and amylopectin adsorb at hydrophobic interfaces, though the level of surface coverage is found to be higher for amylopectin, at the same MW, bulk

concentration and amount of hydrophobic modification. It was also observed that amylopectin formed a somewhat thinner, but subsequently denser interfacial layer than that resulting from amylose. The outer edge of the amylopectin layer was also sharper and better defined, as demonstrated from the calculated results for the biopolymer density profiles away from the surface.

While both the linear and branched modified starch were able to provide a reasonable level of colloidal stability in 1 μm emulsion systems, there were sufficiently large energy wells in the mediated interaction potentials for 10 μm droplets to cause their aggregation. The depth of energy well was distinctly larger for amylopectin. This is understandable given the less extended surface films formed by the branched biopolymer. Furthermore, the sharp boundary of the amylopectin layers means that for separation distances only slightly above the point of the overlap of the layers, there is hardly any steric repulsion present. Hence, any attractive van der Waals forces, as there may be between the surfaces, will be completely dominant at these distances. The molecular weight of our model chains was estimated to be ~ 320 KDa. We have shown that the stabilising property of modified starch improves with increasing size of the biopolymers, but rather more slowly than a linear dependence. Thus for amylopectin at least, where much larger chains with molecular weights of several tens of MDa are usually present, it is not inconceivable that sufficient stability can be obtained even for coarser emulsions. Nonetheless, it is often more useful to achieve such stability by using smaller sized macromolecules, not only to have a faster adsorption kinetics, but also since some degradation of the starch is likely during the homogenisation process.

Amylose films were found to be more diffuse and extended. They provide longer ranged interactions with a more gradual increase of steric forces as the polymer layers overlap. However, the linear chains are also more prone to forming bridges between neighbouring surfaces, where the amylose molecules become simultaneously adsorbed on both interfaces. It is shown that this is due to a small but nonetheless significant number of non-adsorbed hydrophobic attachments, mainly at the extreme ends of the modified amylose. These can adsorb onto other approaching surfaces at sufficiently close separations. This phenomenon leads to an attractive contribution to the polymer mediated interactions.

Perhaps the most interesting prediction of this work is that a suitable combination of hydrophobically modified amylose and amylopectin is able to eliminate both of the above issues. For equal sized chains, with the same degree of modification, amylopectin adsorbs

preferentially, to an extent that it almost completely removes all amylose from the surface. By slightly decreasing the value of DS for the branched chains and increasing it for amylose, so as not to change the average value too much, we have managed to have mixed layers in our model, where ~ 25% of the adsorbed starch on surface was amylose. These mixed layers provide a noticeably longer ranged steric repulsion than seen with either component alone, at the same overall bulk concentration. We suspect that this phenomenon is very similar to that reported by the experimental (Parkinson, et al., 2004, 2007) and theoretical studies of Parkinson et al.(2005). In their system too, a mixture of two biopolymers, sodium caseinate and β-lactoglobulin, were found to be much more effective in stabilising emulsion droplets. Parkinson et al attributed this to the greater extent of stretching of the caseinate protein (Parkinson, et al., 2005), when this protein was simultaneously present with an additional adsorbed compact layer of β-lactoglobulin at a surface. The linear hydrophobically modified starch behaves in an analogous manner as casein does in their system, with the dense but thinner layers formed by adsorption of branched amylopectin playing the role of the β-lactoglobulin films.

It is rather fortunate that in almost all cases starch already consists of a mixture of both amylose and amylopectin. Nevertheless, further theoretical studies, combined with experimental work involving well-defined and carefully controlled mixtures of these two components, should provide us with a deeper insight into possible ways of optimising the surface properties of hydrophobically modified starch, in future.

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Appendix

Accounting for lightly branched polymers within the usual SCF calculation scheme of Scheutjens and Fleer (Scheutjens, et al., 1979, 1985), as was highlighted briefly in section 2, is a relatively straight forward task and has been attempted previously (Akinshina, et al., 2008; Ettelaie, et al., 2003; Fleer, et al., 1993). However, as the number of branching points increases, the numerical calculations become unfeasible. For highly branched structures, such as our model amylopectin, it is necessary to modify the scheme in order to deal more efficiently and systematically with the large degree of bifurcation occurring in the chain. Also we wish to take advantage of the identical nature of the polymer sub-branches that emanate from the same cross-linking point (see Fig. 1). To do so, we recall that every monomer in our model amylopectin has one identical residue on the highlighted subset of monomers shown in Fig. 6. Therefore, the computation of the forward and backward segment density functions, volume fractions and all of the other quantities that enter the SCF calculations need only be performed for the monomers in the marked segment (Fig. 6). The number of monomers in this subset, M_i, is significantly less than all the total monomers, N_i, that make up the amylopectin chain. For the calculation of forward segment density functions, we first label all the monomers in the highlighted section from 1 to M_i consecutively, staring with the monomer (a) in Fig. 6. For each of these residues, we specify a branching number B(s) that provides the number of identical sub-branches that emanate from the monomer s. The values of B(s) for all monomers in the set provide the necessary information specifying the architecture of our biopolymer. If a monomer is not a branch point then B(s) = 1. When bifurcation does occur at any monomer s in our amylopectin representation, then B(s) = 2 (Fig. 6). Of course, for architectures more complex than that of amylopectin, B(s) can be larger than 2 where required. Now, as well as the usual segment density functions $G_i^{(f)}(l;s)$ introduced in section 2, we also define a "branched segment density function", $\Theta_i^{(f)}(l;s)$, for each monomer s such that

$$\Theta_i^{(f)}(l;s) = \frac{[G_i^{(f)}(l;s)]^{B(s)}}{[\exp(-\psi_{t_i^f(s)}(l)]^{(B(s)-1)}} \qquad (6)$$

While $G_i^{(f)}(l;s)$ is the probability of a single branch of the chain, consisting of all the residues up to and including monomer s, ending in layer l (i.e. monomer s to be found in

layer l), $\Theta_i^{(f)}(l;s)$ provides the same data for all such B(s) identical sub-branches terminating in s. Obviously where no splitting occurs at monomer s, then the value of $\Theta_i^{(f)}(l;s)$ and $G_i^{(f)}(l;s)$ should be identical. This is indeed the case as can be seen from Eq. (6), whenever B(s) = 1. The recursive relation, Eq. (2), can be applied to generate the segment density functions for each monomer s, using the knowledge of the functions for the preceding residue, s−1. To account for the possible bifurcations occurring at all the previous monomers prior to s, the recursive relation now takes on the form

$$G_i^{(f)}(l;s) = \exp(-\psi_{t_i^f(s)}(l)) \left\{\lambda_{-1}\Theta_i^{(f)}(l-1;s-1) + \lambda_0\Theta_i^{(f)}(l;s-1) + \lambda_1\Theta_i^{(f)}(l+1;s-1)\right\} . \quad (7)$$

As in section 2, $t_i^f(s)$ evaluates to the type number for the residue s; this being 1 for the monomers with hydrophobic attachments and 2 for unsubstituted hydrophilic ones. Using Eqs. (6) and (7) together, one can readily compute all $G_i^{(f)}(l;s)$ and $\Theta_i^{(f)}(l;s)$, starting from the known initial condition $G_i^{(f)}(l,1) = \exp[-\psi_{t_i^f(1)}(l)]$ for first monomer (i.e. residue (a) in Fig. 6).

Every monomer in amylopectin joins up two otherwise separated parts of the molecule together. This follows from the fact that the amylopectin structure does not involve any loops. While $G_i^{(f)}(l;s)$ denotes the segment density for one of these parts, one can also define a complementary segment density function involving the other section. This provides the probability that a polymer, solely consisting of this other section, will have the monomer labelled s in layer l. For a linear chain this is simply the backward segment density function $G_i^{(b)}(l;s)$ already discussed in section 2. For a branched chain this has to be modified to read

$$\Theta_i^{(b)}(l;s) = \frac{G_i^{(b)}(l;s)[G_i^{(f)}(l;M_i - s + 1)]^{(B(s)-1)}}{[\exp(-\psi_{t_i^b(s)}(l)]^{(B(s)-2)}} . \quad (8)$$

If monomer "s" is not a branch point then we simply have $\Theta_i^{(b)}(l;s) = G_i^{(b)}(l;s)$, as is expected. Once again, it suffices to calculate $\Theta_i^{(b)}(l;s)$ for the indicated subset of monomers of the model amylopectin (Fig. 6). Also note that it is more convenient to use a reverse labelling of monomers for "backward" segment density functions, $\Theta_i^{(b)}(l;s)$ and $G_i^{(b)}(l;s)$,

where now the residue (a) in Fig. 6 becomes the M_i^{th} monomer, and not the first. More generally, a monomer labelled s in the "forward" counting case will be have the sequence number ($M_i - s + 1$) in reference to the "backward" functions $G_i^{(b)}$ and $\Theta_i^{(b)}$. The corresponding recursive relation for the "backward" segment density functions is the same as Eq. (7), except with the suffix (f) now replaced with (b) everywhere. This relation, in combination with Eq. (8) and the initial condition $G_i^{(b)}(l,1) = \exp[-\psi_{t_i^b(1)}(l)]$, is all that is needed to determine the "backward" segment density functions $G_i^{(b)}(l;s)$ and $\Theta_i^{(b)}(l;s)$, at every layer l in the gap between the two surfaces, and for all monomers.

The final necessary modification to the scheme concerns the compositional law from which the volume fraction of each type of monomer in every layer is calculated. This is obtained by considering the probability that the two separated sides of the chain, joined together by residue s, both end up at the same position (i.e. at the position of monomer s) (Evers, et al., 1990; Scheutjens, et al., 1979), thus leading to:

$$\phi^{\alpha}(l) = \sum_i \frac{\Phi_i}{N_i} \sum_{s=1}^{M_i} \frac{n(s) G_i^{(f)}(l;s) \Theta_i^{(b)}(l;M_i - s + 1) \delta_{\alpha, t_i^f(s)}}{\exp[-\psi_{t_i^f(s)}(l)]} \quad , \qquad (9)$$

The forward labelling convention for monomers is adopted in the above equation. The equation also involves an additional set of quantities, n(s). This is simply the number of residues that are identical (with regards to their positional hierarchy in our biopolymer architecture) to each of the monomers in the highlighted section of the chain. Thus for example n(1) = 32 in our model, as there are a further 31 monomers that are exactly identical to monomer (a) and therefore will have the same volume fractions and probabilities of residing in each layer l (see Fig. 6). For monomers (c) and (e) in the marked subset, the values are 16 and 2, respectively, with monomer (f) being the only other one similar to the latter.

The above modifications apart, the rest of the SCF calculation can now proceed in exactly the same manner as the one already described for the linear chains.

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