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Colloidal Interactions Induced by Overlap of Mixed Protein + Polysaccharide Interfacial Layers

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Abstract

Colloidal interaction potentials induced by the overlap of mixed protein + polysaccharide interfacial layers, formed solely as a result of electrostatic attraction between these two biopolymers, have been calculated using the Self Consistent Field Theory. A significant difference between the nature and magnitude of these interactions, depending on the manner in which the charge is distributed along the length of the polysaccharide molecules, was predicted. For chains with an even distribution of charge, the repulsive interactions are in general weaker than those mediated by pure protein layers. For strongly charged polysaccharide chains, these become even attractive at a certain range of particle-particle separations. In part this is due to bridging by polysaccharides, occurring between opposite layers. However, in systems containing strongly charged polyelectrolyte, it is also the result of what in practice may be interpreted as a coacervate of protein + polysaccharide, with a tendency for aggregation, forming interfacial layers on the surface of the particles. In contrast, when the charge of the polysaccharide chains is unevenly distributed, the induced repulsive forces are much enhanced and become longer ranged compared to those for pure protein layers. Once the layers begin to overlap, the electro-steric interactions produced are found to completely overwhelm any van der Waals attraction, thus dictating the inter-particle interactions. We also present some preliminary calculations investigating the competitive adsorption of different polysaccharides onto the protein layer. The initial results, for polysaccharides of the same size and overall charge, suggest that the heterogeneously charged polyelectrolyte completely dominates the adsorption onto the surface, displacing all uniformly charged chains from the interface.

Keywords: Colloidal interactions, Mixed protein + polysaccharide interfacial layers, Multilayers, Self consistent field theory, Non-uniformly charged Polysaccharides
1. Introduction

Understanding the behaviour of mixed biopolymer systems and elucidating the nature of colloidal interactions in food systems have been two fundamental themes of long standing interest to Professor Eric Dickinson, throughout the course of his distinguished research career (E. Dickinson, 2003, 2008, 2009; E Dickinson, 2010; E. Dickinson, 2011, 2013; Radford & Dickinson, 2004; Semenova & Dickinson, 2010). It is a privilege for us to present the current work, which touches upon both of these two areas of research, in a special issue of the journal dedicated to Professor Dickinson. The work is the latest in a series of papers attempting to theoretically investigate the behaviour of mixed interfacial layers, resulting from the electrostatic interactions between proteins and oppositely charged polysaccharides (Ettelaie, Akinshina, & Dickinson, 2008, 2009; Ettelaie, Akinshina, & Maurer, 2012). In particular, it is the nature of colloidal interactions arising from the overlap of two mixed protein + polysaccharide layers, formed during the simultaneous adsorption of these biopolymers, which is the focus of the attention here. The aim is to relate the predicted interactions for different polysaccharides, having contrasting degrees of charge distribution along their back bone, to the interfacial structures that arise for each case. The influence of the charge distribution of polysaccharides on the structure of mixed interfacial films, formed by these and proteins molecules, has already been reported in a separate publication of our own (Ettelaie, et al., 2012) and those of others (Dobrynin, 2005; Patel, Jeon, Mather, & Dobrynin, 2005, 2006).

The idea of depositing different polyelectrolytes from a solution, on top of each other in a sequential manner, with the aid of electrostatic interactions, so as to produce a multi-layered film is widely attributed to Decher (Decher, 1997; Decher, Hong, & Schmitt, 1992). At each stage of the deposition, the charge of the polyelectrolyte used is opposite to that in the previous stage (Jaber & Schlenoff, 2006; Schonhoff, 2003). The procedure has come to be known as the layer-by-layer (LbL) deposition technique. The potentials of the method for the fabrication of films with relatively complex but yet well controlled structures, have been explored in many varied fields of technology, since its introduction (Agarwal, et al., 2010; Aldea-Nunzi, Chan, Man, & Nunzi, 2013; Carosio, Alongi, & Malucelli, 2013; Du, Yang, Zhang, & Jiao, 2009; Johnston, Cortez, Angelatos, & Caruso, 2006; Kochan, Wintgens, Wong, & Melin, 2010; Podsiadlo, et al., 2009; Ramsden, Lvov, & Decher, 1995; Song, et al., 2013). The technique has been extended to include deposition of alternating layers of
polymers and small nanoparticles (B. S. Kim, Park, & Hammond, 2008) and, more recently, vesicles (Roling, et al., 2013).

In the context of food systems, the influence of the complexation process between protein and polysaccharides on the stability of emulsions was recognised a few decades ago (Gurov, Mukhin, Larichev, Lozinskaya, & Tolstoguzov, 1983; Larichev, Gurov, & Tolstoguzov, 1983). Likewise, the interfacial tension and surface rheology of such complexes at oil-water interfaces was investigated by Dickinson & Pawlowsky (E. Dickinson & Pawlowsky, 1996, 1997). However, the possible advantages of using protein + polysaccharide to make more stable emulsions were first explored systematically through a series of studies by McClements and co-workers (Guzey & McClements, 2006a, 2006b, 2007; McClements, et al., 2005; McClements, Decker, Park, & Weiss, 2009; Paliandre, Decker, & McClements, 2007). Using an approach similar to that of the initial stages of the layer-by-layer deposition, a film of polysaccharide was adsorbed onto the surface of emulsion droplets, already stabilised by protein. By appropriate choice of pH, it is possible to insure that the net charge of the protein and polysaccharide are opposite to each other. Thus, this entices the otherwise hydrophilic polysaccharide, driven by the electrostatic attraction to the primary protein layer, to accumulate at what is essentially a hydrophobic interface, namely the surface of the oil droplets. Of course, this is not the only way that one can arrange for polysaccharides to adsorb onto hydrophobic surfaces. Alternative methods have also been investigated. In particular, forming conjugates between protein and polysaccharide, through covalent bonding of the two, is another possibility that has been examined experimentally (Akhtar & Dickinson, 2003, 2007; E. Dickinson & Semenova, 1992) and studied theoretically (Akinshina, Ettelaie, Dickinson, & Smyth, 2008). Yet a different technique involves chemically altering some of the groups in the polysaccharide by making them more hydrophobic. This approach is best exemplified by the hydrophobic modification of starch (Nilsson & Bergenstahl, 2006). Nevertheless, all these other methods, in the strict sense, involve formation of new molecules. It is only LbL deposition that solely explores the physical associations to induce the adsorption of otherwise hydrophilic polysaccharides to hydrophobic surfaces.

It has been argued that polysaccharide layers provide much stronger repulsion between oil droplets. Polysaccharide chains are normally much larger macromolecules than proteins. Hence, their adsorption at interfaces results in much thicker layers, ensuring that repulsion
between emulsion droplets comes into operation at significantly larger separation distances. Secondly, the nature of repulsive interactions induced by the overlap of approaching polysaccharide layers is predominately steric in origin. This is in contrast with protein layers, where a significant component of the induced repulsive force is electrostatic and thus dependent on the charge of the protein. This tends to make the protein stabilised emulsions susceptible to colloidal instability through aggregation and subsequent coalescence, at high salt concentrations and even more noticeably at pH values close to PI of the protein used (around 4 to 5 pH units for casein stabilised emulsions). Initial experimental result on emulsions stabilised by protein + polysaccharide layers have largely confirmed these expectations. For example, emulsions stabilised by β-lactoglobuline and pectin showed a much better resistance to salt induced aggregation than those made with β-lactoglobuline alone (Guzey, Kim, & McClements, 2004; Guzey, et al., 2007). Similarly, emulsions having multi-layers consisting of caseinate + alginate (Paliandre, et al., 2007) or β-lactoglobuline + chitosan (Hong & McClements, 2007) were reported as having a considerably better stability at pH values close to PI of protein, where protein only stabilised emulsions were seen to be unstable. Furthermore, using a third sub-layer of deposited biopolymer, Guzey et al (2006b) demonstrated the superiority of emulsions thus produced under elevated temperatures up to 60 °C. Such emulsions, stabilised by tertiary multi-layers, were found to possess better freeze-thaw cycling properties (Gu, Decker, & McClements, 2007). The use of multi-layered stabilised emulsions has also been explored in relation to the formation of a barrier against the diffusion of lipase and in prevention of oxidation of oils (Gudipati, Sandra, McClements, & Decker, 2010; Li, et al., 2010). It is argued that such layers retard the intake of fats and can potentially provide a way of designing emulsions with specific controlled digestibility.

Apart from its importance in relation to food systems, the above studies of McClements and co-workers were notable in one other aspect. Up to that point, much of the work involving LbL deposition technique concerned macroscopically sized substrates. The above studies attempted to use the surface of oil emulsions as the template for the stacking of the multi-layers. This immediately poses two problems specific to such mesoscopicaly sized surfaces. Firstly, throughout the process of deposition the colloidal integrity of the emulsion system has to be maintained (Guzey, et al., 2006a). It is well known that macromolecules, while capable of inferring stability to colloids, can also cause the aggregation of these, as for example through the mechanisms of depletion or bridging (E. Dickinson, 1992; Hunter,
Secondly, with applications involving relatively large substrate (e.g. fibres, sensors, non-linear optical devices, etc), normally the treated surface is dried once the multi-layer has been deposited on it. This is not the case for food colloids. Drying of the substrate has a dramatic effect on the mobility of the polymer chains and hence their possible intermixing between different sub-layers. Essentially, the interfacial film structure obtained prior to drying is locked in, perhaps indefinitely. In contrast, biopolymers, in various sub-layers on the surface of an emulsion droplet, are subject to significant inter-diffusion. This may cause the structure of the multi-layer to evolve and alter with time. In fact the presence of substantial inter-diffusion in multi-layers, as well as lateral diffusion, has been reported by Yoo et al (2008). Other evidence for changes in the structure of multi-layers come from the work of Jourdain, Schmitt, Leser, Murray, & Dickinson (2009). In this study, interfacial adsorbed films were formed on n-tetradecane-water interfaces in two different ways, involving mixtures of sodium caseinate and dextran sulphate. The first involved a sequential adsorption of the polysaccharide onto an already existing primary layer of protein, much as is normally done in the LbL type deposition. For the second method, the adsorption occurred simultaneously from a mixed solution of dextran and caseinate. The films obtained by these two contrasting procedures initially showed quite different interfacial rheologies. But following ageing of the films for a few days, the rheological behaviour of the two mixed layers evolved towards similar values. This was interpreted as indicating that the two mixed interfacial films, adsorbed in these two different ways, initially had very dissimilar structures, but that in the course of time evolved towards the same equilibrium state. Clearly it is of some advantage if it can be arranged that the equilibrium configurations maintain some of the desired features required from the multi-layers. In the context of the present work this relates to the ability of the interfacial films to continue to provide the strong repulsion between the oil droplets. The molecular size of the polysaccharides, their architecture (e.g. linear or branched), the magnitude of the charge they carry and the manner in which this charge is distributed along the chains, are all possible parameters that can be exploited to achieve this purpose. The work here theoretically examines the last of these two factors. In particular, selective de-esterification of high methoxyl pectin, either through enzymatic means or otherwise, can nowadays be used to produce a variety of pectin molecules ranging from chains with quite uniform charge densities to much more blockwise ones (Y. Kim, Teng, & Wicker, 2005; Limberg, et al., 2000; Lutz, Aserin, Wicker, & Garti, 2009; Willats, et al., 2001).
The paper is organised as follows. In the next section we shall first review and summarise some of the key results we found in our previous study (Ettelaie, et al., 2012) with regards to the structure of single isolated protein + polysaccharide interfacial layers. These are presented first, as they are of particular relevance to the discussion of the data we obtain in the current work. Next, we shall outline our methodology and the model, which again are largely based along the same lines as those reported before (Ettelaie, et al., 2012). In section 4 the results of the calculations showing the interactions mediated by the overlap of mixed layers, involving polysaccharides with a variety of charges and charge distributions, adsorbed on the surface of two approaching colloidal particles, are presented. Finally, the nature of these induced forces and their relation to the structure of the mixed layers, for each one of the model polysaccharides, is discussed in greater detail.

2. Structure of mixed layers: relation to charge and charge distribution of the polysaccharide

The colloidal interactions between two emulsion droplets are the result of the overlap of adsorbed layers on the surface of the two droplets. This leads to steric forces between the surfaces, which together with the electrostatic interaction, resulting from the charge of the interfacial films, are responsible for the colloidal stability of the emulsion. For mixed or multi-layers, the composition of the adsorbed films, their thickness, the configuration of each biopolymer species and the distribution of charge within the layers, are all factors that are strongly influenced by the blockiness and strength of charge carried by polysaccharides chains. Using self consistent field (SCF) calculations, we had studied the structure of protein + polysaccharide films for a single isolated adsorbed layer (Ettelaie, et al., 2012). The model we adopted for the protein was based on the primary structure of αs1-casein. For polysaccharide we considered the chains as consisting of two kinds of monomers, one with a high, more negative charge and the other with a lower value. This allowed for a non-homogeneous charge distribution along the polysaccharide to be specified in the model. The calculations were performed at pH values (pH = 3), below pI of protein where its net charge is positive. The results highlighted a number of interesting behaviour, including several findings that had tentatively been hinted at in other simulation studies (Dobrynin, 2008; Patel, et al., 2005, 2006). These are summarised below.
It was found that the excess number of adsorbed polysaccharide at the interface was not a simple monotonically increasing function of the charge of the polyelectrolyte. At first, beginning with neutral chains, the amount of adsorbed polysaccharide increases as the macromolecules are made more negative. In particular, the polysaccharide charge has to be sufficient to entice the chains to adsorb to the surface, given that in these models no other favourable interaction between protein and polysaccharide was presumed. As the polyelectrolyte deposits on the surface, the polymer chains loose configurational entropy. This is due to the more restrictive environment that is imposed by the interface limiting the number of conformation that chains can adopt. However, this lose is compensated by the enthalpic gains arising from their attraction to the positively charged protein molecules, already present at the interface. If the interaction between the two biopolymers were specific and short ranged in nature, then the amount of adsorbed polysaccharide would continue to increase, eventually only plateauing out due to the packing and other similar steric constraints, as the strength of the interactions were made stronger. This is not what is found for electrostatic interactions, beyond a certain level of charge (Ettelaie, et al., 2012). At this optimum charge, the adsorption attains its maximum and then decreases as chains are made more negative, despite the stronger attractive interaction between the protein and polysaccharide. The result is not all that surprising though. Adsorption of the polyelectrolyte on (or into) the protein layer neutralises and then reverses the electric charge of the interfacial layer. As this happens, the attraction of polyelectrolytes to the interface begins to switch to repulsion. This reversal is achieved by a far smaller number of adsorbed chains when the molecules are strongly charged. This then explains the drop in the amount of adsorbed polysaccharide, predicted by the calculations, as they become more negatively charged.

The SCF calculations also highlight significant differences in the structure and the thickness of the interfacial mixed films between lightly charged and strongly charged polysaccharides. These differences are best summarised pictorially by the schematic diagrams in figure 1. For weakly charged chains, there isn’t much adsorption of the polysaccharide (Fig. 1a). However, the limited numbers of chains that do adsorb tend to make relatively large loops, protruding away from the surface, presumably due to thermal fluctuations overcoming the relatively weak attraction towards the protein sub-layer. As the chains are made more negative (from an average charge density of -0.05e to -0.1e per monomer), the amount of adsorbed polyelectrolyte increases dramatically. Nevertheless, no huge differences were
found between the thicknesses of the films in Figs. 1a and 1b (Ettelaie, et al., 2012). In fact, if anything, the interfacial film in figure 1b was found to be marginally thinner, but of course consisting of a higher density of polysaccharide chains. The polyelectrolyte continues to make large loops extending away from the surface in Fig. 1b. The configurations adopted by the protein chains were also found to be quite similar in these two systems. Fig. 1c shows the change in the structure of the film when the charge density of polysaccharide is made even more negative (-1.0e per monomer). Now the number of adsorbed chains is small once again, with polysaccharide molecules also adopting quite different conformations to those in the previous cases. The chains lie quite flat on the surface, overlapping strongly the protein sub-layer. The strong attraction between protein and polysaccharide prevents any significant protrusion of the adsorbed polyelectrolyte into the bulk. In fact in such cases there are no regions of the interfacial film that consist solely of polysaccharides. Such films are best regarded as mixed interfacial layers, rather than multi-layers, in our opinion. Interestingly, it was also seen that the protein in the mixed layer extended somewhat further in this later system than those involving polyelectrolytes of lower charge. This is likely to be due to the more intimate incorporation of polysaccharide within the protein layer, causing the latter to extend more than otherwise it would do in pure \( \alpha_{s1} \)-casin layers.

The above discussions focused on polysaccharides where the charge was uniformly distributed along the molecules. With blockwise polysaccharides, such as pectin having varying degree of charge for different sections, the lightly charged blocks have to compete with the stronger charged parts for adsorption. As the contrast between the charge densities of different parts becomes more pronounced, it becomes favourable for some of the weakly charged sections to desorb away from the protein layer, thus allowing a larger number of chains with their strongly charged blocks to adsorb in their place. In our calculations we used a simple diblock type model to represent such heterogeneously charged polysaccharides. The chains were considered as comprising of a small, highly charged end, followed by a much longer but lightly charged section. For such a model, the conformation of polysaccharides changed from that of polymers lying relatively flat on the surface, to considerably more extended brush like configurations, protruding well into the bulk. Again the schematic pictures in Fig. 2 capture the main conclusions of our pervious SCF calculations for such systems (Ettelaie, et al., 2012). It is noticed that now the extended, less negative blocks, form a region of the interfacial film further away from the actual surface and on top of the sub-
layer mainly consisting of protein. Note that the SCF calculations deal with the equilibrium states. Therefore, such interfacial films, formed by protein and the heterogeneously charged polysaccharides, should remain multilayered even after aging. In fact, the system should actually evolve towards such multilayered structures in these cases. The general trend of an increasing level of adsorption, followed by a decrease beyond a certain optimum value of the electrical charge of the chains, seems to also apply to the blockwise polysaccharides. However, it was mainly the changes in the charge density of the smaller, more strongly charged end for which this variation in the amount of adsorbed polysaccharide was noticed. The negative charge density of the larger section, so long as it remained low compared to the smaller part, did not seem to have much an effect on the level of adsorption.

Finally, there is one more result in the calculations of Ettelaie et al (2012) that is of relevance to the current work. The most extended layers were predicted when the lightly charged, long sections were in fact neutral. However, the repulsion between the droplets induced by the interfacial layers is a combination of both steric and electrostatic forces. Although the more extended layers may be expected to provide stronger and longer ranged steric forces, the electrically neutral esterified monomers of polysaccharide do not contribute to electrostatic component of the repulsion. We reported on the surface electric potential values, as would be seen from the bulk side, for a single isolated protein + polysaccharide layer. The most significant reversal of the potential from a positive value, for the protein only films, to a negative one, upon adsorption of polysaccharide, occurred when the lightly charged longer blocks of the polyelectrolyte are reasonably extended, but still have a non negligible degree of negative charge (Ettelaie, et al., 2012). This is the situation which is schematically represented in Fig. 2b.

3. Calculation methodology and models for protein and polysaccharide chains

In this section we shall briefly review the basic ideas underlying our SCF calculation of forces between two approaching interfaces, covered by protein + polysaccharide layers. The actual details of the SCF method can be found in a number of key papers (Evers, Scheutjens, & Fleer, 1990, 1991; Fleer, Cohen Stuart, Scheutjens, Cosgrove, & Vincent, 1993; Leermakers, Atkinson, Dickinson, & Horne, 1996; Scheutjens & Fleer, 1979, 1980) as well
as in some of our own work (Akinshina, et al., 2008; Ettelaie, Khandelwal, & Wilkinson, 2014; Ettelaie, Murray, & James, 2003).

In common with other types of mean-field theories, the first step in SCF calculations involves a statistical mechanical averaging of the molecular degrees of freedom. This averaging process results in a coarse-grained free energy for the system, expressed in terms of more accessible macroscopic set of quantities. Such averaging process often necessitates the use of elaborate mathematical methods, such as Hubbard–Stratonovich transformation or similar techniques (Fredrickson, Ganesan, & Drolet, 2002; Lee, Mezzenga, & Fredrickson, 2008; Mezzenga, Lee, & Fredrickson, 2006) which we shall not discuss here. However, the result of such an exercise for our system, consisting of two flat parallel interfaces placed a distance \( L \) apart, in a protein + polysaccharide solution, is a free energy expression given in terms of the density profiles of every species, including the solvent, in the gap between the two surfaces (Grosberg & Khokhlov, 1994; Lifshitz, Grosberg, & Khokhlov, 1978). We shall denote the set of such density profiles as \( \{ \phi_i^{\alpha}(r) \} \), where the index \( i \) represents the type of molecule (i.e. protein, polysaccharide, solvent, ions), while \( \alpha \) indicates the monomeric species, belonging to molecule of type \( i \). In particular, macromolecules (protein and polysaccharide) will consist of several different kinds of monomer species, \( \alpha \). Finally, \( r \) represents the distance in the gap measured away from one of the interfaces, with the other interface placed at \( r = L \), the gap size. The expression for the resulting free energy, per unit surface area, takes the following form

\[
\frac{\Delta F}{k_B T} = -\int_0^L \left[ \sum_i \frac{1}{N_i} \sum_\alpha \left( \phi_i^{\alpha}(r) - \Phi_i^{\alpha} \right) \right] \, dr - \int_0^L \left[ \sum_\alpha \psi_\alpha^{\alpha}(r) \sum_i \phi_i^{\alpha} \right] \, dr \\
+ \frac{1}{2} \int_0^L \left[ \sum_{i \neq j} \sum_{\alpha \neq \beta} \chi_{\alpha \beta} \phi_i^{\alpha}(r) \phi_j^{\beta}(r) \right] \, dr + \frac{1}{2} \int_0^L \left[ \sum_\alpha \psi^{\alpha \text{el}}(r) \sum_i q_\alpha \phi_i^{\alpha} \right] \, dr \\
+ \sum_\alpha \chi_{\alpha \text{s}} \sum_i \left[ \phi_i^{\alpha}(0) + \phi_i^{\alpha}(L) \right]
\]  

(1)
where $\Phi_i^\alpha$ is the bulk concentration of monomer species of kind $\alpha$ belonging to molecules of type $i$ and $q_\alpha$ is the electric charge they carry. The symbol $k_B$ denotes the Boltzmann constant and $T$ the temperature. The size of molecules of type $i$, specified by the number of residues they contain, is denoted by $N_i$. For solvent and simple ions this is 1. The first two terms in Eq. (1) represent the entropy and the rest the enthalpic contributions to the free energy of the system. The chemical nature of each monomer kind in the model is reflected by the nature and the value of the nearest neighbour interactions between this and the other monomer types. These are specified here by the set of Flory-Huggins parameters, $\chi_{\alpha\beta}$, appearing in the third term of Eq. (1). Similarly, the affinity of each monomer type for adsorption onto the surface is represented through their absorption energies, $\chi_{\alpha s}$, given in units of $k_B T$ per monomer. The more negative the value of $\chi_{\alpha s}$ the higher the tendency for adsorption, while monomeric species with positive $\chi_{\alpha s}$ will tend to avoid direct contact with the interface. The longer ranged electrostatic interactions between the charged species are reflected by the fourth term in the equation, where $\psi_{el}(r)$ is the electrostatic potential at each point $r$. The inclusion of this term is essential here, given that it is this electrostatic interaction that is responsible for the adsorption of polysaccharide chains onto the protein layer in the first place. The electrostatic potential, $\psi_{el}(r)$, is itself related to the distribution of charge species in the gap between the two surfaces, determined by Poisson equation as usual:

$$\nabla^2 \psi_{el}(r) = \frac{-e_i e_0}{\varepsilon} \sum_\alpha q_\alpha \sum_i \Phi_i^\alpha(r).$$

We take the relative permittivity of the solution $\varepsilon_i=79$, with $\varepsilon_0$ in the above equation denoting the permittivity of free space. One further set of quantities, $\psi_\alpha(r)$ appear in Eq. (1). These are a set of auxiliary fields that project out a given set of density profiles for which the free energy in Eq. (1) is to be calculated (Grosberg, et al., 1994; Lifshitz, et al., 1978). Each field $\psi_\alpha(r)$ acts on its corresponding monomer kind, $\alpha$. Although these fields appear in the theory as a result of the mathematical averaging process that lead to Eq. (1), they can be given some kind of physical interpretation as follows. They are the set of fields that if applied to an equivalent, but non-interacting set of chains, would result in the desired density profiles $\{\phi^\alpha(r)\}$. By “non-interacting” we mean chains that do not influence the conformation or spatial distribution of their neighbouring molecules and by “equivalent” we refer to chains that have exactly the same primary sequence of monomers, the same number of residues and
the same bulk concentrations as the original system. Thus, for any set of density profiles we have a corresponding set of fields \( \psi_\alpha(r) \). Together these two, through equation (1), provide the required value of the free energy for this particular density profile. Often other physical consideration may impose additional restrictions on the variation of the density profiles. One obvious example, often invoked in calculations of this type, is the incompressibility of the system. This condition demands that the valid sets of \( \phi_\alpha^\alpha(r) \) are those where the total concentration of all monomer species, including solvent, should always be a constant everywhere in the gap and the same as that in the bulk solution. That is to say

\[
\sum_i \sum_\alpha \phi_i^\alpha(r) = \sum_i \sum_\alpha \Phi_i^\alpha. \tag{3}
\]

To obtain the behaviour of the system, one needs to consider all possible variations of the density profiles satisfying condition (3), each having a probability of occurrence related to the value of their corresponding free energy and the resulting Boltzmann factor, \( \exp(-\Delta F(\{\phi_\alpha^\alpha(r)\}; k_B T) \). The central approximation in the SCF calculations is to assume that this probability is overwhelmingly dominated by a particular set of density profiles that minimizes the free energy of the system. All fluctuations about this profile are neglected. In fact, in writing Eq (1), we have already ignored such fluctuation of \( \phi_\alpha^\alpha(r) \) in directions parallel to the plates. This approximation works best for cases where we have dense polymer layers, as happens to be the case here. It becomes less accurate for the more dilute cases, where the relative fluctuations in the concentrations of different species can no longer be considered as small.

Any density fluctuations about the set \( \{\phi_\alpha^\alpha(r)\} \) for which the free energy is lowest, will obviously lead to an increase in the free energy of the system. This simple requirement can be used to show that the density profiles and their corresponding auxiliary fields, for the set \( \{\phi_\alpha^\alpha(r)\} \) that minimises the free energy, are related to each other in the following way:

\[
\psi_\alpha(r) = \psi_h(r) + \left( \sum_\beta \chi_{\alpha\beta} \sum_i \phi_i^\beta \right) + q_\alpha \psi_{el} + \chi_{\alpha\sigma} \left[ (\delta(r) + \delta(r - L)) \right], \tag{4}
\]

where \( \delta(r) \) represents the Dirac’s delta function and \( \psi_h(r) \) is a hard core potential that ensures the incompressibility condition, Eq. (3). This hard core potential is the same for all monomeric species at any given point, although of course it can vary from one point to the
next in the gap between the surfaces. In general the process of calculating the density profile variation for which the free energy attains the lowest value (i.e. satisfies Eq. (4)) has to be performed numerically. This requires that all the equations, Eq. (1) to (4) above, be discretised. A particularly efficient method for such numerical calculations was originally devised by Scheutjens and Fleer (1979, 1980) for homopolymers and subsequently generalised to more complex co-polymers (Evers, et al., 1990). In particular, by taking the grid size to be the same as the nominal size of the monomers, $a_0$, the discretised model becomes one of the polymer chains on a lattice. As such, the model has the advantage of being somewhat more intuitive, but we stress that in principle one may choose other values for the size of the grid, different to $a_0$, if so desired. Also in such lattice models it is useful to take the size of all monomers to be the same as each other, and equal to $a_0$. Again this limitation may be relaxed, though in most cases doing so does not qualitatively change the main conclusions and only serves to increase the complexity of the calculations. The scheme of Scheutjens and Fleer divides the space between the two surfaces into layers parallel to the plates, each with a thickness $a_0$ (Fleer, et al., 1993). The problem then becomes one of obtaining the values of $\phi_i^\alpha$ in each of the $(L/a_0)$ layers, for every type of molecule $i$ and every monomeric species $\alpha$, in accord with Eq. (4). The final step in completing the SCF calculations involves computing the density profiles $\{\phi_i^\alpha(r)\}$ resulting from the application of a given set of fields, $\{\psi_a(r)\}$. This is achieved (Ettelaie, et al., 2003; Evers, et al., 1990; Leermakers, et al., 1996) through the use of so called segment distribution functions $G_i^f(n,z)$ and $G_i^b(n,z)$. These quantities specify the probability that a chain consisting of the first $n$ residues of the molecule $i$ will end up having the $n^{th}$ monomer in the layer $z$, where $z = 1$ to $(L/a_0)$. The suffix “b” or “f” differentiate the two ends of the polymer chain from which the $n$ monomers are chosen. Of course, for symmetrical chains including homopolymers, we have $G_i^f(n,z) = G_i^b(n,z)$. The method of Scheutjens and Fleer (Scheutjens, et al., 1980) exploits the connectivity of the chains and uses a recursive relation between $G_i(n,z)$ and $G_i(n-1,z)$ to rapidly evaluate all the necessary segment distribution functions for any given set of fields, $\{\psi_a(r)\}$. The full detail of the calculations can be found in a number of references (Evers, et al., 1990; Fleer, et al., 1993; Leermakers, et al., 1996; Scheutjens, et al., 1980) including some of our previous work (Akinshina, et al., 2008; Ettelaie, et al., 2014; Ettelaie, et al., 2003). However, with functions $G_i^f(n,z)$ and $G_i^b(n,z)$ at hand, the concentration of each monomer species in every layer is can now be obtained from the composition law (Evers, et al., 1990; Fleer, et al., 1993) as follows:
\[ \phi_i^\alpha (z) = \left( \sum_\alpha \Phi_i^\alpha \right) \sum_{n=1}^{N_i} \left( \frac{G_i^f(n, z)G_i^b(N_i - n, z)\delta_{\alpha, \alpha_i(n)}}{\exp(-\psi_\alpha (z))} \right). \]  \hspace{1cm} (5)

In Eq. (5), the function \( \delta_{\alpha, \beta} \) is the usual Kronecker delta function, which is equal to 1 if \( \alpha = \beta \) and zero otherwise. We have also defined the function \( t_i(n) \) such that it evaluates to the species index number, identifying the group to which the \( n^{th} \) residue of molecules of type \( i \) belongs. The SCF calculations begin with a rough guess of the density profiles that minimises the free energy. These are substitutes in equations (2) and (4) to obtain the set of fields, \( \{ \psi_\alpha(z) \} \). The fields are used to compute \( G_i^f(n, z) \) and \( G_i^b(n, z) \), and through Eq. (5), a new set of concentration profiles. The whole calculation is then started again with this new set \( \{ \phi_i^\alpha(r) \} \) and repeated iteratively, until no substantial change in the values of \( \{ \psi_\alpha(z) \} \) and \( \{ \phi_i^\alpha(r) \} \) between two successive iteration steps is detected.

The values of \( \{ \psi_\alpha(z) \} \) and \( \{ \phi_i^\alpha(r) \} \), obtained in the above manner, are substituted in Eq. (1) to yield the required change in the free energy, \( \Delta F(L) \), resulting from the creation of two interfaces, a distance \( L \) apart. The colloidal interaction between the two surfaces, mediated by the adsorbed layers, is then given as the change in the free energy of the system as two, originally isolated, interfaces are moved to within a separation distance \( L \) of each other:

\[ V(L) = \Delta F(L) - \Delta F(\infty) \]  \hspace{1cm} (6)

The above results are obtained for two flat plates, and represent the interaction potential per unit area (from now on in units of \( k_B T \sigma^2 \), unless stated otherwise). To convert these results to the inter-particle potentials between droplets, we make use of the well known Derjaguin approximation (Hunter, 2000):

\[ V_{\text{par}}(L) = \pi R \int_{\infty}^{L} V(x) \, dx \]  \hspace{1cm} (7)

valid when the radius of the droplets, \( R \), is large compared to the particle surface separation, \( L \). A major advantage of calculating the forces in this manner is that no artificial divisions between the electrostatic, steric repulsion, bridging, depletion, and other colloidal forces mediated by the layers needs to be made. It is often tempting to try and calculate such forces.
separately. This is a reasonable approximation in some cases, but tends to fail for thick layers at overlap separations, particularly for layers involving biopolymers with complex non-uniform structures. We refer to the interaction potentials calculated here as electro-steric interactions from now on to reflect this point.

The models we use for polysaccharide and protein are essentially those we considered in our previous work (Ettelaie, et al., 2012). These themselves were in turn adopted from the original work of Leermakers et al (1996). For the protein, we use a model based on the primary structure of αs1-casein with 199 residues. Two additional residues are included at the end of each chain to reflect the possible charge of the N and C-terminus. In the model of Leermakers et al (Leermakers, et al., 1996), the amino acid residues are divided into six different groupings according to the degree of their hydrophobicity, polar nature and the value of their pKₐ for charged groups. The χ interaction parameters between residues in different groups, as well as those with solvent, ions and the surface, all reflect these differences in the polar, charged or hydrophobic nature of the residues. A full table of the value of these parameters can be found in previous papers (Akinshina, et al., 2008; Ettelaie, et al., 2008) and therefore will not be reproduced here. As for polysaccharide, we take these to consist of 500 carbohydrate moieties. The moieties are negatively charged but have two different values of charge. The low charge sugar groups make up the high methoxyl part of the polysaccharide, while the more de-esterified section, having a higher charge density, is comprised of the more strongly charge monomers. We take the de-esterified part to consist of 20 monomers resides, situated at one end of the polyelectrolyte chains. Apart from their charge, the interactions of the two types of sugar residues with monomers in all the other groups, as well as with solvent molecules and the surface, are exactly identical to each other. Unless stated otherwise, all our calculations were performed at pH=3, where the net charge of the protein is positive and that of polysaccharide negative. We also take the bulk volume fraction of both biopolymers as 10⁻¹¹. Note that these values refer to the bulk volume fractions of biopolymers that remain in the solution, once the majority of chains adsorb at the interface. Low values chosen are based on the expectation that in a typical food emulsion most of biopolymer will eventually be found adsorbed on the surface and will not remain in the bulk. In particular, the low values of these parameters should not be taken as implying the presence of only a very small amount of biopolymer in the whole system. The volume fraction of salt in the solution was 2×10⁻⁴, roughly equating to a molar solution of 0.007 mol/l.
for NaCl, if a_0 is taken to be 0.3 nm. Finally we mention that the polysaccharide model assumes flexible chains. No account of the possible inherent rigidity of the polyelectrolyte has been made in these calculations.

4. Results and discussions

The first set of polysaccharides considered were homopolymers, having a uniform distribution of charge along their backbone. The structure of an isolated adsorbed layer of these polysaccharides + protein is demonstrated in Fig. 1 (Ettelaie, et al., 2012). In Fig. 3a we display our SCF calculated interaction potential, per unit area, as a function of separation distance, when two such layers start to overlap each other. The interaction mediated solely by the “a_{41}-casein” like protein, in the absence of any polysaccharide, is also included as the grey line for comparison. Several polysaccharides with different charge densities ranging from -0.0496e to -1.0e per monomer are considered. We also use these data to obtain the interaction potential between two colloidal particles of size 1 µm, using the Derjaguin approximation of Eq. (7). The latter are shown in Fig. 3b, where we have also added the van der Waals attraction between the particles to the inter-particle potential. The van der Waals interaction (in units of k_B T) for two spherical particles of radius R is given as (E. Dickinson, 1992; Hamaker, 1937; Hunter, 2000)

\[ V_{vw} = -\frac{AR}{12L}, \quad (8) \]

where A is the composite Hamaker constant for the oil in water emulsions (~ 1 k_B T). The closest surface separation between the two particles is as before denoted as L. A quick comparison of the graphs on Fig. 3a with those of Fig. 3b, reveals the relative insignificance of the van der Waals forces. Indeed, once the adsorbed layers begin to overlap, the electro-steric interactions generated as a result far outweigh any van der Waals forces existing between the particles. The most noticeable feature of both sets of graphs is that all interactions, involving these uniformly charged polysaccharides, are less repulsive than those mediated by interfacial layers of pure protein alone. In fact, for systems involving higher charged polyelectrolytes, the interaction induced by the adsorbed protein + polysaccharide develops an attractive energy well. For the most negatively charged of these, with a charge density of -1e/monomer, the depth of the energy well is ~ -130 k_B T (see Fig. 3b). For these
systems, we predict that the particles will become colloidally unstable and aggregate upon the addition of the polysaccharide. Guzey & McClements (2006a) highlight the tendency for aggregation of droplets as the main problem in preparation of multilayer stabilised emulsions. They identify the lack of a sufficient amount of polysaccharide on the surface of the droplets as an important factor exasperating the aggregation process. Obviously one possibility for this to occur is if there is insufficient amount of polysaccharide used in the formulation of such emulsions. However, as already discussed in section 2, even when enough polysaccharide is present, the adsorbed amount can be rather small if the polyelectrolyte chains are very highly charged. The presence of only a small amount of polysaccharide on the surface has been associated with the possibility of bridging flocculation between the droplets (E. Dickinson, 2011; Guzey, et al., 2006a). It is clear from the graphs in 3b that indeed for systems involving the stronger charged polysaccharides, the interaction potentials are much more likely to lead to aggregation. Our results for the density profile variation in the gap between the particles indicate that, at moderate particle separations of 5 to 7 nm, there is still a significant amount of polyelectrolyte present in the gap, well above the concentration in bulk. Given the large size of the polysaccharide molecules, it is highly likely that in such small gaps, a polysaccharide chain is simultaneously incorporated and becomes part of both neighbouring layers. This occurs to a greater or lesser extent for all the uniformly charged polysaccharides (even the weaker charged ones) and is the likely reason why the calculated interactions for all polysaccharide + protein systems in figures 3a and 3b are less repulsive than the one involving protein alone. However, our results also suggest the existence of an additional and even more significant factor, apart from the bridging by polysaccharide, reducing the repulsion between the particles. We believe that it is this factor that leads to the attraction between the particles, in systems with the most highly charged polyelectrolytes (Fig. 3a and 3b). In section 2 we pointed out that in our previous studies we had seen that when the polyelectrolyte chains were strongly charged, the structure of the interfacial layer resembles one of a mixed protein + polysaccharide film (Ette laie, et al., 2012). We find that at those separation distances, where the two such layers first begin to overlap, not only the induced forces are not repulsive but in fact they become attractive. This is perhaps not so surprising if one considers that the interfacial films as made from highly entangled complexes of protein + polysaccharide. It is known that such complexes tend to have a lower solubility than those of their individual constituent, polysaccharide and protein, components (de Kruif, Weinbreck, & de Vries, 2004). These complexes tend to aggregate and in some cases even
precipitate out of the solution (i.e. form coacervates). Thus, it is feasible that films, consisting of such complexes, will have a similar tendency to aggregate. In other words, an interfacial film formed from the adsorption of these complexes would have a preferential tendency to be in contact with another similar film, rather than remaining in contact with the aqueous solution. Furthermore, we had found (Ettelaie, et al., 2012) that the magnitude of the surface electric potential, in the presence of the highly charged polyelectrolyte, with a homogeneous distribution of charge, is noticeably lower than for surfaces covered with protein. Thus, both of the above two effects cause a significant reduction in the repulsive forces in systems containing highly charged polyelectrolytes and contribute to the appearance of the attractive energy wells in the particle-particle interaction potentials.

Additional support for the above view is provided from the examination of the density profiles of both protein and polysaccharide across the gap between the surfaces. We have plotted these in Figs. 4a and 4b for a system with the most negatively charged polyelectrolyte, i.e. -1e/monomer. This is done at a separation distance of 22a_0, where the minimum in interaction potential for droplets in this system occurs (see Fig. 3a). The graphs show that there is a substantial amount of protein and polysaccharide (in comparison to their bulk concentrations) present everywhere in the gap. In particular, nowhere in the gap can a region comprising solely of polyelectrolyte be identified. More interestingly, the density profile for protein extends much further away from each surface, than otherwise would do in the absence of the polysaccharide. This is demonstrated by the graphs in Fig. 4b, where we have displayed the density profile of the protein both in the absence and the presence of the highly charged polysaccharide. The protein layers without the polyelectrolyte are seen to be rather thin and do not substantially overlap at this separation distance of 22a_0. In fact, most of the repulsive interaction, observed for the pure protein system in Fig. 3a and 3b, is the result of electrostatic forces at this distance. In contrast, there is much overlap between the protein chains residing on the opposite interfacial layers, for the system that does indeed contain polysaccharide. Normally such extended protein layers, by their own, should provide a great deal of repulsion as they begin to overlap. However, it seems that the presence of oppositely charged polyelectrolyte, intertwined within the protein interfacial layer, causes the protein molecules to be drawn towards the approaching opposite layer. The situation can roughly be envisaged as one of having two interfaces, being held together by the “glue” formed from protein + polysaccharide complexes. We stress again that all our predictions involve
equilibrium structures for the interfacial layers, maintained at all separation distances. Rapid collisions between emulsion droplets may not leave sufficient time for the conformation of biopolymers in the films to adjust quickly enough. In such cases, the non-equilibrium effects may dominate and cause deviations from the above results.

So far our discussions have focused on polysaccharides with a uniform distribution of charge. One may ask whether having chains, made of blocks with different charge densities, would not be beneficial in leading to better colloidal stability for the emulsion droplets. In suspecting this we are motivated by the fact that diblock polymers, consisting of a part with a strong adsorption affinity and a second longer section with a preference to remain in solution, form the ideal steric stabilisers in most circumstances. Of course, polysaccharide chains are drawn to the surface by their electrostatic attraction for the protein layers. Thus, by varying the degree of the charge of different sections of the molecule, and therefore altering their affinity for protein, a similar situation as the one involving diblock polymers may be engineered. It is also already known (Ettelaie, et al., 2012) that polysaccharides with a heterogeneous distribution of charge along their backbone, make interfacial layers with equilibrium structures that more closely resemble a multilayer. The type of interfacial films formed by these polyelectrolytes + protein is schematically illustrated in Figs. 2b and 2c. The inter-particle interaction potentials mediated by these adsorbed layers has been plotted against the particle separation distance, L, for a series of systems involving such polyelectrolytes, in Fig. 5. Once again the van der Waals attraction has been included in all the inter-particle potential graphs, with the result for the “protein only case” also shown as the grey line. The polysaccharides in the five remaining systems of Fig. 5, labeled (a) to (e), all have exactly the same electric charge of -24.8e, but differ from each other in the location where the charge resides. The system labeled (a) has a homogeneous charge density of -0.0496/monomer, similar to those already considered in Fig. 3. The charge distribution becomes increasingly more non-uniform as we move through different systems from (a) to (e). The polysaccharide chains in the system labeled (e) are the most heterogeneously charged ones, with all the electric charge residing on the first 20 monomers on one end of the molecules. This gives a charge density of -1.24e/monomer for this small section, with the larger block, made up of the remaining 480 residues, being electrically neutral. We denote this system as -1.24e/0e and use the same convention to identify the other systems. The first important feature of the graphs in Fig. 5 is that for all those cases involving the non-uniform
polyelectrolyte, the repulsive interaction has been enhanced due to the presence of the polysaccharide. This is in complete reversal to what was found for the chains with a homogeneous charge distribution. This suggests a strong colloidal stability for the emulsion droplets in all the systems (b)-(e). In particular, note that the repulsive interactions come into operation at fairly large particle separations, ~30 to 40 nm for cases (c), (d) and (e), and quickly dominate over attractive van der Waals forces at closer distances. Secondly, the strongest interaction, once the interfacial layers begin to overlap, is observed for the protein with the polysaccharide which has the largest charge contrast between its small and large blocks, i.e. system (e). As the charge becomes increasingly more evenly distributed, the repulsive forces are reduced. Another interesting feature, although probably of little actual practical significance unless much larger emulsions are considered, is that beyond the overlap separation distances it is the interaction for the system (e) that decays away most rapidly. This is more clearly seen in the inset of Fig. 5, where the part of the interaction graphs, around the overlap distances has been magnified to demonstrate the point. This is done for the three of the systems, -0.7e/-0.0225e, -1.0e/-0.01e and -1.24e/0e. Also at these distances, the van der Waals forces, which at closer separations remain rather negligible in comparison to the electro-steric interactions, start to dominate over the latter. This gives rise to the energy minimum wells in all the three graphs, displayed in the inset. However, at such large particle separations all the interactions are already rather weak and the values of the energy minima found are less than 1k_B and easily overcome by the Brownian motion of the droplets. Given the larger extent of the interfacial film for the -1.24e/0e system (i.e. graph (e)), at first, one may expect the longest ranged repulsion to be found for this system (compare Figs 2b and 2c). However, for the -1.24e/0e system all the repulsion is essentially due to the steric component, since the long block of the polyelectrolyte, forming the brush region of the surface layer, is essentially unchanged. In these cases then the steric forces diminish very rapidly as soon as the particle separation distance becomes larger than twice the thickness of the layers. For systems -0.7e/-0.0225e and -1.0e/-0.01e there is also a small electrostatic component to the repulsion. This persists for slightly longer distances than the steric forces, at distances where the layers have not quite overlapped yet. As discussed in section 2, the largest reversal of surface potential is not observed for the system -1.24e/0e which forms the thickest layers, but rather for system -0.7e/-0.0225e (graph (d)). This was also confirmed by our previous study (Ettelaie, et al., 2012). Thus, not surprisingly the tail end of the interaction potential, which is mainly due to the electrostatic repulsion, is stronger for -0.7e/-
0.0225e system than it is for the -1.24e/0e one. Of course, the reverse becomes true at closer separations, when the layers begin to overlap and the steric component starts to manifest itself. This finding becomes more significant for coarser emulsions. The colloidal interactions between droplets are theoretically predicted to scale linearly with the particle size (Hunter, 2000). Thus, for example for 10 \( \mu m \) sized droplets, the depth of the minimum in the particle-particle interaction potential can be \( \sim 10 k_B T \), as opposed to \( 1 k_B T \) for 1 \( \mu m \) ones studies here. This is sufficient to give rise to the formation of weak flocs. While these can still easily be broken by stirring or upon application of small amount of shear, nevertheless their presence can have a significant impact on the rheological behaviour of the emulsion system.

In Fig. 6 we explore the influence of the magnitude of the charge, carried by the short blocks, on the steric forces mediated by the mixed biopolymer layers. This time we keep the negative charge density of the long, more lightly charged block of the polyelectrolyte constant at -0.01e/monomer, but increasing it for the short block from -0.25e to -0.5e, -1.0e and finally -3.0e. Thus, in all cases the polysaccharides are still quite unevenly charged. Once again the grey curve shows the particle-particle interaction potential in a protein only solution. All the inter-particle interaction potentials are strongly repulsive. However, the strongest interaction occurs for the -1.0e/-0.01e system (graph (c) in Fig. 6), but begins to slightly diminish at a more negative charge density of the small block (graph (d) in Fig. 6). Similarly, the particle-particle interactions are somewhat weaker at a lower degree of charging (graph (b) in Fig. 6). It is therefore concluded that there exist an optimum negative charge for the short, highly charged section of the polysaccharide for which the strongest interactions are observed. It turns out that this is also the charge density at which the maximum adsorption of our heterogeneous polyelectrolyte onto the protein layer occurs (Ettelaie, et al., 2012). The amount of adsorbed polysaccharide above and below this value is lower for the very same reasons as those already explained for the uniformly charged chains. For the weakest charged case, -0.25e/-0.01e, the presence of polysaccharide (graph (a) in Fig. 6) in the solution is seen to have made little difference to the mediated colloidal interactions between the particles. For this system the total charge of a polyelectrolyte chain is only -9.8e, already too small to induce any significant level of polysaccharide adsorption onto the surface of the particles.
We shall complete the discussions in this section by considering the possibility of competitive adsorption occurring between the evenly charged polysaccharides and those with a non-uniform charge distribution. Polysaccharides are highly polydisperse biopolymers. The polydispersity not only permeates the size distribution of the chains, but also in the way that the charged residues are distributed along these biopolymers. It is natural to ask then, given a polydisperse distribution of polyelectrolyte chains, which type of polyelectrolyte will dominate at the interface as part of the mixed protein + polysaccharide layers. The question is an important one from a practical point of view, since even uniformly charged polysaccharides will have a fraction of chains with a less even spread of charge. A complete investigation of this interesting problem, taking into account a representative distribution of such polydisperse polyelectrolytes, is beyond the scope of the present work but will be addressed in the future. However, here we shall consider a mixture of our uniform and non-uniformly charged model polysaccharides, both present simultaneously in the solution, to provide some preliminary results regarding the competitive adsorption in such systems. The two polysaccharides we choose for this purpose are the -1.24e/0e molecules and the evenly charged polyelectrolyte with a charge density of -0.0496e/monomer. Both of these biopolymers have an equal overall charge of -24.8e per chain. The bulk volume fraction of both is also chosen to be equal to each other and the same as our model $\alpha_{s1}\text{-casein}$, at $10^{-11}$. Fig. 7 displays the predicted inter-particle interaction potential, plotted against the separation distance between two particles, for the system containing the protein and the two polyelectrolytes. The colloidal interaction potentials in emulsion systems, consisting of protein + each one of the two different polysaccharides separately, have also been shown for comparison. The potential for the “polydispersed” system follows that for the protein + heterogeneously charged polysaccharide exactly. It shows the same strong repulsion taking effect at large particle separations ~ 35 nm and increasing rapidly as the particles move closer to each other. In both cases the forces are considerably stronger than those predicted for the pure protein layers. That the graph for the mixed polysaccharide system is almost identical to that for the heterogeneously charged polyelectrolyte, but distinct from the one involving the evenly charged chains, is an indication that it is the former polysaccharide type that strongly dominates the equilibrium adsorption onto the protein layer. The examination of the density profile of each biopolymer, in the gap between the surfaces, (not shown here) leads to pretty much the same conclusion. That this should be the case is expected. We have already seen that it is preferential for the more strongly charged, short sections of the heterogeneous...
polyelectrolyte to displace the lightly charged blocks of the same chain. It is therefore just as likely that such strongly charged sections do the same to the uniformly charged polysaccharide, where the even distribution of negative charge along the chain leads to a smaller value for the charge density. In such polydispersed combination of chains, it is interesting to exam how low the concentration of the heterogeneous fraction of the polysaccharide can become before the more evenly charged fraction begins to adsorb onto the surface. We shall address this and similar interesting problems in a future publication. We note that relatively little work, either experimental or theoretical, on the competitive adsorption of different polysaccharides onto protein layers has been carried out in the past. One recent exception is the study by Cho, Decker and McClements (2009) who probed the competitive adsorption between carrageenan and pectin deposited onto the surface of β-lactoglobuline stabilised emulsion droplets. The degree of esterification of pectin was reported to be 60%, but it was not clear how uniformly the charge residues were distributed along the length of the chain. Furthermore, it was found that carrageenan possessed a much higher charge than pectin. This unfortunately makes a comparison of these experimental results with our predictions above more complicated. But it is worthwhile to note that emulsions stabilised by pectin + protein layers were reported to have better stability than those with protein + carrageenan, despite the higher surface charge of the latter.

5 Conclusion and summary

We have theoretically investigated the nature and magnitude of the interactions that are induced between two particles as a result of the overlap of mixed protein + polysaccharide layers, adsorbed on their surface. Our calculations are based on the Self Consistent Field (SCF) theory and its numerical implementation through the use of the well known Scheutjens-Fleer scheme. The method calculates the electro-steric interaction produced between the particles, without the need for an artificial division of forces between different components (e.g. steric and electrostatic). For extended interfaces, made up of complex adsorbed biopolymers, approximations based on such separation of interaction components often becomes invalid and can lead to errors in the prediction of the forces.

In this study we consider systems at a low pH=3, where the net electric charge of the protein is positive. The interfacial layers are formed by electrostatic attraction of negatively charged
polysaccharides to the protein films on the surface of the particles. When the charge of the polysaccharide is evenly distributed throughout the length of the chain, it is found that the inter-particle potential mediated by such mixed layers becomes less repulsive than that produced by films of pure protein. This is seen to be due to some degree of bridging by the polysaccharide chains between the two neighbouring opposite layers. As the negative charge of the polyelectrolyte is increased beyond some optimal value, we observe that the amount of polysaccharide adsorbed at the interface actually falls. At same time, in systems containing such highly charged polysaccharide chains, the particle-particle interaction potentials turn attractive over a certain range of separation distances. This leads to the appearance of an energy minimum in the interaction potential curves with a sufficient depth to cause the aggregation of the particles. Examination of the density profile of the two biopolymers, in the gap between the particle surfaces at these separation distances, indicates that both polymer and polysaccharide permeate the entire gap between the particles. We believe that the situation here consists of two approaching particles, covered with films made of a coacervate of protein + polysaccharide. The layers have a tendency for aggregation with each other. This is perhaps expected, given that the complexes of the two biopolymers often have a lower solubility than the individual molecules themselves, and often precipitate out of solution.

The above situation alters considerably when the charge distribution of polyelectrolyte is made non-uniform. In this study, we have assumed that such a polysaccharide consists of a highly charged short section, together with a much larger lightly charged block. No bridging is found for these types of polysaccharides. Instead, the induced particle-particle repulsive potential for systems involving these polyelectrolytes becomes significantly enhanced, with the forces coming into operation at far larger particle surface separations (~ 30-40 nm) in comparison to cases involving just the protein. This is a reflection of the extended structure of the interfacial layers which now include a distinct sub-layer, comprising solely from the weakly charged regions of the polyelectrolyte molecules. The large qualitative differences predicted between the induced interaction potentials for these two types of polyelectrolyte, should make it that much easier to substantiate these theoretical predictions using direct force measurements by AFM. However, these experiments have to be performed slowly, since all our results are obtained assuming equilibrium configurations for the layers.

Finally, we have briefly considered the issue of competitive adsorption of two polysaccharides, one having a uniform and the other an uneven distribution of charges, with
one another to accumulate on (or into) an adsorbed protein surface layer. We intend to report
on a more detailed study of this phenomenon in a future publication. For now systems
simultaneously containing chains of the same size and the same overall electric charge, but
different distribution of charge along the backbone, were considered here. The result showed
a clear preference for the non-uniformly charged polysaccharide to adsorb onto the protein
film. In fact, the polyelectrolyte chains with a heterogeneous charge distribution almost
completely displace the uniformly charged ones from the surface. As a result the calculated
inter-particle interaction potential in such a solution, containing all the three biopolymers,
turns out to be exactly identical to the one we find in the absence of the uniformly charged
chains. To our knowledge, very few experiments involving electrostatically driven
competitive adsorption of polysaccharides onto protein films have ever been performed thus
far. All such cases have involved different polysaccharide species, with very different sizes
or overall degrees of charge (Cho, et al., 2009). We hope that more systematic experiments
along the same lines, involving polyelectrolyte mixtures with similar sizes and electric
charges, but having different distribution of esterified residues, will provide valuable data
against which some of the predictions here can be validated.


