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# Novel food grade dispersants: Review of recent progress

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**Abstract**

Many foreseen advances in the design of food structures, suitable for ever demanding nutrient delivery systems, tailored controlled release, microencapsulation and protection of active ingredients, require a generation of superior dispersants than those currently provided by proteins. While the most efficient structure for such dispersants is relatively easy to specify, in foods they cannot simply be synthetically manufactured. The review highlights several possible strategies for realising more efficient food colloid stabilisers and summarises the key recent progress for each approach, both experimentally and theoretically. The emphasis is on those methods that lead to macromolecularly adsorbed layers. Practical aspects apart, we also discuss a number of interesting fundamental questions that each approach raises.

**Introduction**

Some years ago we attended a lecture by Professor Dickinson on the general topic of dispersants in food colloids. Three key take home messages from this talk for us where 1) many advocated advances in the way that foods will be designed in future, such as surface engineering, or bottom up approach to food structuring, can only truly be realised if we have a much better control over the nature and magnitude of interactions that operate between food constituents, 2) for structures on mesoscales, these interactions essentially imply those operating between food colloidal particles and emulsions, 3) customary food emulsifiers such as proteins, using which we normally manipulate such forces, have significant shortcomings in providing the required level of control for these envisaged future developments. Yet, due to regulatory and safety issues, for food scientists the problem cannot simply be solved by synthesising a whole new generation of more efficient dispersants - an option that is often available for none-food related colloidal formulations in other industries. Instead, Professor Dickinson emphasised his view in which various existing components may be combined, or manipulated in a minute way, as to provide more superior surface functionality [1]. He provided two possible examples from his own work to demonstrate the principle. One example was based on the preparation and use of conjugates of a protein with a polysaccharide [2*, 3*] and the second relied on the enhanced impact that the presence of a layer of whey protein imposed on stabilising properties of sodium caseinate [4, 5**].

Since Prof Dickinson's talk on the subject, much has happened in this field and the area has rapidly grown into a dynamic branch of food colloid research, with a few other avenues also being actively pursued in the quest for achieving superior food dispersants. Such research is not only important from a practical/industrial point of view, but it has also led to some very interesting and fundamental questions regarding our understanding of the behaviour of mixed surface active biopolymers at interfaces. Our aim in this review is to provide a highlight of several of these strategies, providing an overview of advantages and possible limitations for each. We also briefly discuss the questions that have arisen in the light of the work done so far in the literature, but still need to be resolved in our opinion, in order to fully optimise each method. As with most multicomponent formulations, it is often possible to provide several (and sometimes contradictory) reasons for the observed experimental behaviour of the systems involving mixtures of biopolymer dispersants. In this respect, the theoretical and modelling studies have proved a helpful tool in examining the plausibility of different explanations. Where such work exists in relation to any of the methods discussed here, we will also attempt to summarise the main results of these theoretical studies.

One notable omission from our review is the so called Pickering route to stabilising emulsions, where it is the adsorption of small particles at the surface of the droplets (or bubbles) that is responsible for their colloid stability. This is because firstly the mechanism of stabilisation by particles is quite

different to that induced by molecularly adsorbed interfacial films discussed here. Furthermore, the vast amount of research on Pickering emulsions does not make it possible for us to do justice to the work that requires a separate lengthy review of its own. It only suffices to mention that emulsion droplets stabilised by particles show exceptional stability against almost all modes of colloidal instability. However, it remains a real challenge to produce edible yet sufficiently small (~ 10-50 nm) particles, with the appropriate surface chemistry for adsorption at air-water or oil-water interfaces, suitable for use in food systems. Consequently, most reported work on the potential use of Pickering particles in food systems tends to involve rather coarse emulsions thus far (>10 μm).

It is useful to begin by examining the shortcomings of proteins as dispersants. Most food related proteins are globular compact biopolymers. Their strong amphiphilic nature means that they have a strong tendency for adsorption onto hydrophobic-hydrophilic interfaces. When they do so, they tend to unfold to a larger or lesser extend and form relatively thin adsorbed surface layers. Food proteins also tend to be smallish macromolecules, at least when compared to synthetic polymers typically used as dispersants. Therefore, even relatively disordered proteins, such as casein, do not form particularly thick interfacial films (~ 3-5 nm). When layers overlap, an osmotic differential appears between the regions in the gap separating the particles, where protein concentration is large, and that outside where protein concentration is next to zero. This ideally leads to a strong repulsion. However, any interactions resulting from the overlap of the surface layers only manifest themselves when layers begin to touch. They rapidly decay away as the inter-particle separation is increased further beyond this overlap distance. For protein layers, at such separation distances the van der Waals attraction between colloidal particles or emulsion droplets (say of a size of a couple of microns) is not entirely negligible. The attraction suffices to cause aggregation of the drops. Fortunately, proteins are also charged. The electrostatic repulsion between the layers operates at separations beyond overlap by a further distance of the order of the screening length, as dictated by the concentration of background electrolyte. The combination of both the steric and the electrostatic repulsion is required to allow food protein emulsifiers to perform their function as dispersants. Reduction of either of these components serves to cause colloidal instability. This is nicely demonstrated by acidification and rennet coagulation of otherwise colloidally stable casein micelles in milk, where the electrostatic and the steric repulsions are turned off in each case, respectively. This reliance of proteins on presence of both components, and the many environmental factors such as pH, salt concentration, quality of solvent, temperature, etc., influencing one or both of these repulsion forces, makes the protein stabilised colloids quite susceptible to changes in processing conditions. A further important issue that limits the efficiency of proteins as emulsifiers is their relatively blocky primary structure, with small segments of hydrophobic amino acids followed by equally short trains of hydrophilic ones. In the context of synthetic polymers it has been shown that chains with many small adsorbing and non-adsorbing sections are noticeably inferior in their dispersant stabilising ability compared to those

having long continuous blocks [6]. The presence of many adsorbing segments along the chain increases the possibility of the so called bridging configurations, where chains make multiple contacts with surfaces of two neighbouring droplets. For polymers with many small anchoring segments and where the charge is low, this can even cause the mediated interactions to switch sign and become attractive, rather than the expected steric repulsion.

**Mixed and Multiple layers**

In contrast to proteins, polysaccharides tend to be considerably larger macromolecules. Whereas the number of monomer residues making up a typical food protein may be a couple of hundreds, the sugar moieties comprising say starch can be as many as tens or even hundreds of thousand monomer units. Polysaccharides also tend to be hydrophilic molecules, with water acting as a good solvent for these biopolymers under a wide range of conditions. Thus, when fully dissolved and under dilute conditions the chains are found to be highly swollen with typical radii of gyration that can be as large as 100 nm. The swelling of chains is the result of strong excluded volume interaction between their monomers; precisely the same interactions that is also responsible for provision of strong steric repulsion between interfacial layers, upon their overlap. Thick layers and strong repulsive forces, that are not very sensitive to changes in pH or background electrolyte, make these molecules ideal candidates to act as dispersants. However, the problem is that these biopolymers are not amphiphilic. Most polysaccharides show no affinity for adsorption at hydrophobic-hydrophilic interfaces. The technique discussed in this section, as well as the ones considered in the two following sections, describe several different means by which the polysaccharides can be made to reside on surfaces. They all share the basic approach of using much larger polysaccharides in one way or another to achieve the desired improved dispersant stabilising functionality.

An interesting way of enticing the polysaccharides to adsorb at interface is to use their possible electric charge to attract them to an already deposited layer of opposite charge on the surface. The idea owns its origins to the so called layer-by-layer deposition process, first suggested by Decher [7] to form multi-layers on macroscopic surfaces. In each stage of the process the previous solution is washed. Then a new solution, containing polymers of opposite charge to the existing layer so far, is introduced. This process can be repeated many times to form stacks of alternate layers, one on top of another. The first application of the technique to food colloids is due to McClements and his co-workers [8*, 9**]. These researchers used the idea of L-b-L to deposit a layer of a negatively charged polysaccharide on top of an already adsorbed protein film at low pH, below the isoelectric point of the protein. Under such conditions the protein film is positively charged and thus attracts the anionic polysaccharide. Of course, the method can also be applied at pH values above iso-electric point of the

protein, but this time employing a cationic polysaccharides (e,g. chitosan [10*]). The initial studies by McClements and co-workers [8*, 9**] were also significant in another important respect. They were amongst the early examples of the application of L-b-L method to mesoscopically sized interfaces, using the surface of emulsion droplets as the template instead of a large macroscopic object. However, doing so does pose several unique problems of its own which otherwise are not present during the deposition of multi-layer films on larger objects. The most significant of these concerns the colloidal stability of the emulsions at intermediate stages of the deposition. Polysaccharides can become simultaneously associated with two protein films on the surface of a pair of closely spaced droplets. This induces aggregation and subsequent coalescence of the emulsion drops through the bridging flocculation mechanism [8*, 11, 12]. Another important consideration is the integrity of the multi-layers during large possible swings in pH as for example occurring in the storage period of the product. This may result in the reversal of the charge of the protein layer. With both biopolymers having the same charge polarity the polysaccharides are expected to begin to desorb from the surface. In practice it seems that the once formed, the protein + polysaccharide multilayer can tolerate this effect, provided the swing above (or below for cationic polysaccharides) is not too far away from the isoelectric point of the protein [8*]. This observation may be the result of some degree of inter-diffusion of the two biopolymer sublayers. It is not easy for polysaccharides to desorb immediately if they have become entangled with protein molecules. The kinetics of disentanglement of long polymer chains is known to be a relatively slow processes. This is an interesting point to which we shall return later on in this section.

Despite the above requirement for careful preparation, the potential of multi-layers as a superior means of stabilising emulsions in foods has been well demonstrated in the last decade or so. Multi-layer protein + polysaccharide stabilised emulsions have been shown to exhibit superior stability properties in the presence of high salt concentrations [9**], at pH values close to iso-electric point of protein (where primary emulsions would destabilise) [12, 13], though results contradicting the latter have also been reported in some cases [14]. Similarly, better stability during heating or freeze-thaw cycles [15, 16] is achieved through the use of protein + polysaccharide layers. This is of particular interest in microencapsulation of active ingredients using the emulsification-drying route. Often, in application of this technique to foods, polysaccharide is added to the emulsion dispersion in order to provide bulk to the final dried powder. Given that the emulsions droplets are also normally stabilised using a protein, it is natural to choose the polysaccharide in such a way so as it enhances the emulsion stability by forming multilayers. Research investigating the possible use of multilayers in microencapsulation technologies are increasingly being reported in recent literature [17*, 18]. According to some of these studies there is an optimum level of polysaccharide which best suits the encapsulation process [17*] but the factors determining this value need further work to understand. The use of multilayers consisting of sodium caseinate with pectin, carrageenan, sodium alginate or

gum Arabic itself, has been found to provide the same degree of stabilising performance as that seen from gun Arabic in emulsion based beverages. The latter is the colloid stabiliser of choice in these types of systems but there is a concerted attempt to replace it with more widely available functional ingredients [11].

If the layer of polysaccharide around a droplet is also indigestible to various gastric enzymes, this ought to slowdown the hydrolysis and digestion of the oil in the emulsion formulation. There is indeed good experimental evidence to support this suggestion [10*, 19, 20] and the potential of multilayers is currently an area of great interest both in the design of possible healthier foods and in controlled release application in nutraceuticals and pharmaceuticals [21]. The deposition of four or more consecutive layers is seldom reported in food related literature, but we stress that some of the studies we referred to so far do involve tertiary emulsions [19, 20]. Another interesting recent variation involves enzymatic crosslinking of the adsorbed secondary layer after its deposition [22*]. Superior stabilising properties were observed.

A different possible way of depositing a protein + polysaccharide layers is to opt for a mixed layer, by carrying out the emulsification process in a single step in a solution consisting of both biopolymers simultaneously. Of course, care has to be taken that the concentrations are not above the miscibility gap and that possible complex formation between the biopolymers does not result in precipitation. These types of mixed layers have been deployed in formation of double emulsions [13, 23, 24]. The enhanced surface rheology of the adsorbed layer of complexes has been discussed by Fisher [25]. Rather surprisingly relatively few systematic studies have been carried out to carefully compare the stabilising properties of mixed and multilayer films made of same protein and polysaccharide compositions. A couple of notable exceptions are the interesting works by Jourdian et al [26**] and the more recent investigation by Azarikia and Abbasi [27*]. Both of these studies serve to show that the sequentially deposited multilayers have a somewhat superior stabilising properties, at pH values close to IEP of protein or at high salt concentrations, compared to mixed layers. These studies also brings us to a rather interesting question regarding the final configuration of our protein + polysaccharide layers. Thermodynamic considerations tell us that this configuration is determined solely by the concentration of various components in the system, and the applied external conditions (pH, temperature, etc.). More specifically, the equilibrium state of the adsorbed film is not a function of the procedure by which the deposition is carried out. It stands to reason then that the mixed and the multilayer films both cannot be the equilibrium arrangements for the biopolymers on the surface. Either one of these gradually evolves into the other, or both slowly change towards a common arrangement. Indeed, experiments of Jourdain et al [26**] suggest the latter to be the case. These researchers monitored the dynamic interfacial tension of mixed and sequentially adsorbed films and found that the two approached each other. The same was true of the measured surface viscosity, which

increased for both films with time, rapidly for the mixed case and less slowly for L-b-L, but again towards the same values. The question is then whether the final equilibrium arrangement is closer to a multilayer or a mixed film configuration? The question has clear relevance to the long term stability provided by protein + polysaccharide films. In the last ten years or so there have also been several attempts to study the kinetics of multilayer films, as they are built up and then evolve further, by using molecular dynamic simulations [28, 29]. Some of these studies show clear evidence that the boundaries between different sublayers become increasingly more "fuzzy" as different biopolymers diffuse and interpenetrate each other with time. Unfortunately, these simulations are restricted to relatively short time periods and not sufficient for the evolution of the film to reach its final equilibrium state. A different approach, more suited to dense polymer layers, was one adopted by us based on the use of self-consistent-field calculations [30*, 31**]. These studies do not provide much information on the kinetics and therefore the speed with which the films age. But they do allow for the final equilibrium state to be determined. Using a model of protein based on milk protein $\alpha_{s1}$-casein, it was found that indeed for polysaccharides with a uniform distribution of charge the thermodynamically preferred state of the protein + polysaccharide layers is one more akin to a mixed film. A true stable multilayer was nonetheless possible if parts of polysaccharide had a higher charge density with other parts lightly charged. This was particularly the case when all of the strongly charge segments were located at one end of the chains. In the latter case, the combined electro-steric repulsion was also significantly improved. The equilibrium configuration of the two types of layers, involving homogeneous and non-uniform charged polysaccharides, is depicted schematically in Fig. 1, taken from reference [30*]. How fast will a multilayer revert to a mixed layer, remains an interesting question that deserves more experimental work. It may turn out that multilayer structures are long lasting metastable states that will far exceed the shelf-life of the required food colloid formulation. In that case one need not worry about the eventual state of the interfacial film. But this is unlikely to be the case for every possible polysaccharide and protein combination.

Other interesting and largely unanswered issues related to multilayer films concern the overcharging aspect. When an anionic polysaccharide adsorbs on the primary positively charged protein film, it continues to do so beyond charge neutrality, making the resulting multi-layer negative [32]. Indeed this is exploited in the L-b-L method to lay the next layer of the cationic polysaccharide. Fig. 2, taken from the work of Guzey and McClements [9**], shows the reversal in the surface potential caused by adsorption of pectin onto a β–lactoglobulin laden surface at pH=4. Why does the negatively charged polysaccharide continue to accumulate onto a negative surface? Various suggestion have been put forward. Ettelaie et al [31**] have shown that the charge inhomogeneity of polysaccharide can lead to such a reversal. However, it seems that reversal of surface potential also happens for uniformly charged cases. Alternatively, it may be that other non-electrostatic interactions between

polysaccharides and proteins exist. In the molecular dynamic simulations, such forces need to be assumed a priori and be included in order to generate stacks of sublayers on top of each other [29]. Without them the simulation will not produce more than two sublayers. We note that these interactions need to be rather strong if they are to counteract electrostatic repulsion. This makes their origin somewhat difficult to envisage. For example extensive hydrogen bonding between the two biopolymers can do the trick, but is not so obvious why it will happen to this extent. Another possibility is that some localised areas of a polysaccharide become associated with positively charged part of the protein. This suggestion may also explain why the multilayers persists for one or two pH units on the wrong side of IEP, where both biopolymers have the same charge polarity. Once again, while easy to visualise for a pair of protein + polysaccharide in bulk solution away from other macromolecules, it is difficult to see how an extended object like a polysaccharide chain, once on the interface, ceases to experience the much more uniform field resulting from the average charge of the dense biopolymer film of which it is a part. We should also mention one last possibility that is due to charge regulation of the protein. For example, an anionic polysaccharide, will supress the pH in its locality. It is this pH that a smaller protein molecule will feel in the vicinity of this polysaccharide, and not the actual value in bulk solution. So even at the isoelectric pH, the net charge of a protein chain close to a polysaccharide may remain slightly positive.

A further likely area of future interest is the possible competitive adsorption of several different polysaccharides onto a primary protein layer. The importance of this arise from the likely simultaneous presence of different gums in commercial food formulations. Even a single polysaccharide species will have a range of structural (e.g. level of branching), as well as size distribution. An initial study on the effect of inclusion of two polysaccharides can be found in the work of Chang et al [33*], where competitive adsorption of gum Arabic and fucoidan onto a primary caseinate layer was considered. Perhaps not surprisingly, the more charged fucoidan was found to displace gum Arabic. In a separate theoretical study, Ettelaie et al [30*] predicted that for a mixture of two polysaccharides of the same charge and size, the more non-uniformly charged chains would displace the ones with a more homogenous distribution. This was attributed to short segments with much higher charge density in the case of the former, thus showing the importance of charge density, in addition to the overall electric charge of the chains, in this type of adsorption processes.

Vegetable proteins are known not to be particularly good emulsifiers or steric colloid stabilisers, as they tend to be highly aggregated globular proteins. However, the job of providing colloidal repulsive forces, caused by the overlap of the surface layers, is delegated to the polysaccharides in the multilayer stabilisation technique. Proteins only serve to attract the polysaccharides to the interface and therefore it is feasible to consider vegetable proteins for this purpose. Nonetheless, the primary emulsions made with such proteins do have to be stable for a short but sufficient time until a

secondary layer can be deposited. Several examples of the use of a vegetable protein with a polysaccharide have been reported in last few years [34, 35].

**Conjugates of protein + polysaccharides**

In our discussion in the previous section it was clear that the interactions responsible for accumulation of polysaccharide at the surface of droplets were electrostatic in origin and as such somewhat vulnerable to factors that can drastically alter these forces. To make the system less sensitive to such external parameters, one may attempt to covalently link the protein and polysaccharide molecules. Certain amino acid residues, most notably lysine can easily undergo a Maillard type reaction with the reducing sugar of polysaccharides, resulting in a covalent bond between the two biopolymers. The reactions are promoted under relatively dry condition (i.e. at suitably low water activity) and require heat treatment over an incubation period of a few hours, at very least [2*]. A recent review of the nature of such reactions and conditions promoting them is given by Oliver at al [36]. The amphiphilic nature of protein means that it adsorbs on the surface of the emulsions droplets, thus dragging the attached polysaccharide chains with it to the interface. Just as with the electrostatic complexes in the previous section, the main repulsion between the droplets is expected to be the steric one, mediated by the overlap of thick polysaccharide layers at the interfaces. Early experiments with such conjugates, demonstrated the exceptional emulsion stabilising properties of these hybrid biopolymer molecules [3*, 37] from the very onset. The contrast between the colloidal behaviour of the conjugate and protein stabilised droplets is particularly astonishing at isoelectric pH of protein. The conjugate stabilised emulsion is hardly affected, while the protein stabilise ones show extensive destabilisation and breakup at these relatively low pH values.

It is worth pointing out that protein + polysaccharide conjugates also occur naturally. Glycoproteins such as κ-casein are proteins with a few small side chains, each consisting of 3 or 4 sugar moieties. Though not often considered as such, these are effectively conjugates. The presence of these side chains, all occurring on one side of the protein, is thought to be crucial in providing κ-casein with its functional characteristics, stabilising colloidal casein micelles in milk [38]. Perhaps the best known of the naturally occurring conjugates is the proteinaceous fraction of gum Arabic, making up no more than around 12% of the total polysaccharide in this gum. Gum Arabic is frequently used as an emulsifier and emulsion stabiliser in manufacturing of citrus soft drink products [39]. It owns its ability to act as such to this small portion of the gum. The covalently bonded protein section of the conjugates acts as the agent causing the adsorption of the composite macromolecule onto hydrophobic-hydrophilic interfaces. Other naturally occurring examples are to be found in almond gum [40], Persian gum [41] and cashew tree gum [42]. But in all of these naturally occurring cases

the portion of conjugates remains relatively quite small, making it more efficient to try and produce these "artificially" by reacting proteins with polysaccharides.

The emulsification and emulsion stabilisation properties of conjugates produced during reactions between a wide variety of different proteins and polysaccharides have been studied in the last 15 years or so, with further new combinations being continuously tried and reported all the time. A few recent examples of such work considered α-lactalbumin-acacia gum [43], β-lactoglobulin-gum Acacia Seyal [44] and whey protein-maltodextrin [2*]. More interestingly, the possibility of tertiary conjugates with nutritional as well as emulsion stabilising functionalities have been explored by linking polyphenol with protein and dextran [45*]. As with the discussion in the previous section, since the main functionality in providing the steric interactions is the responsibility of the polysaccharide section of the conjugate, it is possible to use vegetable proteins in place of animal derived ones. Good emulsion stabilising properties have been achieved by reacting vegetable derived proteins with polysaccharides, where such proteins on their own are known to have quite poor emulsifying performance. Such studies have involved peanut protein isolate-dextran [46] and wheat protein-dextran [47].

One way in which the efficiency of the stabilising layer can be further improved is by using conjugates that contain a gel forming polysaccharide. It has been suggested that formation of a gel network by conjugates, accumulating at the interface, can make the layers robust to competitive displacement by small surfactant molecules [48]. This leads to a further advantage for such conjugates compared to proteins, as proteins are normally removed from the surface of droplets in the presence of low molecular weight emulsifiers. This is not desirable as it leads to destabilisation of the emulsion dispersion. Other questions regarding optimising the interfacial properties of conjugates arise by considering the most optimum number of polysaccharide attachments and the location of such linkage along the protein backbone. Akhtar and Dickinson [2*] showed that while the stability of emulsions stabilised by β-lactoglobulin + dextran conjugate improved up to a point, due to attaching more polysaccharide chains to the protein molecule, beyond a certain number the emulsions were less stable. This was attributed to the increasing hydrophilicity of the conjugates with attachment of more chains [2*]. With linkage of more polysaccharide chains to the protein, eventually it becomes preferable for the complex to remain in the aqueous solution, rather than to adsorb at the oil-water interface. The location of the attachment is trickier to control. If it can be realised practically, it can prove an extremely useful way of making even more efficient food grade steric stabilisers. Wong et al [47] have made conjugates of wheat protein with different sized dextran and concluded that the larger sized dextran chains preferentially attach towards the N-terminus end of the protein. Despite a larger number of linked smaller chains, the stabilising properties of conjugates involving larger dextran were

demonstrated in this study [47]. It is certainly true that linking the polysaccharide to one end of the protein is preferential to attaching it to a middle section of the molecule, as was predicted by the SCF based calculations of Akinshina et al [49*]. However, different sizes of the dextran may also have been responsible for the observed differences in the emulsion stabilising behaviour of the two conjugates used in the work of Wong et al. Thicker interfacial films were obtained with conjugates of larger dextran chains [47]. Despite this, in real applications one has to balance the stabilising power of the conjugates with its kinetic of adsorption. Large molecules are slow at diffusing to interfaces and often do not pack as well as smaller molecules. How small can one make the polysaccharide attachments before the conjugate shows no appreciable improvement over the protein? This question was considered in the theoretical work of Akinshina et al [49*], where a conjugate consisting of a "$\alpha_{s1}$-casin like" protein and a relatively short polysaccharide chain was used as a model system. Fig. 3 shows the average calculated distance of each amino acid residue of this "$\alpha_{s1}$-casin like" molecule from the interface, when adsorbed on the surface. Different locations considered for the attachment of a short polysaccharide chain are also shown in the figure. These in most cases correspond to the position of lysine in the primary structure of $\alpha_{s1}$-casin. The protein $\alpha_{s1}$-casein is sometimes though as crudely having a tri-block type structure. The train-loop-train type configuration of the molecule adsorbed at the interface, so typical of tri-block synthetic type polymers, is quite evident in Fig. 3. Due to its tri-block like nature, $\alpha_{s1}$-casein suffer from a tendency to induce bridging flocculation between the emulsion droplets. This is particularly the case at isoelectric point of the protein, where no electrostatic repulsion exists to counteract bridging attraction. The same is thought to be the reason as to why β-casein, with its more di-block type structure, performs better as a colloid stabiliser. What Akinshina et al observed was that attaching a short polysaccharide at middle of the hydrophilic loop (see Fig. 3) of their "$\alpha_{s1}$-casein like" protein increased the tendency for bridging. In contrast, if attached to one end of the protein, the stabilising ability of the conjugate became markedly better than the original $\alpha_{s1}$-casein, especially close to its isoelectric pH. For long chains, the location of the attachment was found to be less critical [49*].

Production of conjugates has largely been performed on a lab scale. The scaling up of the process to an industrial level poses several complications of its own. While attempts have been made to make protein + polysaccharide conjugates in wet form, still the most efficient techniques remain those using dried powder mixtures of the two biopolymers. As such the technique is unfortunately energy intensive and slow, particularly since the dry powder has to also undergo a considerable period of incubation at elevated temperatures. In the light of these results, and since spray drying is a faster technique, it may be preferable to use it in the large scale industrial manufacturing of the conjugates. Other issues worthy of consideration are the impact of the contaminant. For example commercial grade whey protein will most certainly contain some lactose impurity. Even a small amount of this

impurity is appreciable on a molar basis, when compared to much larger polysaccharide molecules. The Maillard reaction between sugar and protein can block many reacting sites on the protein, thus reducing the efficiency of linkage between the two biopolymers. The reactions between polysaccharide and protein may also not be complete in such large scale production. This leaves behind unreacted protein which during emulsification process will most certainly compete with the conjugates for adsorption at the hydrophobic surfaces. How small does the portion of unreacted protein have to be to ensure that the conjugates will be the ones prevailing at the interface? Questions such as these, are likely to become more thoroughly investigated as and when the food industry begins to use protein-polysaccharide conjugates more extensively in the formulation of their products. Issues relating to synthesis and the behaviour of conjugates as food grade dispersants have been the subject of several recent reviews. The very latest of these can be found in the excellent articles of Dickinson [50**] and that of de Oliveira et al [51*].

**Hydrophobically modified polysaccharides**

Polysaccharides are by and large hydrophilic macromolecules. In the techniques discussed in the previous two sections, they were induced to reside on a hydrophobic surface through their favourable electrostatic interaction or covalent linkage with protein chains. However, it is possible to do away with the protein and turn the polysaccharides into amphiphilic molecules, capable of adsorption at air-water or oil-water interfaces, directly. This is achieved by hydrophobic modification of the polysaccharide through covalent attachment of several small hydrophobic groups at different, often random, locations along the biopolymer backbone. Adjusting the number and size of such sites, the amphiphilic nature of the hydrophobically modified polysaccharide can be fine-tuned. The method has most widely been applied to cellulose and its derivatives [52], chitosan [53], dextran and starch [54*-56*], perhaps not surprising given that these are the most abundant polysaccharides. Other notable examples include hydrophobic modification of alginates [57]. 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 anhydrides, such as octenyl succinic anhydride (OSA) and inclusion of fatty acid chlorides with hydroxyl groups in starch molecules [54*, 55, 58*, 59]. The hydrophobic nature of attachments necessities the use of a limited amount of organic solvent during synthesis of such modified polysaccharides, which is not particularly desirable in producing food grade ingredients. Alternative routes for such modification, not required organic solvents are increasingly being explored [56*], where, for example, the locus of the modification reactions are shifted to the centre of micellar structures, made by self-assembly of suitable surfactants [56*]. Despite this, at present only octenyl succinic anhydride (OSA) is currently a permitted food-grade reagent for the modification of starch [59]. This, somewhat synthetic aspect of the modification, is sometimes considered as one of the major disadvantages of hydrophobically modified polysaccharides, compared to other types of food

dispersants discussed in previous sections. Furthermore, the degree of modification (i.e., the number of glycoside monomers of polysaccharide with attachments) allowed for use in foods is limited to a maximum of 3% in many countries, and in some cases even lower. Fortunately, this is still sufficient to ensure the strong adsorption of hydrophobically modified starch to surface of oil emulsion droplets [54*, 55].

While in some cases the modified starch may possess some electrical charge [60], the main mechanism for stabilising the emulsions in these types of modified biopolymers is through provision of steric repulsion. This is nicely demonstrated by the work of Chanamai and McClements [61**] where they compared the behaviour of WPI stabilised droplets to those stabilised by hydrophobically modified starch and also gum Arabic. In particular, modified starch stabilised emulsions were found to exhibit excellent stability at all pH values, including at isoelectric pH for WPI. Furthermore, the measured $\zeta$-potential was found to be very low in the entire range of pH values considered by these researchers [61**]. These results are reproduced here in Fig. 4. It is this reliance on steric, rather than electrostatic stabilisation which gives the emulsions stabilised by modified starch their relative insensitivity to changes in the environmental conditions. This is especially true of variation in pH and background salt concentrations. Nonetheless, there are also certain common features between hydrophobically modified starch and protein based systems. As we mentioned in the introduction, excessive amount of biopolymer remaining in the solution can lead to depletion effects, while too little, to fully cover the surface of droplets, can cause bridging flocculation. This pattern of bridging-steric stabilisation-depletion flocculation, predicted with increasing biopolymer concentration, has been found for protein stabilised emulsions as well as synthetic random copolymers. For modified starch, steric stabilisation followed by depletion has also been reported [62]. However, even higher concentrations of modified starch lead to the formation of gel networks in the bulk solution, resulting in a considerable increase in the viscosity [63]. This stabilises the droplets as it retards their Brownian motion and the rate of inter-droplet collisions. It is interesting to speculate on the nature of such gel networks. For hydrophobically modified starch this is more likely to progress through the association of the hydrophobic groups, as oppose to hydrogen bonding one normally observes for unmodified starch [64*]. As for bridging effect, while we do not know of studies that unquestionably demonstrate this by hydrophobically modified starch, polysaccharides are known to be capable of doing so under other different circumstances (e.g. in L-b-L stabilised emulsions [33*]).

The use of a single functional ingredient which can act as both a stabiliser/emulsifier and also a rheology modifier can be quite useful in certain type of applications. An especially good example of this is the use of hydrophobically modified starch in microencapsulation process, through the emulsification route. In this method, one first homogenises the dispersed phase, containing the active ingredient (flavour, drug, vitamins, etc.), to form an emulsion. This is then dried to remove the

dispersion medium, leaving behind a powder that includes the encapsulated active. To provide the powder with the necessary bulk properties post drying, polysaccharide is also often included in the formulation. In such applications, the hydrophobically modified polysaccharide can simultaneously provide both the above two required functionalities. Hydrophobically modified polysaccharides can also stabilise emulsions in somewhat different way, by acting as Pickering particles capable of adsorbing onto the surface of the droplets. It seems that for the encapsulation purposes this mode of stabilisation is preferred to one involving molecularly adsorbed interfacial layers. The use of hydrophobically modified starch granules in Pickering stabilisation of emulsions has been extensively discussed by Yusoff and Murray [58*], Marefati at al [65*] and Sjöö et al [66]. Other possible techniques for synthesis of such modified polysaccharide based nanoparticles, suitable for use in Pickering stabilisation of food emulsions, have also been reported by a number of researchers [67]. In particular, crosslinking the polysaccharide chains to ensure that the polysaccharide particles will not dissolve over time, is a useful technique worthy of mention in this context [68].

As in previous sections, use of a novel biopolymer as food grade dispersant introduces several interesting questions. Starch is made from both branched (amylopectin) and linear (amylose) chains. Notwithstanding obvious differences in the molecular weight of these two components, which of these would be a more efficient dispersant if suitably modified? One of the few theoretical studies concerning hydrophobically modified starch [69*] suggests that a combination of the two will give a superior stabilising behaviour compared to one expected from each component on its own. The double act performed by hydrophobically modified amylose and amylopectin seems to mirror one involving combinations of globular and disordered proteins, such as whey protein + casein [4, 5**]. A rather lucid account of this combined synergic action of two biopolymers, reinforcing their interfacial properties, can be found in a very recent review by Dickinson [50**]. Another question concerns the position of attachments, particularly onto branched polysaccharides. Bai et al [70] have found that hydrophobic attachments favour locations close to branching points, particularly when the degree of modification is low. It would be interesting to investigate (theoretically and experimentally) how the emulsifying and stabilising ability of the modified amylopectin is affected if the attachments are more uniform, or even biased towards the non-reducing ends. In a somewhat related study, Tizzotti et al [71*] considered the influence of the degree of branching, level of modification and overall molecular weight of the chains. Authors conclude that the trend in emulsifying behaviour of modified starch with these architectural parameters is not all that dissimilar to the one exhibited by low molecular weight synthetic branched surfactants.

Starch begins to be hydrolysed in mouth, while protein is fragmented in stomach. Yet, other polysaccharides are not digested at all. Therefore, it is quite plausible to foresee the use of mixtures of emulsions, stabilised by different types of hydrophobically modified polysaccharides, as well as

with ones by proteins, to achieve tailored controlled realise profiles in food and related industries in future.

**Fragmented proteins**

An alternative approach to design of better food dispersants is to consider polypeptide fragments of proteins for this purpose. The strategy is very different to those considered above. Rather them attempting to make a larger entity (whether an electrostatic complex, conjugate or by hydrophobic attachments), this method results in smaller chains than the original protein. The basic idea is that by hydrolysing a protein to a smaller set of polypeptide fragments, some of these may have more desirable structures, boosting their emulsion stabilizing functionality. Smaller molecules also have the added advantage of faster adsorption kinetics, making it in principle easier to produce very fine stable droplets. This is not only due to their higher diffusion coefficient, resulting from their smaller size, but also the fact that such fragments are more likely to be in a coil-like disordered conformation. However, experimental studies involving such fragments seem to provide a rather mixed picture, with some finding excellent improvement in interfacial properties [72, 73*], while many others reporting very little change, if not a deterioration in dispersant stabilising ability [74] relative to the original protein. Some studies have even indicate a stronger ability of polypeptides to act as a barriers against oxidation [75], presumably due to their better packing at the interfaces, as well as possible antioxidant properties.

Ettelaie at al [76**] applied self-consistent-field (SCF) calculations to fragments of a model protein. The aim of their study was to demonstrate that at least in principle there are situations in which the interfacial layers formed from fragmented proteins, could be shown to provide stronger colloidal repulsive forces than films of the original protein. For this purpose, the authors based their model protein on the primary structure of $\alpha_{s1}$-casein. They also considered a somewhat idealised situation where any one of the 14 possible bonds of $\alpha_{s1}$-casein, susceptible to hydrolysis by trypsin, can be individually and selectively targeted, with all the other peptide bonds remaining intact. They argued that by breaking up the $\alpha_{s1}$-casein from essentially a tri-block like polymer, to one that has a di-block structure (i.e. more like β-casein) a stronger stabilising power would be achieved. Indeed, their numerical calculations supported this view when a peptide bond close to the hydrophilic middle part of the $\alpha_{s1}$-casein, on the N-terminus side, was broken. This is displayed in graphs of Fig. 5, showing mediated colloidal interaction between 1 μm sized droplets arising from the overlap of adsorbed layers. But perhaps more interestingly, when the same calculations were performed for breakage on the C-terminus side of the hydrophilic loop (see Fig. 3), they failed to show any significant improvements. This is despite the fact that in both cases a polypeptide with a more di-block, β-casein

like structure is generated. The clue to resolving this puzzle came from examining the competitive adsorption between the two resulting fragments caused by hydrolysis of a single bond. When the broken bond was on the N-terminus side of the hydrophilic loop, the di-block like polypeptide dominated the surface adsorption [76**]. However, if the cleaved bond was on the C-terminus side, it was the other less desirable fragment, that prevailed on the interface. The result is significant as it shows that not only suitable fragment structure is necessary, but also one needs to take into account how the wanted polypeptides compete for adsorption with all the other possible hydrolysates generated during the breakage of the protein. Given that in practice the fragmentation is likely to involve several bonds, this may explain why the results of experiments could seem rather contradictory and somewhat difficult to reproduce. Even relatively small changes in the degree of hydrolysis can very quickly alter the composition on the surface, due to strong competitive adsorption between all the created polypeptide species.

Despite the above difficulties, experimental attempts to use polypeptide fragments as emulsifiers/stabiliser abound in the literature. Since such chains still need to provide some steric stability, they cannot be too small. One may expect then that the optimum degree of hydrolysis (DH) should occur at a relatively small level. Indeed, Chen et al [72] used power ultrasound, as well as extrusion, to indiscriminately fragment soy bean protein. They found an improvement at first, but beyond a DH of around 1.25% the emulsifying ability of the resulting chains decreased sharply. An optimum level of hydrolysis was also reported by Zhang et al [73*], with higher DH values than 10% having a detrimental impact on both emulsion and foam stability, though not necessary foaming and emulsifying ability. These opposing trends concerning the reduction in interfacial tension versus the emulsion stability, have also been seen for lentil protein isolate hydrolysed with heat + trypsin [77]. In this latter study the authors only considered DH=4, 9 and 20%. Already, at a DH value of 4%, a reduction in emulsion stability index was found.

The nature of the enzyme and thus the bonds that are susceptible to breakage, also has a big impact on the interfacial properties of the fragments, as one may well expect. This was demonstrated by work of Barac et al [78] who used papain and a commercial enzyme to hydrolyse pea protein isolate. While significant improvements in emulsifying properties, at least over some range of pH, was noticed for papain, the same was not true when the commercial enzyme was used. Similar differences in the emulsion stabilising behaviour of hydrolysates, produced by fragmentation of soy protein isolate by neutrase and trypsin, were also reported. It was seen that polypeptides produced by the latter enzyme exhibited superior properties [79*].

Finally we should also mention that much of the research work on protein hydrolysates is not only driven by the interest in their surface adsorption properties, but also due to their potential to act as

antioxidants [80]. Further advantages (and disadvantages) of the use of fragmented proteins, in relation to the sensory aspects of foods, were recently examined recently by Gani [81*].

In summary, it seems that a relatively non-selective of breakage of bonds is only of real benefit for proteins which have a poor initial emulsifying and emulsion stabilising behaviour, and then at relatively low DH values. For proteins with already reasonable interfacial properties (e.g. sodium caseinate) little can be gained by fragmentation. This is unless a very selective cleavage of bonds is performed. Furthermore, it may be necessary to filter out some of the more undesirable hydrolysates, also generated in the process, for a true improvement to be seen in such cases.

**Conclusions**

Food industry still largely uses proteins as natural colloidal dispersants to stabilise emulsions and food grade nanoparticles. However, a bottom up approach to the design of food structure in future, requirements for more targeted delivery of food nutrients during digestion, and a more carefully tailored release profile of flavours during mastication of foods, all require a far better control over interactions that operate between food entities on mesoscale levels. To achieve this, edible superior dispersants with performance better than those currently used in industry, are essential. This review has examined recent progress on several fronts in this direction, namely the use of protein + polysaccharide multilayers, Maillard conjugates between protein and polysaccharides, hydrophobic modification of starch and other polysaccharides and the use of polypeptides obtained gentle hydrolysis of various proteins. We have largely limited the review to dispersants which form molecularly adsorbed layers on interfaces. Thus, for example, the stabilisation by small food grade particles (i.e. Pickering stabilisation) is not considered here. However, this is not to say that much progress involving the realisation of such nanoscale particles, as well as interest in studying the properties of emulsions stabilised by them, has not already been made. Other approaches not discussed but worth mentioning involve the use of combinations of proteins, either with each other or with small molecular weight emulsifiers. Nonetheless, in such cases one has to be much more careful, as often there are additional complications which are not usually conducive to good stabilising properties. Examples are competitive adsorption between different proteins and between proteins and small MW emulsifiers [82], as well as the possibility of phase separation and phase transition in the mixed interfacial films [83].

The optimisation of the strategies discussed in this review also highlight a number of very interesting fundamental questions, a few of which were briefly discussed here. The progress in resolving these questions provides exciting areas of continued and future research, which will need a combination of careful experimental work, guided by underlying theoretical understanding, to fully answer.

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**Figure Captions**

Fig. 1 – A schematic showing the differences between the equilibrium structure of two neighbouring protein + polysaccharide mixed layers, upon their overlap, when a) the charge of the polysaccharide is uniformly distributed along its backbone b) when a section of polysaccharide contains most of its charge [31*].

Fig. 2 – The ζ-potential of a priori adsorbed, positively charged protein layer, upon deposition of a secondary pectin layer, at different bulk concentrations of pectin. Results are taken from [9**] with permission and demonstrate the reversal of the charge of the interfacial layer when the secondary layer is adsorbed.

Fig. 3 – The average distance for each monomer of $\alpha_{s1}$-casein from the surface, as obtained by SCF calculations, for a chain adsorbed as part of a dense protein layer on the interface. Monomers are labelled sequentially starting from 1, beginning with the first amino acid residue at N-terminus side. The arrows indicate the possible positions on protein backbone considered for attachment of a polysaccharide chain in the calculations of Akinshina et al [49*].

Fig.4 – The ζ-potential of emulsion droplets stabilised by hydrophobically modified starch plotted as a function of pH, in solutions with different background salt concentrations. Results are taken from the work of Chanamai and McClements [61**].

Fig.5 – SCF calculated interactions between a pair of oil droplets of size 1 μm, mediated by the adsorbed interfacial films of a) intact $\alpha_{s1}$-casin and b) hydrolysates of $\alpha_{s1}$-casein produced from the breakage of a single bond next to the hydrophilic loop of the protein, on the N-terminus side of the chain. All the results are at a salt volume fraction of 0.01 and pH values of 7 (dash-dotted line), 5 (short dashed line), 4.5 (long dashed line), and 3 (solid line). Data were taken from reference [76**].

**Figure 1**

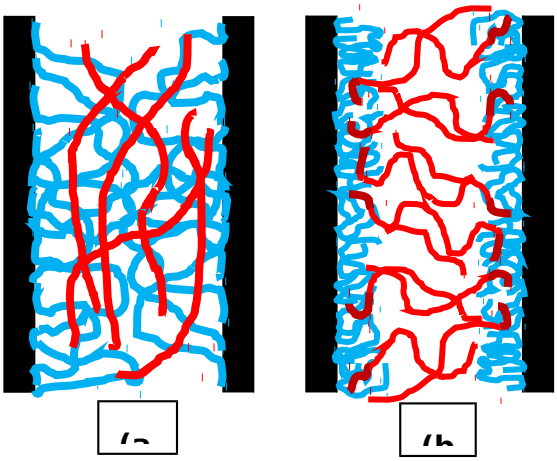


**Figure 2**

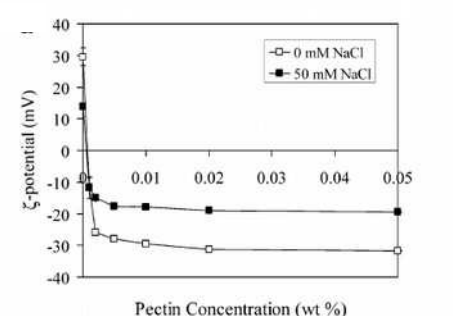

40
30
20
10
0
-10
-20
-30
-40
0.01
0.02
0.03
0.04
0.05
0 mM NaCl
50 mM NaCl
ζ-potential (mV)
Pectin Concentration (wt %)

**Figure 3**

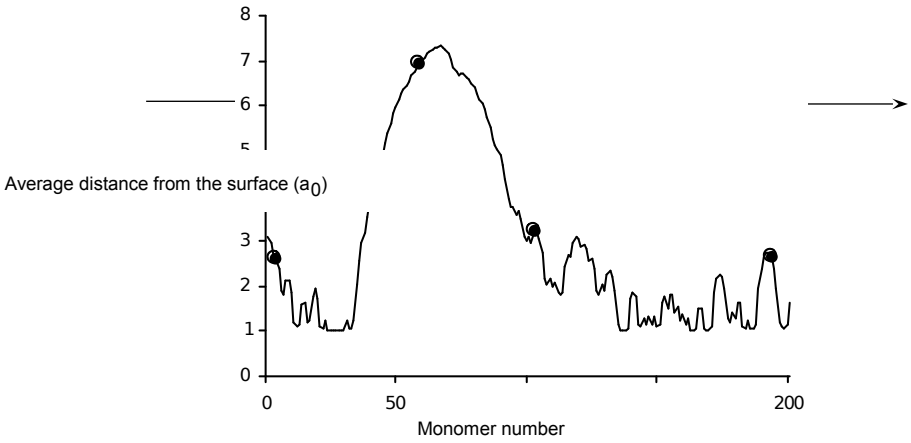

8
7
6
Average distance from the surface ($a_0$)
3
2
1
0
0
50
200
Monomer number

**Figure 4**

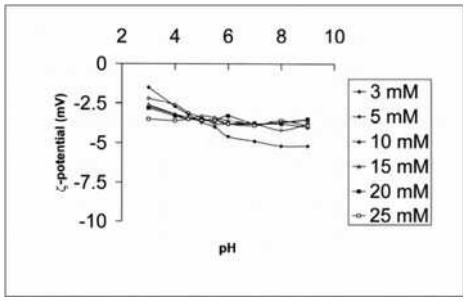

2
4
6
8
10
0
-2.5
-5
-7.5
-10
ζ-potential (mV)
pH
3 mM
5 mM
10 mM
15 mM
20 mM
25 mM

**Figure 5a**

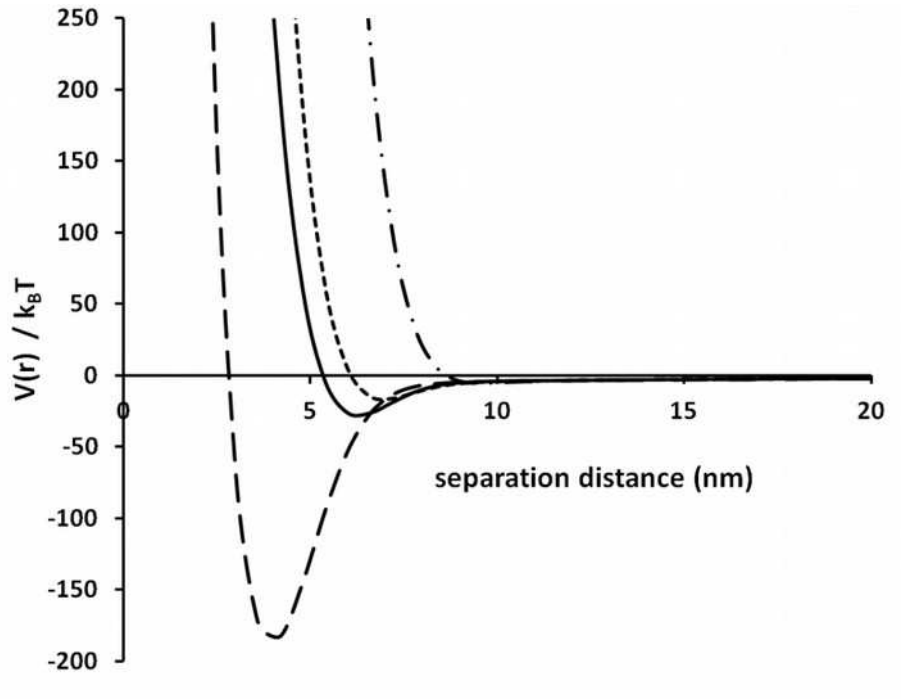
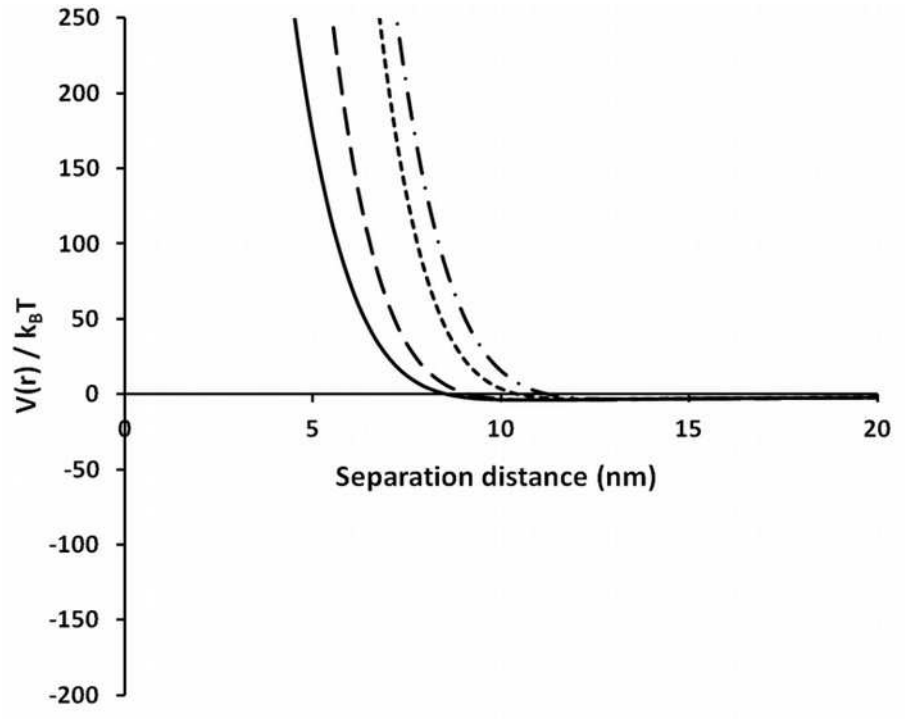

250
200
150
100
50
0
-50
-100
-150
-200
V(r) / $k_B$T
0
5
10
15
20
separation distance (nm)

**Figure 5b**


250
200
150
100
50
0
-50
-100
-150
-200
V(r) / $k_B$T
0
5
10
15
20
Separation distance (nm)