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ARTICLE

# Adsorption of Gum Arabic, Egg White Protein, and Their Mixtures at the Oil−Water Interface in Limonene Oil-in-Water Emulsions

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The adsorption behavior of gum Arabic, egg white protein, and their mixtures at the oil−water interface for 20% limonene oil emulsions has been investigated at pH 3.5 and 7.5. It has been shown that protein-rich fractions of gum Arabic adsorb onto limonene oil droplets and that there is no significant molecular mass dependence. The amount adsorbed was determined from differences in the intensities of the gel permeation chromatography elution profiles of the gum before and after preparing emulsions and was found to be ∼6.5 and ∼5 mg/m$^2$ at pH 3.5 and 7.5, respectively. These values are greater than might be expected for monolayer coverage. The amount of protein associated with the gum Arabic adsorbed was about 0.25 mg/m$^2$, which corresponds to ∼26% of the total protein present in the gum. In comparison, the amount of egg white protein adsorbed was found to be ∼1.2 and ∼ 0.8 mg/m$^2$, respectively, at pH 3.5 and 7.5, which are typical values for monolayer coverage. For gum Arabic−egg white protein mixtures (1:0.05 w/w corresponding to ∼1:1 on a molar basis) at pH 7.5, both species are negatively charged, and there is no interaction between them. On formation of emulsions, they compete with each other for surface sites, and egg white protein molecules are adsorbed preferentially, although not exclusively. At pH 3.5, the molecules have opposite charge and interact and at this ratio form soluble electrostatic complexes, which are adsorbed at the interface during emulsification. The droplet size of emulsions prepared with gum Arabic was significantly less than for egg white protein over the concentration range studied. Gum Arabic−egg white protein mixtures (1: 0.05 w/w) at pH 3.5 produced emulsions with a droplet size similar to gum Arabic alone, while those prepared at pH 7.5 produced emulsions with a significantly larger droplet size.

**KEYWORDS: Gum Arabic; egg white protein; polysaccharide−protein complexes; emulsification; adsorption isotherms; oil−water interface**

## INTRODUCTION

Gum Arabic is a tree gum exudate that is obtained from the stems and branches of *Acacia senegal* and to a lesser extent *Acacia seyal*, which grow widely across the Sahelian belt of Africa (*1*−*3*). The gum from *A. senegal* has been the subject of a number of investigations, and it has been demonstrated that it is a complex polysaccharide consisting of three components that differ principally in their molecular size and protein contents (*4*−*7*). These are commonly referred to as the arabinogalactan−protein (AGP), arabinogalactan (AG), and glycoprotein (GP) fractions, which have molecular mass values of $1-2 \times 10^6$, $2.5 \times 10^5$, and $2 \times 10^5$ and protein contents of ∼10, $<0.5$, and $>20$%, respectively. All three fractions contain a highly branched carbohydrate component that consists of a $\beta$-1,3-galactan core with ramified side chains consisting of galactose, arabinose, rhamnose, and glucuronic acid. The AG is the major component (90% of the total gum), and Sanchez et al. have recently concluded from small angle neutron scattering, transmission electron microscopy, and atomic force microscopy experiments that it has a disklike structure with a diameter of 20 nm and a thickness of 2 nm (*8*). The AGP, which represents ∼10% of the gum, has a "wattle blossom" type structure with carbohydrate residues of molecular mass $\sim 4 \times 10^4$ attached to a polypeptide chain consisting of ∼250 amino acids through serine and hydroxyproline linkages (*9*). It is likely that the carbohydrate residues also have a disklike structure. The GP represents only ∼1% of the gum and has a different amino acid profile and lower glucuronic acid content than the other components (*5*, *6*). Both the AGP and the GP have been shown to possess polyproline II, $\beta$-sheet, and random coil structures from circular dichroism studies, whereas the AG has no secondary structure (*6*).

Gum Arabic is widely used to stabilize flavor oil emulsions for application in beverages, dried soups, cake mixes, etc. It is now generally recognized that the emulsification properties are due to the presence of the proteinaceous fractions, which adsorb onto the oil droplets while the carbohydrate moieties extend out from the surface into the aqueous solution. It has been argued that the

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adsorbed gum arabic surface layer is able to prevent droplet flocculation and coalescence through both electrostatic and steric repulsive forces (*9*−*14*). Although proteins are also used to stabilize oil-in-water emulsions, they are not generally considered as effective as gum Arabic and are more susceptible to flocculation in the presence of electrolytes (*15*).

There has been considerable interest in recent years in the use of polysaccharide−protein complexes as emulsifiers, which can be in the form of Maillard type conjugates (*16*, *17*) or electrostatic complexes (*18*−*31*). One approach that has been adopted to stabilize emulsions is to form multilayers through electrostatic complexation at the surface of the oil droplets. This can be achieved, for example, by preparing an emulsion in the presence of protein and then adding a polysaccharide under conditions where the protein and polysaccharide have opposite charge. Although bridging flocculation may occur initially, if there is sufficient polysaccharide present to provide surface saturation, the aggregation can be disrupted by mechanical shear, yielding stable emulsions (*15*). Rather than this two-stage process, we have shown that it is possible to produce stable oil-in-water emulsions at low pH values (∼pH 3) by the addition of soluble bovine serum albumin−gum Arabic complexes at relatively high protein−polysaccharide ratios (∼8:1) (*32*). Jourdain et al. have also reported that stable emulsions can be prepared using soluble sodium caseinate−dextran sulfate complexes at low pH in which the dextran sulfate was in large excess (*33*).

The aim of this work was to study the adsorption of gum Arabic, egg white protein, and mixtures of the two at the oil−water interface at pH values of 3.5 and 7.5. At pH 3.5, the protein will have an overall net positive charge, and gum Arabic will have a net negative charge; hence, they are expected to form electrostatic complexes. At pH 7.5, both carry a net negative charge; hence, they are not expected to interact. Egg white protein itself is not regarded as a good emulsifier, but it was considered that its properties could be enhanced by complexation with gum Arabic. Kudryashova and de Jongh have recently reported some interesting results for the adsorption of egg white ovalbumin−pectin complexes at the air−water interface (*34*).

## MATERIALS AND METHODS

**Materials.** A kibbled gum Arabic sample from *A. senegal* species was supplied by Agrisales Ltd. (London). The sample had a moisture content of 12.5%, and the sugar composition was determined using high-performance liquid chromatography and found to be 40% arabinose, 34% galactose, and 12% rhamnose. Elemental analysis was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES), and the gum was found to contain 0.72% potassium, 0.66% calcium, and 0.22% magnesium with trace amounts of barium, strontium, and manganese. It also contained 18 ppm sulfur and 3.2 ppm phosphorus. The protein content was found to be 2.15% as determined by amino acid analysis.

Egg white protein was obtained from San-Ei-Gen FFI Inc. (Japan). It consists of a number of proteins, notably ovalbumin, which represents ∼58% of the total and has a molecular mass of 58000 and isoelectric point (IEP) at pH 4.6; conalbumin, which represents ∼13% of the total and has a molecular mass of 80000 and IEP at pH 6.6; and ovomucoid, which represents ∼11% of the total and has a molecular mass of 28000 and IEP at pH 3.9. Other proteins include ovoglobulin, which accounts for 11.5% of the total, and lysozyme (3.5%), which has a molecular mass of 14600 and an IEP at pH 10.7.

The moisture content was determined to be 7.15%. To determine the solubility of the egg white protein, 15 g was dissolved in 100 mL of water, and the solution was stirred overnight at room temperature. It was then centrifuged for 120 min, and the amount of dissolved material in the supernatant was determined by drying aliquots in an oven at 105 °C to constant weight. The solubility was found to be 89%. In the work presented below, the concentrations quoted are based on the amount of dissolved protein. (*R*)-(+)-Limonene and sodium benzoate were purchased from Sigma-Aldrich (Germany).

**Methods.** *Molecular Mass Distribution*. A Superpose 6 column was used to determine the molecular mass distribution of gum Arabic, egg white protein, and gum Arabic−egg white protein mixtures. A 100 μL injection loop was used, and sample elution was monitored using a Dawn DSP Laser photometer, which had a wavelength of 633 nm (Wyatt Technology Corp., United States) together with a Wyatt Optilab DSP Refractometer with 10 mm P100 cell (Wyatt Technology Corp.) and a UV Spectrophotometer (Agilent 1100 series) at 214 and 280 nm. The prepared samples were filtered through 0.45 μm nylon filters prior to injection onto the column. For gum Arabic and gum Arabic−egg white protein mixtures, 0.1 M NaCl eluent was used. For egg white protein, 5 mM phosphate buffer at pH 7.5 was used to maintain the pH of the sample. A constant flow rate of 0.5 mL/min was adopted. The values used for the refractive index (RI) increment ($dn/dc$) of gum Arabic and egg white protein were 0.141 and 0.185 mL/g, respectively. The collected data were processed using ASTRA version 4.5 software.

*Formation of Electrostatic Complexes between Gum Arabic and Egg White Protein*. Stock solutions of 0.5% gum Arabic and 0.5% egg white protein were prepared at pH 3.5 and 7.5. The 0.5% egg white protein was centrifuged at 2500 rpm for 60 min to remove insoluble material, and the supernatant was serially diluted to obtain various concentrations of egg white protein (0.05−0.5%). To 10 mL of 0.5% gum Arabic, 10 mL of various concentrations of 0.05−0.5% egg white protein were added. The turbidity was monitored by measuring the absorbance at 400 nm using a Perkin-Elmer (Lambda 25 UV/vis) spectrophotometer.

*Electrophoretic Mobility*. The electrophoretic mobilities of 5% gum Arabic and 5% egg white protein were measured as a function of pH using Laser Doppler Velocimetry (Zetanano-ZS, Malvern instruments). Stock solutions of 20 mL of 5% gum Arabic and 5% egg white protein were prepared and titrated using the multipurpose titrator. The instrument automatically uses acid or base to adjust the pH; hence, the electrophoretic measurements were measured between pH 2 and pH 9. Measurements were also carried out on 20% limonene oil-in-water emulsions containing 5% gum Arabic, 5% egg white protein, and gum Arabic−egg white protein mixtures at varying mixing ratios and at pH 3.5 (see below) prepared by mixing for 4 min using a Silverson Homogenizer. All of the experiments were performed at a temperature of 25 °C using clear disposable Zeta capillary cells (DTS 1060). The cells were cleaned with distilled water before use and were filled with the sample slowly to avoid air bubbles. The data were analyzed by using Dispersion Technology version 4.2 software (Malvern Instruments).

*Determination of Adsorption Isotherms*. Gum Arabic and egg white protein solutions of various concentrations 0.1−0.5% were prepared at two different pH values, that is, pH 3.5 and pH 7.5 using HCl and NaOH to adjust the pH. Sodium benzoate (0.1%) was added as a preservative. Limonene oil-in-water emulsions were prepared by adding 26.67 g of gum Arabic or egg white protein solution at pH 3.5 and 7.5, and the volume was made up to 32 g with distilled water. Eight grams of D-limonene was added, and 20% w/w emulsions were prepared using an IKA Ultraturrax T25 mixer set at 18000 $min^{-1}$ for 4 min. The emulsions were left to equilibrate overnight before centrifugation, the supernatant was collected, and the molecular mass distribution was determined. The difference in the RI and UV absorbance (280 nm) peak areas before and after emulsification was determined using the Astra software and was indicative of the amount of gum Arabic or egg white protein adsorbed onto the oil droplets. The surface area of the emulsions was determined from the size of the emulsion droplets determined by laser diffraction using the Mastersizer 2000. The density of the limonene was 0.843.

The adsorption of gum Arabic−egg white protein soluble complexes was also investigated. A 0.57% gum Arabic solution and 0.0284% egg white protein solutions were prepared and stirred overnight. Fifty milliliters of each sample was taken, and the pH was adjusted to 3.5 and 7.5. Equal amounts of gum Arabic and egg white protein at the same pH were mixed together to produce 0.3% solutions of gum Arabic−egg white protein mixtures at a ratio of 1:0.05. Ten milliliters of these solutions was passed down the Superose 6 GPC column, and the elution was monitored by RI.

The remaining solution was used to prepare a 20% limonene-oil-in water emulsion by adding 25 mL of limonene oil to the 0.3% solutions of gum Arabic−egg white protein mixtures (1:0.05 at pH 3.5 and 7.5) and mixing for 4 min using the Ultraturrax mixer.

*Determination of Droplet Size*. The droplet size was determined as a function of gum Arabic, egg white protein, and gum Arabic−egg white protein concentration by laser diffraction using the Mastersizer 2000 (Malvern Instruments). The instrument was cleaned by filling the dispersion unit with distilled water, increasing and decreasing the speed of agitation, and flushing water through the system until the laser intensity displayed was ~80%. The emulsion was added dropwise using a plastic pipet to the water in the dispersion unit until the obscuration was about 12%. The measurements were made at room temperature and were performed in triplicate.

## RESULTS AND DISCUSSION

**Gum Arabic. Figure 1** shows the GPC elution profile of gum Arabic monitored by RI and UV absorbance at 280 nm. The elution curves using the two detection systems are different due to

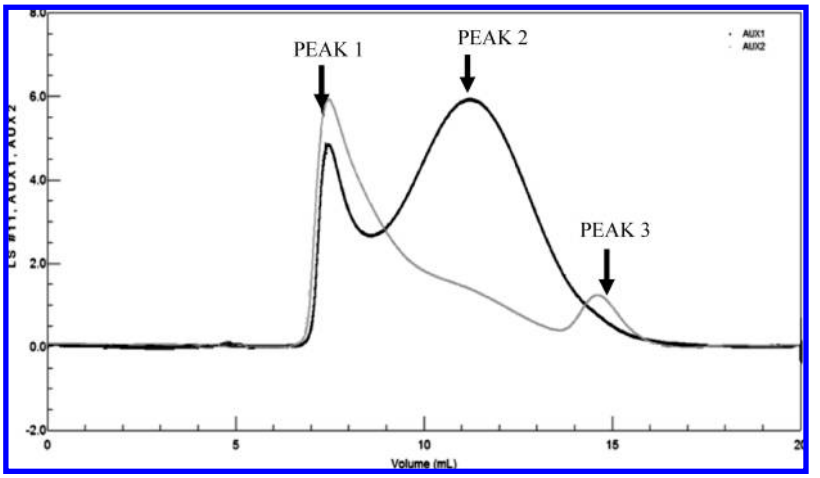


**Figure 1.** GPC elution curves of gum Arabic monitored by RI (dark line) and UV absorbance at 280 nm (light line).

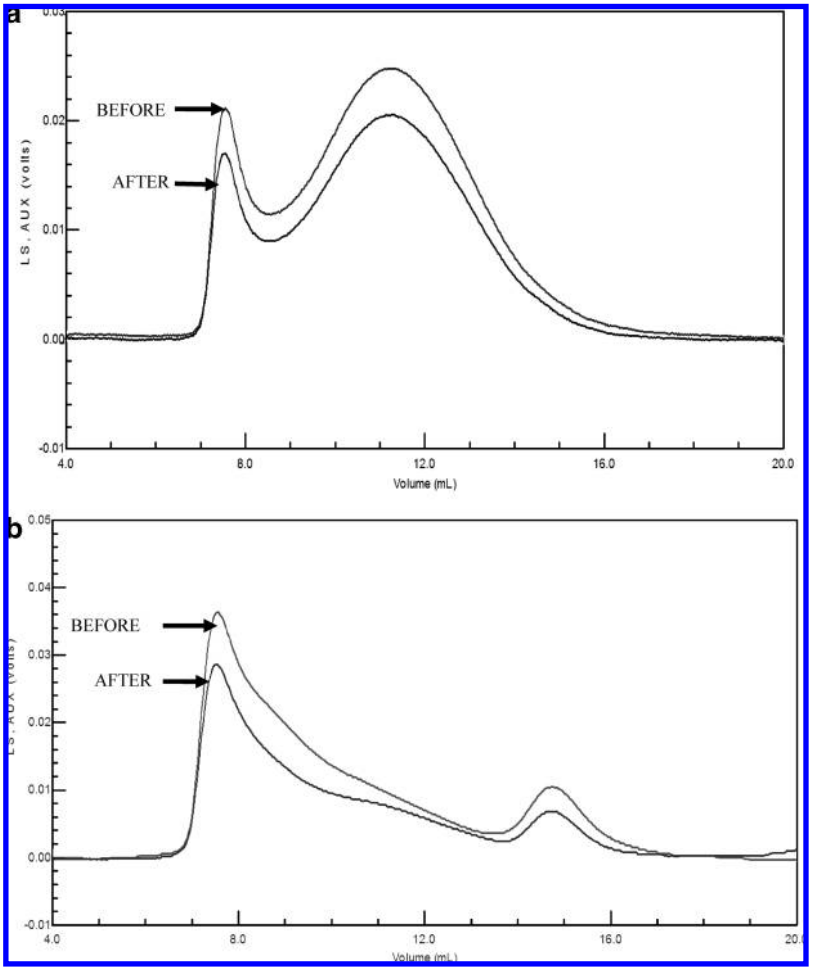


**Figure 2.** (**a**) GPC elution curves of gum Arabic at pH 7.5 monitored by RI and the supernatant recovered after preparing an emulsion. (**b**) GPC elution curves of gum Arabic at pH 7.5 monitored by UV absorbance at 280 nm and the supernatant recovered after preparing an emulsion.

the fact that the RI elution curve is sensitive to the concentration of all of the eluting species, while UV absorbance at 280 nm is sensitive only to the concentration of the proteinaceous material eluting. As has been discussed previously (*1*−*6*), gum Arabic consists of three distinct components, namely, an AGP, an AG, and a GP, which correspond to peaks 1, 2, and 3, respectively. These differ mainly in their molecular mass and protein contents.

The RI and UV absorbance (280 nm) GPC elution profiles of a gum arabic solution at pH 7.5 before preparing limonene oil-in-water emulsions and the supernatant after emulsification are given in **Figure 2a**,**b**, respectively. The difference in the intensities of the peaks represents the amount of gum Arabic adsorbed onto the oil droplets. It is interesting to note that there is no molecular mass dependency for the adsorption process and that all of the fractions adsorbed contained proteinaceous components as noted from the UV absorbance elution profiles. Similar findings were observed at pH 3.5 (data not shown). It has been reported previously that protein-rich fractions adsorb, and it has been argued that the more hydrophobic protein moieties serve to anchor the molecules to the surface, while the hydrophilic carbohydrate component protrudes out into the aqueous phase (*1*−*5*). Further evidence for the role of the protein comes from the fact that if the gum Arabic is treated with proteolytic enzyme, the protein associated with the AGP component is hydrolyzed and the gum loses its ability to form stable emulsions (*35*).

The difference in the intensity of the GPC RI elution profiles was used to calculate the amount of gum Arabic material adsorbed, while the difference in intensity of the GPC UV absorbance (280 nm) profiles was used to calculate the amount of proteinaceous material adsorbed. The adsorption isotherms obtained for the whole gum at pH 3.5 and 7.5 are given in **Figure 3**. The initial slope of the isotherms is indicative of low affinity adsorption. Whereas the isotherm at pH 7.5 reaches a plateau value of ~5 mg/m$^2$, the amount adsorbed at pH 3.5 continues to rise over the concentration range studied up to ~6.5 mg/m$^2$. At the lower pH, the degree of dissociation of the gum Arabic glucuronic acid groups is lower; hence, the molecules are less charged. Consequently, intermolecular electrostatic repulsion between the adsorbed molecules is reduced, enabling more molecules to adsorb at the surface of the oil droplets. This effect is commonly observed for polyelectrolyte adsorption (*36*). The amount adsorbed is similar to the value of ~6 mg/m$^2$ reported by Nakauma et al. who studied the emulsification properties of gum Arabic for medium-chain triglyceride emulsions (*10*). The values are significantly higher than is normally found for monolayer coverage, which is typically 1 mg/m$^2$. This may be due to the conformation adopted by the molecules at the surface of the oil droplets or may possibly be due to the formation of multilayers since it is well-known that gum Arabic has a tendency to self-associate in solution. Sanchez et al., for example, showed that the storage modulus of 6% solutions at pH 4.2 increased significantly with aging, which was attributed to intermolecular association of the gum Arabic molecules (*37*). At pH 4.2, the proteinaceous material (mainly hydroxyproline, proline, and serine) would carry a net positive charge and hence could interact with the negatively charged glucuronic acid residues. Such interaction, however, does not explain the adsorption results at pH 7.5 where the proteinaceous material is likely to carry a net negative charge. We have recently argued that sugar beet pectin forms multilayers at the surface of polystyrene lattices and that this is due to the formation of electrostatic complexes between the

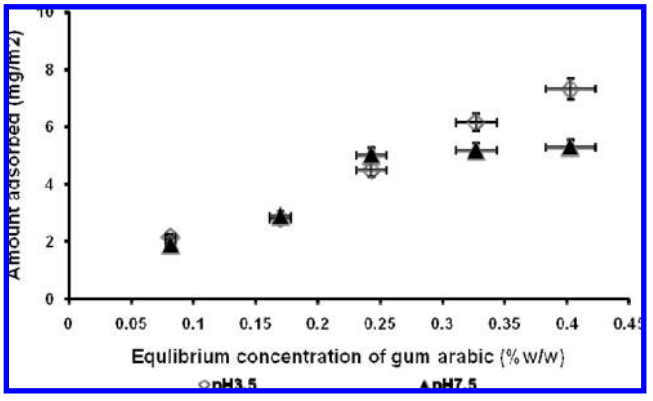


**Figure 3.** Isotherms for gum Arabic adsorbing onto limonene oil droplets from solution at pH 3.5 and 7.5.

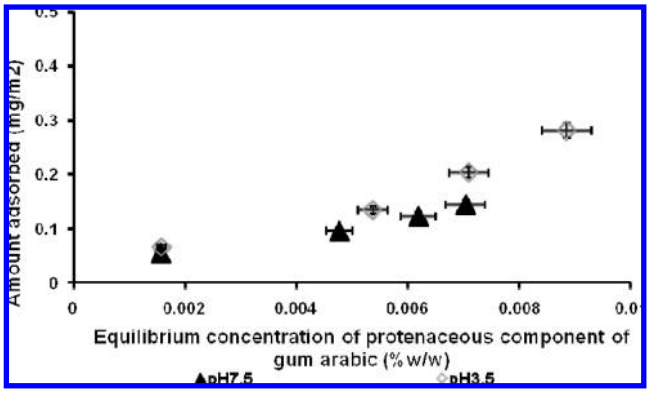


**Figure 4.** Isotherms for the adsorption of the proteinaceous components of gum Arabic onto limonene oil droplets from solution at pH 3.5 and 7.5.

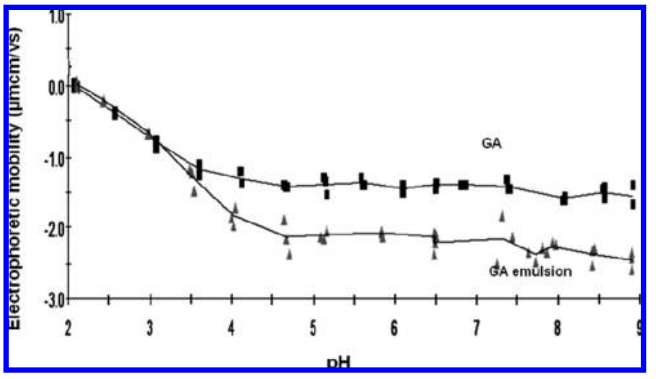


**Figure 5.** Electrophoretic mobility of gum Arabic and gum Arabic-stabilized emulsions as a function of pH.

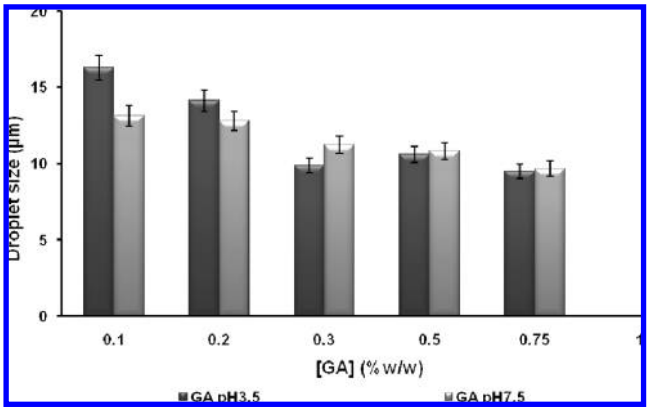


**Figure 6.** Histogram showing the droplet size, $D_{3,2}$, of emulsions prepared with varying concentrations of gum Arabic at pH 3.5 and 7.5.

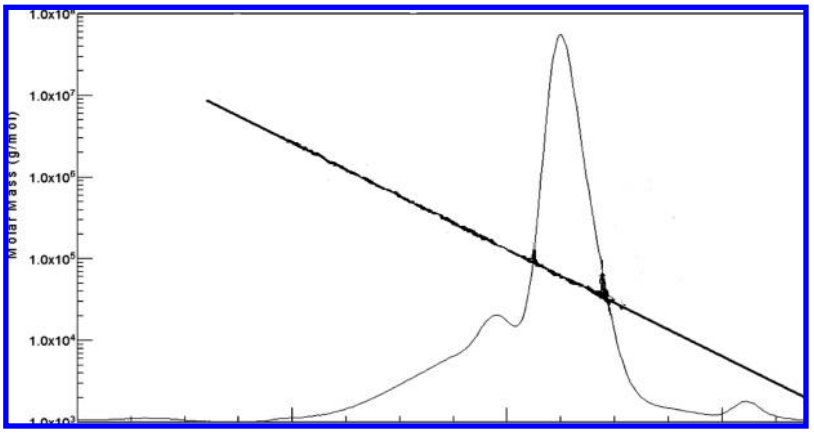


**Figure 7.** GPC elution curves for egg white protein monitored by RI and the $M_w$ of the eluting species.

proteinaceous components of the pectin and the pectin galacturonic acid groups (*38*). The isotherms obtained for the adsorption of the proteinaceous components of gum Arabic at pH 3.5 and 7.5 are given in **Figure 4**. The amount of protein adsorbed is slightly higher at pH 3.5 than at pH 7.5, which is consistent with the fact that more gum is adsorbed at the lower pH (**Figure 3**). The highest amount adsorbed represents ~26% of the total protein present, which is in keeping with previously reported data (*3*).

The electrophoretic mobilities of gum Arabic and gum arabic stabilized emulsions are shown in **Figure 5** as a function of pH. Gum Arabic alone has an electrophoretic mobility of ~−1.5 $\mu$m cm/(V s) above ~pH 4.5, but the value decreases to close to zero as the pH is lowered to ~2. These results are explained by the reduction in the degree of ionization of the carboxylate groups of the glucuronic acid residues as the pH drops below ~4.5. The values for the gum Arabic-stabilized emulsions closely reflect the trend obtained for the gum Arabic in solution alone, although the magnitude of the electrophoretic mobilities differs with values of ~−1.7 and −2.5 $\mu$m cm/(V s) being obtained at pH 3.5 and 7.5, respectively. These correspond to $\zeta$ potentials of −22 and −32 mV using the Smoluchowski relationship. Jayme et al. (*12*) reported values for the electrophoretic mobility of ~2.5 and 2.9 $\mu$m cm/(V s) for gum Arabic-stabilized emulsions at pH 3.5 and 7.5, respectively, in $10^{-3}$ M NaCl. They, however, attributed the charge to the carboxylate groups of the proteinaceous components, which is unlikely since they are present in very small quantities as compared to the carboxyl groups of the glucuronic acid residues. Nakauma et al. (*10*) and Chanamai and McClements (*39*) have reported $\zeta$ potentials for gum Arabic-stabilized emulsions of −60 and −40 mV, respectively, at pH values >4.

The droplet sizes for emulsions prepared using varying concentrations of gum Arabic at pH 7.5 and 3.5 are shown in **Figures 6**. The droplet size is seen to decrease as the gum arabic concentration increases due to the increased amount of gum Arabic molecules available to adsorb at the interface. There does not appear to be any significant difference in the behavior at pH 7.5 and 3.5, despite that fact that the $\zeta$ potentials are significantly higher at pH 7.5.

**Egg White Protein.** The RI and molecular mass GPC elution profiles for egg white protein are given in **Figure 7**. As noted above, egg white protein consists of a number of proteins, and the profile shows two main peaks, which are attributed to ovalbumin and ovomucoid (which elute between 12 and 14 mL) and conalbumin (which elutes at ~11 mL). These proteins account for more than 80% of the proteins present. As shown in **Figure 8**, the electrophoretic mobility of egg white protein decreases from ~−2.0 to ~+2.0 $\mu$m cm/(V s) on decreasing the pH from 9 to 2,

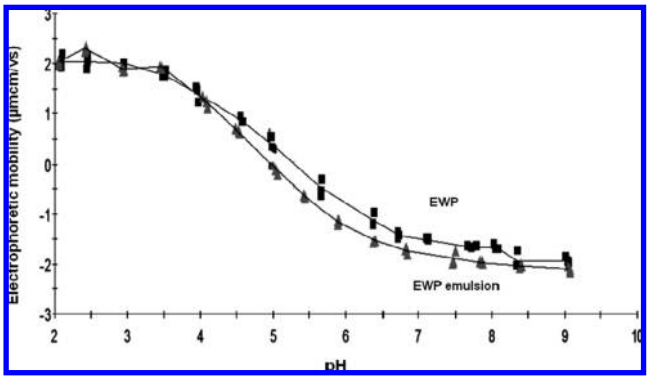


**Figure 8.** Electrophoretic mobility of egg white protein and egg white protein-stabilized emulsions as a function of pH.

and the IEP is at ~pH 5. It is noted that this is an average value since egg white protein consists of a number of proteins. The electrophoretic mobilities of egg white protein-stabilized emulsions are very similar to the protein alone. The RI elution profiles of egg white protein solution at pH 7.5 and 3.5 before emulsification and the supernatant after emulsification are given in **Figure 9a**,**b**, and the difference in the intensities represents the amount of protein adsorbed. The irregular shape of the profiles after adsorption may be due to the preferential adsorption of certain protein species present. The findings are consistent with the results of Drakos and Kiosseoglou, who analyzed the various proteins present before and after adsorption onto corn oil using sodium dodecyl sulfate−polyacrylamide gel electrophoresis (*40*). They showed that the relative proportions of ovalbumin, ovomucoid, conalbumin, and lysozyme in the egg white protein before adsorption were 74.6, 10.2, 8.6, and 6.6%, respectively, while the relative proportions in the adsorbed egg white protein were 86.8, 6.5, 6.7, and nil. The isotherms obtained from the difference in the intensities of the GPC RI elution profiles for the adsorption of egg white protein at pH 3.5 and 7.5 are given in **Figure 10** and also show low affinity. The amount adsorbed at plateau coverage is typical for monolayer adsorption and is slightly higher at pH 3.5 (~1.2 mg/m$^2$) as compared to pH 7.5 (~ 0.8 mg/m$^2$). The differences may be due to reduced intermolecular repulsions at pH 3.5 or may be due to the protein molecules adopting a different configuration at the interface. On adsorption, protein molecules tend to unfold such that the hydrophobic core resides at the interface, while the more hydrophilic moieties protrude into the aqueous phase. The amount adsorbed is significantly less than for gum Arabic.

The droplet size for emulsions prepared using varying concentrations of egg white protein, at pH 7.5 and 3.5, are shown in

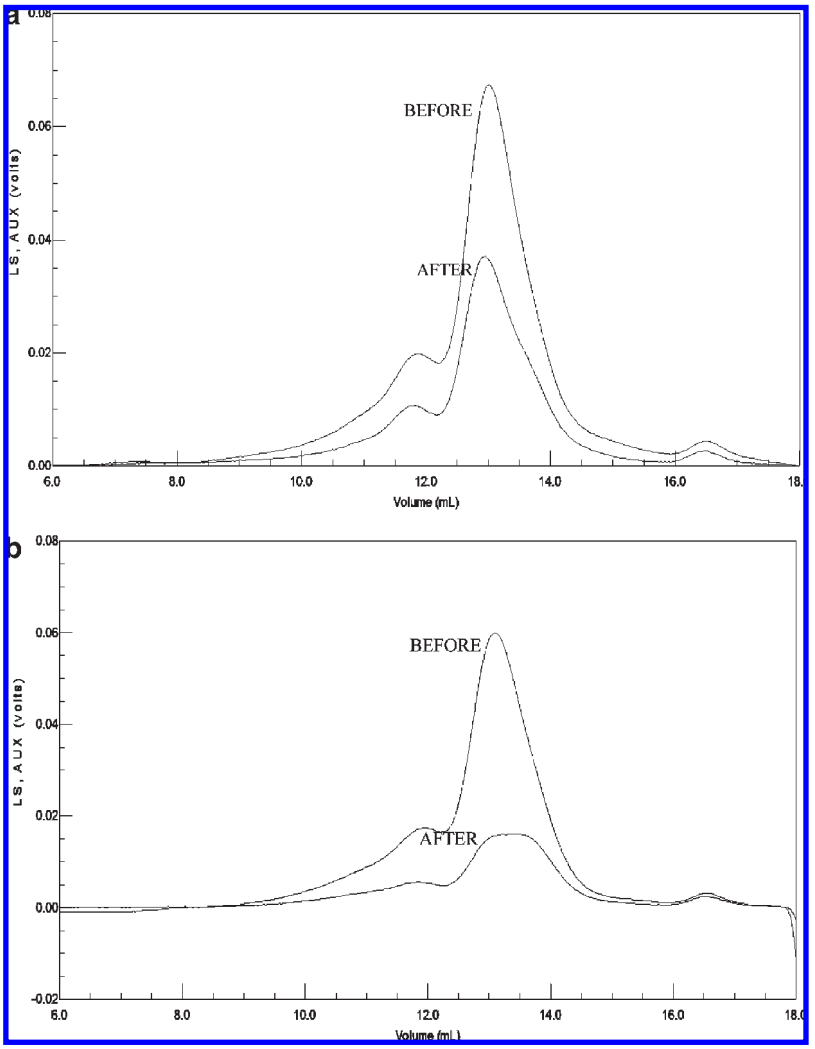


**Figure 9.** (**a**) GPC elution curves of egg white protein at pH 7.5 monitored by RI and the supernatant recovered after preparing an emulsion. (**b**) GPC elution curves of egg white protein at pH 3.5 monitored by RI and the supernatant recovered after preparing an emulsion.

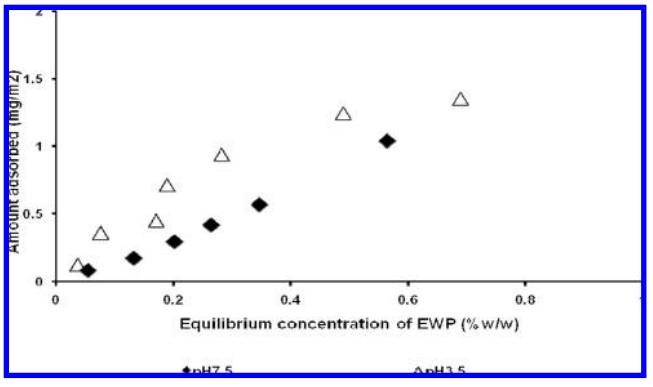


**Figure 10.** Isotherms for the adsorption of egg white protein onto limonene oil droplets from solution at pH 3.5 and 7.5.

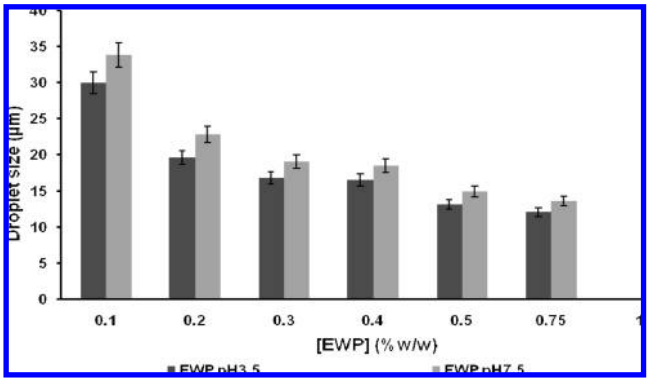


**Figure 11.** Droplet size, $D_{3,2}$, of emulsions prepared with varying concentrations of egg white protein at pH 3.5 and 7.5.

**Figure 11**. The droplet sizes are significantly larger than for gum Arabic emulsions over the same concentration range and are slightly smaller at pH 3.5 where the electrophoretic mobility is $\sim+1.7$ $\mu$m cm/(V s) as compared to pH 7.5 where the electrophoretic mobility has a value of $\sim-1.7$ $\mu$m cm/(V s). These correspond to $\zeta$ potentials of $\pm22$ mV.

**Gum Arabic−egg White Protein Complexes.** The turbidity of solutions of gum Arabic−egg white protein mixtures was monitored by UV absorbance, and the data are plotted as a function of the mixing ratio in **Figure 12**. It is noted that at pH 7.5 the absorbance remains close to zero, indicating that there is no interaction between the polysaccharide and the protein as might be expected because both carry a negative charge. At pH 3.5, however, the absorbance increases significantly above a protein concentration of ~0.1% due to the formation of insoluble electrostatic complexes. At lower protein concentrations, soluble electrostatic complexes are formed. These correspond to gum Arabic:egg white protein ratios of ~1:0.05 on a weight basis and ~1:1 on a mole basis.

The RI and UV absorbance (280 nm) GPC elution profiles of a mixed gum arabic−egg white protein solution (1:0.05 w/w)

solution at pH 7.5 before preparing limonene oil-in-water emulsions and the supernatant after emulsification are given in **Figure 13a**,**b**, respectively. It is seen that the egg white protein peak, which appears at an elution volume of between ~18 and 23 mL, is not present after emulsification, indicating that it is all adsorbed onto the oil droplets. The UV elution profiles indicate also that some of the gum Arabic AGP component, which elutes between 9 and 12 mL, is also adsorbed but that the GP component, which elutes at 17−19 mL, does not. The gum Arabic and egg white protein molecules do not form electrostatic complexes at pH 7.5 and hence will compete with each other for surface sites. The preferential adsorption of egg white protein is attributed to its greater surface activity based on surface tension measurements. It was found that the surface tensions of 2% solutions of gum Arabic and egg white protein were 64 and 47 mN/m, respectively. The results obtained for the same system at pH 3.5 are given in **Figure 14a**,**b**. At this lower pH, the gum Arabic and egg white protein molecules will interact to form soluble electrostatic complexes that adsorb at the oil−water interface rather than the gum Arabic and egg white protein molecules competing for the surface. This is confirmed by the fact that the gum Arabic GP peak (which elutes between 14 and 18 mL), together with a significant proportion of the gum Arabic AGP peak (7 and 10 mL) and the protein peak (16 and 18 mL), are absent after adsorption. The GPC elution profiles show separate gum Arabic and protein peaks because the eluent used to perform the GPC experiments was 0.1 M NaCl, and in such an environment, complexation is suppressed.

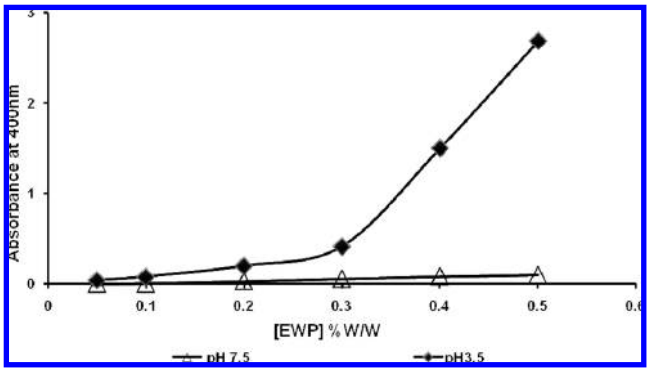


**Figure 12.** Turbidity of solutions of gum Arabic with varying concentrations of egg white protein present at pH 7.5 and 3.5.

The electrophoretic mobility of limonene oil-in-water emulsions at pH 3.5 is shown as a function of the egg white protein:gum

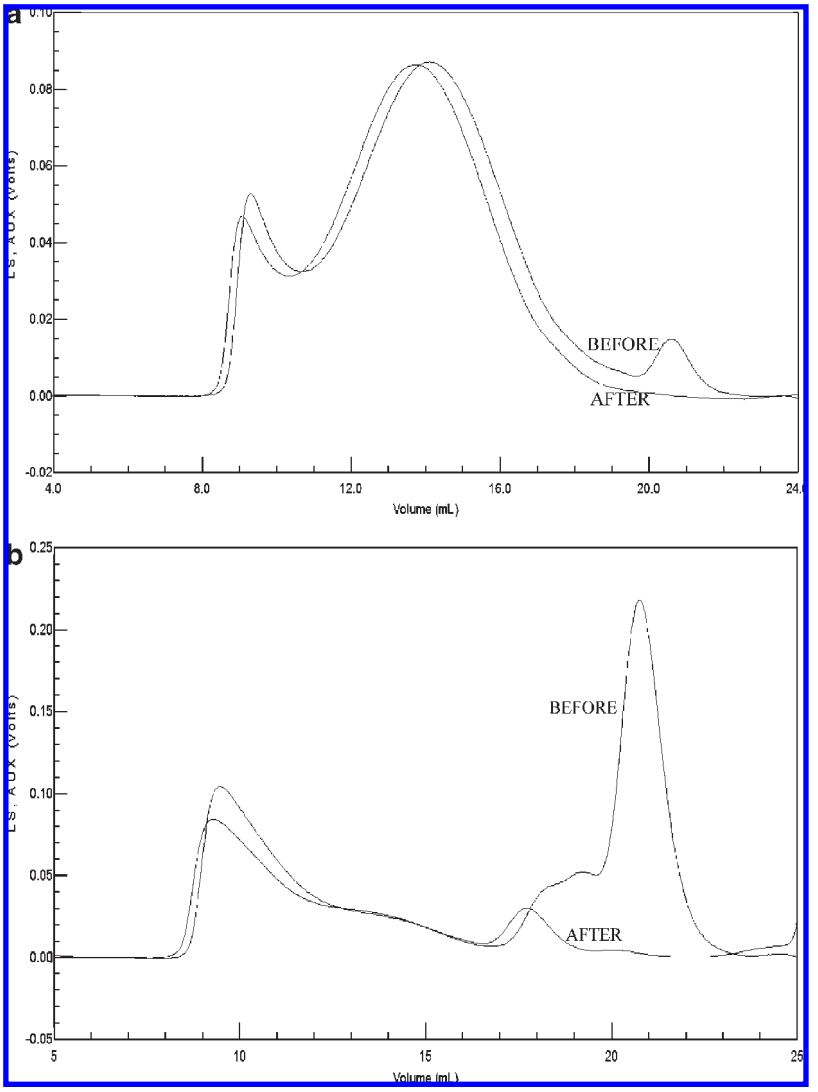


**Figure 13.** (**a**) GPC elution curves for a mixed gum Arabic−egg white protein solution (1:0.05 w/w) at pH 7.5 monitored by RI and the supernatant recovered after preparing an emulsion. (**b**) GPC elution curves for a mixed gum Arabic−egg white protein solution (1:0.05 w/w) at pH 7.5 monitored by UV absorbance at 280 nm and the supernatant recovered after preparing an emulsion.

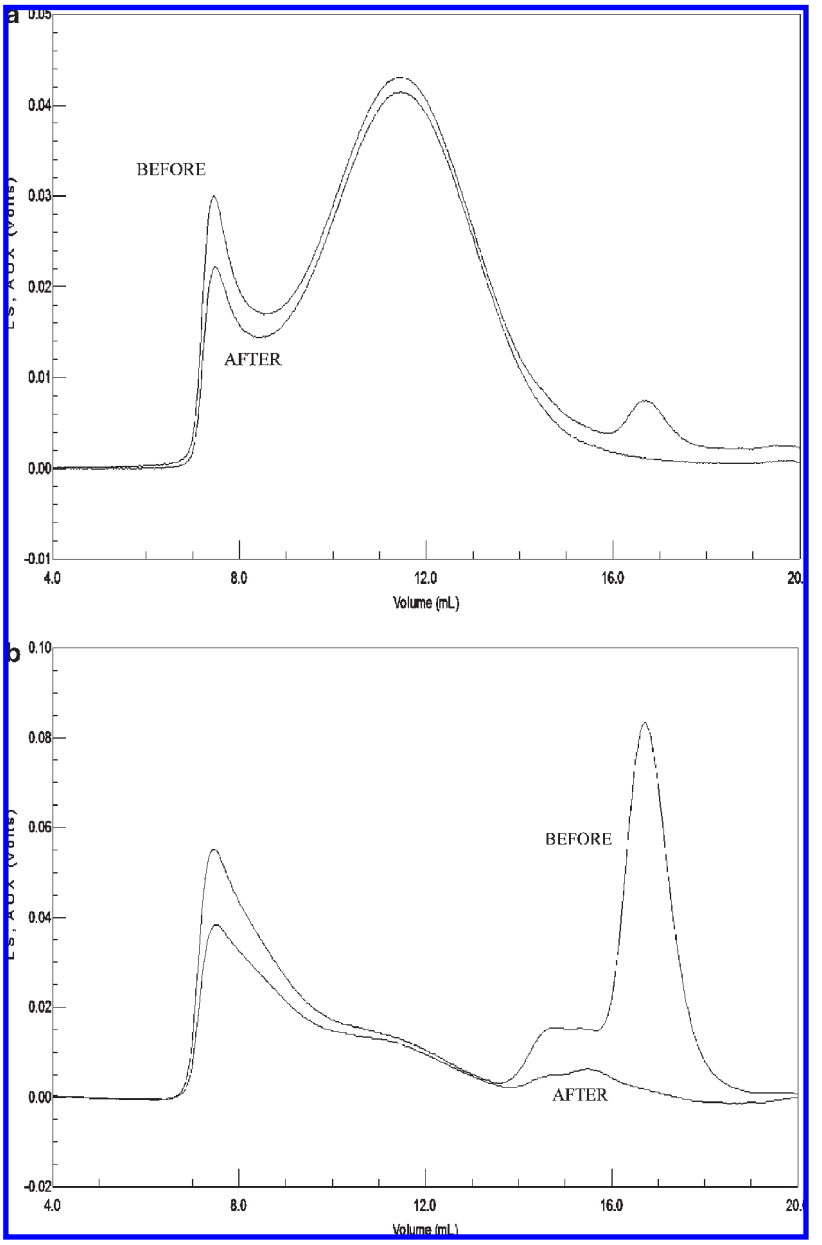


**Figure 14.** (**a**) GPC elution curves for a mixed gum Arabic−egg white protein solution (1:0.05 w/w) at pH 3.5 monitored by RI and the supernatant recovered after preparing an emulsion. (**b**) GPC elution curves for a mixed gum Arabic−egg white protein solution (1: 0.05 w/w) at pH 3.5 monitored by UV absorbance at 280 nm and the supernatant recovered after preparing an emulsion.

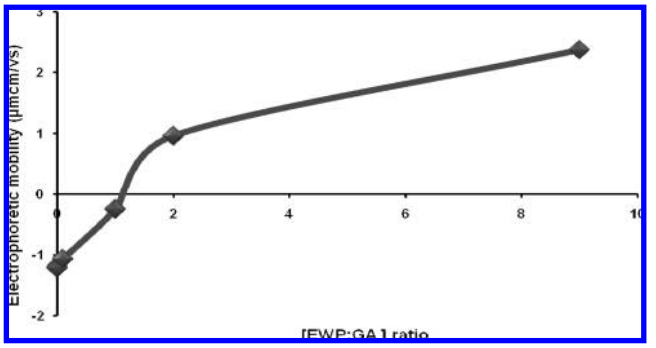


**Figure 15.** Electrophoretic mobility of limonene oil-in-water emulsions at pH 3.5 stabilized by gum Arabic−egg white protein at varying mixing ratios.

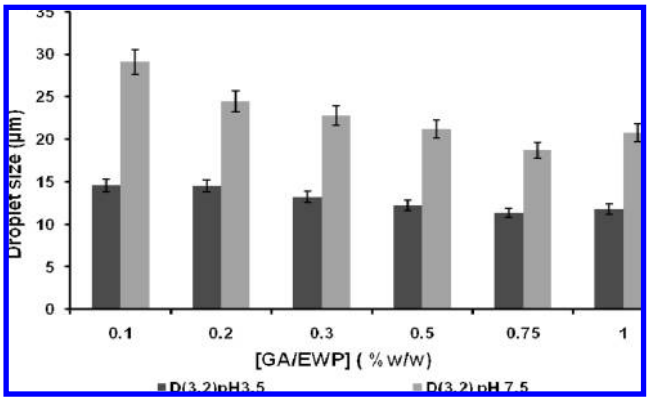


**Figure 16.** Histogram showing the droplet size of emulsions, $D_{3,2}$, prepared using gum Arabic−egg white proteins mixtures (1:0.05 w/w) at pH 3.5 and 7.5.

arabic mixing ratio in **Figure 15**. At low egg white protein:gum Arabic ratios, the droplets carry a negative charge as expected. However, as the proportion of egg white protein increases, the

electrophoretic mobility approaches zero and then becomes positive. The droplet sizes of emulsions prepared using gum Arabic−egg white protein mixtures (1:0.05 w/w) are shown in **Figure 16** and are seen to be significantly larger at pH 7.5 as compared to at pH 3.5. This is explained by the fact that at pH 3.5, soluble electrostatic complexes are formed, which adsorb at the interface, while at pH 7.5, there is no interaction between the gum Arabic and the protein molecules, and competitive adsorption takes place with the protein adsorbing preferentially.

In summary, this work has shown that protein-rich fractions of gum Arabic of varying molecular mass adsorb onto the surface of oil droplets. The amount of gum Arabic adsorbed is higher than might be expected for monolayer adsorption. Egg white protein also adsorbs at the surface of oil droplets but produces emulsions with larger droplet size than for gum Arabic at the same concentration. The amount adsorbed is significantly less than for gum Arabic and is consistent with the formation of a protein monolayer at the interface. For gum Arabic−egg white protein mixtures (1:0.05 w/w) under conditions where they do not interact, the egg white protein adsorbs preferentially due its greater surface activity. In conditions where interaction occurs, the electrostatic complexes are adsorbed at the interface. The droplet sizes of emulsions prepared using gum Arabic−egg white protein mixtures (1:0.05% w/w) are significantly larger at pH 7.5 than at pH 3.5.

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