pH Dependent Polymer Surfactants for Hindering BSA Adsorption to Oil-Water Interface

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ABSTRACT

Bovine serum albumin (BSA) adsorption to oil-water interfaces was studied in the presence and absence of two linear polymers poly(dimethylamino)ethyl methacrylate (PDMAEMA) and PDMAEMA-block-[poly butyl acetate]$_{38700}$-PDMAEMA$_{33000}$ (PBuA). As the hydrophobicity of the polymers is pH dependent, the pH was varied using pH 6.6, 7.0 and 7.4 during the measurements. Pendant drop and a rheometer with double wall ring geometry were utilized for surface tension and oscillatory shear measurements, respectively. The presence of both the PDMAEME and the PBuA resulted in decreased adsorption of BSA with increasing pH where PBuA was more efficient. The greatest reduction of the interfacial complex viscosity was reached in the presence of PBuA at pH 7.4.

INTRODUCTION

Pharmaceutically active proteins are introduced to various interfaces in most pharmaceutical preparations both during processing and in the final product. The amphiphilic nature of proteins, folding the hydrophobic groups towards the center in aqueous medium, can result in unfolding when introduced to hydrophobic interfaces, e.g. oil-water interfaces, in order to reduce the energy in the system. This unfolded form of the proteins can have reduced therapeutic efficacy and in worst cases can induce unfavorable immune reactions.

Protein adsorption to oil-water interfaces occurs in three different regimes[1,2]. The first regime (I) is a lag phase, mostly dependent on the diffusion of the protein to the interface[3]. During this regime the interfacial tension stays unchanged and the rheological properties cannot be detected due to the absence of a continuous network. Regime I can only be detected for very low protein concentrations. The second regime (II) is governed by accelerated adsorption of the protein to the interface, the already adsorbed proteins attracting the bulk proteins to the interface. Regime II can be recognised by a drop in interfacial tension and the dynamic moduli slowly increases. At the end of regime II the interface is covered with a protein monolayer[2,4]. Regime III is attributed to a multilayer formation due to intermolecular interactions and unfolding of the protein towards more favourable conformations in the interface. This can be detected as a viscoelastic network structure of the film with a distinct change in the dynamic moduli, whereas the interfacial tension is only mildly affected[4]. The crossover from where the monolayer is fully formed and evolvement of a multilayer starts is the crossover from regime II to regime III and this has been defined to be where the drastic increase in G' is observed and where G'' levels off[5,6].

Protein adsorption to interfaces is inevitable but can be reduced using various excipients. In this study two pH dependent polymers are introduced to the system in order to hinder the model protein, bovine serum albumin (BSA), from adsorbing to the oil-water interface.

MATERIALS AND METHODS

The BSA adsorption was studied in the presence and absence of two linear polymers poly(dimethylamino)ethyl methacrylate (PDMAEMA) and PDMAEMA-block-[poly butyl acetate]$_{38700}$-PDMAEMA$_{33000}$ (PBuA).

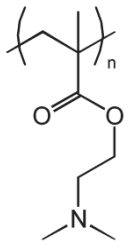

Figure 1. Chemical structure of PDMAEMA.

The chemical structure of PDMAEMA is shown in figure 1. PDMAEMA is water soluble with M_n (GPC) = 93,4 kDa (PDI = 1,5). The chemical structure of PBuA is shown in figure 2. PBuA is a triblock polymer with two PDMAEMA as the end blocks and a poly butyl acetate as a center block. The butyl acetate groups give additional hydrophobicity to the chain and thus the polymer is water insoluble. The molecular weight has been measured to M_n (GPC) = 107,3 kDa, (PDI=1,28 (1:2)).

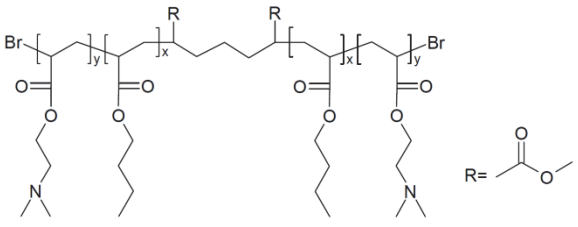

Figure 2. Chemical structure of PBuA.

Phosphate buffer at pH of 6.6, 7.0 and 7.4 (50 mM) was used as the aqueous phase and Miglyol 812 (fractionated coconut oil; medium chain triglycerol) as the oil phase. The BSA and the PDMAEMA were dissolved whereas PBuA was suspended in the aqueous phase prior to the measurements. The protein adsorption was monitored by the utilization of Pendant drop for surface tension measurements, and interfacial rheology using double wall ring (DWR) geometry to study the shear deformation. Schematic illustration of the DWR is shown in figure 3.

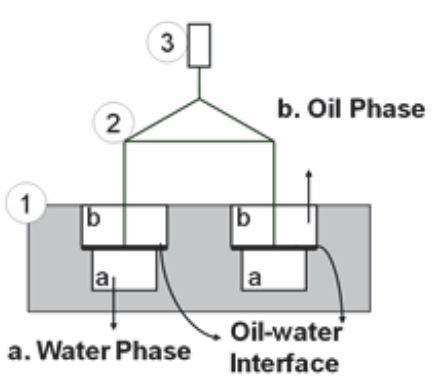

Figure 3. Schematic illustration of the DWR geometry: 1) Delrin® trough, 2) the DWR and 3) geometry holder.

The oscillatory shear measurements were conducted at 0.1 Hz and strain of 0.1% (within the linear viscoelastic regime). The secondary structure of BSA was investigated using Fourier Transform infrared (FTIR) spectroscopy.

RESULTS AND DISCUSSIONS

A typical rheological profile of BSA adsorption can be seen in figure 4.

At the concentration applied in this study, regime I cannot be detected and the crossover from regime II (monolayer) to regime III (multilayer) occurs within the first minute. Protein adsorption is affected by the pH as all proteins are charged. At the isoelectric point the protein is neutrally charged and thus the electrostatic forces are at the lowest[7] resulting in increased affinity towards oil-water interfaces. The isoelectric

point of BSA is at pH 4.7 and therefore the protein is negatively charged at physiologic pH[8].

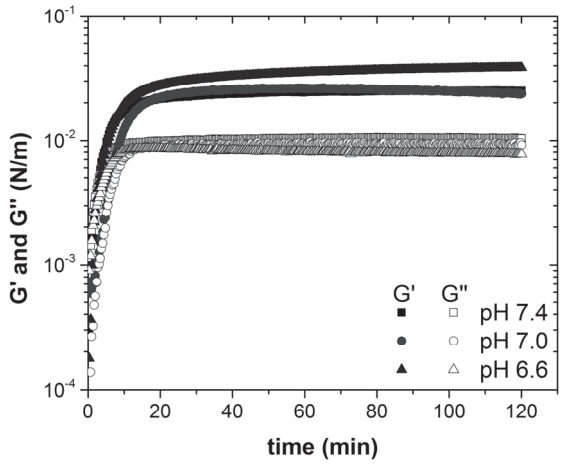

Figure 4. pH dependence of the dynamic moduli, G' and G'', for BSA alone.

In this study the pH is changed from 6.6 to 7.4 which does not have much influence on the total charge of the protein and therefore the interfacial adsorption should not be affected. Accordingly, the interfacial adsorption of BSA is not largely influenced by the pH, with only a small trend towards decreasing adsorption of the BSA with increasing pH (figure 3).

The effect of PDMAEMA

pK_a of 100 kDa PDMAEMA has been measured to 7.5[9]. At this pH 50% of the dimethylamino groups are protonated and thus the polymer becomes more and more neutral when the pH is increased. Additionally, the pK_a is reduced with increased molecular weight of the polymer as the presence of protonated side chains decreases the protonability of the remaining dimethylamino groups[9]. This indicates that the PDMAEMA is less surface active at pH below the pK_a and thus the efficacy of the PDMAEMA in hindering protein adsorption should be increased with increasing pH. Additionally, the polymer is not able to interact at the interface and thus cannot produce viscoelastic film detected by the interfacial rheology measurements (data not shown). The theory is well supported with these findings as the BSA adsorption is reduced in the presence of PDMAEMA and the effect is increased with increasing pH (figure 5).

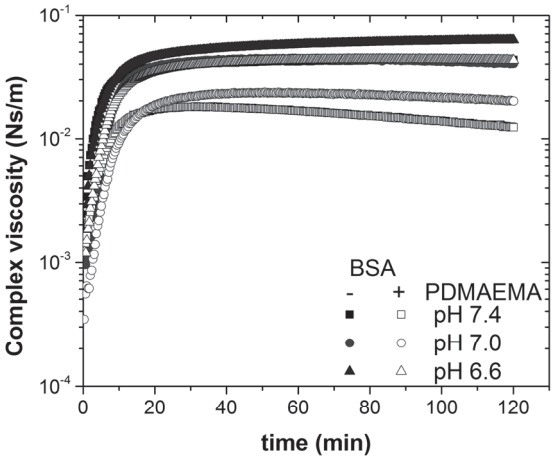

Figure 5. The complex viscosity monitored during BSA adsorption in the presence and absence of PDMAEMA at pH of 6.6, 7.0 and 7.4.

This reduction is seen in the lowering of the complex viscosity, indicating less viscoelastic film. The crossover from the more dominant viscous behaviour to the more elastic appears within 8 min regardless of the presence of the polymer or the pH and thus the multilayer is formed at similar time points (data not shown).

Furthermore, at pH 7.0 and, even more pronounced, at 7.4 the complex viscosity reaches a maxima after 30 and 40 min, repectively, and thereafter a slow decrease is observed. This weakening of the interfacial film could either be attributed to changes in the behaviour of the protein film in the presence of the PDMAEMA or a compitition between BSA and PDMAEMA at the interface where it seems that the BSA is desorbing, giving space for PDMAEMA at the interface.

These two theories are further supported by the interfacial tension measurements shown in figure 6. Neither the protein alone

nor the polymer-protein combinations showed any changes in the interfacial tension regardless of the pH.

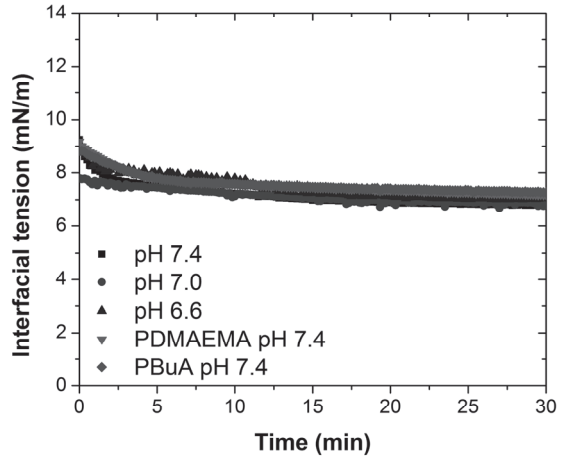

Figure 6. Interfacial tension as a function of time during adsorption at pH of 7.4, 7.0 and 6.6 for BSA alone, and in the presence of PDMAEMA and PBuA at pH of 7.4.

This indicates that similar interfacial tension is detected independent of both the pH of the bulk solution, and whether the polymers are present in the system.

Effect of PBuA

The pK_a of PBuA is similar to the PDMAEMA due to the similar molecular weight of the polymer. However, the water insoluble polybutylacetate group increases the interfacial activity of the molecule. The presence of PBuA at pH of 6.6 slightly decreases the complex viscosity reached, similar to PDMAEMA (figure 7).

The increase of the pH to 7.0 shows more decrease in the complex viscosity and the developement of the viscoelastic film is clearly slowed down. Additionally, an indication of desorption can be observed after 1 hour. Further increase of pH to 7.4 completly hinders the formation of the viscoelastic film in the interface, indicating no BSA adsorption (figure 7).

As shown in figure 6 the interfacial tension is unaffected by the presence of PBuA. The polymer and the protein give similar interfacial tension reduction and thus no distinction can be made whether the polymer or the protein is present, unless a combination of the methods is used.

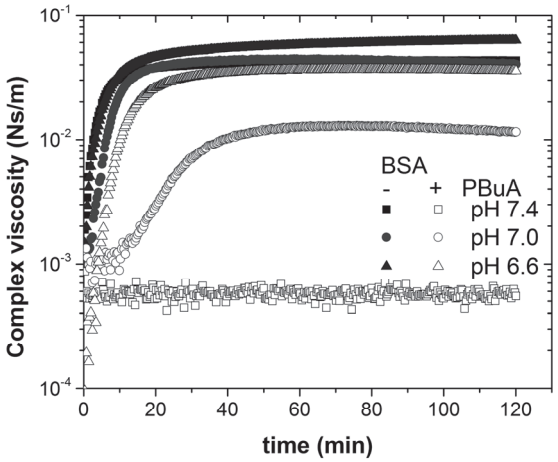

Figure 7. Complex viscosity over time during BSA adsorption in the presence and absence of PBuA at pH of 6.6, 7.0 and 7.4.

For more detailed information on the crossover from monolayer to multilayer, the dynamic moduli are depicted in figure 8.

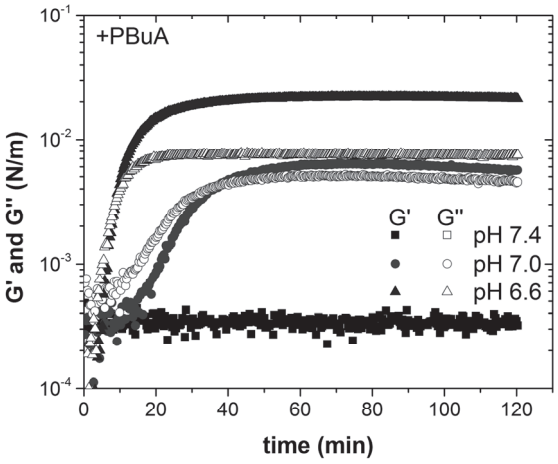

Figure 8. pH dependence of the dynamic moduli, G' and G'' for BSA in the presence of PBuA.

A clear delay in the multilayer formation, from under 10 min to 40 min, is seen in the presence of PBuA at pH of 7.0. This is yet another indication of the competition between the polymer and the

protein at the interface where at lower pH the BSA appears to integrate at the interface whereas at pH of 7.4 the increased affinity of the PBuA towards the interface displaces the BSA.

PDMAEMA vs PBuA

Polymeric surfactants have been proven to be more efficient at preventing protein adsorption[10] and thus the efficacy of PDMAEMA and PBuA should be greater that similar monomeric or oligomeric substances. If protein is interacts with surfactant prior to arrival at the interface, this allows opportunity for protein-surfactant complexes to form, thus altering the adsorptive properties of the protein[11]. However, the results from this study show that the water solubility of the PDMAEMA is not the controlling factor as the PBuA is more efficient in the prevention of BSA adsorption (figure 9).

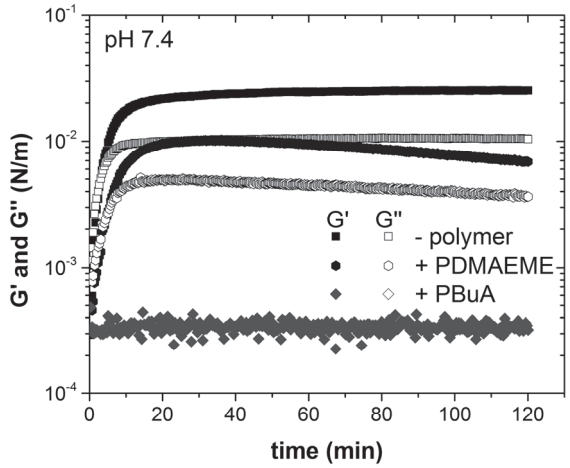

Figure 9. Complex viscosity as a function of time during BSA adsorption at pH of 7.4 for BSA alone and in the presence of PDMAEMA and PBuA.

As the protein is negatively charged and the polymer surfactants are positively charged, increased charge density could give rise to complexation between the BSA and the polymers. However, the decrease in the pH and thus the increased positive charge of the polymer decreased the efficacy for hindering the protein adsorption. Additionally, FTIR measurements show that the secondary structure of BSA is not affected by the presence of either of the polymers (data not shown), supporting the conclusion that no protein-polymer complex is formed.

The increased hydrophobicity by the addition of polybutylacetate in the PBuA gave improved prevention of BSA adsorption, indicating that the affinity towards the interface gives rise to the polymer adsorption rather than BSA adsorption.

CONCLUSIONS

The presence of the PDMAEMA and the PBuA resulted in decreased adsorption of BSA with increasing pH where PBuA was more efficient. The greatest reduction of the interfacial complex viscosity was reached in the presence of PBuA at pH 7.4. This effect was not detected by the interfacial tension method and thus combination of IFT and rheology methods is recommended.

PDMAEME and PBuA show promising effect on the hindering of BSA adsorption to oil-water interfaces. This effect is, as expected, dependent on the pH of the formulation. Therefore, the strategic choice and design of polymers can minimise protein accumulation at the interface and this may reduce the tendency for protein aggregation in these types of systems.

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