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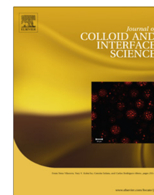

Interaction of *Moringa oleifera* seed protein with a mineral surface and the influence of surfactants

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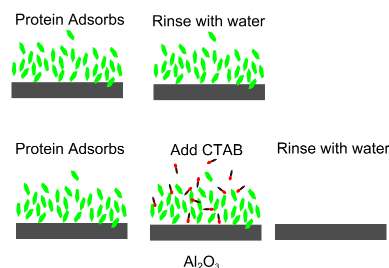
GRAPHICAL ABSTRACT



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ABSTRACT

The paper describes the adsorption of purified protein from seeds of *Moringa oleifera* to a sapphire interface and the effects of addition of the anionic surfactant sodium dodecylsulfate (SDS) and the cationic surfactant hexadecyltrimethylammonium bromide (CTAB). Neutron reflection was used to determine the structure and composition of interfacial layers adsorbed at the solid/solution interface. The maximum surface excess of protein was found to be about 5.3 mg m^{-2}. The protein does not desorb from the solid/liquid interface when rinsed with water. Addition of SDS increases the reflectivity indicating co-adsorption. It was observed that CTAB is able to remove the protein from the interface. The distinct differences to the behavior observed previously for the protein at the silica/water interface are identified. The adsorption of the protein to alumina in addition to other surfaces has shown why it is an effective flocculating agent for the range of impurities found in water supplies. The ability to tailor different surface layers in combination with various surfactants also offers the potential for adsorbed protein to be used in separation technologies.
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1. Introduction

Protein adsorption to surfaces is common in many biological and industrial processes. Knowledge of the mechanism of adsorption and the structure of the adsorbed protein layers is important in areas relevant to biology (protein chromatography, cellular adhesion), medicine (biomedical materials), food processing (stabilization of foams and emulsions, fouling of equipment) and biotechnology [1]. Protein adsorption is a common phenomenon; wherever proteins come into contact with a solid interface, they are very likely to adsorb to it. The adsorption phenomena of protein molecules include a number of interactions at solid–liquid interfaces [2,3]. To understand the interaction between protein molecules and solid surfaces it is important to consider many factors,

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such as the physical and chemical properties of solid surfaces for example the roughness and chemical dissociation, properties of the protein molecules like the folding and isoelectric point, and the solution conditions for the protein [4]. Many proteins are amphiphilic because they contain a mixture of amino acids with hydrophobic chains and ionic or polar side chains. They aggregate in solution and may be surface active even at very low concentrations.

This study addresses the adsorption from dilute aqueous solution of protein extracted from *Moringa oleifera* (MO) seeds to aluminum oxide surfaces. There is a large body of literature investigating the MO protein as an effective flocculating agent in water purification [5–9]. Although the details of the mechanism of water purification are not yet well understood, there is evidence that the role of the protein is directly related to adsorption [10,11]. There are general reviews about the chemistry of *Moringa* products that also relate to economic and environmental issues [12,13]. Information on the amount of material that is adsorbed at the surface, the structure of the adsorbed layer and how this relates to concentration in solution is obviously of crucial importance in making efficient use of the protein and providing water with low levels of both impurities and additives. To understand the adsorption mechanism, well-defined interfaces are required to avoid any ambiguity originating from the substrate surface. The binding of a water purification protein to silicon oxide (SiO_2) and its interaction with an anionic surfactant sodium dodecylsulfate (SDS) has been investigated previously using neutron reflection over a range of solution conditions with different concentrations of surfactant using an in-situ solid/liquid adsorption cell [10]. This technique is able to measure not only the adsorbed amounts but also provides information about the surface/interfacial structure of the adsorbed protein at a resolution that cannot be obtained currently using other methods. Interaction of adsorbed proteins with surfactants is of interest as displacement of functional layers may be significant. In the case of water treatment, natural surfactants may occur in the water to be treated. In food applications, stabilization of foams and emulsions may depend on the interaction of proteins, as steric stabilizers, with other surfactants.

The use of the *Moringa* seed protein as a flocculating agent and the unusual dense flocs with a high fractal dimension that are formed has been studied using small-angle and ultra small-angle neutron scattering [14]. Results were discussed primarily in the context of water purification. The present work has been designed to investigate the range of materials that will interact with the protein and thus removed in a purification process. The study with added surfactants suggests how selectivity of adsorption can be achieved and could be exploited in processes such as separation of different types of particles, for example in mineral processing.

Crystals of sapphire (α-Al_2O_3) are convenient substrates for studies of adsorption to alumina because it has an advantageous combination of optical and mechanical properties e.g. [15,16]. The chemical properties of sapphire surfaces are important factors when the surfaces are brought into contact with an aqueous solution [17–20]. For the present study, sapphire was chosen as a good model for a mineral surface that does not have strong negative charge in a solution at neutral pH. The isoelectric point (iep) of alumina, which is the pH at which the surface has a net charge of zero, is reported in literature to be between pH 6–8.5 and depends on the crystal plane [21]. This is close to neutral pH which makes it easy to achieve either a positively or negatively charged surface. Silica, SiO_2, another commonly used substrate for surface adsorption studies has an iep of about 2, making only a neutral or negatively charged surface practically available. The study by Isono et al. [22] has reported the point of zero charge for single-crystal sapphire to be below pH 7. The surface charge arises from deprotonation and protonation of hydroxyl groups on the surface. Measurements of the contact angle of water show that the sapphire surface is macroscopically hydrophilic [21].

This paper reports the results of adsorption of MO protein to sapphire substrates and the effect of added surfactant using neutron reflection. We have recently studied the adsorption of different surfactants to sapphire [17–20]. In the present study, the surfactants were chosen as simple proxies for materials that might occur in real applications and we used sodium dodecylsulfate (SDS) and cetyltrimethylammonium bromide (hexadecyltrimethylammonium bromide or CTAB), which are anionic and cationic, respectively. In a previous study of the adsorption of the MO protein on SiO_2, SDS was found to co-adsorb to the irreversibly bound protein layer [10]. The SDS did not displace the protein even at a concentration above the critical micelle concentration (cmc). The adsorbed layer of protein apparently binds SDS into a denser layer at the surface. Although the protein has a net positive charge and is thus likely to bind to a negatively charged silica surface, SDS does not simply cause desorption by neutralizing the protein.

2. Experimental principles and interpretation of neutron reflection data

Neutron reflectometry is widely used to study adsorption on flat solid substrates and at air–liquid interfaces [23,24]. The measured signal depends on the variation of the refractive index, n, in the direction perpendicular to the surface. The experiment involves determination of the reflectivity of an interface as a function of the wavelength or angle. The data allows quantitative, structural, and compositional information about the adsorbed material to be obtained at molecular length scales. In specular neutron reflection, the ratio of the intensity of the reflected beam to that of the incident beam is measured as a function of the momentum transfer, Q normal to the reflecting surface. The specular condition occurs when the angle of the incident beam is equal to the angle of the reflected beam and Q is given by

$$Q = (4\pi/\lambda)\sin\theta \tag{1}$$

where λ is the incident neutron wavelength and θ is the angle of incidence. Measurements can be made using combinations of different wavelengths and incident angles. The scattering is a nuclear interaction, and information is derived via the scattering length density of the materials, given by

$$\rho = \sum N_i b_i \tag{2}$$

where N_i is the number density of the element or isotope i, and b_i is the coherent neutron scattering length of the species. The scattering length density, ρ, determines the refractive index, n, for the neutrons, which is given by:

$$n = 1 - (\lambda^2/2\pi)\rho \tag{3}$$

A particular advantage of neutron reflection is that b can vary between isotopes of an element and there is a large difference for normal hydrogen (^1H) and deuterium (^2H or D). Table 1 shows the scattering length densities of materials used in the present study. By matching ρ for the solvent with that of the substrate, one can obtain a reflection signal that depends only on the interfacial layer. Making additional measurements with different hydrogen and deuterium composition in the solvent allows one to verify the composition of surface layers as ρ is related to the volume fraction of each component in the layer by

$$\rho = \phi_p\rho_p + \phi_w\rho_w \tag{4}$$

and the constraint $\phi_p + \phi_w = 1$ is used. ρ_p and ρ_w are the scattering length densities of protein and water, respectively, and ϕ_p and ϕ_w are their respective volume fractions. If a protein molecule has a

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