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Sticking particles to solid surfaces using *Moringa oleifera* proteins as a glue☆

Shirin Nouhi[a], Marc Pascual[a,1], Maja S. Hellsing[a], Habauka M. Kwaambwa[b], Maximilian W.A. Skoda[c], Fredrik Höök[d], Adrian R. Rennie[a,*]

[a] *Centre for Neutron Scattering, Uppsala University, Box 516, 751 20, Uppsala, Sweden*
[b] *Namibia University of Science and Technology, Faculty of Health and Applied Sciences, Private Bag 13388, 13 Storch Street, Windhoek, Namibia*
[c] *Rutherford Appleton Laboratory, Harwell, Didcot OX11 0QX, United Kingdom*
[d] *Department of Applied Physics, Chalmers University of Technology, Gothenburg, Sweden*

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ABSTRACT

Experimental studies have been made to test the idea that seed proteins from *Moringa oleifera* which are novel, natural flocculating agents for many particles could be used to promote adhesion at planar interfaces and hence provide routes to useful nanostructures. The proteins bind irreversibly to silica interfaces. Surfaces that had been exposed to protein solutions and rinsed were then exposed to dispersions of sulfonated polystyrene latex. Atomic force microscopy was used to count particle density and identified that the sticking probability was close to 1. Measurements with a quartz crystal microbalance confirmed the adhesion and indicated that repeated exposures to solutions of *Moringa* seed protein and particles increased the coverage. Neutron reflectivity and scattering experiments indicate that particles bind as a monolayer. The various results show that the 2S albumin seed protein can be used to fix particles at interfaces and suggest routes for future developments in making active filters or improved interfaces for photonic devices.

1. Introduction

Proteins from *Moringa* seeds have attracted wide scientific attention in recent years largely because of their potential applications rather than seeking to understand the biological role. There are 14 species of *Moringa* [1,2] and some are widely cultivated, in particular, *Moringa oleifera*, although native to the Indian subcontinent is exploited for its leaves, oil and as a source of food [3]. A particular interest has been the exploitation of crushed seeds for water purification that has been known traditionally for many years but attracted scientific studies since the 1980′s [4–6].

The *Moringa* seed proteins have been identified as effective flocculating agents for a wide range of impurities (see e.g. [7]). They can replace the usual industrial produced materials that are normally either cationic polymers [8] or polyvalent salts. Major advantages for the use of *Moringa* seeds in this application arise from its low toxicity and negligible environmental impact. This allows water treatment processes to be developed that can be used on a domestic or village scale in remote areas without trained technical supervision. In many countries with major needs for such technologies, the *Moringa* trees can be grown readily even under conditions of low rainfall.

Some more recent studies have focused on understanding the details of interactions of the protein molecules with various surfaces and with other molecules [9–14]. Although these may be dominated by the overall net positive charge at neutral pH, there are strong indications that self-association of the proteins is important and that adsorption occurs at a range of interfaces [11,14]. These interactions strongly encourage flocculation and heteroflocculation of a wide range of materials. At some surfaces, such as alumina, displacement of the protein by rinsing with a cationic surfactant was observed [14].

It is now established that a major component of the proteins is a 2S albumin. A crystal structure has been established [15] but the behaviour of this protein is not identical to the mixture found in the crude extract as it has been found not to adsorb to alumina interfaces at neutral pH [16]. Understanding the details of

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☆ This article has been prepared to mark the 65th birthday of Professor Piero Baglioni. Many of the authors have enjoyed scientific discussions with Piero and benefited from his enthusiasm for solving interdisciplinary practical challenges by application of fundamental principles of colloid and surface science.

* Corresponding author.
E-mail address: adrian.rennie@physics.uu.se (A.R. Rennie).

[1] Present address: UMR CNRS Gulliver 7083, ESPCI ParisTech, 10 rue Vauquelin, F-75005 Paris, France.

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the interactions offers the prospect of designing materials that could separate specific types of particles dispersed in water, either alone or when combined with specific other surfactants. The use of sand that has been prepared with pre-coated layers of *Moringa* protein as antimicrobial filters has been suggested by Jerri et al. [17]. However, the specific range of direct antimicrobial activity and its mechanism is still the subject of ongoing studies. It is known from a number of studies that the proteins extracted from seeds can vary according to the conditions of sample preparation. Some explicit descriptions of various materials with different molecular mass have been described previously [18–20]. As practical applications of seed products, such as for water purification, would use a crude extract, most studies have been conducted with samples that contain a range of proteins.

Binding particles at interfaces can be of a great importance not only in respect of filters but also to retain self-assembled structures that may be formed at interfaces. For example, colloidal particles have been shown to form large domains of crystalline structure at solid/liquid interfaces [21] which can be used directly for low-cost technique manufacture of photonic devices [22]. However, it is common that the formed structures crack and particles tend to move during the drying process. For such applications, it is also valuable to develop means to fix defined nanostructures in place.

The present study uses a range of techniques to investigate the use of an extract of *Moringa* protein that is pre-adsorbed on silica surfaces as a means to bind particles in a controlled manner at interfaces. Experiments have been made to explore the binding of polystyrene latex particles, of two sizes, with a small negative charge to silica surfaces at which they would not normally attach.

2. Methods and materials

2.1. Apparatus

Atomic force microscopy, AFM, uses a physical tip attached to a flexible cantilever to move across the surface of the sample and provide topography images at the nanometre scale [23–25]. In this study, the tapping mode of AFM was used on dried samples. This method did not provide images of the substrate/protein interface but was used primarily to investigate the number of particles that are stuck to the surface and compare the distribution of the particles in the presence and absence of a pre-adsorbed protein layer in different particle/protein concentrations. A Nanosurf Mobile-S with 190 Al-G tips was used and most images were 5 mm × 5 μm with 256 lines scanned.

A quartz crystal microbalance (QCM) consists of a quartz crystal which is oscillated at its resonant frequency in a shear mode. If mass is added or removed from the crystal, a frequency shift, Δf is observed which according to the Sauerbrey [26,27] law is linearly related to the changes in mass, Δm, as:

$$\Delta f = -C_f \Delta m \quad (1)$$

where C_f is a constant that depends only on the sensitivity of the crystal.

Quartz crystal microbalance with dissipation monitoring (QCM-D) provides a simultaneous measurement of changes in the dissipation factor, D, as well as the oscillation frequency of the crystal at the fundamental frequency and higher overtones. The dissipation is the ratio of the energy lost per cycle to that of the elastic energy and is determined from the decay of amplitude after excitation [28]. A Q-Sense E4 instrument was used. Information contained in combined Δf and ΔD measurements at multiple overtones was used, which for rigid nanoparticles has been previously shown to offer information about film thickness [29].

Neutron reflectometry was used to quantify, in-situ, the adsorption of proteins to the surface and binding of particles to the protein layer. Neutrons interact with the nuclei of atoms and this allows them to penetrate deep into the sample with few interactions. The high penetration depth of neutrons makes some materials such as silicon to be so-called transparent for neutrons. This is an advantage of neutrons over, for example, AFM technique since one can probe the structure at the solid-liquid interfaces by illuminating the interface from the solid side. The neutron reflectivity experiment was performed on the reflectometer INTER at the ISIS facility, Rutherford Appleton Laboratory, UK [30] with a reflecting sample surface that is close to horizontal. The time-of-flight mode uses wavelengths from 2 to 14 Å to provide data in a momentum transfer, Q, range between 0.01 and 0.34 $Å^{-1}$, however, the samples in this study showed measurable signal up to about 0.12 $Å^{-1}$. The collimation slits and the data collection were chosen to provide resolution in momentum transfer, $\Delta Q/Q$, of about 2.5 percent. Specular reflectivity was measured using a point detector at 0.5 and 2.3°. Off-specular scattering data were measured with a linear detector at each angle to record the scattering above and below the reflected beam as a function of neutron wavelength.

The sample holder has been described previously [31]. The solution was sealed with a PTFE gasket between the quartz crystal and a polycarbonate base with injection and outlet ports. The base contains a small stirring magnet to keep the samples spread uniformly within the cell. The temperature was maintained at 25 °C during the measurements using a Julabo bath that circulated water through the metal parts that clamp the crystal and the base.

2.2. Interpretation of neutron reflection data

In this experiment, neutron reflection was used in both specular ($\theta_i = \theta_f$) and off-specular ($\theta_i \neq \theta_f$) conditions, where θ_i is the angle of the incident beam and θ_f is the angle of the outgoing beam, in order to investigate the out-of-plane and in-plane structure of particles stuck to the surface. Neutron reflectivity, $R(Q)$, is defined as the ratio between the intensity of the reflected beam over the intensity of the incident beam, at the specular condition. Reflectivity in the specular condition, where Q is perpendicular to the interface, is commonly shown as a function of magnitude of momentum transfer, Q, and it provides information such as the thickness, composition, and roughness of layers parallel to the interface. The momentum transfer perpendicular to the interface is given by:

$$Q = (4\pi/\lambda)\sin(\theta_i) \quad (2)$$

where λ is the wavelength of the neutrons and θ_i is the grazing angle of incidence. Reflectivity is calculated using the characteristic optical matrix of the stratified layers which is defined by scattering length density, ρ:

$$\rho = \Sigma n_i b_i \quad (3)$$

where n_i is the number density for atoms of element, i, b_i is the scattering length and the sum is taken over all of the elements found in a layer. Different isotopes have different scattering lengths for neutrons and this allows one to increase the contrast and choose the scattering interface, by carefully choosing the dispersion phase. For example, it is convenient to study the adsorption of *Moringa* proteins or polystyrene particles in D_2O rather than H_2O (see Table 1 for the values of scattering length density). The total scattering length density, ρ, of each layer is given as:

$$\rho = \rho_w \varphi_w + \rho_d \varphi_d \quad (4)$$

where ρ_w and φ_w are the scattering length density and volume fraction of water, and ρ_d and φ_d are those of the dissolved or dispersed material.

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