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Structure of flocs of latex particles formed by addition of protein from Moringa seeds☆

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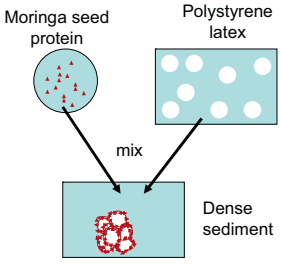
HIGHLIGHTS

- *Moringa oleifera* seed protein binds strongly to polystyrene latex.
- Seed protein is an effective flocculent creating very dense flocs.
- Fractal dimensions of flocs increase with particle concentration.
- Floc densities are higher than those found with simple ionic coagulants.

GRAPHICAL ABSTRACT



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ABSTRACT

Proteins extracted from the seeds of *Moringa* trees are effective flocculents for particles dispersed in water and are attractive as a natural and sustainable product for use in water purification. Studies with a model system consisting of polystyrene latex particles have shown that the protein adsorbs to the surface and causes flocculation as unusually dense aggregates. Small-angle neutron scattering that exploits contrast matching of deuterated latex particles dispersed in D_2O to highlight bound protein has shown that the adsorbed amount reaches about 3 mg m^{-2}. The particles form very compact flocs that are characterized by fractal dimensions that approach the theoretical maximum of 3. Ultra small-angle neutron scattering allows these flocs to be characterized for a range of particle and protein concentrations. Proteins from two species of *Moringa* trees were investigated. The protein from *Moringa stenopetala* seeds gave rise to slightly lower fractal dimensions compared to *Moringa oleifera*, but still much larger than values observed for conventional ionic or polymeric flocculents that are in the range 1.75–2.3. Compact flocs are desirable for efficient separation of impurities and dewatering of sludge as well as other applications. A trend of increasing fractal dimension with particle concentration was observed when *M. stenopetala* seed protein was used and this resembles the behaviour predicted in Brownian dynamics simulation of flocculation.

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1. Introduction

The study of flocculation is of wide practical importance as it forms part of many industrial processes, for example in production of ceramics, mineral flotation and the manufacture of coatings. Aggregation of impurities is an important first stage in water purification so that contaminant particles can sediment, cream or be removed easily with filters. The aggregation of particles to form clusters has also been of theoretical interest and many models are based on the early ideas of Smoluchowski [1] about the kinetics of colloidal coagulation. This relates the diffusion of component particles in a fluid to the growth of aggregates. More recently, the idea of self-similar structures [2] on many length scales that are known as fractals has helped in the categorisation of colloidal flocs [3,4]. The probability of finding a particle in an aggregate at a given distance from the centre depends on the density of packing and can be related to an exponent known as the dimension, d, of a mass fractal.

A first stage in most procedures for drinking water treatment and purification is the coagulation and flocculation of particulate impurities [5,6]. The particular motivation for the present work has been to understand the mechanism by which a novel, natural flocculent is effective in water purification. A number of studies have identified that seeds from *Moringa oleifera* trees can be used to clarify water [7,8], and that the active ingredient for this purpose is the seed protein that has an important component with molecular mass of about 6.5 kDa [9]. Recent neutron reflection experiments [10] have identified that the protein binds strongly to silica surfaces and a plateau in the adsorbed amount is reached at solution concentrations as low as 0.3 mg ml^{-1}. Interactions of the protein with surfactants have also been studied. Dynamic light scattering [11] has shown that the protein disperses in water with a hydrodynamic radius of 1.5–2 nm in dilute solution but tends to aggregate when solutions are concentrated. The strong tendency to associate and to bind to a wide variety of interfaces provides an obvious mechanism for the protein to act as a flocculent [10]. Recent reports describe the use of the *M. oleifera* protein in connection with water purification and these include both its role as a coagulant [8,12] and possible antimicrobial action [13]. The requirement to maintain low levels of organic carbon in treated water with added protein has also been addressed specifically [14]. A comparative study has indicated that *M. oleifera* protein is, at present, one of the most effective plant materials that has been tested [15].

The low molecular mass suggests that the protein does not act in the same way as most polymeric flocculents. The present work was initiated to understand the physical processes that are involved in the flocculation of particles by investigating samples in situ using ultra small-angle neutron scattering. The model system of particles chosen for the study was polystyrene latex. Complementary measurements of the adsorption to the surface of these particles are presented. An understanding of the physical processes that govern aggregation under simple quiescent conditions is essential in order to comprehend more complicated behaviour in stirred and flowing dispersions. It should be noted in this respect that the application of protein flocculent has been advocated for use under a very wide range of conditions such as sedimentation in small pots on a domestic scale and in larger treatment plants. The results of the present work are discussed in terms of fractal dimensions that can describe the density of flocs that arise from an aggregation process.

2. Background

2.1. Fractal aggregates

It is convenient to investigate the structure of fractal objects using X-ray, neutron or light scattering as these techniques probe the three-dimensional distribution of density within samples. These experiments are similar in that the intensity, I, is measured as a function of scattering angle, θ, and wavelength, λ. Data is usually reported as a function of the momentum transfer, Q that is given by $Q = (4\pi/\lambda)\sin(\theta/2)$. The mass, m, within a sphere of radius R for a mass fractal is simply given by $m(R) \sim R^d$ and the intensity of scattering is given by [16] $I(Q) \sim Q^{-d}$. There are several well-established models for aggregation that predict specific values of the dimension d. These distinguish whether the property that controls the growth is primarily the diffusion rate or if the probability of a particle sticking to a cluster (reaction) provides the limit. It has been recognized since early experiments of Lin et al. [17–19] that there is likely to be polydispersity in most systems and that small clusters can diffuse and aggregate with other clusters. This has given rise to the terms diffusion limited colloid aggregation (DLCA) and reaction limited colloid aggregation (RLCA). The exponents d for these cases have been identified from experiment, theory and computational models as about 1.85 and 2.1 respectively [17]. RLCA provides a higher density and consequently larger value for the dimensionality, d, than the DLCA process. The higher value of d for RLCA can be understood qualitatively as occurring because particles or small clusters are able to explore more of the free space within an aggregate if the reaction probability rather than diffusion limits the 'sticking'.

Meakin and Jullien [4] have pointed out that mechanisms such as rearrangement within clusters can change the observed dimensionality and give some overlap between the ranges of expected exponents. The paper warns that making only analysis of fractal dimensions of aggregates is inadequate to determine the mechanism and kinetics of their formation. This caveat would apply whether the aggregates are measured using scattering or real-space techniques such as microscopy. However, even if full details of the mechanism that gives rise to a floc may be ambiguous from a structural analysis, the results are important as for example, properties such as mechanical strength [20,21], the sol to gel transition, and the ability to pass through filters [22] will depend on the structure.

There are some further complications to the simple interpretation of scattering from aggregates. A review by Sorensen [23] discusses a number of important points such as the effects of the finite size of the components that form the aggregate, as well as the overall aggregate size that can strongly limit the range in which a power-law scattering behaviour is observed. As the fractal dimension reaches the limiting value of 3 that corresponds to a uniform density in a three-dimensional space, the scattering will necessarily change as there would then be sharp boundaries between clusters. The intensity would then vary as Q^{-4}, as expected in the Porod limit of small-angle scattering. In order to understand the aggregation, it is clearly helpful to measure over a wide range of Q to provide information about the limits at both small and large length scales. The model proposed by Teixeira [24] has been widely used to describe scattering from fractal aggregates of spherical particles.

2.2. Polystyrene latex as model particles

A number of particles have been used as model materials for studies of flocculation. For example, gold particles, silica sols and polystyrene latex have all been demonstrated [17] to fall on the same master curves of scattering data for aggregates if the appropriate conditions as regards stability are chosen for the different dispersions. Most studies that exploit light scattering or microscopy have been restricted to rather dilute samples to avoid strong multiple scattering that would limit investigations to regions very close to walls of containers. The size and concentration of particles have been identified as of considerable interest. Gravitational settling was observed [18,25] and this motion may predominate over diffusion so as to alter the aggregation. Early studies have investigated

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