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INFLUENCE OF SOLUBLE PROTEINS ON THE ADHERENCE OF PARTICULATE SOILS

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ABSTRACT

Adsorbed compounds from food and pharmaceutical mixtures may influence interactions at interfaces and thus fouling and cleaning. In this study, quartz particles (10 to 30µm) were used as a model soil for examining the effect of dissolved proteins on the cleanability of substrates after soiling and drying. Glass and stainless steel pretreated by UV-Ozone (StSteel-UVO) were used as model hydrophilic substrates, while hydrophobic substrates were represented by stainless steel cleaned with ethanol (StSteel-Eth) and polystyrene. BSA and β-LGB were used as proteins. The quartz suspensions used for soiling were prepared in pure water and in a solution of each protein. After soiling and drying, the cleanability was evaluated using a radial-flow cell, with pure water as the cleaning fluid.

The presence of proteins in the suspension used for soiling hydrophilic substrates (Glass and StSteel-UVO), decreased the adherence of quartz particles. Its effect was less marked and tended to be opposite for less hydrophilic substrates (StSteel-Eth, Polystyrene). The adherence cannot be explained by a simple relation with the contact angle. Other factors may be the solution surface tension itself and the protein behavior at the interfaces created by drying and by rehydratation during cleaning.

When considering the influence of substrate on soiling, it must be kept in mind that high surface energy solids (metals, oxides) are readily contaminated in contact with air and lose their hydrophilicity. This may improve the substrate behavior regarding cleanability with respect to particulate soil.

INTRODUCTION

Fouling involving particle deposition and drying is of major concern for surfaces exposed to natural environments or surfaces of industrial equipments. Particulate soils may occur in storage tanks, in the ducts or on the plates of heaters and coolers. The problem is particularly severe in food and pharmaceutical processing, where fouling deposits may endanger microbial sterility and product purity (Stephan et al., 2004; List and Müller, 2005). The efficiency of a plant is lowered by the formation of fouling deposit on heat transfer and other surfaces (Bott, 1995).

Polysaccharides, proteins, lipids and other biopolymers are the main constituents of food and pharmaceutical mixtures. Proteins on the outer surface of bacteria are known to play an important role in the initial attachment to solid surfaces (Dufrêne et al., 1996; Flint et al., 1997; Caccavo, 1999; Lower et al., 2005). Baking has a major effect on the subsequent removal of soils as illustrated by tomato paste used as food model deposit (Liu et al., 2006). In this case, a micromanipulation probe made it possible to directly observe the influence of substrate surface properties on the resisting interfacial tension in adhesive failure conditions, and to differentiate this process from failure within the deposit. Details in the mode of drying a starch deposit were found to influence adherence measured with a radial flow cell, which was attributed to the properties of soluble macromolecules accumulated during drying and forming an adhesive joint between the substrate and the soiling particles (Detry et al., 2011).

The aim of the present work is to improve the understanding of mechanisms affecting soiling and cleanability by assessing the role of dissolved proteins. Quartz particles were taken as a simplified particulate soil model. Glass, stainless steel and polystyrene were chosen as model substrates to examine the influence of substrate hydrophobicity. Albumin from bovine serum (BSA) and β-Lactoglobulin (β-LBG) from bovine milk were chosen as models of dissolved proteins in the soiling suspension.

MATERIALS AND METHODS

Material

Stainless steel (AISI304-2R, 1mm thick) plates were provided by Arcelor (France). The face used for the study had a mirror finish and was protected with a plastic sheet which was removed before substrate preparation. Glass slides (50 mm × 50 mm × 1 mm) and polystyrene sheets (300 mm × 300 mm × 0.25 mm) were purchased from

Menzel-Gläser (Germany) and Goodfellow (United Kingdom), respectively. MilliQ water was produced by a MilliQ-50 system from Millipore (France). Absolute ethanol was purchased from Sigma-Aldrich (Wisconsin, USA). Albumin from bovine serum (BSA) (lyophilized powder, ≥98%, essentially fatty acid free and globulin free; molar mass 66000) and β-Lactoglobulin (β-LBG) from bovine milk (lyophilized powder; ≥85%,) were purchased from Sigma-Aldrich (Wisconsin, USA).

Substrate preparation

The substrates were first cleaned with ethanol and dried with Kimtech Science paper. Secondly, stainless steel and glass substrates were sonicated (ultrasonic cleaner Branson 3200, USA) in ethanol for 15 min, while polystyrene substrate was immersed for 30 min in ethanol. The substrates were then rinsed thoroughly with ethanol, dried with a gentle flow of nitrogen and wrapped in an aluminum foil until use. These substrates will referred to as Glass, Polystyrene and StSteel-Eth. Certain coupons of stainless steel (named StSteel-UVO) were submitted to an additional UV-Ozone treatment for 20 min (UVO-cleaner, Model 42, Jelight Company, USA) to improve the removal of organic contamination, and used immediately.

Contact angles and liquid surface tension measurement

Static contact angles of water and of BSA and β-LGB solutions were measured on 16 mm × 10 mm coupons of the substrates, using the sessile drop method with a goniometer (Krüss, France). The measurements involved at least 10 drops. The surface tensions of the liquids were measured with a Prolabo Tensiometer (Tensimat n°3) using the Wilhelmy plate method with a platinum foil.

Particulate soil model

Quartz particles with a size about 10 to 30 µm were separated as described by Touré et al. (2011) and used as particulate soil model. Three kinds of quartz suspensions were prepared at a concentration of 150 g/L: (i) suspension in a BSA solution (8 g/l), (ii) suspension in a β-LGB solution (8 g/l), (iii) suspension in water. For preparing the suspensions in BSA and β-LGB solutions, 7.5 g samples of quartz were first mixed with 25 ml of MiliQ water and stirred for 30 min at room temperature. Then, 25 ml of a BSA or β-LGB solution in distilled water (16 g/l) were added and the entire mixture was stirred for 1 h. The suspensions were kept at 4 °C (BSA and β-LGB storage temperature) for 72 h.

Soiling and cleanability assessment

The substrates (coupons of 50 mm × 50 mm) were soiled with the quartz suspensions brought at room temperature. Suspension droplets were deposited by 4 successive manual aspersions using a sprayer located at a distance of 40 cm. They did not lead to the formation of a continuous film and no drainage occurred. Water was evaporated by drying for 30 min in a dark cupboard at 20.6±1.9 °C and 39±3% relative humidity (Detry et al., 2007, 2011; Touré et al., 2011). The coupons were then submitted within 2 min to the cleaning test.

Cleanability assessment was performed at 20 °C in a radial-flow cell (RFC). This consisted of an upper disk with a 2 mm diameter central inlet and a lower disk in which the soiled square sample was fitted to be cleaned. The distance between the upper disk and both the sample and the lower disk was set by three adjustable micrometric screws and controlled to be 1.00±0.02 mm with calibrated steel spacers. A complete description of the device, its hydrodynamics and its utilization can be found elsewhere (Detry et al., 2009, 2011). Distilled water was flown during 5 min with a flow rate of 40 ml/min. The sample was then removed and dried at room temperature. Pictures of the substrate were taken before and after cleaning, using an epifluorescence stereomicroscope (ZX9 Olympus, Belgium) equipped with a CCD camera. After cleaning, a circular zone with a lower density of aggregates was observed at the center of the sample. The pictures of the sample before and after cleaning were processed with a specific application of the Matlab software (The Mathworks Inc.), which gave the ratio of the number of aggregates initially present on the surface to the number of aggregates remaining after cleaning, as a function of the radial position. The radial position at which the residual density of aggregates became ≥50% was considered as the critical detachment radius (Goldstein and DiMilla, 1997, 1998). The value of the critical radius was checked for consistency in the LUCIA software to insure the absence of artifacts such as the nucleation of air bubbles on the surface. At least 10 repetitions of each experiment (soiling-cleaning) were made, being distributed in at least three independent series.

The critical radius of detachment, the output parameter of the cleaning experiments reflects the critical wall shear stress which corresponds to the minimal hydrodynamic drag force required to detach a soil from surface under given experimental conditions (Jensen and Friis, 2004). With the radial flow chamber used, the conversion of critical radius into critical wall shear stress is not reliable above flow rates of 20 ml/min (Detry et al., 2011). Therefore, the results of the present study are expressed in terms of critical radius, keeping in mind that for a defined flow rate, the higher the critical radius, the lower the adherence.

RESULTS

The critical detachment radii measured on Glass, StSteel-UVO, StSteel-Eth and Polystyrene, soiled with a quartz suspension in water and in BSA and β-LGB solutions are presented in Fig.1A. For all the substrates, the critical detachment radius was measured after cleaning for 5 min at a flow rate of 40 ml/min. Remember that the higher the critical detachment radius the lower the particle adherence (Detry et al., 2011). For Glass, no detachment radius could be measured when the quartz particles were suspended in pure water, but a detachment of a few particles occurred near the inlet. As regards the substrates soiled with quartz particles suspended in pure water, the critical detachment radius increased according to the order Glass (not measurable) < StSteel-UVO (about 2.5 mm) < StSteel-Eth (about 6.7 mm) < Polystyrene (about 7.5 mm). For Glass and StSteel-UVO, the critical detachment radius dramatically increased when soiling was made with quartz suspensions in BSA or β-LGB solutions. The critical detachment radii were in the same range for Glass and StSteel-UVO soiled with a suspension in BSA solution and for StSteel-Eth and Polystyrene soiled with a suspension in pure water. In contrast, the presence of proteins decreased the critical detachment radius for StSteel-Eth. No test was made for Polystyrene soiled with a suspension in a β-LGB solution owing to the low effect observed for BSA solution compared to water.

Fig. 1B presents the contact angles measured on the four substrates using pure water and BSA and β-LGB and solutions. There was not much influence of the nature of the liquid on the contact angle. Glass and Polystyrene gave a low and a high contact angle, respectively, as expected. StSteel-UVO presented a lower contact angle than StSteel-Eth, with a difference which was particularly marked with water.

In order to assess the influence of substrate preparation on surface properties, the water contact angle was measured at different stages of the preparation of the inorganic substrates. The results presented in Table 1 show that cleaning with ethanol decreased slightly the water contact angle and that UVO treatment decreased it much more, both for glass and for stainless steel.

The surface tensions (with standard deviations) of the liquids were 72.9 (0.3), 30 (1.1) 48.8 (0.2) mN/m for water, BSA solution and β-LGB solution, respectively.

Table 1. Water contact angle measured on glass and stainless steel after the indicated preparation stage.

Substrate	Contact angle (°)		
	not treated	ethanol treated	UVO Treated
Glass	24.2 ± 2.2	17 ± 1.2	< 10
Stainless steel	43.2 ± 3.9	30 ± 1.5	10.3 ± 1

*Mean ± standard deviation

DISCUSSION

The surface analysis of inorganic solids by X-ray photoelectron spectroscopy always shows the presence of carbon (Gerin et al., 1995; Caillou et al., 2008; Rouxhet, 2013). This is due to organic compounds which may originate from material processing, from artifacts due to sample preparation, or from contamination by adsorption from the surrounding, either the ambient atmosphere or the spectrometer vacuum chamber. Table 1 shows that initial surface cleaning with ethanol does not bring the water contact angle to low values expected for oxides or oxihydroxides present at the surface of glass and stainless steel, indicating that it did not remove the totality of organic surface contaminants. An additional UVO treatment insured a more effective removal of organic contaminants by oxidation, as revealed by the low contact angle achieved.

It must be noted that high energy surfaces cleaned by UVO treatment and left in ambient air show a quick increase of the water contact angle due to adsorption of organic compounds. For gold and stainless steel, the increase is quite steep, the contact angle reaching values above 20 ° in a few hours, and above 60 ° in less than 1 day for gold and a few days for stainless steel. For silica, the increase is significant only after several days, providing a contact angle of the order of 20 ° (Rouxhet, 2013). Such decrease of wettability may improve cleanability after soiling and drying, as observed when a tomato paste was baked on oil-coated stainless steel compared to bare stainless steel (Liu et al., 2006).

The robustness of the observations regarding adherence was demonstrated by the reproducibility of results obtained with Glass and Polystyrene, in experiments performed at a time interval of 17 months.

Fig. 1 shows that the adherence of the quartz particles brought by a suspension in water is much higher on the most hydrophilic substrates, Glass and StSteel-UVO having a water contact angle in the range of 10 to 17 °, than on the two other substrates, StSteel-Eth and Polystyrene. According to previous observations (Detry et al., 2011), this may be due to better droplet spreading and increase of capillary forces which affect the shape of the adhering aggregates and the efficiency of shear forces exerted by the cleaning fluid, and the strength of the particle-substrate contact. However the relation between soil adherence and substrate contact angle is far from monotonous as indicated by StSteel-Eth and Polystyrene, which give a similar adherence, with water contact angles of 30 and 75 °, respectively.

The relationship between soil adherence and contact angle fails if the soils made from suspensions in protein solutions are considered. As shown in Fig. 1, the presence of proteins decreases strongly the adherence on the two hydrophilic substrates and increases slightly the adherence on the two other substrates while it hardly affects the contact angle of each substrate.

For the two hydrophilic substrates, the adherence decreases with the liquid surface tension, which may be attributed to lower capillary forces and consequently to looser contact between the quartz particles and the substrates after drying. However this relation fails and even seems to be reversed for the two other substrates.

In the study of starch particles adhesion on different substrates, drying was shown to provoke an accumulation of dissolved macromolecules, forming a bridge between the particles and the substrate. Accumulated protein or adsorbed proteins may affect particle removal as do surfactants in

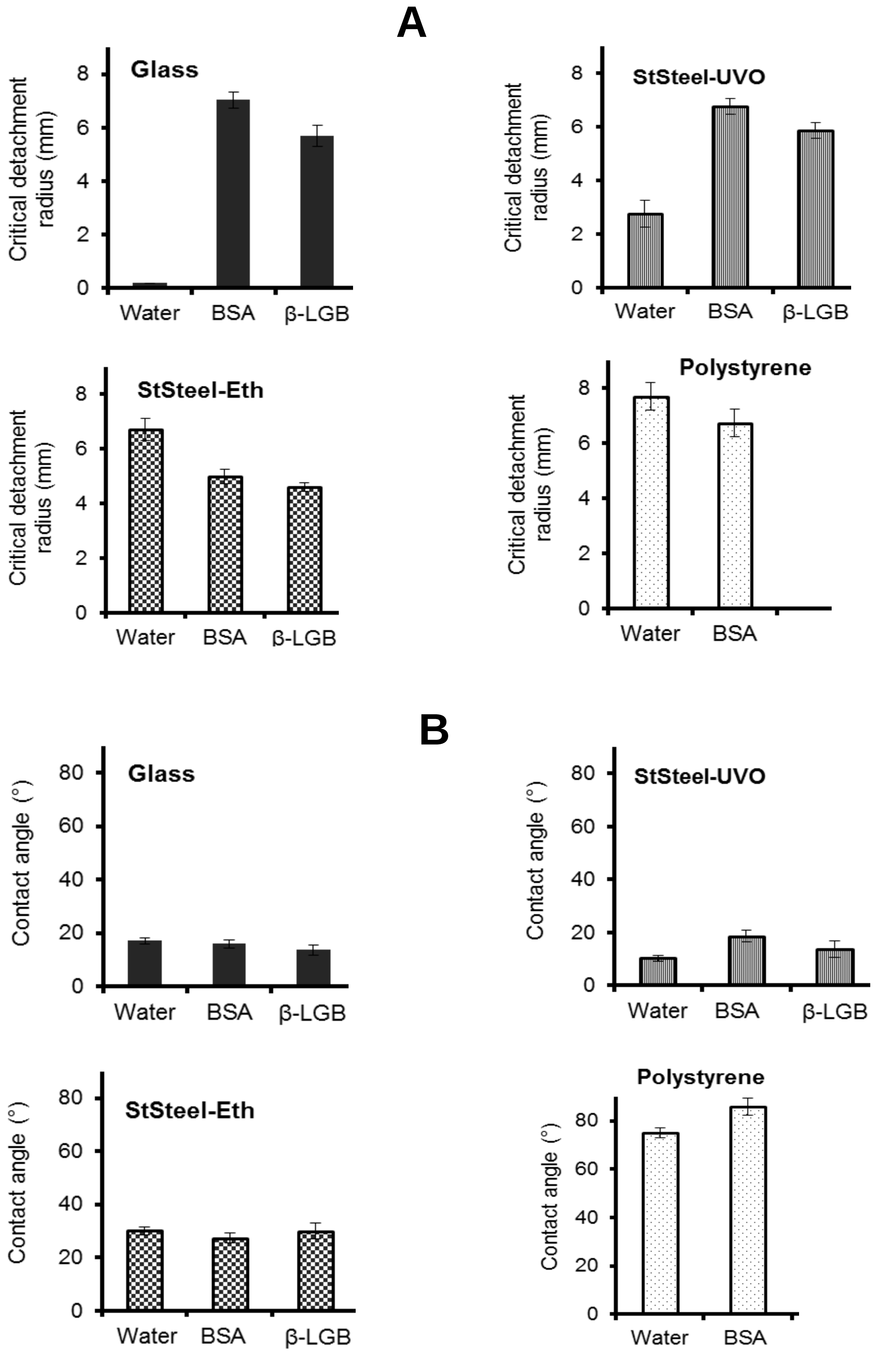

Fig. 1 A. Critical detachment radii measured on Glass, StSteel-UVO, StSteel-Eth and Polystyrene substrates soiled with suspensions of quartz particles in water and in BSA and β-LGB solutions. B. Contact angles measured on the same substrates with water and with BSA and β-LGB solutions.

particulate soil detergency (Rojvoranum et al., 2011), e.g. by creating electrostatic or steric repulsion. A possible explanation for the difference observed between Glass or StSteel-UVO, on one hand, and StSteel-Eth, on the other hand, may be a different behavior of proteins according to the substrate. It is indeed known that protein adsorption (affinity, adsorbed amount, reversibility, mobilitity in the adsorbed phase, behavior upon drying) is strongly influenced by substrate hydrophobicity (Andrade and Hlady, 1987; Jacquemart et al., 2004; Van Tassel, 2006; Gurdak et al., 2006; Rabe et al., 2011). This may also influence the distribution and the state (native, denaturated) of proteins at the quartz-substrate interface and the possibility that they act as a linking or as a dispersing agent.

CONCLUSIONS

When soiling with hydrophilic particles such as quartz was made in pure water, the soil adherence increased with substrate hydrophilicity. This is in agreement with the expected influence on droplet spreading and capillary forces created upon drying, which affect the shape and compactness of the adhering aggregates. The presence of proteins in the suspension used for soiling hydrophilic substrates (Glass and StSteel-UVO), decreased the adherence of quartz particles. Its effect was less marked and tended to be opposite for less hydrophilic substrates (StSteel-Eth, Polystyrene).

The comparison of different substrates and two proteins showed that the adherence cannot be explained by a simple relation with the contact angle. Other factors may be the solution surface tension itself and the protein behavior at the interfaces created by drying and by rehydratation during cleaning. A broader study, including protein denaturation, is under way to elucidate the effect of soluble protein on the adhesion of particulate soils.

When considering the influence of substrate on soiling, it must be kept in mind that high surface energy solids (metals, oxides) are readily contaminated in contact with air and lose their hydrophilicity. Amazingly this may improve the substrate behavior regarding cleanability with respect to particulate soil.

ABBREVIATIONS

BSA	bovin serum albumin
β-LGB	beta-lactoglobulin
RFC	radial-flow cell
StSteel-Eth	stainless steel cleaned with ethanol
StSteel-UVO	stainless steel cleaned with ethanol and an additional UVO treatment
UVO	ultraviolet–ozone

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