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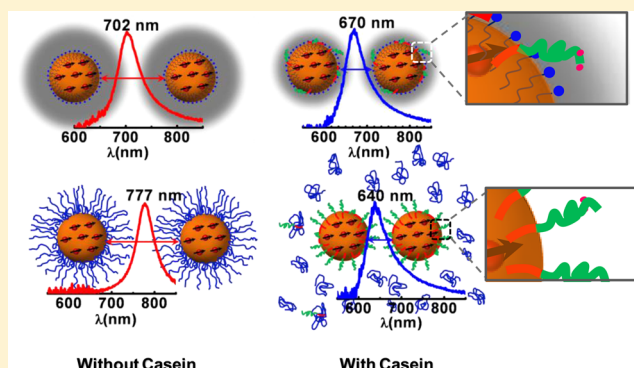
Probing of Competitive Displacement Adsorption of Casein at Oil-in-Water Interface Using Equilibrium Force Distance Measurements

V. Mahendran, J. Sangeetha, and John Philip*

SMARTS, Metallurgy and Materials Group, Indira Gandhi Centre for Atomic Research, Kalpakkam 603 102, Tamil Nadu, India

S Supporting Information

ABSTRACT: The equilibrium force distance measurement is employed for the first time to probe the competitive and displacement adsorption of casein at an oil–water (O/W) emulsion interface that was initially adsorbed with either a diblock polymer or an anionic surfactant. A significant change in the force–distance profile was observed under the competitive displacement adsorption of casein, which is further confirmed from the hydrodynamic diameter and zeta potential measurements. A decrease in the onset of repulsion and decay length are observed on competitive adsorption of smaller size casein molecules at O/W interface. With addition of casein in PVA-vac diblock polymer stabilized emulsion, the onset of repulsion decreases from 88 to 48 nm whereas the magnitude of force increases from 1 to 19 nN. The force decay length is reduced from 10.5 to 4.5 nm upon addition of casein. Our results suggest the complete replacement of adsorbed diblock polymers by casein molecules. The hydrodynamic diameter and zeta potential measurements corroborate the casein mediated polymer displacement and the competitive adsorption of casein at the O/W interface. In the case of anionic surfactant covered O/W interfaces, casein molecules weakly associate at the interface without displacing the smaller size surfactant molecules where no significant changes in the onset repulsion and force profiles are observed. These results suggest that the casein molecules are effective displacers for replacement of adsorbed macromolecules from formulations, which has several important practical applications.



■ INTRODUCTION

Understanding the nature and reversibility of competitive displacement adsorption of smaller molecules at interfaces covered with macromolecules is important from practical applications point of view.¹ Such competitive displacement adsorption provides a unique possibility to reuse adsorbents.² As the competitive interactions between different adsorbing species determine the stability and product lifetime, several studies have been carried out on the interaction of protein with other molecules at the air–water and oil–water interface.^{3–17} The recent neutron reflectivity studies show that a synergistic interaction between certain nonionic surfactants and proteins can lead to spontaneous self-assembly at the air–water interface to form layered surface structures.¹⁴ Such protein–anionic surfactant interactions can also effectively be used to tune electrostatic interactions.¹⁵

The protein adsorption at the oil–water interface is important in manufacturing industries such as pharmaceutical and food emulsion industries.^{14,18–20} Milk proteins are used as a stabilizer in food hydrocolloids because of their exceptional amphiphilic nature and stabilizing properties.^{18,21} In bovine milk, casein consists of four major proteins, α_1 -casein, α_2 -casein, β -casein, and κ -casein, which are known to adsorb rapidly at the oil–water interface and provide stability via

electrosteric stabilization.^{22,23} α_1 -casein and β -casein are the major contents (~75%) of milk casein.²² The average molecular weight of β casein is ~24 kDa with a net charge of $-15e$ at neutral pH. β -Casein is an unstructured protein and has no internal covalent cross-links and has 209 amino acid residues, and the nonuniform distribution of hydrophobic and hydrophilic residues gives rise to a distinctly amphiphilic molecular structure.^{24,25} β -Casein possesses a strong negative charge with four of the five phosphate groups being located at the N-terminal end between amino acid residues 13 and 21.²⁵ The average molecular weight of α_1 is ~23.5 kDa with a net charge of $-22e$ at neutral pH.^{25,26} α_1 -Casein is also amphiphilic with nonuniform and more randomly distributed 199 hydrophobic and hydrophilic residues than that of β -casein.²⁴ β -Casein is more hydrophobic and surface active than α_1 -casein.²² The casein resembles a block copolymer with alternating charge and hydrophobicity, that is a charged phosphopeptide loop and a hydrophobic train.²⁷ α_1 -Casein has an N-terminal hydrophobic train, followed by a charged loop and a final C-terminal hydrophobic train.²⁸ The κ -casein

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micelle diameter is ~ 23 nm and consists of approximately 30 monomers, and the CMC value varies from 0.02 to 0.05 wt %²⁹.

The stability aspects of protein stabilized emulsions, under various conditions like temperature,³⁰ pH,²⁴ cations,¹⁶ and biochemical environment,^{9,31,32} have been studied in food hydrocolloids. In general, the polymer adsorption at solid interfaces is strongly influenced by solution properties such as pH and ionic strength.³³ The experiments suggest that the ionizable character of silica plays a key role in the adsorption of neutral polymer chains.³⁴ Though nonionic polymer adsorption (e.g., poly(ethylene oxide) and poly(*N*-vinylpyrrolidone)) onto charged solid surfaces in water has been widely studied, measurements at the oil–water interface are limited.^{35–38}

An understanding of competitive displacement adsorption is important for extending the shelf life and reuse of many products such as pharmaceutical formulations, drug delivery globules, cosmetics, paints, detergents and pesticides. Irrespective of intense research on protein adsorption behavior at interfaces, the understanding is still vague because of the complex interaction between the protein molecules and surfaces through van der Waals, electrostatic, hydrophobic, and hydrogen bonding interactions. Measurement of interaction forces in the presence of adsorbing species at a solid interface is studied using atomic force microscopy³⁹ and surface force apparatus.⁴⁰ We attempt to obtain better insight into these complex interactions between proteins and oil–water (O/W) interface in the presence of diblock polymer mediation using insitu colloidal force measurement. Here, we probe the competitive displacement adsorption efficiency of casein at an oil–water interface stabilized with either negatively charged surfactant or neutral polymer by employing intermolecular force, zeta and hydrodynamic diameter measurements. The questions we tackle from this study are the following. (a) Are casein proteins effective in displacing adsorbed diblock copolymers from O/W interface? (b) How casein-diblock polymer interaction affects the force parameters and stability of O/W emulsions? (c) What is the effect of polymeric size on nature of forces during and after competitive displacement adsorption?

■ EXPERIMENTAL SECTION

Materials Used. The anionic surfactant used in our experiments is sodium dodecyl sulfate ($C_{12}H_{25}-SO_4 Na$), hereafter referred as SDS. The purity of SDS was 99.9%, and it was obtained from Sigma. The polymer used in the experiments is a statistical copolymer of vinyl alcohol-vinyl acetate (CH_2CHOH [88%]- CH_2CH ($OCOCH_3$) [12%]), with average molecular weights of 40 000 and 115 000 (referred as PVA-vac 40K and PVA-vac 115 K, respectively), obtained from Aldrich and used as such. PVA-vac is a water-soluble polymer at room temperature, and its θ temperature in water is around 97 °C. The unperturbed radius of gyration of the polymer (R_g) of PVA-vac measured at a concentration well below the overlap concentration (C^*) is found to be ~ 8 and 11 nm for 40K and 115K, respectively. Ultrapure Milli-Q water with a resistivity value of 18 $M\Omega\cdot cm$ was used in the preparation of emulsions and polymeric solutions.

Ferrofluid Emulsion Preparation. The magnetic nano-emulsion has three major constituents: ferrofluid oil, surfactant, and water. The preparation of ferrofluid oil and emulsion is discussed in detail in our earlier publications.^{41,42} For clarity, the details are briefly discussed. The oil-in-water nanoemulsion

is prepared by shearing ferrofluid, consisting of functionalized ferrimagnetic nanoparticles of size ~ 10 nm.⁴³ From the concentrated crude polydisperse emulsion, highly monodispersed emulsion with hydrodynamic diameter ~ 190 nm is produced by fractionation crystallization technique.⁴⁴ These emulsion droplets are adsorbed with an anionic surfactant SDS and PVA-vac by a washing procedure.⁴⁵ To study the effect of surface charge and casein interaction, initial emulsion stabilized with 0.8 mM SDS is used. The pH of the emulsion was 6.4. To study the effect of adsorbed polymer and casein interaction, initial emulsion is stabilized with 0.6 wt % PVA-vac (of average molecular weight 40K and 115 K) and incubated for 48 h. The SDS and PVA-vac stabilized emulsions were characterized for the hydrodynamic diameter and zeta potential using dynamic light scattering and electrophoretic mobility measurements, respectively. The average hydrodynamic diameters for SDS, PVA-vac 40K, and PVA-vac 115 K stabilized emulsions were 190, 293, and 500 nm, respectively, and the corresponding zeta potential values were -46 , -14 , and -2 mV, respectively. The force profile measurements were done at 25 °C.

Pasteurized cow milk (fat ~ 4.5 g/100 g, protein ~ 2.9 g/100 g, carbohydrate ~ 4.9 g/100 g, and calcium ~ 114 mg/100g) was used to prepare casein. In cow milk, casein comprised of 80% of the total protein and the remaining protein components are α -Lactalbumin, β -lactoglobulin, serum albumin, immunoglobulin and other proteins. Casein is extracted from skimmed milk.⁴⁶ Whole milk is centrifuged at 6000 rpm for 20 min to remove fat (due to its lower density, it forms a creamy layer). Then the pH of the skimmed milk was brought down to pH 4.6, the isoelectric point of casein where the casein starts precipitating and the filtrate (α -lactalbumin and β -lactoglobulin, bovine serum albumin) is called whey protein. Casein was separated from whey protein by using filter paper. The other possible residuals α -lactalbumin and β -lactoglobulin do have a tendency to adsorb at the hydrophobized interface; however, β - and α_{s1} -casein have a more preferential adsorption.⁴⁷ The concentration of the isolated casein was determined by thermogravimetric analysis as 4.2 wt %. From the concentrated casein, the desired wt % is prepared by diluting it in Milli-Q water. The isolated casein is characterized by hydrodynamic diameter and electrophoretic mobility measurements.

Intermolecular Force Measurement. Magnetic chaining technique (MCT) is a popular technique for probing the intermolecular interactions between the magnetic emulsions at nanoscales.^{41,48} Typical experimental setup and working principle are discussed in detailed in our earlier publications.⁴⁵ The MCT experimental setup has three major components: a white light source, a fiber optic reflection probe-spectrometer, and a solenoid coil. The fiber optic reflection probe coupled to a spectrometer records the reflected spectra from the samples, which are kept inside a solenoid coil. By controlling coil current, the magnetic field strength inside the emulsion is varied (additional information on the MCT technique is available in the Supporting Information, Figure S5). The emulsion droplets are either electrostatically (SDS) or sterically (PVA-vac) stabilized against agglomeration. When an external magnetic field is applied, droplets form one-dimensional (1D) chainlike structure due to overwhelming magnetic dipolar attraction. For an infinitely long droplet chain, the dominant dipole–dipole attractive force is $F_{\text{chain}} = -\Sigma n(6m^2/(nd)^4)$ and $m = \mu_0 4\pi a^3 \chi_s H_T / 3$. Here m is the magnetic moment of each droplet, μ_0 is magnetic permeability of free space, and χ_s is the magnetic susceptibility, and the total magnetic field $H_T =$

$H_{\text{applied}} + H_{\text{induced}}$. As the periodicity of 1D chain lies in the visible wavelength region, Bragg reflection is seen when the sample is illuminated with a white light. The diffraction from a 1D chain follows Bragg's law, $2nd \sin \theta = N\lambda$, where n is refractive index of continuous medium (water), d is the distance between the centers of adjacent droplets, N is the order diffraction, θ is the diffraction angle, and λ is the diffraction wavelength. For 180° geometry, the first order Bragg's condition is $2nd = \lambda$ and the corresponding interdroplet spacing is given by $h = d - 2a$, where a is the droplet radius. By controlling applied magnetic field, d can be varied precisely.

Hydrodynamic Diameter and Zeta Potential Measurements. The hydrodynamic diameter and zeta potential values were measured using a Malvern Zeta nanosizer. The hydrodynamic diameter measurement is based on dynamic light scattering (DLS) principle. In DLS, the scattered intensity fluctuations are measured as a function of time, which depends on the size and diffusion coefficient of particles under Brownian motion. The autocorrelation function relates the scattering intensity at time t and after a small time interval of τ , as $g^{(2)}(\tau) = \langle I(t)I(t + \tau) \rangle / \langle I(t) \rangle^2$. The $g^{(2)}(\tau)$ is related to the first order correlation function of the electric field as $g^{(1)}(\tau) = [g^{(2)}(\tau) - 1]^{0.5}$, which decays exponentially with a delay time τ for a suspension containing spherical, monodisperse, and Brownian particles, $g^{(1)}(\tau) = A e^{-Dq^2\tau} + B$,⁵⁰ where A and B are amplitude of the correlation function and baseline, respectively, and D is the translation diffusion coefficient. For spherical particles, the translational diffusion coefficient and hydrodynamic radius (R_h) are related by the Stokes–Einstein equation $D = kT/6\pi\eta R_h$. The refractive index values of octane and casein are 1.398 and 1.3317, respectively. Zeta potential (ξ) is obtained from electrophoretic mobility (U_e) measurements, using Henry's equation, $\xi = 3\eta U_e / 2\epsilon f(Ka)$, where η , ϵ , and $f(Ka)$ are the viscosity, dielectric constant of the medium, and Henry's function, respectively.

RESULTS AND DISCUSSION

SDS Stabilized Emulsion–Casein Interaction. Figure 1 shows the hydrodynamic diameter and zeta potential of pure

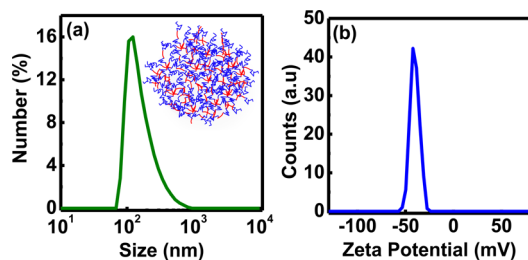


Figure 1. Distribution of (a) hydrodynamic diameter and (b) zeta potential values of 0.42 wt % casein. Vverage values of hydrodynamic diameter and the zeta potential values are found to be ~ 120 nm and ~ -40 mV, respectively, at pH 6.69. The schematic representation of casein micelle is shown in the inset of (a).

casein. The average hydrodynamic diameter and the zeta potential values are found to be ~ 120 nm and ~ -40 mV at a pH value of 6.69, respectively, which is in agreement with the reported values.⁴⁶

To study the casein interaction at a negatively charged interface, the initial emulsion was washed and stabilized with 0.8 mM SDS. Because of the adsorbed DS[−] headgroup at the

oil–water interface, the droplet surface is negatively charged; this was evident from the negative zeta potential (-35 mV) observed in SDS stabilized emulsions. The molecular dynamic simulation and sum frequency studies show that SDS headgroup is fully surrounded by water molecules and the SDS alkyl tail is in contact with oil, where at least two chain defects are needed to bend the alkyl tail toward the surface.⁵¹

Figure 2 shows the force–distance profile of SDS stabilized

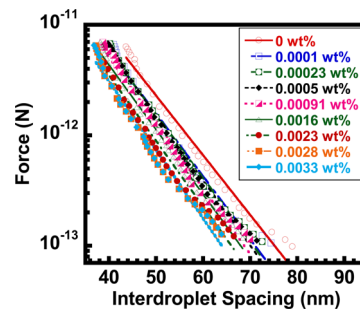


Figure 2. Force distance profiles at different casein concentrations for 0.8 mM SDS stabilized emulsion. Solid lines correspond to the fit to the experimental data using electrical double layer repulsion equation.

emulsion at different casein concentrations ranging from 0 to 0.0033 wt %. The reported value of casein CMC is 0.05 mg/mL. The force profile at zero casein concentration decays exponentially with the interdroplet distance, where the decay length is found to be 8 nm, which is comparable to the theoretical Debye length (~ 10 nm) for 0.8 mM SDS. With the increase in casein concentrations, the force profiles are akin to that of the one stabilized with SDS but with slightly different slopes, onset of interaction, and magnitude of force. From the best fit obtained from the electrostatic repulsion equation (solid lines in Figure 2), we extracted three parameters: magnitude of force decay or the pre-exponential factor (K) dictated by the surface potential, Debye length (κ^{-1}) from the slope of the curve, and the first interaction length or onset repulsion ($2L_0$), corresponding to the distance at which magnitude of force is 2×10^{-13} N. The κ^{-1} decreases from 8 to 7 nm as the casein concentration is increased from 0 to 0.0033 wt % and the $2L_0$ is found to decrease from 67 to 57 nm in the above concentration range. For all the casein concentrations, the force profiles are repulsive in nature and decay exponentially with interdroplet distance. Without casein, the observed decay length of 8 nm was close to the theoretical decay length value for SDS (0.8 mM). The radius of gyration, R_g , of β -casein micelles is about 12 nm.²⁹ As the concentration of casein used here is much lower than the CMC value of casein, free micelles are not present in the emulsion. The decay length comparable to the decay length at 0.8 mM SDS shows that the O/W interface is still intact with SDS molecules. However, from the force measurement data, we cannot completely rule out the possibility of the presence of some casein molecules at O/W interface.

The amphiphilic behavior with high proportion of nonpolar and polar residues of casein seems to enable them to adsorb at the oil–water interface.²² A recent Monte Carlo simulation study showed that the unstructured β -casein adsorbs to both positively and negatively charged surfaces through several interactions such as direct electrostatic, hydrophobic interactions, structural rearrangement, and charge regulation due to proton uptake and release.⁵² With increasing casein concen-

tration up to 0.0023 wt %, the droplet starts interacting at shorter interdroplet spacing than the SDS stabilized interfaces.

For a thin electrical double layer, $\kappa a > 5$, where κ is the inverse Debye length, which determines the range of electric double layer interaction and is represented as $\kappa = 1/[(4\pi q^2/\epsilon kT)^2 C_s]^{-0.5}$, where C_s is the salt concentration, kT is the thermal energy, and q is the charge; for 0.8 mM SDS ($\kappa a = 9.34$), the electrostatic repulsion as a function of distance is given by the Derjaguin approximation $F_r(d) = 2\pi\epsilon\psi_0^2 a \kappa [(\exp[-\kappa(d-2a)])/(1 + \exp[-\kappa(d-2a)])]$,^{53,54} where ϵ is the dielectric permittivity of the carrier medium, ψ_0 is the surface potential, a is the droplet radius, and d is the center to center distance between droplets. In this approximation, the surface potential is assumed to be constant and independent of interparticle spacing h .

By increasing casein concentration from 0 to 0.0033 wt %, the range of repulsion is found to decrease while the magnitude force decreases slightly from 1.15 to 0.95 nN. The observed repulsive nature with increasing casein concentration indicates that some casein molecules may form a patchy coverage at the interface and increases the surface charge of oil–water interface through hydrophobic interactions as reported by Evers et al.⁵² Similar force profile was observed with increasing SDS concentration, where the added SDS monomers coadsorbs at the interface and screen the surface charges, resulting in a reduction in the onset of repulsion.⁴¹ With increasing casein concentration beyond 0.0023 wt %, the force–distance profiles did not show much change. The measured autocorrelation function, hydrodynamic diameter and zeta potential for 0.8 mM SDS stabilized emulsion (pH 6.24) at different casein concentrations are shown in Figure 3. The hydrodynamic

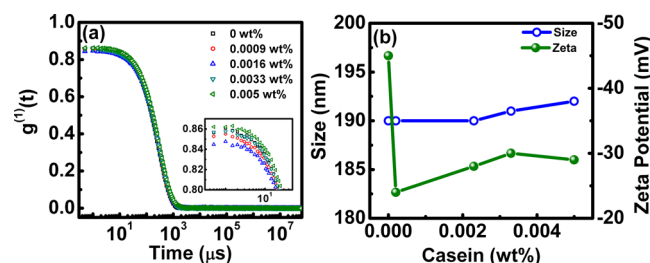


Figure 3. (a) Electric field autocorrelation function of scattered light (inset shows an enlarged view at shorter time interval) from SDS stabilized emulsion at different casein concentrations (pH 6.24). (b) Hydrodynamic diameter (open symbols) and zeta potential (solid symbols) for 0.8 mM SDS stabilized emulsion at different casein concentrations.

diameter of emulsion droplets varies slightly from 190 to 192 nm as the casein concentration is increased from 0 to 0.005 wt %. However, a drastic reduction in the zeta potential from -45 to -30 mV is observed when 0.005 wt % of casein is incorporated in the emulsion. Here, the variation in the zeta potential and hydrodynamic diameter of the mixed dispersion roughly follow the superposition of size/zeta distribution.

PVA-vac–Casein Interaction. To study the effect of casein adsorption on the intermolecular forces between the polymer stabilized droplets, the emulsion is washed five times with 0.6 wt % of PVA-vac solution and incubated for a period of 48 h for equilibrium adsorption.⁴⁵ The hydrophobic vac goes into the oil phase, and the hydrophilic PVA extends into water. Due to finite size of confined segments (excluded volume effect) and hydration layers, the steric force magnitude and

range can be much greater than $10R_g$ in aqueous solutions.⁵⁵ Figure 4a shows the force distance profile for PVA 40K

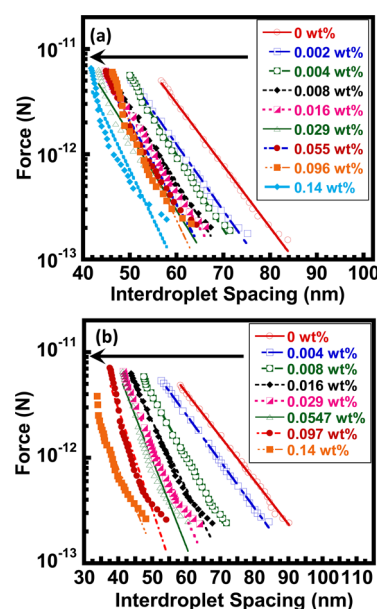


Figure 4. Force–distance profiles at different casein concentrations for PVA-vac (a) 40K and (b) 115 K stabilized emulsion. Solid lines correspond to the theoretical fit.

stabilized emulsion at different casein concentrations. At all the studied casein concentrations, force profiles decay exponentially with interdroplet distance. The force profile without added casein is taken as a reference curve, which was exponentially decaying. It has been shown that the force profile between polymer covered particles is repulsive and can be represented by a simple exponential function, $F_r(h) = (k_b T \pi R / \lambda^2) \exp[-h/\lambda]$,⁵⁶ where h is interparticle spacing and λ is decay length. From the best fit to above equation, we have extracted three parameters: decay length (λ) from the slope and the $2L_0$ or the first interaction length, that corresponds to the interdroplet spacing at a force magnitude of 2.8×10^{-13} N, and magnitude of force (K) which is dictated by the adsorbed polymer layer. Without casein, the characteristic decay length $\lambda = 7.5$ nm was close to the unperturbed R_g value of free polymer measured using viscometry (~ 8 nm).

With the addition of 0.004 wt % casein, the λ decreases from 7.5 to 7 nm. The λ values were 6, 5, and 4.5 nm for 0.016, 0.054, and 0.14 wt % casein, respectively. Interestingly, the $2L_0$ decreases drastically from 77.5 ($\sim 10R_g$) to 57 nm ($\sim 7R_g$) when 0.14 wt % casein is present in the emulsion where the force magnitude also increases from 10 to 30 nN with casein. Figure 4b shows the force–distance profile measured at varying casein concentrations for PVA-vac 115 K stabilized nanoemulsion. The force distance curves were exponentially decaying with interdroplet distance, though slight deviations are observed at the higher casein concentrations. Without casein, $\lambda = 10.5$ nm, which is close to the unperturbed R_g value of PVA 115 K measured from viscometry (~ 12 nm). The decay lengths were 9.8, 7.4, 6.7, 6, and 4.5 nm for 0.004, 0.008, 0.016, 0.029, 0.096, and 0.14 wt % casein, respectively. In this concentration range, the onset repulsion decreases from 88 ($\sim 7.3R_g$) to 48 nm ($\sim 4R_g$), whereas the magnitude of force increases from 1 to 19 nN.

Figure 5 shows the change in $2L_0$, λ , and K as a function of casein concentration. For both 40K and 115K stabilized

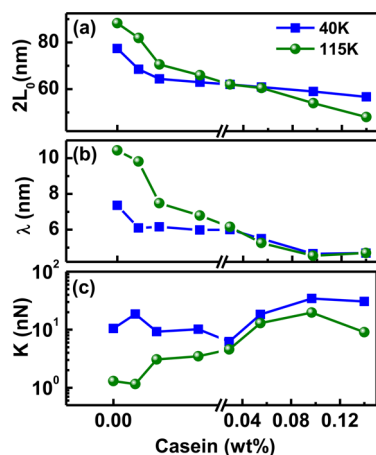


Figure 5. (a) First interaction length ($2L_0$), (b) decay length (λ), and (c) force magnitude (K) for PVA-vac 40K (square) and PVA-vac 115 K (circle) as a function of casein concentration.

emulsion, λ and $2L_0$ values decrease with casein, whereas an increase in the force magnitude is observed. The significant diminution in the λ and $2L_0$ value suggests that the adsorbed polymer is replaced by casein through competitive interaction. If the polymer remains at the interface along with the casein molecules, the decay length would not have reduced to 4.5 nm. The increase in force magnitude with casein concentration suggests that the casein volume fraction at the droplet interface also increases.

As expected, such adsorption of casein molecules at an interface leads to an electrostatic repulsive force due to the negative charges of α_{s1} - and β -casein ($-22e$ and $-15e$, respectively).⁵⁷ To understand the competitive displacement adsorption phenomena, we have carried out zeta potential measurements in PVA-vac 40K stabilized emulsion at different casein concentrations.

Figure 6 shows the autocorrelation function, hydrodynamic diameter, and zeta potential for different casein concentrations

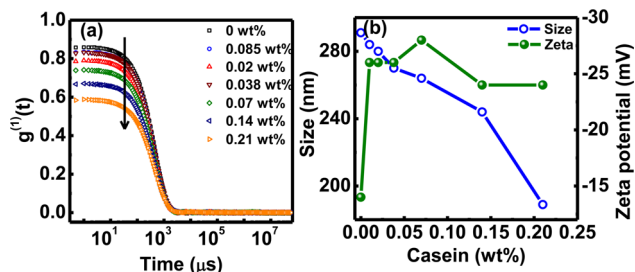


Figure 6. (a) Autocorrelation function as a function of time at different casein concentrations. (b) Hydrodynamic diameter (open symbols) and zeta potential (solid symbols) for PVA-vac 40K stabilized emulsion (pH 6.53) as a function of casein concentration. Arrow in (a) indicates the increase in casein concentrations.

for PVA 40K stabilized magnetic nanoemulsion. All the measurements were carried out at 25 °C, and the results are plotted as the average of 45 measurements. The average hydrodynamic diameter of the emulsion droplets with 0, 0.01, 0.02, 0.04, 0.07, 0.14, and 0.21 wt % casein are 295, 284, 280,

270, 264, 244, and 189 nm, respectively. Up to 0.04 wt % casein, the hydrodynamic diameter distribution was narrow and monomodal, but on further increase in casein concentration, the distribution became broad (100–300 nm), though the peak remains monomodal. The significant reduction in the hydrodynamic diameter (~ 120 nm less) with 0.21 wt % of casein, unambiguously confirms the competitive adsorption of casein by displacing the adsorbed polymer molecules at the oil–water interface. Obviously, such displacement of adsorbed polymers should lead to a reduction in the $2L_0$ values due to absence of extended polymer trains, loops and tails from the O/W interface, which is in excellent agreement with our results. To confirm this further, we have carried out the zeta potential measurements with different casein concentration and the results are shown in Figure 6b. Though the oil-in-water emulsion droplets are washed and incubated with polymers, some residual negative charges are present at interfaces due to anionic surfactant used during the emulsification process, which gives rise to the observed negative zeta potential value of ~ -15 mV (pH 6.53). When the casein concentration is increased to 0.01 wt %, the zeta potential value increases to -26 mV and remains constant thereafter. Among the four major protein components (α_{s1} -casein, α_{s2} -casein, κ -casein, and β -casein), α_{s1} -casein and β -casein are the main individual components coexisting at oil–water interfaces.²² Our results are in good agreement with the earlier findings that both α -casein and β -casein molecules can absorb strongly and coexist at oil–water interfaces.^{5,27}

Therefore, the adsorption of casein molecules at the O/W interface by replacing the preadsorbed PVA-vac molecules is evident from the increase in the zeta potential and the decrease in hydrodynamic diameter with increasing concentrations of casein. From earlier studies, it was concluded that the adsorbed β -casein has an extended long tail in aqueous medium, whereas the α -casein forms a short loop at the interface and the interaction between β -casein adsorbed interfaces is strongly repulsive at all ionic strengths due to contributions from steric and electrostatic forces.^{22,58,59} The net repulsive interaction observed in the present study shows that the β -casein adsorbed at the oil–water interface contributes to the repulsion, as the interaction between α -casein covered interface is attractive.

Figure 7 shows the hydrodynamic diameters of pure casein, SDS stabilized emulsion without and with casein, and PVA 40K stabilized emulsion without and with 0.21 wt % casein. The average hydrodynamic diameters of pure casein, SDS, and PVA 40 K stabilized emulsion are 115, 190, and 294 nm,

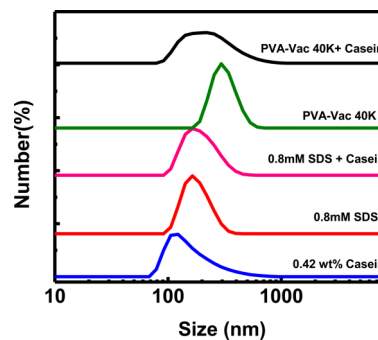


Figure 7. Hydrodynamic diameter distribution of casein, 0.8 mM SDS stabilized emulsion without and with 0.004 wt % casein, and PVA 40K stabilized emulsion without and with 0.21 wt % casein.

respectively. On addition of 0.21 wt % casein, the average hydrodynamic diameter of PVA 40K stabilized emulsion is reduced and the distribution becomes broader. Here, the average diameter was 190 nm, with a distribution ranging from 100 to 300 nm. No change in the hydrodynamic diameter is observed on adsorption of casein at SDS stabilized emulsion. The larger hydrodynamic diameter distribution for PVA40K with casein shows the conformational changes of adsorbed PVA-vac, which originally forms a loop-train-tail conformation where the vac part the polymer chains remains inside the oil core and the PVA segments forms a thick adsorbed layer and extend into the solution as loops and tails. Here, some loosely bound polymers may bind to other free polymer coils through casein linkers. The observed variation in the hydrodynamic diameters with different additives and adsorbing moieties can also be explained in terms of the mixing of two different sizes.

Why do the low molecular weight casein molecules displace the preadsorbed PVA-vac and adsorb at the oil–water interface? It is known that the competitive displacement adsorption at an interface preadsorbed with polymer chains can be initiated by smaller or chemically different displacer molecules where the conformation and the nature of interactions between surfaces and polymer chains, surfaces and solvents, and solvents and polymer chains are the important parameters influencing such competitive displacement adsorption.⁶⁰ Casein has a disordered structure, which resembles a random coil polymer.²⁷

Cohen Stuart et al.⁶¹ showed that the complete displacement of adsorbed polymer by displacer molecule is favorable, if the desorption energy of the displacer molecules is greater than or equal to the adsorption energy of the polymer segment, which was derived from the critical displacer concentration, ϕ_{cr} at which the polymer surface excess vanishes. According to theory, the displacer adsorption energy is given by^{35,61}

$$\chi_s^{pd} = \ln \phi_{cr} + \chi_{sc} - \lambda_1 \chi^{pd} - (1 - \phi_{cr})(1 - \lambda_1) \Delta \chi^{do} \quad (1)$$

where λ_1 represents the fraction of contacts a lattice site makes with sites in one of the adjoining layers and $\Delta \chi^{do}$ is the combined solvency parameter defined as

$$\Delta \chi^{do} = (\chi^{po} - \chi^{pd} - \chi^{do}) \quad (2)$$

The superscripts of the χ -parameters po, pd, and do correspond to the contact free energies between polymer and solvent, polymer and displacer, and displacer and solvent, respectively. The adsorption of flexible polymers at interfaces is governed by the free energy of mixing of polymer and solvent, represented by Flory–Huggins parameter, and the free energy associated with the formation of polymer-surface contacts and the concomitant breaking of solvent-surface contacts.

The hydrodynamic diameter and zeta potential data suggest that a very low concentration of casein (0.01–0.2 wt %) is sufficient for the complete displacement of adsorbed polymers (~0.6 wt %) from the O/W interface. The force measurement, hydrodynamic diameter, and zeta results suggest that casein forms a patchy coverage on SDS covered O/W interface whereas they vividly adsorb at O/W by displacing the larger PVA-vac polymer molecules. Based on our results, the conformation of polymer, surfactant, and casein at the O/W interface along with the experimentally obtained Bragg peak positions at various concentrations of casein are schematically represented in Figure 8. Figure 8a–c shows the diffraction peak

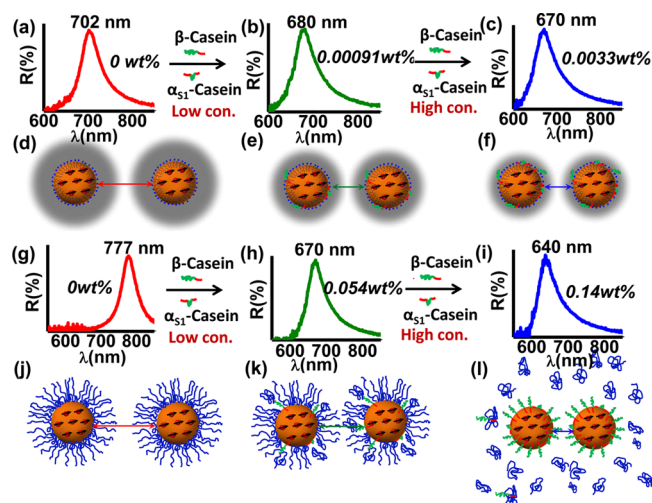


Figure 8. Bragg peak at different casein concentrations at a fixed magnetic field of 126 G and the schematic representation of β -casein and α_{s1} -casein conformation at the oil–water interface. (a–c) diffraction peak at 0, 0.00091, and 0.0033 wt % casein; (d–f) corresponding schematic representation of the interdroplet spacing; (g–i) diffraction peaks and at 0, 0.054, and 0.14 wt % casein; and (j–l) schematic representation of casein conformation at PVA-vac interface.

without and with different casein concentrations in SDS stabilized emulsion and Figure 8d–f show the corresponding changes in the interdroplet spacing. With increasing concentrations of casein in the dispersion, casein molecules form a patchy coverage at the O/W interface due to the hydrophobic interactions.⁵² Figure 8g–i shows the diffraction peak without and with different casein concentrations in PVA-vac stabilized emulsion, and Figure 8j–l shows the corresponding changes in the interdroplet spacing. With increasing concentrations of casein in the dispersion, casein displaces PVA-vac and adsorb at the O/W interface.

Our equilibrium force, hydrodynamic diameter, and zeta potential results suggest that casein molecules are excellent displacers for replacement of adsorbed macromolecules at O/W interface.

CONCLUSIONS

The competitive displacement adsorption of casein at polymer stabilized O/W emulsion droplet interface has been studied using in situ equilibrium intermolecular force measurements. We observe a significant change in the force distance profiles under competitive and displacement adsorption of casein at an O/W interface. With the addition of casein in PVA-vac diblock polymer stabilized emulsion, the onset of repulsion decreases from 88 to 48 nm whereas the force decay length is reduced from ~10.5 to 4.5 nm upon the addition of casein, which provides unambiguous evidence for the replacement of extended polymer coils by smaller size casein molecules. The hydrodynamic diameter and zeta potential results corroborate the observed casein mediated polymer displacement and adsorption of casein at the O/W interface. In the case of anionic surfactant covered interfaces, a feeble change in the onset of repulsion and force profiles is observed, which suggests that some casein molecules coexist with the surfactant covered O/W interface. Our results suggest that the casein molecules are effective displacers for replacement of adsorbed macro-

molecules from emulsion interfaces, which may find several important practical applications for reuse of formulations.

■ ASSOCIATED CONTENT

■ Supporting Information

XRD, VSM, FTIR, TGA hydrodynamic diameter distribution and TEM of the Fe_3O_4 nanoparticles (Figure S1), hydrodynamic diameter and zeta potential values for SDS, PVA40K, 115 K stabilized emulsions (Figure S2), cryo-freeze fracture TEM images of two nanoemulsion droplets (Figure S3). Phase contrast optical microscopic images of these droplets (a) without and (b) with magnetic field show Brownian droplets and aligned droplets in 1D, respectively (Figure S4). Details of the working principle of MCT and schematic experimental setup (Figure S5). Details of hydrodynamic diameters with different additives and adsorbing moieties (Figure S6). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b02612.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: philip@igcar.gov.in. Tel: 00 91 44 27480232. Fax: 00 91-44-27450356.

Notes

The authors declare no competing financial interest.

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