Tunable attractive and repulsive interactions between pH-responsive microgels

Jae Kyu Cho,[a] Zhiyong Meng,[b] L. Andrew Lyon[b] and Victor Breedveld[a]

Received 19th June 2009, Accepted 16th July 2009
First published as an Advance Article on the web 31st July 2009
DOI: 10.1039/b912105f

We report direct measurements of the pairwise interparticle potential between poly(*N*-isopropylacrylamide-*co*-acrylic acid) (pNIPAm-*co*-AAc) colloidal microgels as a function of pH, as determined through Ornstein–Zernike analysis of the pair distribution function of quasi-2D dilute colloidal suspensions. The interaction potential ranges from purely repulsive at high pH due to electrosteric interactions to weakly attractive at low pH due to hydrogen bonding, which explains previous observations on the unique phase behavior of these particles in concentrated suspensions.

Colloidal suspensions display various phases and microscopic structures, ranging from random disordered to self-assembled ordered structures.[1] The phase behavior is determined by the effective volume fraction of the suspension and interparticle forces, such as van der Waals attractions, Coulombic interactions, steric repulsions and hydrogen-bonding. Understanding the interplay between these intermolecular forces is essential to control colloidal phase behavior, for example to fabricate ordered colloidal structures for specific applications. The majority of studies to date have been devoted to hard-sphere systems, which have simple intermolecular forces dominated by entropic excluded volume effects[2] and to other colloidal systems with relatively well-understood additional interactions, such as Coulombic repulsion and depletion attractions.[3] Only fairly recently, soft and deformable particles, in particular star polymers and microgels, have attracted significant attention under the impulse of improved synthesis techniques. These soft colloidal systems are characterized by tunability of their softness and complex interparticle interactions, which result in rich phase diagrams and unique hydrodynamic behavior.[4,5] In particular, poly(*N*-isopropylacrylamide) (pNIPAm)-based microgels have been studied extensively because of their reversible temperature-responsiveness.[6,7] By incorporating ionizable functional groups, *e.g.* acrylic acid (AAc), as co-monomers, one can synthesize ionic microgels that respond not only to temperature, but also to changes in pH and ionic strength.[8]

In spite of several studies on the phase behavior of colloidal microgels as a model system for soft spheres, both from our groups and from other researchers,[7,9–12] direct measurements of the underlying interparticle interactions have not been reported. However, previous experimental observations in our groups have indicated that these interactions are non-trivial, especially in pNIPAm-*co*-AAc microgels. While the phase behavior of pure pNIPAm microgels can be explained relatively well by defining an effective volume fraction of particles and using hard-sphere-like interactions, the incorporation of AAc adds significant complexity to the system. Hard sphere theory is not sufficient to explain the dynamics of these pH-responsive microgels, as illustrated by the following examples from our own research. First, pNIPAm microgel suspensions are liquid-like below the hard sphere freezing volume fraction of 0.494, but pNIPAm-*co*-AAc microgels were found to exhibit close-packed crystalline structures under experimental conditions that yield effective volume fractions below 0.40.[9] In another study, we observed two unexpected phenomena: irreversible adhesion of pNIPAm-*co*-AAc microgels to glass substrates in very dilute suspensions, and enhanced thermal stability of pNIPAm-*co*-AAc colloidal crystals at temperatures well above the volume phase transition temperature.[10] These observations were specific for low pH conditions, where AAc groups are protonated and Coulombic repulsions are minimized, and strongly suggest the existence of an attractive interparticle potential. A third set of experiments provides further clues about the interparticle interactions: the microstructure of concentrated pNIPAm-*co*-AAc microgels was found to slowly evolve from diffusive to sub-diffusive liquid and ultimately to crystalline over several weeks of aging.[12] This last set of experiments suggests that the attractive interaction must be weak ($<k_BT$); otherwise microgels should have formed aggregates or crystals on much shorter timescales.[13] On the other hand, if the attractive potential is only a fraction of k_BT, multi-particle interactions are required to form stable structures. The coordination of multiple particles into specific organized structures is statistically unlikely, which significantly slows the kinetics of formation. Based on all of these experimental results and for lack of other explanations, we have hypothesized previously that the interaction between pNIPAm-*co*-AAc microgels is pH-dependent: weakly attractive at low pH due to hydrogen bonding and repulsive at high pH due to electrosteric forces.[12] In particular the hypothesized existence of weakly attractive forces has been somewhat controversial, because there is no other colloidal system in which these forces are known to play a dominant role. We therefore aimed to measure the interparticle potentials experimentally to clarify this sticky issue. In this Communication, we present measurements of the pairwise interparticle potential between colloidal pNIPAm-*co*-AAc microgels as a function of pH. The results were obtained from optical video microscopy of core–shell (C–S) microgel particles under quasi-2D confinement. To the best of our knowledge, no previous study has attempted to measure the interactions between stimuli-responsive microgels as a function of environmental conditions.

Direct measurements of intermolecular forces have been achieved by a variety of methods, for example, surface force apparatus (SFA),[14] total internal reflection microscopy (TIRM),[15] atomic force microscopy (AFM),[16] and optical tweezers (OT).[17] However, each of

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[a] *School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, 30332-0100, USA*
[b] *School of Chemistry & Biochemistry, The Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, 30332-0400, USA*

these techniques has specific limitations that make it difficult to apply them to colloidal microgels. For example, AFM and SFA are well suited for interactions that are stronger than several k_BT. TIRM measures the equilibrium probability distribution of particles relative to a wall under the influence of gravity and thus requires density contrast between particle and fluid, which is not present in microgel systems. Furthermore, TIRM yields particle-wall interaction rather than particle–particle interactions. OT require a minimum refractive index difference between particles and suspending medium in order to trap them in the laser beam, which highly swollen microgels do not offer. An alternative method is to extract the equilibrium pair interparticle potential between colloidal particles from measurements of the spatial particle distributions in suspension, based on liquid structure theory.[18,19] Key advantages of this method are its high sensitivity and the ability to measure particle–particle interactions without external perturbations, which is important for soft materials like pNIPAm-*co*-AAc microgels that easily deform and compress upon external forces and are hypothesized to have weak interactions. The pair distribution function can be determined *via* optical microscopy, but this technique provides two important challenges. First of all, accurate detection of particle positions requires good imaging contrast, even though the demands are less stringent than for OT. Individual pNIPAm-*co*-AAC microgel particle can be tracked in differential interference contrast (DIC) mode, but the optical contrast is insufficient to accurately determine the positions of microgel particles at close separation, which is crucial to achieve pair distribution measurements that are suitable for potential calculations. In order to solve this problem, we synthesized core–shell (C–S) microgel particles with a fluorescent polystyrene (PS) core and pNIPAm-*co*-AAc shell. These C–S particles can be tracked individually even at interparticle contact. Secondly, it is desirable to confine the colloidal particles to a quasi-2D geometry in order to prevent artifacts due to the projection of the actual particle positions in three dimensions to a two dimensional video microscopy image.[20] We achieve this by utilizing mono-disperse colloids as spacers between glass slides to create a quasi-2D geometry with precisely controlled separation between the glass slides across a large sample area.

The C–S colloidal microgels were synthesized *via* aqueous precipitation polymerization, using fluorescently labeled carboxylate-modified polystyrene (PS) microspheres (FluoSphere®, 0.50 μm diameter, Invitrogen) as seeds and creating a layer of poly (*N*-isopropylacrylamide-*co*-acrylic acid) (pNIPAm-*co*-AAc) hydrogel as shell. NIPAm (Sigma-Aldrich) was purified by recrystallization from hexane (J.T. Baker) before use. AAc (Sigma-Aldrich), cross-linker (*N*,*N*′-methylene bis(acrylamide) (BIS); Fluka) and initiator (ammonium persulfate (APS); Sigma-Aldrich) were used as received. NIPAm (0.1333g; 81.3 mol%)/AAc (0.0148g; 14.2 mol%)/BIS (0.0100g; 4.5 mol%) were dissolved in 30 mL DI water containing purified microspheres. The colloidal suspension with reaction mixture was pre-heated to 60 °C and purged with N_2, followed by initiation by adding APS after 30 minutes. The reaction was performed at 60 °C for 4 hours and the synthesized C–S microgels were purified extensively by dialysis against DI water over 3 weeks to remove unreacted components. The polydispersity of the C–S microgels was determined to be less than 8% by dynamic light scattering measurements (DLS, Protein Solutions). The hydrodynamic radius R_H of the C–S microgels as a function of pH was characterized by particle tracking video microscopy (PTVM), which identifies trajectories of individual particles *via* image analysis algorithms to determine their mean squared displacement (MSD).[21] If the temperature T and viscosity η are known, R_H follows directly from the Stokes-Einstein relation $\text{MSD}/2\tau = dk_BT/6\pi\eta R_H$, where d is the dimensionality of the trajectories (usually 2) and τ the correlation time. We selected PTVM over DLS to determine R_H, because the latter is highly sensitive to the presence of impurities and small aggregates, which can explicitly be ignored during image analysis. The results are shown in Fig. 1, where R_H has been plotted as a function of pH. Ionization of AAc in the microgels, induced by pH increase, results in reversible volumetric swelling of the hydrogel shell as a result of the Coulombic repulsion between deprotonated AAc groups, ingress of cations from the medium, and a resultant osmotic pressure difference between the microgel and medium.[8]

A uniform quasi-2D suspension in a confinement cell was created by using monodisperse hard colloidal particles as spacers between two glass slides, as previously described by our group[4] (see Fig. 2(a)). A dilute suspension of core–shell colloidal particles and 2.0 μm diameter polystyrene (Invitrogen) spacer particles was prepared in various pH buffers from pH 3–6 at constant ionic strength $I = 15$ mM. Prior to mixing, both species of particles were extensively purified with DI water by repeated centrifugation and washing steps. To ensure efficient and robust convergence of numerical calculations during data analysis, concentrations of the particle suspensions were chosen so that all samples had a projected area coverage of *ca.* 10% for each pH. A drop of the suspension was placed between a glass coverslip and microscope slide, excess fluid was removed to reduce the spacing between slides to the diameter of the spacer particles, and the cell was sealed with fast curing epoxy resin. To prevent adhesion between the C–S particles and glass walls, we gently sonicated the confinement cell and all measurements were made within 1 h of sonication. An inverted optical microscope (Leica DM-IRB) with 100 × N.A. 1.30 oil immersion objective and Peltier-cooled CCD camera (Cohu, Poway, CA) was used to record movies of the suspension and every measurement was performed at 23 °C. Typically, images of particles within a ~64 × 48 μm field of view were captured for 10 min at 30 frame/s and analyzed using image analysis routines in IDL software.[21] As explained above, imaging of the fluorescent PS cores is not affected by swelling-deswelling of the microgel shell and one can locate the position of particles accurately at all pH values; a representative image from video microscopy is shown in Fig. 2(b). The absence of out-of plane motion of the particles confirmed that the colloidal particle suspensions are quasi-2D.

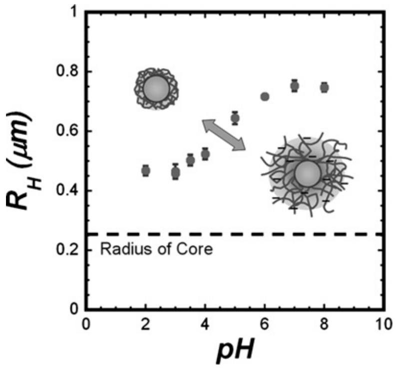

Fig. 1 The hydrodynamic radius of stimuli-responsive C–S microgel particles as a function of pH at a concentration of ~0.01 wt%. The dashed line represents the radius of the fluorescent core, 0.25 μm.

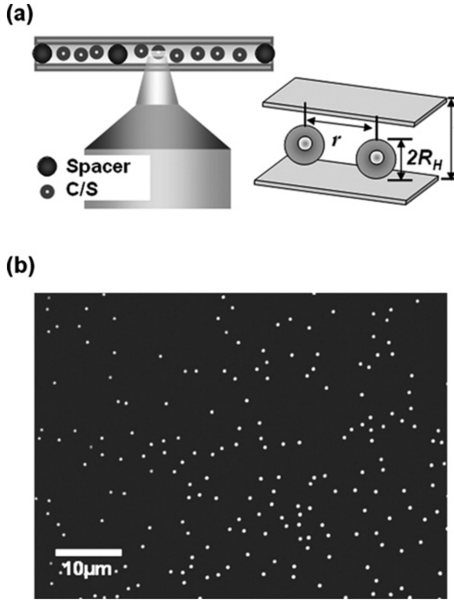

Fig. 2 (a) A schematic of the experimental set-up in quasi-2D where r is the center-to-center separation between C–S particles. (b) A representative image from fluorescence microscopy of C–S colloidal suspension in the quasi-2D confinement cell at pH 6.0 and $I = 15$ mM. For all pH conditions, C–S colloidal suspensions of ~10% area density were used; only the PS core is visible in fluorescence microscopy.

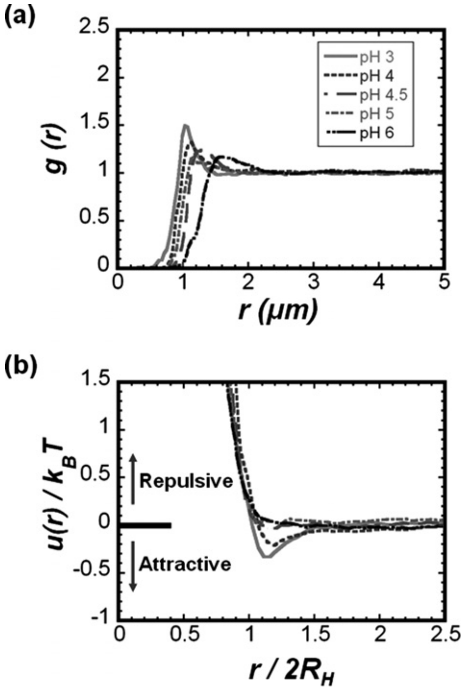

Fig. 3 (a) The pair distribution function $g(r)$ of C–S colloidal suspensions as a function of r and pH. (b) The pairwise interaction potential between C–S colloidal suspensions as a function of normalized separation and pH.

The pairwise interaction potential between colloidal particles in a medium can be determined from the equilibrium structure of particle suspensions.[18,19] In our case, this is quantified *via* the two-dimensional radial distribution function $g(r)$, which provides the probability of finding neighboring particles at specific separation distances as shown in Fig. 3(a). Since single images only contain a moderate number of particles (*ca.* 200 within field of view), $g(r)$ was obtained by averaging over 20 000 frames to get meaningful statistics. Theoretically, in the infinitely dilute regime $g(r)$ can be related directly to the pairwise interparticle potential $u(r)$ *via* the Boltzmann distribution as $\lim_{n\rightarrow 0} g(r) = \exp[-u(r)/k_BT]$ where n is the areal density of particles. However, in reality, under the experimental conditions that are required to measure $g(r)$ accurately, multi-body interactions play a role and the multi-particle potential $w(r)$ is found rather than $u(r)$: $g(r) \approx \exp[-w(r)/k_BT]$. It is possible to extract $u(r)$ from $w(r)$ using the Ornstein–Zernike convolution integral equation in combination with a suitable approximate closure relation. The hypernetted chain (HNC) approximation (eqn (1)) is known to be appropriate for soft spheres:[22]

$$u(r) = w(r) + nk_BTI(r) \qquad (1)$$

The convolution integral can be solved iteratively from the experimental $g(r)$ data:

$$I(r) = \int_A \Big[g(r') - 1 - n\,I(r)\Big]\Big[g(|r' - r|) - 1\Big]d^2r' \qquad (2)$$

Our experimental results of $g(r)$ for C–S colloidal microgels as a function of pH appear in Fig. 3(a). It is important to note that the microgels change their size as a function of pH. As expected, the position of the nearest-neighbor peak therefore shifts towards larger separation r as the microgels swell at high pH. It is also apparent from the lack of structure of $g(r)$ that the quasi-2D C–S colloidal suspensions are liquid-like. Equations (1) and (2) were applied iteratively until convergence was achieved. We also confirmed that $u(r)$ is independent of areal density n by performing experiments at slightly different areal coverages. The final results for $u(r)$ as a function pH appear in Fig. 3(b). It is reasonable to assume that $u(r)$ accurately reflects the interparticle potential between pNIPAm-*co*-AAc microgels, since the core (PS) is covered by a thick pNIPAM-*co*-AAc hydrogel layer. Clearly, $u(r)$ is pH-sensitive and changes from attractive to repulsive as the pH increases.

Although pNIPAm microgels have soft repulsive interactions, our experiments confirm that the incorporation of AAc to the polymer network alters not only the chemistry of the material, but also the physical behavior of microgels. At low pH values, the potential is attractive, but weakly, as we hypothesized; this is indicated by the depth of the potential well of less than k_BT for pH 3.0 and 4.0. The attractive interaction is observed only below the pK_a (~4.15) of the AAc moieties, where the C–S particles have negligible surface charge. Additionally, the ionic strength of our buffers is 15 mM, so that the Debye screening length $\kappa^{-1} \approx 3$ nm and Coulombic interactions should not play a major role in $u(r)$ on the lengths scales shown in Fig. 3(b) and the attractions in our system therefore differ from the attractions between like-charged particles previously reported by Grier group.[19,23]

Attractive interactions in our system can be attributed to three forces: van der Waals attraction, hydrogen bonding between protonated AAc groups and amide groups, and hydrophobic interactions between isopropyl groups. The fact that the attractions are most pronounced at low pH leads to the conclusion that the major contribution comes from hydrogen bonding between AAc groups (*e.g.* salt-bridges) and acid-amide interactions. Another interesting feature is that the interactions are apparently long-range (∼50 nm), which can be explained with two phenomena: (1) the ambiguity between the physical size of the microgel particle, as defined by the outer dimensions of the fuzzy microgel interface, and the particle hydrodynamic radius and (2) the presence of dangling polymer chains at the microgel surface, which can be extended if the enthalpic gain of multiple hydrogen bonds overcomes the entropy penalty of stretching. At high pH (above the $\mathrm{p}K_\mathrm{a}$ of AAc) where most of AAc moieties are deprotonated, the interaction between microgels is purely repulsive. This repulsive behavior is expected in the presence of charged, dangling chains protruding from the surface, providing electrosteric repulsion between C–S microgels. The current results are in excellent agreement with our previous observations that were discussed above: Fig. 3(b) clearly shows that attractive interparticle interactions of magnitudes less than $k_\mathrm{B}T$ exist in pNIPAm-*co*-AAc microgel systems and that these attractions can be attributed to hydrogen bonding.

In this Communication, we have directly measured pairwise interparticle potentials $u(r)$ between pNIPAm-*co*-AAc microgel colloids in quasi 2-D. We confirmed the existence of weak attractive potential below the $\mathrm{p}K_\mathrm{a}$ of AAc, which is consistent with our previous observations and the interparticle potential changed to repulsive beyond the $\mathrm{p}K_\mathrm{a}$. This pH-dependent long-range interaction implies that the origin of attractive interaction arises mainly from rearrangement/reorientation of multiple weak hydrogen bonds between acid/amide groups on dangling polymer chains. Our result clearly demonstrates that by incorporating additional functional groups in the network one can enhance tunability of interactions between colloidal microgels. It is likely that this tunable potential will open up new avenues for self-assembly of soft building blocks for advanced materials by tuning colloidal interactions.

Acknowledgements

VB acknowledges financial support from the National Science Foundation (CAREER award, CTS-0547066). LAL acknowledges financial support from the Office of Naval Research, and the ONR DURIP program for this work (N00014-03-1-0225 and N00014-04-1-0488). The authors are grateful to Dr Behrens (Georgia Institute of Technology) for providing the computer code for Ornstein–Zernike calculations.

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