Clustering in polar media

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Clustering prevails in water-soluble polymers and biological macromolecules. It has also been observed in polar solvent mixtures. The possible causes of clustering are discussed. A systematic investigation of clustering in poly(ethylene oxide)/d-water solutions has been undertaken using the small-angle neutron scattering method. The poly(ethylene oxide) monomer is formed of an oxygen atom and an ethylene group. Using the random phase approximation, partial Flory–Huggins interaction parameters for the three pairs (oxygen/d-water, ethylene/d-water, and oxygen/ethylene) are derived. Results show that the first two (oxygen/d-water and ethylene/d-water) are characterized by a lower critical solution temperature phase behavior (whereby phase separation occurs upon heating), while the third one (oxygen/ethylene) is characterized by an upper critical solution temperature phase diagram (whereby phase separation occurs upon cooling). It is argued that clustering is caused by the increasing repulsive interaction between oxygen and ethylene for decreasing temperature and increasing polymer volume fraction. This leads to increasing attractive interactions between ethylene groups that stick together. © *2010 American Institute of Physics*. [doi:10.1063/1.3484235]

I. INTRODUCTION

Clustering is pervasive in polar media. The majority of water-soluble molecules cluster to form large aggregates when their volume fraction is high enough. These include macromolecular systems (such as water-soluble polymers or biological macromolecules in solution)[1–8] as well as liquids consisting of solvent mixtures.[9,10] The cause of clustering has been elusive. A possible origin of clustering is investigated here.

Clustering has been observed in poly(ethylene oxide) (PEO)/water solutions; PEO is the simplest water-soluble polymer. Its monomer contains a hydrophobic group $–CH_2CH_2–$ and oxygen –O– which is hydrophilic. Many investigations have discussed the possible causes of clustering in PEO/water solutions. The clustering of PEO in water has been investigated by scattering methods. Dynamic light scattering (DLS) has shown that PEO is characterized by two modes when dissolved in water: a slow mode due to clustering and a fast mode due to polymer-solvent interactions. Cluster sizes are of order of microns in semidilute polymer solutions. An investigation has proposed that clustering may be caused by impurities in water;[1] filtering of a PEO/water solution (not just water) causes clusters to break down. Other investigations of the same PEO/water solution have shown, however, that clusters reform over a couple of days.[2,3] Clusters break down upon filtering but reform over time. This shows that impurities are not causing clustering and that clustering is kinetically driven.

Small-angle neutron scattering (SANS) has also been used to investigate clustering which shows up as a strong signal at very small angles. SANS has shown that clustering can be caused by chain-end units;[4] PEO chains end-capped by –OH groups at both ends do not form clusters in water (due to chain-end effect) but do form clusters in benzene. Moreover, PEO chains end-capped by $–OCH_3$ groups at both ends form clusters in water but not in benzene. This shows that clustering can be caused by solvent-phobic end units that stick to parts of the polymer chains, thereby forming network clusters. End group clustering is, however, not the dominant form of clustering.

Clustering takes place in protein solutions as well. The clustering of lysozyme in semidilute water solution has also been investigated by SANS.[5] It was concluded that protein-protein interactions are characterized by a short-range attractive and a long-range repulsive Coulomb interaction potentials. It was later argued, however, that those studies were hampered by impurities in the protein samples.[6] What is interesting is that the same scenario that played out for PEO/water solutions[1] got repeated for protein/water solutions.[6] Filtering the solutions before performing DLS measurements caused clusters to disappear, which lead to the same conclusion that clustering may be due to impurities. This is an erroneous conclusion since clusters reform after a while.

Using the SANS technique, we discuss a possible major underlying cause of clustering in what follows.

II. SANS QUALITATIVE INVESTIGATIONS

Clustering shows up as a strong low-Q SANS signal, Q being the scattering variable. Figure 1 shows clustering in two synthetic polymers and two biological macromolecules in deuterated water solution. Deuteration is used in order to enhance the neutron scattering length density contrast. Poly(acrylic acid) is a charged polyelectrolyte while poly(ethylene oxide) is a neutral synthetic polymer. DNA is formed of

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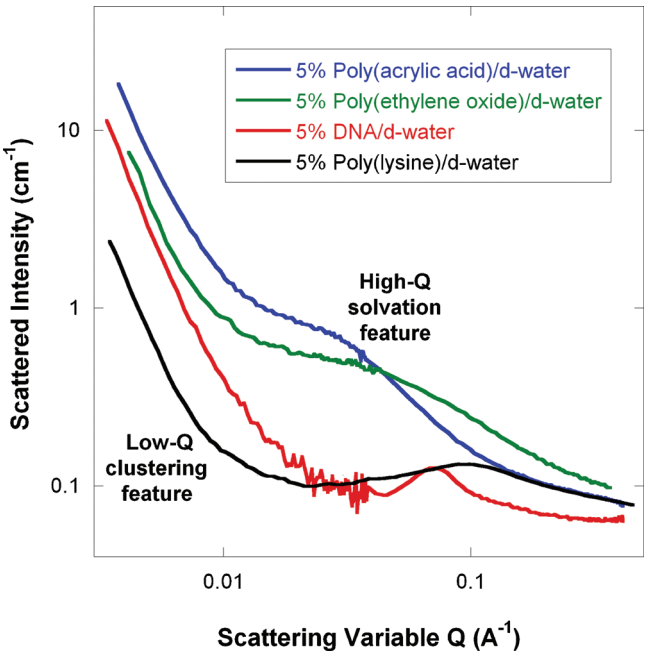

FIG. 1. SANS data from two synthetic polymers and two biological macromolecules dissolved in deuterated water at ambient temperature (25 °C).

nucleotides while poly(lysine) is a poly(amino acid). Amino acids are the building blocks of proteins. All four SANS spectra are characterized by a strong low-Q signal due to clustering and a smaller high-Q signal due to solvation characteristics (solvent/monomer interactions). The high-Q signal contains a polyelectrolyte peak for charged systems (such as DNA) or smooth variation for neutral systems (such as PEO). In the case of poly(acrylic acid), the clustering feature is so strong that it has merged with the polyelectrolyte peak. Cluster sizes are larger than the measurement window of the SANS instrument so that only the tail (Porod region) of the low-Q signal is observed (with no visible Guinier region).

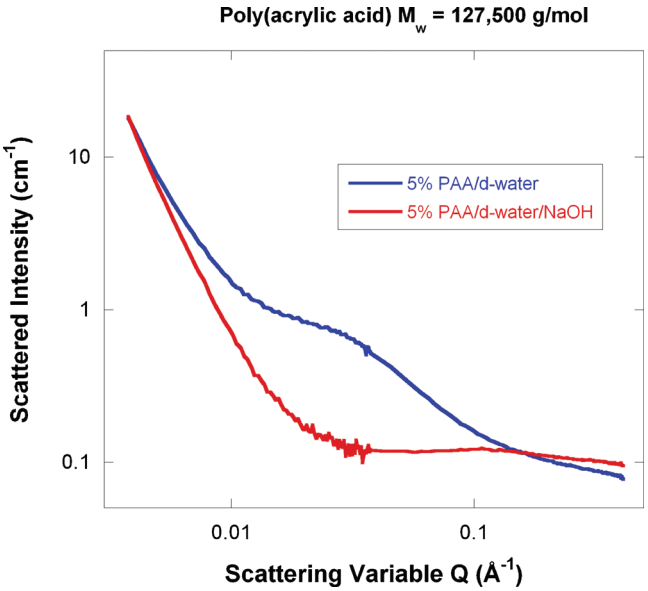

FIG. 2. SANS from a semidilute solution of poly(acrylic acid) in d-water with and without the addition of a charge neutralizing base (NaOH) at ambient temperature (25 °C).

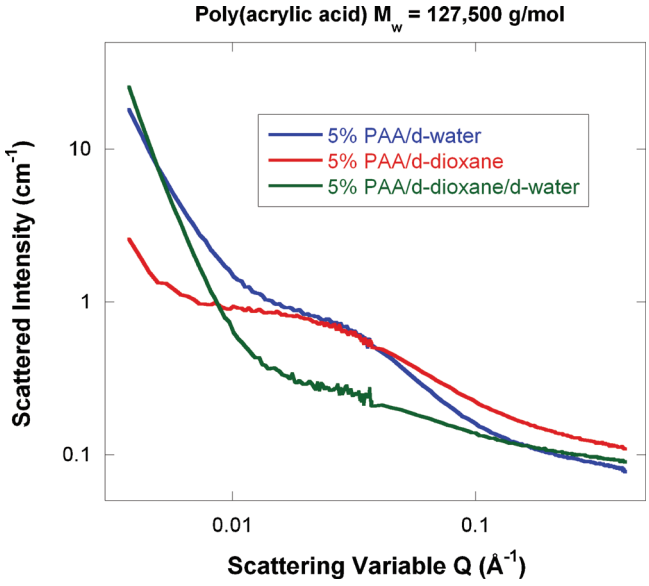

FIG. 3. SANS from a semidilute solution of poly(acrylic acid) in d-water, d-dioxane, or in a 50%/50% d-water/d-dioxane solvent mixture at ambient temperature (25 °C).

Clusters can be seen to form in charged as well as in neutral solutions. In order to assess whether charge interactions are a dominant factor in cluster formation, SANS data were taken from the PAA/d-water semidilute polyelectrolyte solution with and without the addition of a charge neutralizing base (NaOH) in Fig. 2. It is noted that the low-Q clustering feature changes very little while the high-Q solvation feature changes drastically. This observation points to the clue that clustering may not always be due to Coulomb interactions. Changing the charge-charge interaction environment changes the solvent quality which may indirectly affect the clustering feature.

In order to evaluate the effect of solvent quality on clustering, poly(acrylic acid) is dissolved in two different solvents (d-water and d-dioxane) in Fig. 3. The low-Q clustering feature is seen to change drastically. This observation points to the conclusion that solvent quality is a determining factor for cluster formation. Figure 3 also shows SANS data from PAA in (50%/50% mass fraction) mixtures of d-water and d-dioxane. Both the low-Q clustering and the high-Q solvation features change in a nonideal mixing way. Solvent mixtures are better solvating agents but produce larger clusters. This clue suggests that clustering and solvation vary in opposite trends.

The qualitative observations gathered so far are (1) clustering is not always due to Coulomb interactions, (2) solvation effects play an important role, and (3) clustering and solvation seem to vary in opposite trends. These observations are solidified in Sec. IV with a systematic investigation of clustering in PEO/d-water using the SANS technique.

III. SANS FROM PEO/D-WATER

SANS measurements have been taken from a series of PEO/d-water solutions at various polymer volume fractions (0.5%, 1%, 2%, 3%, 4%, 5%, and 10%) and for various

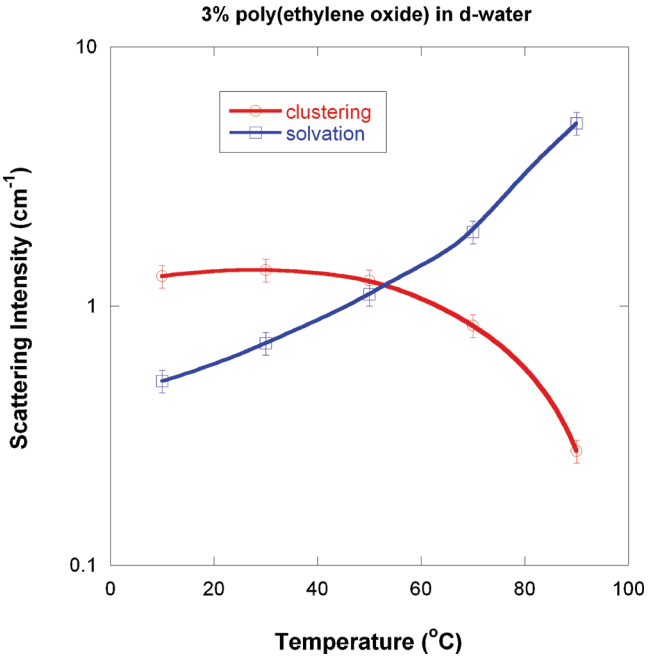

FIG. 4. Variation of the clustering (A/Q^n) and solvation (c) components for increasing temperature for the 3% PEO/d-water solution at the lowest measured Q=0.004 $Å^{-1}$. Statistical error bars correspond to one standard deviation.

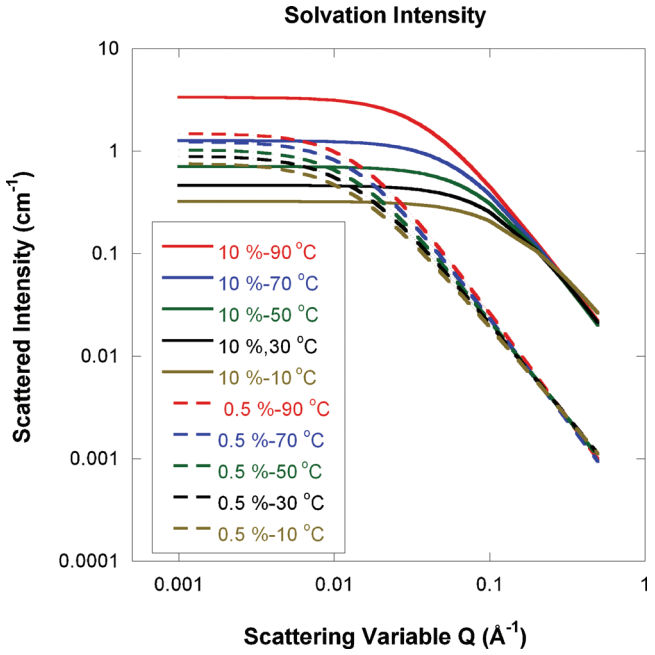

FIG. 5. Plot of the Q-dependent solvation intensity C(q) obtained using the fit parameters for the 0.5% and the 10% PEO volume fractions and for varying temperature.

temperatures (10, 30, 50, 70, and 90 °C). The PEO molecular weight is M_n=96 000 g/mol (polydispersity index =1.04).

The low-Q clustering and the high-Q solvation components of the SANS signal are separated out using the following empirical functional form:

$$I(Q) = \frac{A}{Q^n} + \frac{C}{1 + (Q\xi)^m} + B. \quad (1)$$

Here A and C are the clustering and solvation scale factors, ξ is a correlation length (average distance between entanglements in the semidilute region), n and m are Porod exponents, and B is a constant (Q-independent) incoherent scattering background. Results of the nonlinear least-squares fits show that the low-Q clustering and the high-Q solvation contributions vary in opposite trends for increasing temperature and for increasing polymer volume fraction. Figure 4 shows the variation of the clustering component A/Q^n and the solvation scale C for increasing temperature for the 3% PEO/d-water solution. The lowest measured Q value (Q =0.004 $Å^{-1}$) is used. This confirms the opposite trend for increasing temperature.

Increase in the solvation scale factor (parameter C) with temperature shows that the PEO/d-water system is characterized by a lower critical solution temperature (LCST) phase diagram; i.e., it phase separates upon heating. The Q-dependent solvation intensity $C(Q)=C/[1+(Q\xi)^m]$ has been isolated and plotted for the 0.5% and the 10% PEO/d-water samples at the various temperatures. The trends are reasonable as shown in Fig. 5.

The random phase approximation (RPA) formalism is often used to analyze SANS data from homogeneous polymer mixtures. This is a mean-field approach that works better for polymer blends but is used here to analyze SANS data from the PEO/d-water solution. The only unknown fitting parameter is the Flory–Huggins polymer-solvent interaction parameter (Fig. 6) which is determined to be[7]

$$\chi_{PS} = 1.10 - \frac{132}{T}. \quad (2)$$

Here T is the absolute sample temperature. The minus sign points to a LCST phase behavior as mentioned.

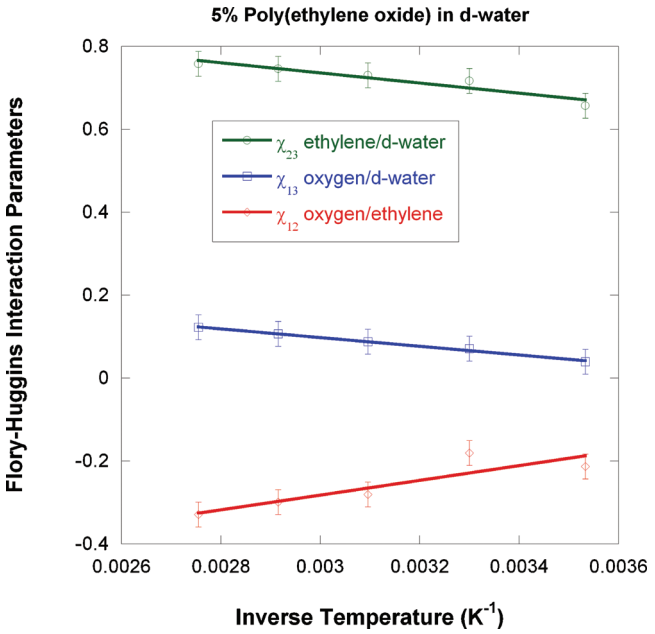

FIG. 6. Variation of the Flory–Huggins interaction parameters for the ethylene/d-water, oxygen/d-water, and the oxygen/ethylene pairs with inverse temperature for ϕ_P=0.05.

In order to appreciate the origin of clustering, the PEO monomer is considered to be a copolymer consisting of an oxygen atom –O– and an ethylene group $–CH_2CH_2–$. The PEO chain becomes a regularly alternating copolymer of oxygen groups (defined as component 1) and ethylene groups (defined as component 2) dissolved in d-water (component 3). The ternary RPA formalism is used to extract the only fitting parameters, the three Flory–Huggins interaction parameters χ_{12}, χ_{13}, and χ_{23}. This formalism is too lengthy to reproduce here; the details of this approach are reproduced elsewhere.[7] The chi-square values for the fits were low enough to believe that the global minimum was reached in each case. No constraints were used.

Fits of the SANS data to Eq. (1) yield a value for the solvation intensity (parameter C) for each of the seven samples (corresponding to the different polymer volume fractions) and for each of the five temperatures measured. For each fixed temperature, there are seven (ϕ_P, C) data sets (here ϕ_P is the polymer volume fraction). Performing nonlinear least-squares fits using the ternary RPA formalism produces the three unknown parameters χ_{12}, χ_{13}, and χ_{23}. Fits are improved when composition-dependent chi parameters are assumed. The results are

$$\chi_{12} = \left(-0.46 + \frac{27}{T}\right) + \left(-7.14 + \frac{3014}{T}\right)\phi_P,$$

$$\chi_{13} = \left(0.59 - \frac{31}{T}\right) + \left(-3.51 - \frac{1477}{T}\right)\phi_P, \quad (3)$$

$$\chi_{23} = \left(0.54 - \frac{50}{T}\right) + \left(11.25 - \frac{1425}{T}\right)\phi_P.$$

Here also, T is the absolute temperature. These results show that the oxygen/d-water and the ethylene/d-water systems are characterized by LCST phase diagrams (they phase separate upon heating), while the oxygen/ethylene system is characterized by an upper critical solution temperature (UCST) phase diagram (phase separation occurs upon cooling). Most water-soluble polymers and biological macromolecules in solution exhibit a LCST behavior. The result that the oxygen/d-water mixture phase separates upon heating was not a surprise. It is hard to predict the behavior of the ethylene/d-water mixture since pure hydrocarbons do not mix with water. The oxygen/ethylene mixture turned out to be characterized by a UCST mixing behavior. The opposite (LCST/UCST) trend observed for the various chi parameters helps explain the opposite trend for the solvation/clustering parts of the SANS signal.

IV. DISCUSSION

The χ_{13} (oxygen/d-water) and χ_{23} (ethylene/d-water) interaction parameters describe the high-Q solvation behavior well while the χ_{12} (oxygen/ethylene) interaction parameter gives insight into the nature of clustering. The χ_{12} (oxygen/ethylene) interaction parameter increases with decreasing temperature and with increasing polymer volume fraction but remains negative. Oxygen/ethylene repulsive interactions get stronger with decreasing temperature. This means that ethylene/ethylene attractive interactions get stronger at low temperatures. Since ethylene groups "like" other ethylene groups more than they like oxygen; they tend to stick together each time they are in close proximity. The chance for proximity increases at entanglement points in semidilute solutions. Behavior of the oxygen/ethylene interaction parameter provides a possible explanation for what is driving clustering.

PEO remains amorphous when dissolved in d-water but crystallizes when dissolved in d-ethanol. The crystalline structure, however, melts at high temperatures (above 35 °C for a 5% PEO/d-ethanol solution). Above the melting temperature, the PEO/d-ethanol system does not show any clustering. This solution is also characterized by a UCST phase diagram.[8] In this case, the Flory–Huggins interaction parameters for the three pairs (oxygen/d-ethanol, ethylene/d-ethanol, and oxygen/ethylene) are all characterized by USCT phase behaviors. They all mix together (at high temperatures) and demix (phase separate) together (at low temperatures). This explains the nonexistence of clustering in this system.

Monomers repel each other in good solvent conditions. Intramonomer interactions (such as between oxygen and ethylene in PEO) could favor hydrophobic "sticking" of these groups (ethylene sticking to other ethylene groups in the case of PEO) which causes clustering. This has been demonstrated here for a neutral synthetic polymer in solution but is expected to hold for other systems such as proteins in solution. Proteins tend to form complex structures (such as helical conformations) in order to hide hydrophobic groups and keep them away from contact with water. Hydrophobic groups that remain exposed tend to stick to other hydrophobic sites, thereby forming clusters. Clustering is believed to be due to mixing/demixing arguments and not to charge/charge interactions. Proteins have been described as interacting via a simple potential with short-range attractive and long-range repulsive interactions.[5] Our conclusions show that these are not mean interprotein interactions, but interactions between specific groups (for example, hydrophobic groups) within the proteins.

Nonmacromolecular polar media such as, for example, mixtures of water and dioxane[9] or water and glucose[10] are also known to form clusters. This effect was referred to as the Stockmayer fluid,[11,12] whereby clusters form due to dipolar interactions. Polarity is understood in terms of charge distribution. The results reported in this paper show that there is no need resorting to charge polarity in order to understand the origin of clustering. Clustering can be caused simply when the subunits of a molecule tend to demix while the entire molecule dissolves well. It is noted that the charge polarity and the demixing thermodynamics concepts are opposite in nature since opposite charges attract each other while different subunits repel each other. For instance, in the Stockmayer fluid concept, the ethylene and oxygen subunits (forming the dipolar group) would attract to form clusters, while in the thermodynamic scheme described in this paper ethylene groups would stick to ethylene groups instead to form clusters.

ACKNOWLEDGMENTS

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