

## Aggregation number distributions and mesoglobules in dilute solutions of diblock and triblock copolymers

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**Abstract.** – We investigate the aggregation number and size distributions for inter-molecular clusters of amphiphilic diblock and triblock copolymers in poor solvent at very low concentrations. Diblocks and triblocks with hydrophilic ends are shown to possess narrow distributions corresponding to formation of monodispersed mesoglobules. Diblocks with hydrophobic ends are found to produce inter-cluster multimers due to bridging by the hydrophilic middle blocks, resulting in polydisperse distributions. Implications of these observations for the preparation of monodispersed nanoparticles and, potentially, for the understanding of the quaternary structure of proteins are discussed.

*Introduction.* – The phenomena of coil-to-globule transition for a single polymer molecule and phase separation in polymer solutions are foundations of the modern polymer science [1, 2]. In recent years there were numerous studies of the behaviour of water soluble polymers near the lower critical solubility temperature (LCST). Typical systems include poly-N-isopropylacrylamide (PNIPAM) homopolymer and block copolymers [2] of the poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), or PEO-PPO systems briefly [3]. These copolymers are widely used in pharmaceutical, agricultural and food industries due to their low toxicity and surfactant characteristics (EO is hydrophilic and PO is hydrophobic). Block and more complex random heteropolymers in solutions and melts at higher concentrations exhibit a variety of ordered micro-phase separated and disordered glassy phases [4, 5]. Block copolymers also play the role of surfactants in ternary mixtures of two otherwise immiscible liquids such as water and oil, and these mixtures also produce micelles and lamellae [6].

There are two types of triblock copolymers having rather distinct properties. First, a PEO-PPO-PEO polymer with hydrophilic ends and a hydrophobic middle is the standard Pluronic [3, 7], which forms stable micelles. Second, a polymer with the inverse structure would tend to network hydrophobic end “stickers” by hydrophilic bridges. These are so-called telechelic associating polymers forming physical gels with nontrivial rheological properties at higher concentrations [8].

The aggregation number distribution during micellisation has been a matter of intensive studies for amphiphilic molecules [7]. The classical situations, in which there is a clear phase separation transition from  $M_{cl} = 1$  to  $M_{cl} = M_{max}$ , or where one could introduce notions of CMC (critical micelle concentration), and possibly also CAC (critical aggregation

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concentration), are well known. Some cases of molecular self-assembly, in which relatively monodispersed distributions arise around CMC, have also been seen. It is a conventional wisdom that in solutions of amphiphilic polymers the notion of CMC does not really exist since single-chain micelles already form in an infinitely dilute solution if the primary sequence of the polymer permits micro-phase separation within the collapsed globule.

The phase diagrams of diblock and in some cases triblock copolymers have been closely investigated [7]. At low concentration these systems form micelles, but the question of their aggregation number is not so well understood as it appears to be quite sensitive to various parameters of the system, although it is generally believed that such a distribution should be fairly polydispersed. This situation is in marked contrast with a two-phase separation onto the macro-aggregate and a gas of single globules in the simpler case of homopolymer solutions, which are well described by the mean-field Flory-Huggins-type theories [9], and their extensions based on scaling arguments [10], self-consistent treatments [1] and the Lifshitz approach [11].

Ordinary micellisation and more complex aggregation number distributions in copolymer solutions are realised due to the complex interactions favouring micro-phase separation of hydrophobic and hydrophilic units. Since the CMC of these polymers is essentially zero as in the case of polysoaps [12], one would like to see how the aggregation number of the micelles would behave nearby, *i.e.* in a very dilute solution. Indeed, normally the character of such a distribution has a clearly distinct character around CMC *vs.* other regimes.

In attempting to resolve this interesting question, unfortunately, both experiment and theory had considerable difficulties and little success. The aggregation phenomenon significantly obscures experimental observation of a single-chain collapse by light scattering techniques, leading often to a diverse range of theoretical interpretations, particularly as it may be hard to separate purely equilibrium issues from the kinetic ones [13]. Indeed, the rather skewed shape of the Flory-Huggins diagram requires extremely low concentrations to see single globules [14], thus making the scattered light intensity too low for a reliable observation.

In a number of recent experiments on PNIPAM polymers in aqueous solution, in which some of the units are either hydrophobically modified or replaced by ionomers [15, 16], it has been observed that stable size monodispersed nanoparticles can be formed on heating above the LCST instead of chains simply aggregating. The mean size of these particles is thermodynamically controlled and it increases with increasing polymer concentration and increasing strength of inter-monomer attraction. Electron microscopy further confirms the existence of such spherical particles with mean size and distribution in agreement with the light scattering. We called such an unusual type of mesoscopic structures *mesoglobules* as they tend to have a compact spherical shape and a rather monodisperse size distribution. To be explicit then their precise definition is as follows. *Mesoglobules are essentially equally sized globules obtained by association of more than one and less than all polymer chains in the system* and corresponding to, at least, a local minimum of the free energy. Thus, the term mesoglobules refers to the globules possessing a well-characterised size distribution, but not necessarily a well-defined conformational structure such as, *e.g.*, in micelles.

In our previous paper [17] based on the Gaussian variational theory and lattice Monte Carlo simulation we have shown that in very dilute solutions the histograms of cluster distribution favour size monodispersed structures for a certain class of heteropolymer sequences and only in some narrow strips of the phase diagram. We have also seen that the mean size of the mesoglobules is related to a characteristic scale of the micro-phase separation. Thus, generally the larger this scale (*e.g.*, block size) the larger the mesoglobules produced.

Significantly, in the situation when the mesoglobules occur there is a quite narrow single-peak distribution in the aggregation numbers and the mean-squared radius of gyration, so

that the populations of single globules or the macro-aggregate are absent. Moreover, we have demonstrated that the mesoglobules are truly thermodynamically stable, *i.e.* the clusters of exactly equal size possess the lowest free-energy value. Clusters of slightly unequal size obtained from these ideal mesoglobules have a higher free-energy value and the barriers separating the mesoglobular minimum from other local minima tend to grow quickly as more and more asymmetric divisions of the system into macromolecular clusters are considered. This situation is quite unusual and novel for spherical micelles and it occurs due to a rather delicate competition of energetic (micro-phase separation) and entropic (translational entropy) terms under the connectivity constraints. For the mesoglobules to form the degree of amphiphilicity (distinction in the interaction energies between “a” and “b” monomers with the solvent) and the mean strength of the monomers attractions should be in a certain relation. In ref. [17] we have seen that this situation is possible for alternating monomers, repeating blocks (having smaller mesoglobules), as well as for a number of randomly generated sequences, but in all cases at rather low concentrations. Mesoglobules can form at infinitesimally small concentrations, corresponding to essentially zero CMC in polymeric surfactants. The mean size of the mesoglobules grows with increasing concentration, but when the latter reaches a certain value, which perhaps one may view as an analog of CAC, a polydisperse distribution of clusters with a large population of the macro-aggregate results, recovering a more conventional regime.

Our understanding of the diblock copolymers, however, was not complete in paper [17]. We believed that a finite-size effect was significant there due to the chosen chain lengths. Thus, in this letter we shall revisit the diblocks and also consider the triblocks of both possible types.

*Results.* – As in ref. [17] we adopt the Metropolis technique in the lattice model of ref. [18]. This model, apart from the connectivity and excluded-volume constraints, includes short-ranged pairwise interactions between lattice sites. The system is completely characterised by three Flory interaction parameters,  $\chi_{aa}$ ,  $\chi_{ab}$  and  $\chi_{bb}$ , along with  $N$ ,  $M$  and linear lattice size  $L$ . In addition to local monomer moves [18], we include translational moves representing

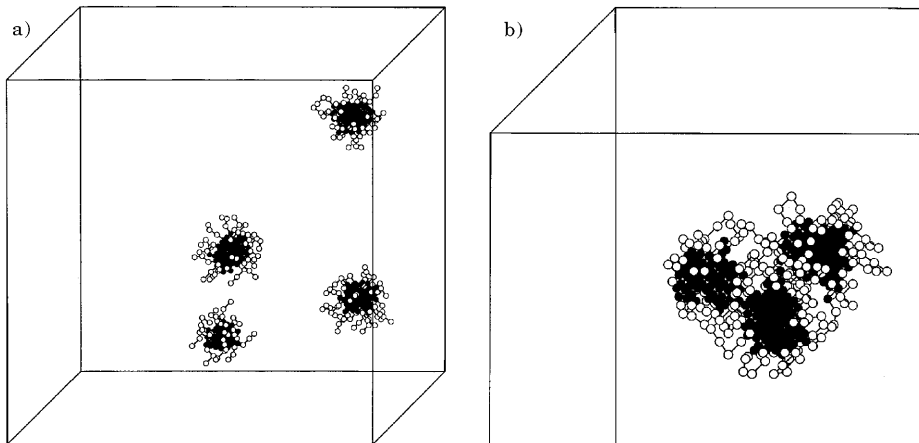


Fig. 1 – Snapshots of typical conformations from simulation for different sequences. a) corresponds to the P-ends sequence  $b_3a_6b_3$  for which mesoglobules are formed. b) corresponds to the H-ends sequence  $a_3b_6a_3$ , where networks of locally collapsed clusters connected by hydrophilic bridges are formed. Here the equilibration time was  $1.92 \cdot 10^9$  of attempted Monte Carlo moves and the other parameters are:  $L = 60$ ,  $N = 12$ ,  $M = 40$ ,  $\chi_{aa} = 1$ ,  $\chi_{ab} = 0.4$  and  $\chi_{bb} = -0.2$ . Black (white) circles correspond to hydrophobic (hydrophilic) monomers.

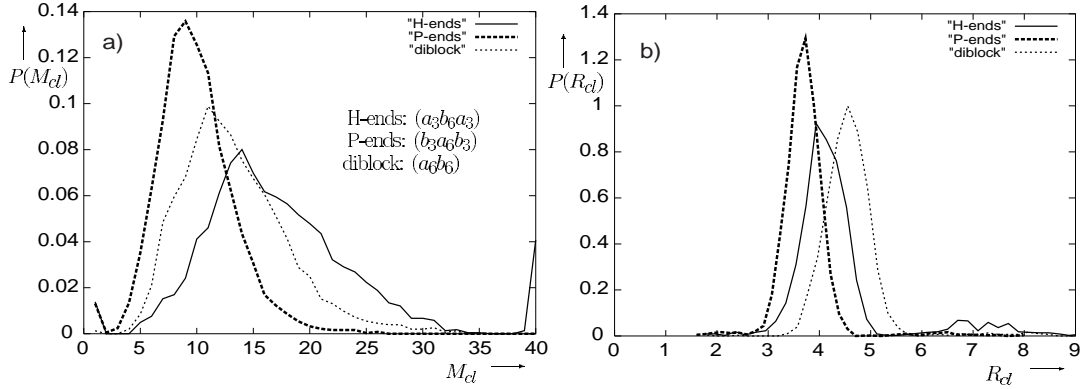


Fig. 2 – Probability densities of the number of chains in a cluster (aggregation number),  $M_{cl}$  (a), and of the radius of gyration of a cluster (size),  $R_{cl}$  (b), for diblock (thin dashed curve) and triblocks (thick dashed and solid lines) sequences. These results have been obtained by analyzing data for the ensemble size  $Q = 1000$ . The other parameters are as in fig. 1.

diffusion of chains. The latter moves are applied to all clusters of chains with a probability inversely proportional to the number of monomers within (Stokes law). We shall consider sequences consisting of strongly hydrophobic and slightly hydrophilic units. However, while in paper [17] the number of monomers in each chain was  $N = 24$  and the total number of chains was  $M = 20$ , here we shall analyse the case of twice the number  $M = 40$  of half-long chains  $N = 12$  in the same simulation box of linear size  $L = 60$ . Thus, the monomer concentrations  $c \equiv NM/L^3$  are the same in both cases, and we shall also keep the numbers of the hydrophobic and hydrophilic monomers equal in each chain.

In fig. 1 we present snapshots of two typical types of structures observed for triblock copolymers in the region of the phase diagram where mesoglobules are normally seen [17]. Figure 1a corresponds to triblocks with the hydrophilic (or P- for polar) ends and the hydrophobic (or H-) middle block, and we shall call these the P-ends triblocks briefly. These structures clearly have essentially equal size and thus could be considered as mesoglobules. Moreover, they possess a pronounced micellar character with a hydrophobic (black circles) core and a hydrophilic (white circles) corona. Figure 1b corresponds to the inverted case of the H-ends triblocks. In this case typical structures often have two, three (as in this figure), or more large clusters inter-connected by the hydrophilic middle block bridges. Such dimers, trimers and multimers are indeed to be expected at very low concentrations as it is known that this type of associating polymers forms telechelic physical gels at higher concentrations [8].

To investigate the aggregation number and size distributions we have obtained a large ensemble of  $Q = 1000$  independent equilibrium states. In fig. 2 we present the calculated histograms of the aggregation number (mass) and size probability densities for the diblock ( $a_6b_6$ ) and triblock sequences (H-ends and P-ends). We have seen in fig. 7b of ref. [17] that the diblock sequence s4 had a large population of the macro-aggregate there. We have argued in ref. [17] that this might be due to a finite-size effect since the total number of chains  $M = 20$  was comparable to the mean aggregation number of the mesoglobules. In fig. 2a, however, one can see for the diblocks that their mean aggregation number in the mesoglobules is about 12, whereas the total number of chains is  $M = 40$ , so that there is no population of the macro-aggregate any more. Therefore, the diblock sequences do indeed produce mesoglobules in a certain region of the phase diagram in dilute solution and the corresponding size distribution in fig. 2b is quite narrow.

Similarly to diblocks, the P-ends triblocks produce a clear single-peak distribution for the aggregation number (see fig. 2a) corresponding to the mesoglobules of a somewhat smaller size and the respective cluster size distribution is also rather narrow in fig. 2b. On the contrary, the aggregation number distribution for the H-ends triblocks is rather broad and has a certain population of the macro-aggregate. The width of this distribution is explained mainly by formation of dimer and multimer clusters, so that one can no longer define a preferred cluster size. Figure 2b shows that the size distribution of the H-ends has two peaks: a very broad multimeric peak (near the region  $6 < R_{cl} < 8$ ) and a still reasonably narrow peak corresponding to the mesoglobules and their dimers (near  $R_{cl} \approx 4$ ). It is interesting to note that the mean size of the latter objects for the H-ends triblocks is considerably smaller than for the diblock sequence of the same length, although the situation is reverse for the mean aggregation numbers. This could be easily understood by comparison of figs. 6d in ref. [17] and 1b. One could see that the P-corona in the former case is quite extended, whereas in the latter case it is more dense and compact, containing a considerable number of loops.

Finally, it is also instructive to compare the mesoglobules for diblock  $a_6b_6$  with those of twice longer sequence  $(a_6b_6)_2$  (see s3 in figs. 7a, 8a in ref. [17]) formed by merging every two  $a_6b_6$  chains at the same monomer concentration  $c$ . By a careful analysis of these data one can conclude that the mean cluster mass increases and the mean size decreases slightly for  $(a_6b_6)_2$  as compared to  $a_6b_6$ . The effect of an increase in the mean cluster mass is rather weak and does not have a simple explanation, but the decrease of the mean size is clearly due to a more compact size of loops in mesoglobules of  $(a_6b_6)_2$  than the size of free P-ends for  $a_6b_6$ , similarly to the discussion in the previous paragraph.

*Conclusion.* – In this letter we have addressed the question of sequence specificity for forming mesoglobules in dilute solutions. The aggregation number and size distributions for inter-molecular clusters in a poor solvent have been found to be monodisperse for diblocks and triblocks with outer hydrophilic ends, while rather polydisperse for triblocks with hydrophobic end “stickers”. Comparative analysis of chains of varying length but at a fixed total monomer concentration has elucidated nontrivial dependences of the mesoglobules mass and size on the copolymer chain length and sequence and a special role of hydrophilic loops and bridges in determining the compactness of the resulting structures.

The main conclusion from these studies is that the mesoglobules formation thermodynamics is rather delicate and that such stable structures are only possible for certain heteropolymer sequences and in a narrow region of the phase diagram. Our results also suggest that multimeric clusters of a certain size may have a stabilisation mechanism similar to that of mesoglobules, and again only for certain heteropolymer sequences.

We believe that the observations of this letter may shed some light on the understanding of the problems of competition aggregation *vs.* folding in protein solutions and, possibly, on self-organisation of the quaternary structure in multimeric proteins. It appears that for the latter process to take place a considerable number of “sticky” hydrophobic amino acid residues should be exposed on the exterior of each of the folded subunits. The resulting association produces a well-defined quaternary structure, which is biologically functional as in, *e.g.*, hemoglobin, rather than a disordered aggregate. Hopefully, work in this direction would also help unravel the mechanism of association of the so-called Bence-Jones proteins believed to be related to amyloid fibril formation.

More importantly, detailed understanding of the mechanism of mesoglobules formation is important for being able to select polymers from which nanoparticles of required size and polydispersity can be prepared. The main advantage of this new approach is that nanoparticles can be formed reversibly in mild conditions from a prepurified polymer. It also seems

quite reasonable that even a weak electrostatic repulsion may play a crucial role for further stabilisation of mesoglobules and improving their monodispersity [16]. Nanoparticles carrying functional groups properly located in the mesoglobule can be obtained, which could be further modified. Such nanoparticles with well-controlled size distribution can find a broad range of applications in pharmaceutical, biotechnological and cosmetic industries, where the technological control is one of the major problems.

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