The formation of mesoglobular phase of heteropolymers in dilute solution

SIU Man Hin (蕭敏騫)

Supervisor: Prof. WU Chi

A Thesis Submitted in Partial Fulfilment

of the Requirement for the Degree of

Master of Philosophy

in

Chemistry

©The Chinese University of Hong Kong
July 2002
Abstract

Pairs of poly(N-isopropylacrylamide-co-vinyl pyrrolidone) P(NIPAM-co-VP) copolymers with a similar VP content and chain length, but different VP distribution on the chain backbone, were prepared at temperatures below and above the lower critical solution temperature (~32 °C) of poly(N-isopropylacrylamide) homopolymer.

The temperature induced coil-to-globule transition of individual chains in dilute solution was investigated by laser light scattering (LLS). Interestingly, for the copolymers with similar VP content, the result showed that the chain prepared at 60 °C had a lower transition temperature and could form denser globules than those prepared at 30 °C.

This was presumably due to the copolymer prepared at 60 °C had a globular protein-like segmented vinyl pyrrolidone distribution while those prepared at 30 °C had a random vinyl pyrrolidone distribution. In other words, the folding of the chains prepared at higher temperature was easier as they could “memorize” or “inherit” their parent globular core-shell structure once the hydrophobic interaction (attraction) between the poly(N-isopropylacrylamide) segments is switched on. Globule with collapsed PNIPAM core and swollen shell made of small VP was formed and this phenomena was reflected in a ratio of the radius of gyration to hydrodynamic radius ($<R_g>/<R_h>$) as it was much smaller than 0.774 predicted for a uniform sphere. The result is in analog with the folding of protein to its native state in nature.

The aggregation process at different temperatures of the copolymers were also investigated. We observed that if the copolymer was placed at certain temperature for a long time, monodispersed nanoparticle would be obtained with stable particle size and weight-average molar mass ($M_w$). Such type of mesoscopic structures were
defined as mesoglobule.

It was observed that the mesoglobule made of copolymer synthesized at 60 °C would always have a larger aggregation number than that prepared at 30 °C. The main reason contributed to this phenomenon was the distribution of VP in the copolymer chain affected the mechanism of the aggregation of the copolymer. This indicated that the thermodynamics of mesoglobule formation was not solely affected by the competition between intrachain shrinking and interchain aggregation, but also by the chain sequence of the copolymer chain. Therefore, by varying the chain sequence, mesoglobules with different sizes and molar masses could be obtained at certain temperature, which resembles the formation of the precise quaternary structure of proteins via the assembly of polypeptides.

We also studied the fast and slow aggregation process of poly(N,N-diethylacrylamide-co-N,N-dimethylacrylamide) (P(DEA-co-DMA)) and poly(N,N-diethylacrylamide-co-N-ethylacrylamide) (P(DEA-co-EA)) with different mol% of DMA and EA, respectively. P(DEA-co-DMA) with 30 and 50 mol% DMA could form stable mesoglobule in sudden and gradual heating to their LCST. But among the three samples of P(DEA-co-EA), only the one with 40 mol% EA could form stable mesoglobule in both heating mode. Precipitation or dissociation of the associates occurred in the aggregation of P(DEA-co-EA) with 60 and 80 mol% EA. This result indicated that stable aggregate could only formed for copolymer composed of certain ratio of monomers. More important is, by varying the comonomer ratio of a copolymer, stable mesoglobule with different size and density could be obtained.

The physical properties of the copolymers we studied might provide some inspiration about the problems in folding and assembly of proteins. It should be emphasized that the copolymer chains studied here are much simple than proteins.
However, this study is one step forward in a long journey towards a better understanding of the protein folding.
摘要

在低於和高於聚(\(N\)-異丙基丙烯胺)均聚物的臨界溶解溫度 (32°C) 下合成了兩種聚(\(N\)-異丙基丙烯胺-co-乙烯基略烷酮)共聚物 P(NIPAM-co-VP); 其中 VP 含量相同，但其在共聚物分子鍵上的分布則不一樣。

用激光光散射技術研究了稀溶液中聚合物分子鍵因受溫度的影響從‘無規線團’蜷縮成‘單鍵小球’的轉變。令人感興趣的是，VP 含量相同時，60°C 較 30°C 合成的 P(NIPAM-co-VP) 共聚物的轉變溫度更低，而且能夠形成緊密小球。

大概是因為 60°C 下合成的 P(NIPAM-co-VP) 共聚物的序列分布類似於蛋白質分子，乙烯基略烷酮以長序列鍵段形式存在；而 30°C 下合成的 P(NIPAM-co-VP) 共聚物分子鍵中乙烯基略烷酮鍵節則呈無規分布。換句話說，高溫下合成的 P(NIPAM-co-VP) 共聚物分子鍵中聚(\(N\)-異丙基丙烯胺)鍵段之間一旦產生親水性作用(吸引)，他們就能夠‘記憶’或者‘繼承’核-殼雙親球狀結構，因此分子鍵更容易折疊，形成了具有塌陷的 NIPAM 核和膨脹的 VP 殼的小球。這一現象表現在其旋轉半径和流體力學半徑比 \(\langle R_g\rangle/\langle R_h\rangle\) 比預計的均勻硬球的值 0.774 小很多，這個結果與天然蛋白質分子折疊為它本來的狀態相類似。

我們亦研究了 30°C 和 60°C 下合成的含 5 mol% 和 10 mol% 乙烯基略烷酮的兩種 P(NIPAM-co-VP) 共聚物聚集時的溫度依賴性。我們觀察到如果共聚物在特定溫度下長時間放置，能夠得到尺寸和重均分子量穩定、分布均勻的納米粒子。這種介觀結構被定義為介觀小球。

研究表明 60°C 較 30°C 下合成的 P(NIPAM-co-VP) 共聚物的介觀小球有更多的聚集數目。產生此現象的主要原因是乙烯基略烷酮在共聚物分子鍵上的序
列分布影響共聚物的聚集機理。實驗結果顯示介觀小球形成的熱力學不僅受分子鍵蜷縮與分子鍵間聚集相互競爭的影響，而且也受共聚物的序列結構的影響。因此，改變共聚物的序列結構，在特定溫度下能夠得到大小和摩爾質量不同的介觀小球，類似於蛋白中多組裝形成的精確四級結構。

此外，我們也研究了 N, N-二甲基-丙烯胺和 N-乙基-丙烯胺摩爾含量不同的聚(N, N-二乙基-丙烯胺-co-N, N-二甲基-丙烯胺)共聚物 (P(DEA-co-DMA)) 和聚(N, N-二乙基-丙烯胺-co-N-乙基-丙烯胺)共聚物 (P(DEA-co-EA)) 的聚集過程。DMA 摩爾含量分別為 30 mol% 和 50 mol% 的 P(DEA-co-DMA) 逐步加熱到他們的 LCST 溫度能夠突然形成穩定的介觀小球。但是，在三個 P(DEA-co-EA) 樣品中，只有 EA 含量為 40 mol% 的 P(DEA-co-EA) 按兩種方式加熱，才能形成穩定的介觀小球。EA 含量為 60 mol% 和 80 mol% 的 P(DEA-co-EA) 聚集時產生分解或沉澱。此結果表明只有特定組成比的共聚物才能形成穩定地聚集；更重要的是，改變共聚單體的比例，可以得到不同大小和密度的穩定介觀小球。

我們研究的共聚物的物理性質能為探索蛋白質分子鍵的折疊和組裝帶來啟示。需要強調的是，雖然我們所研究的共聚物分子鍵比蛋白質要簡單得多；可是該研究對於準確理解蛋白質分子鍵的折疊提供了進一步的依據。
Acknowledgement

I am deeply grateful to my supervisor, Prof. WU Chi, for his invaluable guidance and encouragement during the entire period of my study. His innovative idea is indispensable for my research projects. I have learned a lot from him and always being inspired by his dedicatory attitude to science.

I would like to express my sincere thanks to all members in the LLS group for their useful discussion and kindly help. I am pleased to work with them for the past two years. They are Chau Kin Chiu, Cheng He, Ngai To, Niu Aizhen, Peng Shufu, Tu Yingfeng, Wang Chengqing, Zhang Guangzhao and Zhu Fangming.

My sincerely thanks was also extended to all the staff members in the Department of Chemistry and Graduate School of The Chinese University of Hong Kong for their help and support offered during the course of my study. The Hong Kong Research Grants Council and The Chinese University of Hong Kong were also greatly acknowledged for their financial support and Postgraduate Studentship offered respectively.

Finally, I would like to express my whole-hearted thanks to my family and friends for their support and endless love all the time.

SIU Man Hin
Department of Chemistry
The Chinese University of Hong Kong

July, 2001
# Table of Contents

Abstract i  
Abstract in Chinese iv  
Acknowledgement vi  
Content vii  

## Chapter 1  
**Introduction**  
1.1 Protein folding – Coil-to-globule transition 1  
1.2 Quaternary structure of proteins – Aggregation 5  
1.3 The effect of fast and slow heating rate to the aggregation of copolymers 11  
1.4 Main goal in thesis 14  
1.5 References 18  

## Chapter 2  
**Fundamentals of light scattering and instrumentation**  
2.1 Static laser light scattering 21  
2.2 Dynamic light scattering 22  
2.3 Correlation function profile analysis 24  
2.4 Molar mass distribution and conformation of polymers 25  
2.5 Instrumentation  
2.5.1 Light source 27  
2.5.2 Cell design 28  
2.5.3 Detector 28  
2.5.4 Differential refractometer 29  
References 30
<table>
<thead>
<tr>
<th>Chapter 3</th>
<th>The effect of comonomer distribution on the coil-to-globule transition of a single $AB$ copolymer chain in dilute solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>3.2</td>
<td>Experimental</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Sample preparation and characterization</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Differential Scanning Calorimeter</td>
</tr>
<tr>
<td>3.3</td>
<td>Results and discussion</td>
</tr>
<tr>
<td>3.4</td>
<td>References</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 4</th>
<th>Formation of mesoglobular phase of amphiphilic copolymer chains in dilute solution: 1. Effect of comonomer distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>4.2</td>
<td>Sample preparation and characterization</td>
</tr>
<tr>
<td>4.3</td>
<td>Results and discussion</td>
</tr>
<tr>
<td>4.4</td>
<td>References</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 5</th>
<th>Formation of mesoglobular phase of amphiphilic copolymer chains in dilute solution: 2. Effect of comonomer distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>5.2</td>
<td>Sample preparation and characterization</td>
</tr>
<tr>
<td>5.3</td>
<td>Results and discussion</td>
</tr>
<tr>
<td>4.4</td>
<td>References</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Biological function of proteins appeared to be more correlated to their macromolecular geometry than their chemical details. Activity of proteins greatly relied on the particular amino acid sequence of protein which should be correctly and uniquely translated from the genetic message in the cell. The specific amino acid sequence in the polypeptide chains gave protein a characteristic three-dimensional arrangement of the polypeptide segments and thus a functional native structure was resulted. Moreover, proteins usually adapt a cooperative stabilization arrangement of its structure in order to prevent the competition of strong interactions between solvent molecules and amino acids and the ionic or hydrogen bond interaction among amino acids.

Each proteins are so quaint designed and therefore how the specific and precise amino acid sequence of each protein controlled its activity and assembly is still a big question in science. The relationship between structure and function of proteins was too complex and a conclusive explanation still cannot be figured out.

1.1 Protein folding – Coil-to-globule transition

In structural biology, the reasons for the difference in the folding behavior of individual protein chains and detailed mechanism of protein folding to its native state is still unknown. It was interesting to find that the polypeptide chain could reach their unique native conformation during folding in a relatively fast time scale although there are numerous energy minimal states for the protein to convert to.

Various experiments and computer simulations were performed to investigate this problem. Anfinsen et al. showed that a denatured protein could renature to its native
state rapidly. When the fully reduced ribonuclease, with eight SH groups, was allowed to reoxidize under denaturing conditions, a mixture containing many or all of the possible 105 isomeric disulfide bonded products was obtained. But when the inactivated mixture was exposed to a small amount of sulfhydryl group-containing reagent, a homogeneous product which was indistinguishable from the native ribonuclease was obtained by the conversion of the ribonuclease back to their native state with the formation of correct, native disulfide pairing. Similar result was also obtained with other kinds of proteins. They proposed that such refolding process of proteins should take place along a limited number of ‘pathways’ and it was driven entirely by the free energy of conformation gained by the protein in going to the stable, native structure by the retention of native structure ‘memory’.

But the system studied above involves too many uncontrollable uncertainties from the experiment processes and the complicated structure of proteins. Computer statistics was done to a pool of proteins in order to find out the common features in their structures. Lamarine et al. investigated a set of protein representatives with a variety of distinct globular folds from the Protein Data Base (PDB) by computer analysis. They found that the topohydrophobic position, i.e., the position occupied by hydrophobic amino acid along the chain, would always constitute the deep core of proteins. They act as the anchor of protein folding and were not regularly distributed along the sequence. This indicated that the topological properties of hydrophobic position along the chain play an important role in the folding and assembly of proteins.

Further simplification of the system by computer simulation of protein was done with various approaches and different focus of attention. The steps and kinetics involved in protein folding from a coil to its native state globule were found and the
nature of folding transition state and its relationship to the native structure of proteins were investigated. Zhou et al. found that there were four significant transition states for protein folding, namely as collapsed transition, disordered to ordered (molten) globule transition, globule to native-state transition and the transition of the protein active native state to a frozen inactive state. Michele and coworkers revealed how a nucleation mechanism involving a small number of key residues could lead to the folding of a polypeptide chain to its unique native state. An ensemble of protein structures with different levels of restraints from the native-like inter-residue contacts was generated and their result showed that the overall fold of the transition-state ensemble was mainly determined by the native-like contacts of the key residues in their protein model.

Thirumalai et al. showed that the minimum energy compact structure in protein folding always acted as basin of attraction of most transition pathways of proteins and its quantity was far less than the number of compact structures that could be generated in the folding process. Moreover, by using lattice models of proteins, different folding kinetics were obtained for the copolymers constructed with different collapse transition temperature $T_\theta$ and folding transition temperature $T_f$. For the copolymers with small difference in the above two temperatures, the folding kinetic appeared to be a fast and all-or-none process and the native conformation was reached by a specific nucleation collapse mechanism. But for the copolymers having greater differences in $T_\theta$ and $T_f$, the folding time increased dramatically. $T_\theta$ and $T_f$ could greatly affect the foldability of the sequence and Thirumalai concluded that the kinetic assembly of the native state of a protein should be encoded in its primary sequence. The folding time for various sequence of the protein could be correlated extremely well with the difference between collapse and folding transition
temperature which was intrinsic to the sequence of the lattice models of proteins.

Copolymers with hydrophilic and hydrophobic units were also constructed in order to imitate proteins in a more simplified way. Particularly, the coil-to-globule transition of different types of copolymer chains was simulated by Khokhlov et al.\(^8\)-\(^{10}\) in order to demonstrate how the comonomer distribution, i.e., the sequence difference in structure, could greatly influence the folding of a single copolymer chain. Their results suggested that a protein-like copolymer could "memorize" or "inherit" some special functional properties of their parent collapsed state and proceed the coil-to-globule transition easier than the random copolymer although they have identical composition and chain length. This is in analog with the speculation that specific amino acid sequence in different proteins could greatly affect its folding behavior and hence it's biological activity. Their result led us to a hypothesis that proteins should have specific sequence effect for their particular collapse mechanism and native conformation.

Experimentally, Tenhu et al.\(^{11}\) proved that difference exist in the lower critical solution temperature (LCST) for poly(N-isopropylacrylamide)-g-poly(ethylene oxide) (PNIAPM-g-PEO) prepared at temperatures below and near the LCST of poly(N-isopropylacrylamide) homopolymer. Although the copolymer chains contains similar amount of poly(ethylene oxide), different LCST was observed for the copolymers as evidenced by the differences in the aggregation behavior of the graft copolymers. Their result supported that the arrangement of monomers (or amino acid in polypeptides) would affect the coil-to-globule transition of a copolymer other than just by the variation of chemical composition of the chain.

However, the chain aggregation is a complicated process involving the intrachain contraction and the interchain association. Therefore, the ultimate test and
experimental challenge would be the study of the effect of comonomer distribution on the coil-to-globule transition (folding) of individual copolymer chains without involving any interchain aggregation in dilute solution. The concentration of the copolymer solution should be very small or aggregation would happen instead of a single chain process.\textsuperscript{12} For such low concentration, a highly sensitive instrument should be used in order to study the slightly change of the conformation of the copolymers.

1.2 Quaternary structure of proteins - Aggregation

Another interesting property of protein is the assembly of individual polypeptide chains or proteins into an ordered and stable quaternary structure at certain condition, like enzymes, without further aggregating with other existing polypeptides or chemical substances surrounding them.\textsuperscript{13} Moreover, the interior of a biological cell is an extraordinary crowded environment containing many kinds of proteins, therefore how proteins have such ordered interactions in an highly concentrated protein solution is still an unsolved problem to scientists. It seems that each proteins would have its native state and specific way of assembling together by their specific amino acid sequence generated from genetic code.

Furthermore, aggregation would occur if the solvent quality surrounding a globular protein in solution was changed. The protein may adapt a conformation which is different from its native form and aggregated together due to its structural instability. This can be resulted from the change of temperature, salt concentration or pH, even the addition of cosolvent. Aggregation of protein is governed by a dedicated balance of hydrophobic interaction, hydrogen bonding, disulfide bonding, electrostatic interactions, etc. among amino acids and polypeptides. Different kinds of protein
would also have different levels of tolerance towards changes of environment, some can still exist as its native form even at 100 °C, but some can only stable at a maximum temperature of 2 °C.  

These physical properties of proteins had attracted many attentions from researchers and investigations were carried out in different points of views. For instance, using ribonuclease, Anfinsen et al. investigated the reversible aggregation of proteins. They found that the proteins could return to its shape after denaturation if certain position of the globular protein is still reserved. This result gave us an important enlightenment that the ordered assembly of proteins was greatly depended on certain critical interacting position of the protein chain. Once this specific native position is preserved, denaturation of protein would only exert a minor blockage on proteins in returning to their native state.

Interaction between proteins during assembly or aggregation was also investigated by many scientists. Beretta et al. analyzed the protein-protein interaction by two globular proteins possessing similar size and net charge. In general, the interactions and stability of globular proteins is in terms of a composition of their van der Waals attraction and electrostatic repulsion. They found that although the two proteins did not show large difference in their net charge and number of charge residue per surface unit, difference in effective attractive interactions of these two proteins was observed. It was concluded that the charge imbalance on proteins would be a determining factor in protein interaction. For the protein with charge evenly distributed on surface, condensation of hydrated counterions on protein would create large repulsive hydration forces between them. Smaller effective van der Waals attractive interaction was resulted and the attraction of protein was retarded. But for the other protein possessing less hydrated counter ions, stronger protein-protein
interaction was resulted and thus larger associate was formed. Therefore, the native structure of globular protein would exert distinctive effect on their protein-protein interaction as it determines how the charged groups were distributed on the protein globule, even for the protein pairs with similar size and net charge.

Aymard et al.\textsuperscript{16} also observed similar behavior in their study as they found that the structure of aggregate formed was highly depended on the ionic strength of the proteins. The large-scale structure of aggregates was governed by the balance between electrostatic interactions and the degree of screening between proteins. But apart from this, they also discovered the presence of specific protein binding site of proteins. These sites would partly restruct when the electrostatic interaction between proteins is screened out and the aggregation of proteins would also be initiated by them. Thus the structure factor of proteins should contain information about the relative position of the proteins should have in the aggregate and this research work indicated that variation of aggregation behavior of proteins should not attributed solely to electrostatic interactions.

The system of proteins was also simplified by copolymers constructed with hydrophilic/charged and hydrophobic monomers in different conformations. Various physical properties of proteins were mimicked by these specially engineered copolymers. Timoshenko et al.\textsuperscript{17-19} studied the collapse and aggregation of specially constructed copolymers by computer simulation. Copolymers with same amount of hydrophilic monomers but distributed differently along the chain were created and their aggregation were simulated. Interesting result was obtained as they found that when the diblock or triblock copolymer chains were mutated and the hydrophilic groups were arranged along the chain with different degree of blockiness, the size and the average number of chain of 'aggregates' formed would be smaller when the
length of hydrophilic block along the copolymer decreased. The result was out of their expectation because they predicted that the copolymer with diblock or triblock copolymers should form the smallest size ‘aggregates’ by assembling as a micellar-like structure. Moreover, it was surprised to find that small size and narrowly distributed ‘aggregates’ were formed instead of large aggregates for certain copolymers. Their mean size and monodispersity were extremely sensitive on the thermodynamic parameter of the system and the particular monomer sequence they possessed. Such mesoscopic ‘aggregates’ were then defined as mesoglobule. Timoshenko also mentioned that although there were other mesoglobular states with a lower free energy for the mesoglobule to convert to at a given thermodynamic parameter, the mesoglobule could still stay in the trapped state which it was initially formed. This was due to the activation energy of changing from one state to another state is very high in the energy profile, and in principally, the existing mesoglobule system could be trapped in its metastable state for a very long time.

The work of Timoshenko had introduced us the question of the copolymer’s sequence specificity on forming mesoglobule in dilute solution. Their comparative analysis of copolymer aggregation with fixed total monomer concentration but varying chain length had elucidated us an idea that the mesoglobule formation with certain mass and size was greatly depended on the chain length and sequence of the original copolymer.

Experimentally, copolymers with different block arrangement along the chain were also synthesized and studied. Volpert et al.\textsuperscript{20,21} had prepared a series of acrylamide polymers modified with low amounts of alkyl- or alkylarylacrylamides by aqueous micellar copolymerization. This copolymerization method allows one to control the microstructure of the resulting copolymer by varying the hydrophobe to micelle
ratio. Copolymers would be synthesized from a very blocky structure to an almost random arrangement of monomer. Therefore, copolymers possessing same comonomer ratio but with hydrophobes randomly and single distributed or blocky partitioned along the polyacrylamide backbone could be synthesized.

Upon the viscosity measurement of the copolymer solutions, they found that the blocky copolymer showed much greater associative behavior than that of the statistical one. This was further proved by more pronounced increase in viscosity of the copolymer solution when the hydrophobic block size of the copolymer increased. They found that the increase in viscosity was a consequence of the formation of aggregates by hydrophobic intermolecular association.

Their result clearly showed that the hydrophobic group distribution in copolymer is a key parameter in controlling the thickening ability of the copolymer, which in other words, the associating ability of the copolymers. The longer the blocks, the greater the thickening efficiency and thus the larger extent of the association of copolymers.

Interesting copolymers with different arrangement of polystyrene (PS) and polyisoprene (PI) blocks along the chain were synthesized by Hodrokoukes and coworkers. Triblock copolymers with normal tapered, inverse tapered, random middle block, normal tapered and random structure were synthesized with diblock copolymers as reference material. The copolymers practically had the same composition of polystyrene and their micellization behaviors were investigated in \textit{n}-decane, which is a bad solvent for polystyrene. It was surprised to find that random copolymer could form unimolecular micelles in \textit{n}-decane. Moreover, when they compared with the micelle formed by diblock copolymer, the average aggregation number and size of the micelle formed by triblock copolymer having an inverse tapered middle block or tapered copolymer was smaller. They attributed to the reason
that the existence of a middle block with variable composition of PS and PI of inverse tapered middle block or tapered triblock copolymer had made the interfacial region between the core and shell of the micelles more diffused in compare with the diblock micelles. This facilitated the penetration of solvent molecules into the micelles and thus the solubility behavior of the whole micelle could be altered. The results of Volpert and Hodrokoukes supported the computer simulation done by Timoshenko in a sense because decrease in blockiness along the chain of a copolymer could generally cause a decrease in average aggregation number and size of the micelles formed.

The extraordinary result from research works discussed above indicated that the location or distribution of monomers with different physical properties along a copolymer chain could greatly affect its behavior in various chemical and thermodynamic environment, especially in their assembly process.

Apart from the blockiness of copolymer, the rate of interchain aggregation and intrachain collapse during the heating process of the chain would also exert effect on the self-assembling behavior of copolymers. Many researches had been done to investigate on this competition and significant results were found. Peng et al.\textsuperscript{23} demonstrated the competition result of these two opposing process by laser light scattering study on the calcium induced aggregation of poly(N-vinylcaprolactam-co-sodium acrylate) (P(VCL-co-NaA)). Since PVCL is a temperature sensitive polymer and it would become insoluble at about 35 °C, increase in temperature higher than 35 °C would lead to both interchain complexation and intrachain shrinking of the thermosensitive PVCL. It was found that at low aggregation temperature, individual collapsed chains were able to interpenetrate each other to form a uniform structure as the shrinking of individual PVCL was less eager. But when the aggregation
temperature increased, the collapse of individual chains became so fast that the chance for the copolymers to undergo the interchain complexation in a dilute solution was greatly reduced. Therefore, the density of aggregates formed would increase but the size of the aggregate decreased as interchain penetration was retarded at higher aggregation temperature.

1.3 The effect of fast and slow heating rate to the aggregation of copolymers

Many investigations on self-aggregation of amphiphilic copolymers have been done in recent years. Their usage in industrial applications as well as biomedical substances were widely developed for their interesting tendency of self associating to micelles or aggregate particles with various size and structures in aqueous medium. For instance, the water soluble poly(ethylene oxide)-block-poly(propyl-ene oxide)-block-poly(ethylene oxide) (PEO-PPO-PEO) or commercially called Pluronics (BASF) were block copolymers which can form micelles in water. They are potentially applicable in protecting cells from drugs such as doxorubicin by its hydrophobic drug sequester ability. Moreover, they were also commonly used in drug delivery, detergency, emulsification and many other applications.

The commercially available copolymers were usually constructed with at least two kinds of monomers with distinct chemical nature and physical properties. Colloids with well-controlled structure and morphology could be generated by tuning the solvent condition in order to create certain specific interactions between these specially designed copolymers. Solvent condition could be altered by changing the solvent quality, concentration, temperature, monomer composition and chain sequence of the copolymer.
Others from copolymers which were intrinsically constructed with hydrophilic and hydrophobic monomers, many investigations have been done on the thermosensitive copolymers. These copolymers consist at least one kind of the monomer which have lower critical solution temperature (LCST) or upper critical solution temperature (UCST) properties once it was polymerized. Therefore, by copolymerizing them with different amount of hydrophilic or hydrophobic monomers, LSCT or UCST of a copolymer could be altered.

Miyazaki et al. synthesized a series of poly(N,N-dimethylacrylamide-co-N-phenylacrylamide) (P(DMA-co-PA)) with different mol% of PA by free radical copolymerization. They determined the LCST of the copolymers from the solution’s turbidity at 500 nm by UV-VIS spectrometer. Sharp change in turbidity at the copolymer’s LCST was observed. When the PA content increased in the copolymers, the LCST of the copolymers decreased due to the hydrophobic nature of PA. The formation and disappearance of the coacervate were completely thermo-reversible in the system they studied.

Polyelectrolytes were also frequently investigated for their stability during aggregation at elevated temperatures. Qiu et al. synthesized a series of poly(N-isopropylacrylamide-co-acrylic acid) (P(NIPAM-co-AA)) with different mol% of acrylic acid distributed randomly along the chain. They found that by introducing a very small amount of ionic groups into PNIPAM, stable nanoparticles would be formed in water at temperature higher than PNIPAM’s LCST. LCST of the copolymer was also elevated by increase in the AA mol% along the copolymer chain. It was suspected that the stability of nanoparticles formed during aggregation was came from the strong ionic interaction of AA and water molecules. Since the formation of such nanoparticle required a dedicated balance of hydrophilic and
hydrophobic interactions of the copolymer chains, the competition between intra- and interchain interaction would exert great influence on the size and density of the resultant nanoparticles formed. The rate of such competition could be shifted by changing the ionic content, temperature, pH and salt concentration of the solution and various size of nanoparticles would then be obtained.

Other than chemical composition, the rate of change in solvent condition would also exert great effect on the molar mass, density and size of the resultant nanoparticles formed. Various combinations on the rate of intra- and intermolecular association would lead to the formation of diverse possible structures of associates by amphiphilphilic copolymer.

The association behavior of poly(N,N-dimethylacrylamide)-graft-poly(methyl methacrylate) (PDMA-g-PMMA) dissolved in solvents with different methanol/water ratio were studied by Itakura et al. with laser light scattering. They found that the structure of the associate formed would dramatically change with the rate of change in solvent quality. When they changed the solvent quality from good to poor for one component of the copolymer in a short time span (less than 60 minutes), aggregates with small association number and unimodal particle size distribution were formed. But when the change rate was extended to over 120 minutes, large associates with bimodal distribution were resulted. This was because in the fast change of solvent quality, the intramolecular association of PMMA would dominate over the intermolecular bonding, making the copolymer chain become stable from further aggregation. But when the solvent was gradually changed from good to poor for PMMA in a long time interval, the associate formed at marginally bad solvent would continue to grow and stabilized by the association rearrangement.
between them before the solvent quality gradually became very bad for PMMA. Thus larger associates could be formed at slower change rate of the solvent quality.

Qian et al.\textsuperscript{31} also studied the aggregation of ethylene-vinylacetate (EVA) random copolymer possessing UCST in two different thermal process. One is quickly quenching the copolymer solution from 70 °C to 0 °C, the other is slowly cooling the solution from 70 °C to room temperature and annealed at room temperature for 24 hours before further quenching to 0 °C. It was found that the aggregate formed in the fast cooling process was mainly through the ‘packing’ of individual collapsed chains. While in the slow cooling of the EVA copolymers, microgel like particles were formed by the winding of EVA chains and fibrous-like aggregates. Therefore, the particle size of EVA aggregates could be controlled by varying the initial EVA concentration, the annealing time at room temperature and the cooling rate. For instance, among the three different concentration of EVA samples they studied, only the sample with highest concentration of EVA had a larger value of $R_v(\theta)/C$ in the fast cooling process than that in the slow cooling process. While for the other samples with lower EVA concentration, since $R_v(\theta)/C \propto M_w$, molar mass of the aggregates formed in the slow cooling process were generally higher than that formed in the fast cooling process.

From the results obtained in the investigation of copolymer aggregation described above, constructing amphiphilic copolymers with special properties may be helpful in solving a variety of interface and particle stabilization problems. Change of the copolymer’s chemical structure could alter the cohesion-energy densities of the blocks along the chain as well as the special chemical interaction between copolymer and solvent molecules. While the size of the self-associates phase structure would be greatly related to the physical properties of the copolymer such as their length, block
size and architecture. Also, the rate of change in solvent quality would also exert
great effect on the association mode of the copolymers.

1.4 Main goals in thesis

From the numerous research works mentioned above, we noticed that some features
of copolymers were similar with proteins in many aspects. For example, the
arrangement of monomers along copolymer chain would affect its aggregation
behavior, which is in analog with the affection of amino acid sequence in
polypeptide chain association. Therefore, it is noteworthy that the strangeness of
proteins would be explored by copolymers with 'protein-like structure' carrying
particular properties of a protein. Similarity did exist between the amino acids
proteins and monomer in polymer chemistry.

With the proper choose of comonomers and synthetic method, certain characteristic
of proteins could be mimicked by specially engineered copolymers. Their chemical
and physical behavior could also be studied at various experimental conditions. For
instance, copolymers with different isomerization or various conformation similar to
proteins could be synthesized and studied without the arise of undesirable influence
from the complex structure of proteins. Moreover, it is usual for only one or two
properties of protein were imitated by copolymers, clearer comprehension of how
these properties affected the behavior of protein could then be concentrative studied.

In this thesis, we tried to investigate how the basis of a protein, its amphilicity,
affects their folding and association behavior by constructing copolymers with
hydrophilic and hydrophobic groups. The copolymers mimicked the polypeptides
composed with certain sequence of amino acids by different monomer arrangement
along the chain and their coil-to-globule transition and aggregation behavior at
various conditions were studied.
We focus our study on the physical properties of thermosensitive poly(N-isopropylacrylamide-co-vinyl pyrrolidone) P(NIPAM-co-VP) copolymers using Laser Light Scattering (LLS). The copolymers were prepared with similar vinyl pyrrolidone mole ratio but synthesized below and above the lower critical solution temperature (~32 °C) of poly(N-isopropylacrylamide) homopolymer. We proposed to generate the copolymers with different chain conformations and they should exhibit distinctive behavior on their coil-to-globule transition and aggregation at different temperatures although they have similar amount of vinyl pyrrolidone.

However, it is a rather difficult experimental challenge to prepare a pair of AB copolymers with a similar composition and chain length, but different comonomer distributions on the chain backbone. It has been well-known that poly(N-isopropylacrylamide) (PNIPAM) is a thermally sensitive polymer with a lower critical solution temperature (LCST ~ 32 °C).\textsuperscript{12,32-34} This interesting thermal property has made PNIPAM a simple model for the simulation of protein denaturation in aqueous solution even though real proteins are much more complicated. From the past experience on preparation of narrowly distributed long PNIPAM homopolymer chains and studies of the coil-to-globule transition of a single homopolymer chain in dilute solution,\textsuperscript{12,34,35} we are enabled to use the difference between the chain conformations at different temperatures to incorporate a second monomer into PNIPAM to obtain different sequences. In this study, hydrophilic vinyl pyrrolidone (VP) was copolymerized into PNIPAM at 30 °C and 60 °C, which are, respectively, below and above the LCST of PNIPAM homopolymer. It was expected that at 60 °C, hydrophilic comonomer VP would segregate on the periphery of the collapsed PNIPAM segments, while at 30 °C, the copolymerization would lead to a more random distribution of VP on the PNIPAM chain. At each temperature, the
copolymers with two different VP/NIPAM ratios were prepared. Their coil-to-globule transition and aggregation behavior were then studied by laser light scattering (LLS).

The fast and slow aggregation of poly(N,N-diethyl-acrylamide-co-N-ethylacrylamide) P(DEA-co-EA) and poly(N,N-diethylacrylamide-co-N,N-dimethylacrylamide) P(DEA-co-DMA), with different mol% of EA and DMA respectively, were also studied by LLS. Aggregation was carried out in the fast and slow heating of the copolymer samples to their LCST and the results were presented and discussed in chapter 5.

We believed that by studying the thermal properties of these specially engineered copolymers, more about the folding and assembly of proteins can be understand even though the system we investigated is much more simplified when compared to the proteins in nature. Also, analyzing the result from the aggregation of copolymers composed with different monomer and comonomer ratio would also help to excavate out the mysterious in the formation of mesoglobule with desired molar mass and particle size.
References


(13) http://www.expasy.ch

(14) http://www.brenda.uni-koeln.de


Chapter 2

Fundamentals of light scattering and instrumentation

We always encountered with the examples of light scattering in everyday life. In sunny day, the blue component of white sunlight is predominately scattered by the ozone layer in the atmosphere, giving us the sky with beautiful blue color. Or when there is a fog, the light scattering of small water droplets in air made us have poor vision even at daytime. The color of milk is resulted from the scattering of all colored light by protein molecules in solution. Actually, the phenomenon described above is the result of time-averaged intensity of scattered light, that is, static light scattering.

In laser light scattering, when a monochromatic, coherent light is incident into a dilute macromolecule solution, light will be scattered in all direction if the refractive indices of the solvent molecules and solute are different. Theoretically, the scattered light intensity at each direction would be constant if all the macromolecules or particle are stationary when time passes.

But in reality, these macromolecules are undergoing constant Brownian motion and this leads to the fluctuation of the scattering intensity when time elapsed. This fluctuation rate can be related to the relaxation processes of the molecules, such as diffusion (translation and rotation) and internal motion. Therefore, by measuring this fluctuation of intensity $I(t)$ in time domain, i.e. dynamic laser light scattering and the time-average scattered intensity $I$, i.e. static laser light scattering, information of the macromolecules can be obtained if the detection area is small enough.

In polymer science, static laser light scattering is always used to investigate the static properties of macromolecules in solution, such as molar mass, radius of
gyration and conformation. While the dynamic laser light scattering is used to study the dynamics of the macromolecules in solution, such as the translational and rotational diffusion process. Combination of static and dynamic measurement gives us invaluable information about the physical properties of macromolecules.

2.1 Static Laser light Scattering

For a dilute macromolecule solution measured at small scattering angle (θ), the weight-average molar mass (Mw) and the z-average root-mean square radius of gyration (\(<R_g^2>^{1/2}\) or written as \(<R_g>\)) of polymer chains can be obtained by the following equation,

\[
\frac{KC}{R_w(q)} = \frac{1}{M_w} \left( 1 + \frac{1}{3} <R_g^2> q^2 \right) + 2 A_2 C
\]

where \(K = 4\pi^2 n^2 (dn/dC)^2/(N_A\lambda_0^4)\) and \(q = (4\pi n/\lambda_0)\sin(\theta / 2)\) with \(N_A, dn/dC, n, A_2\) and \(\lambda_0\) being the Avogadro number, the specific refractive index increment, the solvent refractive index, the second-order virial coefficient and the wavelength of the light in vacuum, respectively. Knowing the angular dependence of the excess absolute scattering intensity, or Rayleigh ratio \(R_\omega(q)\), is essential in calculating the molar mass and radius of gyration of the macromolecules.

Practically, this \(R_\omega(q)\) is determined by measuring the scattering intensity of a standard such as toluene and then calculated based on the equation as

\[
R_\omega(q) = \frac{<I>_{\text{solution}} - <I>_{\text{solvent}}}{<I>_{\text{standard}}} R_{\omega,\text{standard}}(q) \left( \frac{n}{n_{\text{standard}}} \right)^a
\]

\(I\) and \(n\) are, respectively, the time-averaged scattering intensity and the refractive index and \(a\) is a constant between 1 and 2, depending on the detection geometry of the light scattering instrument. If the scattering volume were determined by a slit \(a\)
would equal to 1. But if the scattering volume were selected by a pinhole much smaller than the diameter of the incident beam at the center of the scattering cell, \( a \) would be 2. Therefore, if the pinhole size is comparable to the mean diameter, \( a \) would lie between 1 and 2 which is not favored. In practice, we should avoid this situation by choosing either a slit or a smaller pinhole.

### 2.2 Dynamic Laser light Scattering

When the scattered field is a Gaussian random process, the correlation functions  \( g^{(1)}(t) \) and  \( g^{(2)}(t) \) are connected through the Siegert relation

\[
g^{(2)}(t) = 1 + |g^{(1)}(t)|^2
\]  

(2.2.1)

where \( g^{(1)}(t) = \frac{<E(0)E^*(t)>}{<E(0)>^2} \) and \( g^{(2)}(t) = \frac{<I(0)I(t)>}{<I(0)>^2} \) are the normalized field-field and normalized intensity-intensity autocorrelation functions, respectively. By evaluating the above equations, the intensity time autocorrelation function is obtained

\[
G^{(2)}(t) = <I(0)I(t)> = <I(0)>^2 g^{(2)}(t) = <I(0)>^2 [1 + |g^{(1)}(t)|^2]
\]  

(2.2.2)

It is important to relate the \( g^{(2)}(t) \) with \( g^{(1)}(t) \) by the above equation as the \( G^{(2)}(t) \) and \( <I(0)> \) can be obtained experimentally. But an instrumental parameter \( \beta (<1) \) is introduced in Equation (2.2.2) as the detection area can not be zero no matter how small it is and the scattered light detected can not be purely coherent. Thus Equation (2.2.2) becomes

\[
G^{(2)}(t) = A(1 + \beta |g^{(1)}(t)|^2)
\]  

(2.2.3)

where \( A(= <I(0)>^2) \) is the baseline, \( t \) is the delay time, \( \beta \) is a parameter depending on the coherence of the detection optics, and \( I(t) \) is the detected scattered intensity or
photon counts at time \( t \), which includes contributions both from the solvent and from the solute.

In general, the relaxation of \( |g^{(1)}(t)| \) includes both diffusion (translation and rotation) and internal motions of the particles. If we only consider the translational diffusion relaxation of a polydispersed polymer sample with a continuous distribution of molar mass \( M \),

\[
|g^{(1)}(t)| = \int G(\Gamma) e^{-\Gamma t} d\Gamma \tag{2.2.4}
\]

where \( G(\Gamma) \) is called the line-width distribution and \( G(\Gamma) d\Gamma \) is the statistic weight of the particles or macromolecules which possess line-width \( \Gamma \). Since the measured \( G^{(2)}(t) \) can be converted to \( |g^{(1)}(t)| \) by the Laplace inversion program CONTIN, the line-width \( \Gamma \) can be obtained. And \( \Gamma \) can be related to \( C \) and \( q \) by

\[
\Gamma/q^2 = D(1 + k_d C)(1 + f R_e^2 \sigma q^2)
\]

where \( D \) is the translational diffusion coefficient of the solute molecule at \( C \to 0 \), \( k_d \) is the diffusion second virial coefficient, and \( f \) is a dimensionless parameter depends on the polymer chain structure, polydispersity and solvent quality. \( D \), \( f \), and \( k_d \) can be obtained from the plots of \( (\Gamma/q^2)_{C \to 0} \) vs. \( q^2 \) and \( (\Gamma/q^2)_{\theta \to 0} \) vs. \( C \), respectively. Since both thermodynamic and hydrodynamic interactions contribute to \( k_d \), it be further expressed as

\[
k_d = 2A_2 M_w - C_D N_A R_b^3 / M_w \tag{2.2.6}
\]

where \( C_D \) is an empirical positive constant.

When \( C \to 0 \) and \( \theta \to 0 \), \( \Gamma/q^2 \) can be further simplified to

\[
\Gamma/q^2 \to D \tag{2.2.7}
\]
By the definition of $|g^{(1)}(t)|$, $G(D) = q^2G(\Gamma)$, $G(\Gamma)$ can be converted to the translational diffusion coefficient distribution $G(D)$ and further to hydrodynamic radius ($R_h$) by the Stokes-Einstein equation

$$R_h = \frac{k_BT}{6\pi\eta D}$$

In the equation, the $k_B$, $T$, and $\eta$ is the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively. While rest of the parameters are measurable by other method. Calibration is no need and thus the particle sizing of polymer chains by dynamic laser light scattering can be considered as an absolute method.

### 2.3 Correlation function profile analysis

By obtaining $G^{(2)}(t)$, $|g^{(1)}(t)|$ can be determined through Equation (2.2.3). $G(\Gamma)$ and $G(D)$ can then be computed from the Laplace inversion of $|g^{(1)}(t)|$. Laplace inversion programs were helpful in determining desired information, especially in terms of the average line width $\bar{\Gamma} = \int_0^\infty \Gamma G(\Gamma) \, d\Gamma$ and the relative width ($\mu_2/\bar{\Gamma}^2$) of the line-width distribution ($G(\Gamma)$) with

$$\mu_2 = \int_0^\infty (\Gamma - \bar{\Gamma})^2 G(\Gamma) \, d\Gamma$$

But the Laplace inversion also has its limitation and ill-conditioned problem. Due to the limited number of data points and the bandwidth limitation of photon correlation instruments, some unavoidable noises in the measured time correlation function appeared. This would cause the $g^{(1)}(t)$ obtained could not always provide necessary and sufficient information to determine $G(\Gamma)$ uniquely. Therefore, it is important to keep the sample solution thoroughly cleaned by dust-free process as reducing noise is
very important in the measured intensity intensity time correlation function. Or the noise would cause large error in the Laplace inversion. Many computation program of Laplace inversion were developed and improved due to the rapid increase of personal computer speed. Among many programs, the CONTIN program is one of the most widely used and accepted programs for this computation. This program contains safeguarding constraints to avoid the ill-posed nature of the inversion. An early method of analysis was based on a cumulant expansion of the correlation function

\[
\ln|g^{(1)}(t)| = 1 - \Gamma t + \frac{1}{2!} \mu_2 t^2 - \frac{1}{3!} \mu_3 t^3 + \frac{1}{4!} [\mu_4 - 3\mu_2^2] t^4 + \ldots
\]

\[
= 1 + \sum_{m=1}^{\infty} k_m(\Gamma) \frac{-t^m}{m!}
\]

where \( k_m = \left[ (-1)^n \frac{d^n}{dt^n} \ln|g^{(1)}(t)| \right]_{t=0} \) is the \( m \)th cumulant of \( g^{(1)}(t) \) and 

\[
\mu_i = \int_0^\infty (\Gamma - \bar{\Gamma})^i G(\Gamma) \, d\Gamma.
\]

Equation (2.3.2) may be fitted by a least squares routine to the correlation function and values for \( \mu_2, \mu_3, \ldots \) obtained. The average width \( \bar{\Gamma} = \int_0^\infty \Gamma G(\Gamma) \, d\Gamma \) is the mean relaxation time. The variance is \( \mu_2 / \bar{\Gamma}^2 \), where \( \mu_2 = \int_0^\infty (\Gamma - \bar{\Gamma})^2 G(\Gamma) \, d\Gamma \). For low \( q, qR < 1 \) and \( \bar{\Gamma} = \bar{D}q^2 \), where \( \bar{D} \) is a z-average translational diffusion coefficient.

The use of CONTIN could yield reliable \( \bar{\Gamma} \) and \( \mu_2 / \bar{\Gamma}^2 \) values under all conditions as long as the measured time correlation function was obtained within a proper bandwidth range and the photon counts have sufficient statistics, e.g., the baseline (A) has a total counts over \( 10^6 \).
2.4 Molar mass distribution and conformation of polymers

Another important part of polymer characterization by laser light scattering is the determination of molar mass distribution. According to the definition in dynamic LLS,

\[
[g^{(1)}(t)]_{t \to 0} = \langle E(t)E^*(0) \rangle_{t \to 0} = \int G(\Gamma)d\Gamma \propto \langle I \rangle \tag{2.4.1}
\]

where \(\langle I \rangle = \langle I \rangle_{\text{solution}} - \langle I \rangle_{\text{solvent}}\) is the net average scattering intensity. On the other hand, in static LLS, when \(C \to 0\), and \(q \to 0\),

\[
R_{\nu \nu}(q \to 0) \propto \langle I \rangle \propto M_w \propto \int f_w(M)M\,dM \tag{2.4.2}
\]

where \(f_w(M)\) is a differential weight distribution. A comparison of Equation (2.4.1) and (2.4.2) leads to

\[
\int G(\Gamma)d\Gamma \propto \int f_w(M)M\,dM \tag{2.4.3}
\]

where \(G(\Gamma) \propto G(D)\) and \(d\Gamma \propto dD\) because \(\Gamma = Dq^2\). Therefore, Equation (2.4.3) can be rewritten as

\[
\int G(D)\frac{dD}{dM}dM \propto \int f_w(M)M\,dM \tag{2.4.4}
\]

It has long been theoretically predicted and experimentally proven that the translational diffusion coefficient \(D\) and be related to the molar mass according to the scaling relationship as\(^\text{16}\)

\[
D = k_D M^{-\alpha_D} \tag{2.4.5}
\]

where \(k_D\) and \(\alpha_D\) are two scaling constants. The conformation of polymer chains can be seen from the value of \(\alpha_D\).\(^\text{17}\) For a flexible polymer chain, \(0.5 < \alpha_D < 0.6\) in a good
solvent whereas $\alpha_D = 0.5$ for polymer chain in a Flory $\Theta$ solvent. On the basis of Equation (2.4.5), Equation (2.4.4) can be rewritten as

$$f_w(M) \propto \frac{G(D)}{M} \frac{dD}{dM} \propto G(D)D^{1+(2/\alpha_D)}$$

(2.4.6)

where all proportional constants have been omitted because they are irrelevant to a given distribution. According to Equation (2.4.5) and (2.4.6), the values of $k_D$ and $\alpha_D$ are needed to transform $D$ to $M$ and $G(D)$ to $f_w(M)$.\(^{18}\)

By combining the $z$-average root-mean square radius of gyration ($<R_g^2>^{1/2}$ or written as $<R_g>$) and the hydrodynamic radius $<R_h>$ from the static and dynamic laser light scattering measurement respectively, a very useful dimensionless parameter was generated. For example, a polymer with linear coil chain in good solvent would have $<R_g>/<R_h>$ value of about 1.5. Or if it is a non-draining uniform sphere, the $<R_g>/<R_h>$ would be $(3/5)^{1/2}$. While for a normal hyperbranched polymer cluster, the $<R_g>/<R_h>$ is about 1.0-1.3 depends on the branching degree.\(^{19-22}\)

### 2.5 Instrumentation

A modern commercial light scattering spectrometer used in our laboratory mainly consists of light source, the optics, the cell holder and the detector. One set of our light scattering spectrometer was equipped with a differential refractometer designed by Wu et al.\(^{23}\) for the measurement of the specific refractive increment ($dn/dC$) of polymer solutions.

#### 2.5.1 Light source

The light source we used is a He-Ne laser with wavelength of 632.8 nm and an output power of 22 mW. Typically, as a light source in Laser Light Scattering
measurement, the beam amplitude noise should be lower than 0.5 percent or the noise level of the intensity-intensity time correlation function in dynamic Laser Light Scattering will be affected. While for time-averaged scattered light intensity measurement, long-term amplitude stability less than \( \pm 1 \) percent is required. Moreover, both the beam point and intensity stability are also important since a very good alignment of the light beam is always crucial for normal static Laser-Light Scattering measurement.

2.5.2 Cell design

The mechanical parts of our Laser Light Scattering instrument is called goniometer including a cell holder and an co-axial and accurately angular-controlled rotatable arm on which the fast and sensitive avalanche photo diode (APD) detector is located.

The cell holder consists of a hollow Teflon block with an inside diameter of 17 mm to hold the small scattering cuvette. The block is placed inside an index matching vat cylinder polished in cylinder-lens quality and filled with a refractive index fluid (toluene) whose refractive index close to that of cuvette glass. Thus the surface scattering and the curvature of the scattering cuvette could be reduced. The temperature of index matching vat is precisely controlled by water circulation form a thermostat.

2.5.3 Detector

A high quantum efficiency avalanche photo diode (APD) detector in Geiger mode is used. APD detector has a higher photon count rate than a conventional photo multiplier tube (PMT), leading to a faster and more sensitive response to photon irritations. Modern APDs normally have a maximum performance in the wavelength
range of 600 nm to 750 nm, even can reach an overall quantum efficiencies of 70 percent at 633 nm which is very suitable for the light source we are using. The output signal is then treated by amplifier before it is connected to the multiple tau digital time correlator situated in a PC computer. The APDs show a very low dark count contribution and response to signal pulse quickly enough for dynamic light scattering sampling. The rotatable arm makes it possible for the APD detector to get both dynamic and static data at different angles.

2.5.4 Differential refractometer

The specific refractive index increment \((dn/dC)\) is an important parameter in static Laser Light Scattering measurement. From the definition of \(K\) in basic equation of static Laser Light Scattering, the relative error in \((dn/dC)\) would lead to a double error of molar mass calculated. Therefore, precise determination of \((dn/dC)\) value is a necessary. In our refractometer, a small pinhole with a diameter 400\(\mu\)m is illuminated with laser light. The illuminated pinhole is imaged to a position sensitive detector by a lens located at an equal distance \((2f-2f)\) form the pinhole and the detector. A temperature-controlled refractometer cuvette is located in front of the lens. When sample was injected into the cuvette, the displaced light beam refracted at the boundary between the sample and reference liquid from the center of the detector was transferred into output voltage and measured by a digital voltmeter.

Since the laser source of the set up can be changed, by choosing a laser source with the same wavelength of the source used in Laser Light Scattering experiment, correction is no needed for the refractive index increment measured. This novel design has made the measurement of \(\Delta n\) much easier and provides reliable and accurate values of \((dn/dC)\) from the instrument’s stability.
2.6 References


Chapter 3

The effect of comonomer distribution on the coil-to-globule transition of a single AB copolymer chain in dilute solution

3.1 Introduction

For a long time, protein folding has attracted much attention and still remains as a mystery. Protein chains contain hydrophobic, hydrophilic and/or charged amino acid residues. The intra- and inter-chain hydrogen bonding and other interaction lead to some complicated bio-active structures. Different theories were proposed to explain various properties of proteins from a biological point of views. Recently, computer simulation was also used to construct copolymers with hydrophobic and hydrophilic units to imitate proteins. Particularly, the coil-to-globule transition of different types of copolymer chains was simulated to demonstrate how the comonomer distribution, i.e., the sequence difference in structure, could greatly influence the folding of a single copolymer chain.

Khokhlov et al. simulated three AB copolymer chains with an identical composition and length, but different comonomer distributions on the chain backbone. Their results showed that for the chain with a globular protein-like structure in which soluble comonomer B was incorporated on the periphery of a collapsed A chain backbone, the chain folding would be easier than that of a random copolymer without a designed sequence. Moreover, the resultant globule was stable and its chain density was higher. The simulation suggested that such a chain could ‘memorize’ or ‘inherit’ some special functional properties of the parent collapsed state. Timoshenko et al. also showed that for a given degree of amphiphilicity, the folding of a AB copolymer chain with a segmented comonomer distribution was
easier and the resultant mesoglobular phase was more stable in comparison with a random copolymer chain under the same condition.

However, it is a rather difficult experimental challenge, if not impossible, to prepare a pair of AB copolymers with a similar composition and chain length, but different comonomer distributions on the chain backbone. It has been well-known that poly(N-isopropylacrylamide) (PNIPAM) is a thermally sensitive polymer with a lower critical solution temperature (LCST \( \sim 32 \) °C).\(^{10-14}\) This interesting thermal property has made PNIPAM a simple model for the simulation of protein denaturation in aqueous solution even though real proteins are much more complicated.\(^{15}\)

Using two PNTPAM-g-PEO prepared at temperatures below and near the LCST of PNIPAM, Virtenen \textit{et al.}\(^{16}\) studied the comonomer distribution dependence of their chain aggregation. The result showed that the copolymer chains prepared at different temperatures possessed different lower critical solution temperatures, which supports the computer simulation performed by Khokhlov in a sense.

However, we should not forget that the chain aggregation is a complicated process, which involves the intrachain contraction and the interchain association. Therefore, the ultimate test and experimental challenge would be the study of the effect of comonomer distribution on the coil-to-globule transition (folding) of individual copolymer chains without involving any interchain aggregation in dilute solution.

From our experience in the preparation of narrowly distributed long PNIPAM homopolymer chains and the study of the coil-to-globule transition of a single homopolymer chain in dilute solution, we are able to use the difference between the chain conformations at different temperatures to incorporate a second monomer into PNIPAM to obtain different sequences. In this study, hydrophilic vinyl pyrrolidone (VP) was copolymerized into PNIPAM at 30 °C and 60 °C, which are, respectively,
below and above the LCST of PNIPAM homopolymer. It was expected that at 60 °C, hydrophilic comonomer VP would segregate on the periphery of the collapsed PNIPAM segments, while at 30 °C, the copolymerization would lead to a more random distribution of VP on the PNIPAM chain. At each temperature, the copolymers with two different VP/NIPAM ratios were prepared. A proper fractionation led to long narrowly distributed copolymer chains with a similar chain length and VP/NIPAM ratio, but different comonomer distributions. The coil-to-globule transitions of these copolymer chains in dilute aqueous solution were studied by a combination of static and dynamic laser light scattering (LLS) as well as microcalorimetry.
3.2 Experimental

3.2.1 Sample Preparation and characterization

N-isopropylacrylamide (NIPAM) was purified by recrystallization in a benzene/n-hexane mixture. 1-vinyl-2-pyrrolidone (VP) comonomer was distilled at reduced pressure prior to use. Potassium persulfate (KPS) was purified in a mixture of water and methanol. Other chemicals were used without purification. NIPAM-co-VP copolymers with 5 or 10 mol% of VP were, respectively, prepared at 30 °C and 60 °C by free radical polymerization in water, in which KPS/N,N,N',N'-tetramethylethylenediamine (TEMED) redox was used as initiator. Each copolymer was harvested by precipitation, i.e., pouring the reaction mixture into an equal volume of methanol. Each resultant copolymer was further purified by four cycles of redissolution in water and precipitation in methanol to ensure a complete removal of residual monomers. The final product was dried under reduced pressure at 40 °C. The copolymer was further fractionated by precipitation from acetone solution to n-hexane at the room temperature. Its structure was shown in Figure 3.1.

\[ \text{Figure 3.1 Poly}(N\text{-isopropylacrylamide}-\text{co-vinylpyrrolidone}) \]
In each case, only the first fraction was used in LLS measurement. $^1$H NMR (DPX 300 NMR spectrometer) was used to characterize the chain composition. The ratio of the peak areas of the methine proton of the isopropyl group in NIPAM and the two protons neighboring to the carbonyl group in VP was used to determine to the VP content. The results are summarized in Table 3.1.

Table 3.1 Characterization of poly($N$-isopropylacrylamide-co-vinylpyrrolidone)

<table>
<thead>
<tr>
<th>Samples</th>
<th>NIPAM-co-VP/60/5</th>
<th>NIPAM-co-VP/30/5</th>
<th>NIPAM-co-VP/60/10</th>
<th>NIPAM-co-VP/30/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{synthesis}} / ^\circ C$</td>
<td>60</td>
<td>30</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Area of $\delta_H$</td>
<td>$H^a$</td>
<td>0.874</td>
<td>0.875</td>
<td>0.775</td>
</tr>
<tr>
<td></td>
<td>$H^b$</td>
<td>0.042</td>
<td>0.042</td>
<td>0.075</td>
</tr>
<tr>
<td>$[\text{VP}] / \text{mol}%$</td>
<td>4.8</td>
<td>4.8</td>
<td>9.7</td>
<td>11.4</td>
</tr>
<tr>
<td>$M_w / (g/mol)$</td>
<td>$2.9 \times 10^6$</td>
<td>$4.2 \times 10^6$</td>
<td>$5.6 \times 10^6$</td>
<td>$7.9 \times 10^6$</td>
</tr>
<tr>
<td>$T_{\text{transition}} / ^\circ C$</td>
<td>33.5</td>
<td>35.0</td>
<td>36.0</td>
<td>37.5</td>
</tr>
<tr>
<td>$&lt;R_h&gt;_{\text{collapsed}} / \text{nm}$</td>
<td>22.9</td>
<td>28.7</td>
<td>31.2</td>
<td>45.7</td>
</tr>
<tr>
<td>$&lt;\rho&gt;_{\text{globule}} / (g/cm^3)$</td>
<td>$9.6 \times 10^{-2}$</td>
<td>$7.0 \times 10^{-2}$</td>
<td>$7.3 \times 10^{-2}$</td>
<td>$3.3 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

The composition of each copolymer was close to the feeding monomer ratio prior to the copolymerization. The nomenclature used hereafter for these copolymers is NIPAM-co-VP/x/y, where x and y are the copolymerization temperature ($^\circ C$) and the VP content (mol%), respectively. The solution with a concentration of $3.0 \times 10^{-6}$ g/mL was clarified with 0.45 μm Millipore Millex-LCR filter to remove dust before the LLS measurement. The resistivity of deionized water used was 18.0 MΩ cm.
3.2.2 Differential Scanning Calorimeter

The copolymer solutions were measured by a VP-DSC micro-calorimeter (MicroCal Inc) at an external pressure of ~180 kPa. The cell volume was 0.157 mL. The heating rate was 1.5 °C min⁻¹ and the instrument response time was set at 5.6 sec. All the DSC data were corrected for instrument response time and analyzed using the software in the calorimeter. The polymer concentration used in DSC was kept at 1.0 mg/mL.
3.3 Results and Discussion

Figure 3.2 shows typical angular dependence of Rayleigh ratio \([KC/R_{\nu}(q)]\) of the copolymer chains in water at temperatures, respectively, below and above the transition temperature. On the basis of Equation (2.2.1), the slope change as the temperature increases reflects the decrease of \(<R_g>\), i.e., the chain shrinking. The extrapolation of \(KC/R_{\nu}(q)\) at two different temperatures led to the same intercept, clearly indicating that there was no change in the weight average molar mass (\(M_w\)) during the shrinking process, or in other words, the process involves only individual chains.

![Figure 3.2](image)

**Figure 3.2** Scattering vector \((q)\) dependence of Rayleigh ratio \(R_{\nu}(q)\) of copolymer NIPAM-co-VP/60/5 in water, where \(K\) is a constant and copolymer concentration (C) was \(3.0 \times 10^{-6}\) g/mL.
Figure 3.3 directly shows the shrinking of the copolymer chains at a higher temperature in terms of the shift of the hydrodynamic radius distribution of the copolymer chains in water. For each given temperature, we could obtain one $<R_g>$ from the slope of $KC/R_{yy}(q)$ vs $q^2$ and one $<R_h>$ from via $\int_0^{\infty} f(R_h) R_h dR_h$.

![Figure 3.3](image)

**Figure 3.3** Temperature dependence of hydrodynamic radius distribution $f(R_h)$ of copolymer NIPAM-co-VP/60/5 in water, where copolymer concentration was 3.0 x 10^-6 g/mL.

Figure 3.4 shows the temperature dependence of both $<R_g>$ and $<R_h>$ of two copolymers synthesized at two different temperatures with a similar chain length and identical VP content (4.8 mol%).
As expected, both $<R_g>$ and $<R_h>$ decrease sharply during the transition, revealing the chain collapse at higher temperatures. Note that in each case, the average size of the collapsed chains remained nearly a constant even when the temperature was higher than 40 °C at which water is a very poor solvent for PNIPAM. It indicates that
such formed single-chain globules were stable. In contrast, our previous study showed that for PNIPAM homopolymer chains, stable single-chain globules could only be observed within a limited temperature range.\textsuperscript{12}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.5.png}
\caption{Temperature dependence of z-average root-mean square radius of gyration ($<R_g>$) and average hydrodynamic radius ($<R_h>$) of copolymers NIPAM-co-VP/60/10 and NIPAM-co-VP/30/11 in water.}
\end{figure}
The formation of such stable single-chain globules can be attributed to the existence of hydrophilic comonomer VP. This is why the copolymers with hydrophilic comonomer VP have a higher transition temperature than PNIPAM homopolymer. Similar result was also obtained for another pair of copolymer synthesized at two different temperatures with comparable chain length and identical VP content (~10 mol%) as shown in figure 3.5.

Table 3.1 summarizes the coil-to-globule transition temperatures and average hydrodynamic radii in the collapsed state for two pairs of NIPAM-co-VP copolymers with different VP contents. It is not surprising to see that the chains with a higher hydrophilic comonomer VP content have a higher transition temperature. However, it is rather interesting to see that for each pair of the copolymers with a similar VP content, the copolymer prepared at 60 °C has a lower transition temperature than its counterpart prepared at 30 °C. In order to make sure such a shift in the transition temperature, we also measured the partial heat capacity ($C_p$) of these copolymers in solution using micro-calorimeter. Figure 3.6 shows that for the two copolymers prepared at 60 °C, the temperatures at which the maximum heat capacity ($T_{max}$) occurs are indeed lower. Such a difference between the transition temperatures should be attributed to different comonomer distributions on the PNIPAM chain backbone because the copolymers in each pair have a similar chain length and composition.
Figure 3.6 Temperature dependence of partial heat capacity ($C_p$) of NIPAM-co-VP copolymers in water, where the increasing rate of the temperature was 1.5 °C/min and pressure was maintained at 180 kPa.

As we mentioned before, at lower temperatures, water is a good solvent for PNIPAM and the PNIPAM segments formed during the copolymerization exist as random coil. In this way, NIPAM and VP were copolymerized into the chain in a more random fashion to form a statistical copolymer. In contrast, water at 60 °C
becomes such a poor solvent that the PNIPAM segments formed must collapse and hydrophilic VP can only be incorporated into the chains on the periphery of the collapsed PNIPAM segments, leading to a segregation of VP, or in other words, a segmented comonomer distribution. Therefore, the average length of the PNIPAM segment between two neighboring VP segments has to be longer in comparison with a random copolymer chain with a similar VP/NIPAM ratio, as schematically shown in Figure 3.7.

![Figure 3.7](image-url)

**Figure 3.7** Schematic of different chain structures and the coil-to-globule transition of NIPAM-co-VP copolymers prepared at two temperatures, respectively, lower and higher than the lower critical solution temperature (LCST ~ 32 °C) of PNIPAM homopolymer.
The lower transition temperature indicates that the folding of the copolymer chains prepared at higher temperatures is easier, or in a sense, these chains could "memorize" their parent collapsed globular state.

The conformational change of a polymer chain can be better viewed in terms of the ratio of \(<R_g>/R_h>\) because for a coil chain and a uniform non-draining sphere, \(<R_g>/R_h> \approx 1.5\) and \((3/5)^{1/2}\), respectively.\(^\text{13}\) In Figure 3.8, the decrease of \(<R_g>/R_h>\) from ~1.65 to ~0.6-0.8 clearly shows the coil-to-globule of the copolymer chains.

![Figure 3.8](image)

**Figure 3.8** Temperature dependence of ratio of average radius of gyration to average hydrodynamic radius \(<R_g>/R_h>\) of copolymer NIPAM-co-VP with 4.8 mol% VP prepared at two different temperatures.
Just like PNIPAM homopolymer in water, before fully collapsing into a uniform dense globule, the copolymer chain with a coil conformation first crumples into a molten globule characterized by a value of $<R_g>/<R_h>$ smaller than $(3/5)^{1/2}$ predicted for a uniform sphere, i.e., the dip of $<R_g>/<R_h>$ at ~35-36 °C. It is worth noting that even in the fully collapsed state at ~40 °C, the single-chain globule made of NIPAM-co-VP/60/5 has a ratio of $<R_g>/<R_h>$ much smaller than 0.774 predicted for a uniform sphere. This suggests that the globule have an uneven density distribution inside, as previously observed by Khokhlov et al.\(^8\) in computer simulation. Similar result was also obtained for another pair of copolymer synthesized at two different temperatures with comparable chain length and identical VP content (~10 mol%) as shown in Figure 3.9.

![Figure 3.9](image)

**Figure 3.9** Temperature dependence of ratio of average radius of gyration to average hydrodynamic radius ($<R_g>/<R_h>$) of copolymer NIPAM-co-VP with ~10 mol% VP prepared at two different temperatures.
As we mentioned before, the average length of the PNIPAM segments on a chain prepared at 60 °C is longer. Therefore, the collapse of these long PNIPAM segments could force short hydrophilic VP segments out and stay on the periphery, leading to a core-shell structure with a denser PNIPAM core and a swollen VP shell presumably made of small loops. On the other hand, the random copolymer chains prepared at 30 °C lack such segregated VP segments so that the average length of the PNIPAM segments is much short. The collapse of these short PNIPAM segments at high temperatures inevitably pulls hydrophilic comonomer VP inside, resulting in a less compact, but more uniform, globule with a high ratio of $\langle R_g \rangle / \langle R_h \rangle$ similar to uniform latex particles.

The structural difference between the collapsed globules made of the chains prepared at different temperatures can also be evidenced in their size and density. Note that in Figure 3.4 and Table 3.1, when the chain is fully collapsed at ~40 °C, NIPAM-co-VP/60/5 has a smaller size than NIPAM-co-VP/30/5 even though they have a similar $\langle R_g \rangle$ and $\langle R_h \rangle$ in the coil state at lower temperatures. Therefore, the average densities ($<\rho>_{\text{globule}}$) of the single-chain globules made of the chains prepared at 60 °C are higher. The values of $<\rho>_{\text{globule}}$, defined as $M_w/(4\pi<R_h>N_A/3)$, are listed in Table 3.1. The difference in $<\rho>_{\text{globule}}$ also indirectly reflects that the copolymer chains prepared at higher temperatures can "memorize" their parent collapsed globule state and fold to a compact structure, confirming the computer simulation and prediction of Khokhlov et al.\textsuperscript{5} and Timoshenko et al.\textsuperscript{9} It should be stated that $<\rho>_{\text{globule}}$ listed in Table 3.1 is much lower than that of the single-chain globule made of PNIPAM homopolymer (~ 0.3 g/cm\textsuperscript{3}).\textsuperscript{13} This is reasonable because the incorporation of a few per cent of hydrophilic comonomer VP into PNIPAM retards its close packing during the transition.
Using the difference between the chain conformations at different temperatures, we are able to design and prepare a pair of poly(\(N\)-isopropylacrylamide-co-vinyl pyrrolidone) (NIPAM-co-VP) copolymers with a similar composition and chain length, but different comonomer VP distributions on the chain. Especially, the copolymerization of hydrophilic VP into a PNIPAM chain at its collapsed temperature can result in a globular protein-like segmented VP distribution. The study of the temperature-induced coil-to-globule transition of these specially engineered copolymer chains have confirmed recent computer simulations; namely, the folding of a \(AB\) copolymer chain with a globular protein-like structure is easier than the folding of a random \(AB\) copolymer chain with a similar composition and chain length.

Our results also indicate that during the coil-to-globule transition, the protein-like chains can fold into a compact core-shell structure with a higher average chain density at high temperatures. Presumably, the core is made of collapsed long PNIPAM segments and the shell consists of swollen short VP segments in the form of small loops. In a sense, the copolymer coil chain formed in the collapsed state can “memorize” or “inherit” its parent globular structure when the hydrophobic interaction (attraction) between the PNIPAM segments is switched on at higher temperatures. It should be emphasized that the copolymer chains studied here are much simple than proteins. However, this study is one step forward in a long journey towards a better understanding of the protein folding.
3.4 References


Chapter 4

Formation of mesoglobular phase of amphiphilic copolymer chains in dilute solution: 1. Effect of comonomer distribution

4.1 Introduction

It is well-known that polypeptide or protein chains can assemble into different ordered and stable quaternary structures without macroscopic precipitation under a proper condition. Such a limited aggregation induced by a variation of experimental conditions, such as temperature, salt concentration, pH, and cosolvent, has attracted much attention. For example, Aymard et al. showed that the presence of some specific restructureable protein binding sites can initiate the association of proteins when electrostatic interaction is properly screened out. On the other hand, it is also known that the interior of a biological cell could be extraordinary crowded and contains different kinds of proteins. How they are packed inside is still not completely understood at this moment. In general, these special properties have been attributed to their native states and specific amino acid sequences generated from different genetic codes.

In polymer researches, amphiphilic copolymers prepared from hydrophilic and hydrophobic monomers with different comonomer distributions are often used to mimic protein chains, especially in computer simulation. For example, Timoshenko et al. studied the collapse and aggregation of copolymers simulated with an identical comonomer composition, but different comonomer distributions on the chain backbone. For a given composition, statistical random and block (diblock or triblock) copolymers are two extreme cases. Their association in selective solvents have been extensively studied. In contrast, the association of segmented amphiphilic
copolymers in solution is much less understood. The simulation results of Timoshenko et al.\textsuperscript{7-9} showed that even without any added stabilization, a limited number of neutral copolymer chains with a proper comonomer distribution could associate to form narrowly distributed stable mesoglobules existing between single-chain collapsed globules and macroscopic precipitates. The average aggregation number increased with the length of hydrophobic segment. This is because the activation energy of moving away from the mesoglobular phase is so high that the aggregates can be stable for a long time after they are trapped in such a metastable state. However, the formation of such a stable mesoglobular phase has not been experimentally seriously established.\textsuperscript{7}

Experimentally, the preparation of a pair of copolymers with a similar comonomer composition and chain length, but different comonomer distributions, is rather difficult. However, the attempt in this direction has never stopped. Volpert et al.\textsuperscript{10,11} prepared a series of acrylamide polymers modified with a small amount of alkylamides by micelle copolymerization. Their results showed that for a given copolymer concentration, the solution of a segmented copolymer was more viscous than that of a statistical random copolymer. It is generally known that the distribution of hydrophobic comonomers on an amphiphilic copolymer chain can greatly affect its thickening ability, but the detail is missing. It is known that statistical random and block copolymers as two extreme cases are not as effective as a segmented copolymer with the same chain composition and length in thickening a solution. Then, what the optimal length of hydrophobic segments is for a given chain composition and length? Such a question is not only a scientific curiosity, but also important for industrial applications because it is necessary to keep the effect/cost ratio higher.
Besides the effect of comonomer distribution, the rate of changing solvent quality from good to poor in the phase transition also influence the formation and structure of mesoglobules. Peng et al.\textsuperscript{12} experimentally revealed a competition between interchain association and intrachain contraction in a laser light scattering study of the calcium-induced aggregation of poly(acrylamide-co-sodium acrylate) in water. Such a competition can be off balanced by the rate of changing solvent quality or by dilution. Our previous study showed that individual poly(N-isopropylacrylamide-co-vinyl pyrrolidone) (P(NIPAM-co-VP)) chains can undergo a coil-to-globule transition to form collapsed single chain globules stable in a very dilute solution even at temperatures much higher than its lower critical solution temperature (LCST, \textasciitilde36 °C) and the copolymer chains prepared at higher temperatures can ‘remember’ their parent collapsed state.\textsuperscript{13} In the present study, we focused on whether neutral amphiphilic copolymer chains can form a predicted stable mesoglobular phase instead of macroscopic precipitation and how the comonomer distribution affect the formation of such mesoglobules.
4.2 Sample preparation and characterization

N-isopropylacrylamide (NIPAM) was purified by recrystallization in a benzene/n-hexane mixture. 1-vinyl-2-pyrrolidone (VP) comonomer was distilled at reduced pressure prior to use. Potassium persulfate (KPS) was purified in a mixture of water and methanol. Other chemicals were used as received. P(NIPAM-co-VP) copolymers with 5 or 10 mol% of VP were, respectively, prepared at 30 °C and 60 °C by free radical polymerization in water. KPS/N,N,N',N'-tetramethylethylenediamine (TEMED) redox was used as initiator. Each copolymer was harvested by precipitation, i.e., pouring the reaction mixture into an equal volume of methanol. Each resultant copolymer was further purified by four cycles of re-dissolution in water and precipitation in methanol to remove residual monomers. The final product was dried under reduced pressure at 40 °C. The copolymer was further fractionated by precipitation from a mixture of acetone solution and n-hexane at the room temperature. The chain composition was characterized by $^1$H NMR (DPX 300 NMR spectrometer). The ratio of the peak areas of the methine proton of the isopropyl group in NIPAM and the two protons neighboring to the carbonyl group in VP was used to determine the VP content. The results are summarized in Table 3.1. It shows that the composition of each copolymer was close to the feeding monomer ratio prior to the copolymerization. The nomenclature used hereafter for these copolymers is NIPAM-co-VP/x/y, where x and y are the copolymerization temperature (°C) and the VP content (mol%), respectively. All the solutions with a concentration of $4.0 \times 10^{-5}$ g/mL were clarified with 0.45 μm Millipore Millex-LCR filter to remove dust before the LLS measurement. The resistivity of deionized water used was 18.0 MΩ cm.
4.3 Results and Discussion

Figure 4.1 shows a typical intrachain collapse of the coil-to-globule transition of individual PNIPAM homopolymer and P(NIPAM-co-VP) copolymer chains in an extremely dilute aqueous solution.

Figure 4.1 Typical temperature dependence of relative average hydrodynamic radius of individual PNIPAM homopolymer and P(NIPAM-co-VP) copolymer chains in water during the coil-to-globule transition, where $C = 6.7 \times 10^{-7}$ g/mL and $3.0 \times 10^{-6}$ g/mL, respectively.

Note that the lower critical solution temperature (LCST) shifts to a higher temperature and the extent of chain contraction decreases after the incorporation of a few mol% of VP comonomer. This is understandable because VP is hydrophilic in the temperature range studied. Figures 4.2 and 4.3 show the temperature dependence of the association of P(NIPAM-co-VP) copolymers in water in terms of the changes of weight average molar mass ($M_w$) and average hydrodynamic radius ($<R_h>$) of the
resultant copolymer aggregates. Both $M_w$ and $<R_h>$ increase as the time elapses and approach corresponding constants after a certain time. Such formed aggregates were stable for a long time, indicating that the interchain association was stopped at a certain stage.

![Diagram showing the time dependence of weight average molar mass ($M_w$) of mesoglobules formed at different aggregation temperatures.](image)

**Figure 4.2** Time dependence of weight average molar mass ($M_w$) of mesoglobules formed at different aggregation temperatures, where $C = 4.0 \times 10^{-5}$ g/mL.

A comparison of Figures 4.2 and 4.3 shows that $<R_h>_{t \rightarrow \infty}$ decreases as the aggregation temperature ($T_{\text{aggregation}}$) increases, while $(M_w)_{t \rightarrow \infty}$ increases when $T_{\text{aggregation}} < \sim 37 \, ^{\circ}C$, but increases when $T_{\text{aggregation}} > 37 \, ^{\circ}C$. The fact that stable aggregates formed at $36 \, ^{\circ}C$ have a smaller $M_w$, but a larger $<R_h>$, clearly indicates that they have a loose structure. This is because the copolymer chains are only partially collapsed at $36 \, ^{\circ}C$. 

55
Figure 4.3 Time dependence of average hydrodynamic radius ($<R_h>$) of mesoglobules formed at different aggregation temperatures, where $C = 4.0 \times 10^{-5}$ g/mL.

Figure 4.4 Typical hydrodynamic radius distributions ($f(R_h)$) of resultant stable NIPAM-co-VP/60/5 mesoglobules formed at different aggregation temperatures.
Figure 4.4 shows that resultant stable aggregates are narrowly distributed. Similar results were also obtained for other three P(NIPAM-co-VP) copolymers. It also shows that these aggregates are relatively small, implying that they are made of a limited number of chains. It is helpful to note that no precipitation was observed even after a long time, reflecting in no change in the scattering intensity. As for the structural information of these aggregates, we examined the ratio of the radius of gyration to hydrodynamic radius $<R_g>/<R_h>$, as shown in Figure 4.5.

![Graph showing $<R_g>/<R_h>$ vs. $T_{aggregation}$](image)

**Figure 4.5** Aggregation temperature dependence of ratio of average radius of gyration to average hydrodynamic radius ($<R_g>/<R_h>$) of resultant stable mesoglobules made of different copolymers.

The data points are scattered due to experimental uncertainties, especially in the measurement of $<R_g>$ for large aggregates. However, the decrease of $<R_g>/<R_h>$ from ~1.5-1.7 to ~0.8 reveals a change from random-coil chains to uniform spherical aggregates, i.e., mesoglobules. The mesoglobules made of NIPAM-co-
VP/30/11 have the highest ratio of $<R_g>/<R_b>$. This is because NIPAM-co-VP/30/11 have the highest hydrophobic VP content and a random distribution of VP on the chain backbone so that its contraction is hindered. We will come back to this point later. The formation of such stable mesoglobules is in analog with what described by Timoshenko et al.\textsuperscript{3,5} Only after confirming the formation of stable mesoglobules, we can turn our attention to the effect of comonomer distribution.

Figure 4.6 shows the temperature dependence of the average aggregation number ($N_{\text{chain}}$) of the mesoglobules made of different P(NIPAM-co-VP) copolymers, where $N_{\text{chain}}$ was obtained from the ratio of the weight average molar masses of the resultant stable mesoglobules and individual copolymer chains.

![Figure 4.6](image_url)

**Figure 4.6** Aggregation temperature dependence of average aggregation number ($N_{\text{chain}}$) of resultant stable mesoglobules made of different copolymers, where $N_{\text{chain}}$ is defined as $M_{w, \text{mesoglobule}}/M_{w, \text{chain}}$.

Note that for the copolymers with 10 mol\% VP, $N_{\text{chain}}$ reaches its maximum at a
higher temperature because they are more hydrophilic. For each pair of copolymers with a similar VP content, the mesoglobules made of the copolymer chains prepared at 60 °C have a higher $N_{\text{chain}}$. On the other hand, for each pair of copolymers prepared at the same temperature, the copolymer with a higher VP content has an expected smaller $N_{\text{chain}}$ because the average length of the PNIPAM segments is shorter and the copolymers are more hydrophilic.

It is helpful to note that the copolymers prepared at 60 °C have a less random distribution of comonomer VP because most of hydrophilic VP monomers are copolymerized on the periphery of long PNIPAM segments collapsed at 60 °C during the reaction. In other words, the copolymer chains prepared at 60 °C have a more segmented structure in comparison with those prepared at 30 °C. In this way, for a given VP content, the average length of the PNIPAM segments between two neighboring VP segments should be longer than that of a statistic random copolymer prepared at 30 °C. It is expected that at high temperatures NIPAM-co-VP/60/y copolymers with longer PNIPAM segments can provide a stronger hydrophobic interaction, resulting in larger aggregates.

Another feature of Figure 4.6 is the initial sharp increase of $N_{\text{chain}}$ followed by a gradual decrease. Note that for each copolymer, the temperature at which $N_{\text{chain}}$ reaches its maximum roughly corresponds to the temperature at which individual copolymer chains reach their fully collapsed states. It is easy to understand the increase of $N_{\text{chain}}$ with the aggregation temperature because long PNIPAM segments become more and more hydrophobic. Before reaching the collapse temperature, less compact chains can interpenetrate with each other so that the interchain association is dominate. At higher aggregation temperatures, intrachain contraction becomes dominate and short hydrophilic VP segments tend to stay on the periphery of long
collapsed PNIPAM segments to minimize the interfacial energy. In this way, the interchain association is retarded, which explains why \( N_{\text{chain}} \) decreases in the high aggregation temperature range.

Thermodynamically, the formation of stable mesoglobules instead of macroscopic precipitation requires a dedicate balance between enthalpic and entropic contributions. In comparison with macroscopic precipitation, the existence of many small mesoglobules must gain in both translational entropy (\( \Delta S > 0 \)) and interfacial energy (\( \Delta H > 0 \)). For homopolymers in a poor solvent, the gain of \( \Delta H \) is always larger than \( T\Delta S \), i.e., \( \Delta G = \Delta H - T\Delta S > 0 \). Therefore, the equilibrium moves towards the direction of forming macroscopic precipitation. For amphiphilic copolymers in a selective solvent (often water), a microphase separation can occur, in which the association of hydrophobic segments leads to intrachain contraction and interchain aggregation, but hydrophilic segments tend to stay on the periphery. Under a proper condition, the gain of \( T\Delta S \) can offset that of \( \Delta H \), i.e., \( \Delta G = \Delta H - T\Delta S < 0 \), so that further interchain aggregation stops. It is expected that more hydrophilic groups on the periphery would lead to smaller mesoglobules. However, due to the chain connectivity, a perfect arrangement to expose all hydrophilic VP components on the periphery is impossible. For a given type of copolymers with a similar composition, longer hydrophobic (i.e., short hydrophilic) segments should make the arrangement easier. As discussed before, the copolymer synthesized at 60 °C has longer PNIPAM segments than its counterpart synthesized at 30 °C for a given comonomer composition. Longer PNIPAM segments at high temperatures provides a stronger hydrophobic attraction so that the copolymer chains prepared at 60 °C have a lower LCST and a higher \( N_{\text{chain}} \) than its counterpart prepared at 30 °C.

On the other hand, we should consider the kinetic and viscoelastic effects on the
formation of mesoglobules. Once the temperature is raised, the copolymer chains simultaneously undergo intrachain contraction and interchain association. The relative length of the interaction (contact) time ($\tau_C$) of two approaching aggregates and the relaxation time ($\tau_R$) of copolymer chains inside each aggregate will determine whether they can be fused together to form a larger aggregate. Only when $\tau_C > \tau_R$, the merging of two colliding aggregates becomes possible. Otherwise, two aggregates will act as two elastic glass balls and bounds away after the collision.

The hydrophilic VP segments on the periphery reduce the interaction time, while the intrachain contraction increases the local concentration inside the aggregates, resulting in a longer relaxation time. A quick increase of the solution temperature above the coil-to-globule transition temperature promotes the intrachain contraction and suppresses interchain association. This is why $N_{\text{chain}}$ decreases as the aggregation temperature increases in the range of temperatures higher than the LCST. As shown in Figures 4.2 and 4.3, $M_w$ and $<R_g>$ nearly instantly reach their stable values after the solution temperature was raised over 40 °C. In contrast, at lower aggregation temperatures, it took a much longer time (~10 hrs) to form stable mesoglobules because individual partially contracted copolymer chains have much chance to associate with each other. On the other hand, dilution is another way to reduce interchain association.

The competition between intrachain contraction and interchain association can be better viewed from the temperature dependence of the average hydrodynamic radius $<R_h>$, as shown in Figure 4.7. A comparison of Figures 4.6 and 4.7 shows that such a temperature dependence can be roughly divided into three regions. In the lower temperature range, where $N_{\text{chain}}$ remains constant (~1), $<R_h>$ decreases as the solution temperature increases, reflecting the contraction of individual chains. In the
middle temperature range, \( N_{\text{chain}} \) and \( <R_h> \) increase before reaching their maximum values, showing that interchain association becomes dominate. In the higher temperature range, both \( N_{\text{chain}} \) and \( <R_h> \) decrease as the aggregation temperature increases.

![Graph showing the aggregation temperature dependence of average hydrodynamic radius (\( <R_h> \)) of resultant stable mesoglobules made of different copolymers.](image)

**Figure 4.7** Aggregation temperature dependence of average hydrodynamic radius (\( <R_h> \)) of resultant stable mesoglobules made of different copolymers.

It should be noted that the decrease of \( <R_h> \) in the lower and higher temperature ranges are caused by completely different reasons. In the higher aggregation temperature range, intrachain contraction happens prior to interchain association. The higher the aggregation temperature the faster the contraction rate. Therefore, individual collapsed copolymer chains have a much less chance to undergo interchain association. This is why both \( N_{\text{chain}} \) and \( <R_h> \) decrease in this region.

**Figure 4.8** shows the temperature dependence of the average chain density (\( <\rho> \)) of resultant stable mesoglobules, where \( <\rho> \) is defined as \( M_w/(4\pi<R_h>^3N_A/3) \). For
all the copolymers studied, $\langle \rho \rangle$ always increases with the aggregation temperature. Noted that intrachain folding normally results in a lower chain density than interchain penetration because the chains are not infinitely flexible. For the copolymer pair, NIPAM-co-VP/60/10 and NIPAM-co-VP/30/11, the average chain density of NIPAM-co-VP/60/10 mesoglobules is higher because the copolymer prepared at 60 °C have longer PNIPAM segments and tend to form stronger interchain association as discussed before. However, for the copolymer pair with a lower VP content, the average chain density of NIPAM-co-VP/60/5 mesoglobules is lower. On the basis of our previous study, we know that the coil-to-globule transition of individual NIPAM-co-VP/60/5 chains is easier. The lower chain density reflects that NIPAM-co-VP/60/5 mesoglobules consist of many small collapsed single-chain globules, i.e., intrachain contraction is dominate in the formation of the mesoglobular phase.

Figure 4.8 Aggregation temperature dependence of average chain density ($\langle \rho \rangle$) of resultant stable mesoglobules made of different copolymers, where $\langle \rho \rangle$ is defined as $M_w/(4\pi \langle R_b \rangle^3 N_A/3)$.
Poly(N-isopropylacrylamide-co-vinyl pyrrolidone) (P(NIPAM-co-VP)) copolymers are amphiphilic at higher temperatures. Using two pairs of Poly(N-isopropylacrylamide-co-vinyl pyrrolidone) copolymers with a similar chain composition and length, but different VP distributions, we have confirmed that a limited number of such amphiphilic copolymer chains can associate to form stable mesoglobules existing between single-chain collapsed globules and mesoscopic precipitates, even though these chains are neutral and no stabilizer is added. Further, we have shown that the comonomer VP distribution on the chain can greatly influence the formation of such a mesoglobular phase. The degree of amphiphilicity increases with the aggregation temperature, resulting in a competition between intrachain contraction and interchain association. Such a competition depends on the chain composition as well as the rate of changing the solution temperature. Specifically, our results indicate that the copolymers with a segmented VP distribution have a higher tendency to undergo interchain association. When intrachain contraction happens fast and prior to interchain association, smaller mesoglobules are formed. A proper adjustment of the rates of intrachain contraction and interchain association can lead to a desired particle size. This study provides a different view of the formation of polymeric nanoparticles in dispersion as well as a model system to imitate the aggregation of proteins-like chains in solution.
4.4 References

Chapter 5

Formation of mesoglobular phase of amphiphilic copolymer chains in dilute solution: 2. Effect of comonomer distribution

5.1 Introduction

The self-assembly of amphiphilic block copolymers have been extensively studied in recent years because of its fundamental importance and industrial and biomedical applications. The self-assembly in a selective solvent normally results in spherical polymeric micelles with different sizes and structures in dilute solution. Such formed micelles are commonly used in drug delivery, detergency and emulsification. For instance, poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) (PEO-PPO-PEO) can form micelles in warm water and one of its potential application is to protect cells from drugs. However, most of commercially available amphiphilic copolymers are prepared by a random polymerization of two or more kinds of monomers with different chemical natures and physical properties. The association of these random copolymers in a selective solvent has also been extensively studied, but is much less understood, because of its complicate nature. For example, what is the effect of comonomer composition and comonomer distribution on their association behaviors? On the other hand, some of biopolymers, such as proteins, can generally be considered as amphiphilic copolymers. Their special sequence of amino acids often leads to specified folding/association in biological system.

It has been known that association of copolymers in solution can be induced by varying a range of experimental conditions, such as solvent, polymer concentration, ionic strength and pH. Particularly, for a thermally sensitive copolymer, in which at
least one monomer component has a lower or higher critical solution temperature (LCST or UCST), we can conveniently use temperature to adjust the degree of its amphiphilicity. For examples, Miyazaki et al. synthesized a series of poly(N,N-dimethylacrylamide-co-N-phenyl-acrylamide) (P(DMA-co-PA)) with different amounts of PA by free radical copolymerization. They showed that the LCST of such copolymers determined from a sharp change of the solution turbidity was controllable and decreased with increasing the hydrophobic PA content. The transition was thermally reversible.

Qiu et al. studied poly(N-isopropylacrylamide-co-acrylic acid) P(NIPAM-co-AA)) with different amounts of ionic AA. They also showed that the controllable LCST increased with increasing the hydrophilic AA content. Moreover, they demonstrated that the copolymer with a very small amount of ionic AA could form stable nanoparticles in water at temperatures higher than its LCST. The stabilization was attributed to static repulsion from anionic AA groups on the periphery of such nanoparticles. Itakura et al. studied the solvent composition dependence of the association of poly(N,N-dimethylacrylamide)-graft-poly(methyl-methacrylate) (PDMA-g-PMMA) in a methanol-and-water mixture and found that a quick change of solvent quality from good to poor could lead to smaller aggregates with a unimodal size distribution, while slowly changing the solvent quality resulted in larger aggregates with a bimodal size distribution. Qian et al. studied the association of ethylene-vinylacetate (EVA) random copolymers with a UCST in two different thermal processes. If the solution was quickly quenched from 70 °C to 0 °C, the aggregates were mainly made of individual collapsed chains. However, when the solution was slowly cooled from 70 °C to room temperature and annealed for 24
hours before further quenching to 0 °C, large microgels and fiber-like aggregates were formed via the interchain winding.

The examination of literature shows that the formation of stable aggregates made of neutral copolymer chains without any stabilizer has been proposed and predicted, but not been experimentally seriously established yet. The effect of comonomer composition on the chain association have not been systematically investigated. This is partially because this kind of studies require a serious chemical synthesis to control all the parameters so that only the comonomer composition is varied. In the present study, two series of thermally sensitive random copolymers, poly(N,N-diethylacrylamide-co-N-ethylacrylamide) P(DEA-co-EA) and poly(N,N-diethylacrylamide-co-N,N-dimethylacrylamide) P(DEA-co-DMA) with a similar chain length, but different EA and DMA contents were synthesized. PDEA homopolymer changes from hydrophilic to hydrophobic when the temperature increases higher than 32 °C, while PEA and PDMA homopolymers remain hydrophilic as long as the temperature is not higher than ~82 °C. Heating the solution temperature to the range 32 °C - 82 °C makes these copolymers become amphiphilic so that they can associate in water through hydrophobic interaction between the PDEA segments. The association of these copolymers in water under different heating rates was studied by laser light scattering (LLS).
5.2 Sample preparation and characterization

Poly(N,N-diethylacrylamide-co-N-ethylacrylamide) P(DEA-co-EA) and poly(N,N-diethylacrylamide-co-N,N-dimethylacrylamide) P(DEA-co-DMA), respectively, with 40, 60 and 80 mol% of EA and 50 and 70 mol% of DMA were synthesized by free radical polymerization. P(DEA-co-EA) copolymers were prepared in THF with 2,2'-azobis(2-methylpropionitrile) (AIBN) as initiator (1 mol%). The solution was bubbled with dry nitrogen for 15 minutes prior to polymerization. The temperature was gradually raised to 68 °C in a period of 2 hrs. and maintained for ~18 hrs. Each reaction mixture was precipitated in ether or hexane. P(DEA-co-DMA) copolymers were prepared in methanol in a similar fashion. The copolymer compositions determined by $^1$H NMR spectra were very close to the feed ratio of monomers prior to polymerization. The nomenclature used hereafter for these copolymers is P(DEA-co-x/y), where x is EA or DMA and y denotes the mol% content of x. Before the association study, the copolymers were characterized by laser light scattering and the results are summarized in Table 5.1. Their structures were shown in Figure 5.1 and 5.2. The copolymer solutions (6.0 x 10$^{-4}$ g/mL) were clarified with 0.45 μm Millipore Millex-LCR filter to remove dust before the LLS measurement. The resistivity of deionized water used was 18.0 MΩ cm.

In the fast heating process, the copolymer solution at ~25 °C was placed in the LLS cell holder which was pre-heated to a desired temperature with a precision of ± 0.05 °C. Time dependence of the scattering light intensity and intensity-intensity time correlation function were measured during the association. Note that cooling the solution down to 4 °C can completely redissolve the association. In the slow heating process, the copolymer solution was heated slowly from 25 °C to the desired
temperature inside the LLS cell holder at a rate of 0.3 °C/min. LLS measurement started only after the solution temperature reached the desired temperature.

Table 5.1 Laser light scattering characterization of P(DEA-co-EA) with 40, 60 and 80 mol% of EA and P(DEA-co-DMA) with 30 and 50 mol% of DMA

<table>
<thead>
<tr>
<th>Samples</th>
<th>$M_w$ / (g/mol)</th>
<th>$&lt;R_i&gt;$/ nm</th>
<th>$T_{LCST}$/ °C</th>
<th>$T_{aggregation}$/ °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEA-co-DMA/30</td>
<td>$1.24 \times 10^5$</td>
<td>12.5</td>
<td>~45</td>
<td>43.2</td>
</tr>
<tr>
<td>DEA-co-DMA/50</td>
<td>$1.45 \times 10^5$</td>
<td>15.3</td>
<td>~58</td>
<td>58.8</td>
</tr>
<tr>
<td>DEA-co-EA/40</td>
<td>$1.70 \times 10^4$</td>
<td>5.6</td>
<td>~35</td>
<td>35.7</td>
</tr>
<tr>
<td>DEA-co-EA/60</td>
<td>$1.49 \times 10^4$</td>
<td>5.8</td>
<td>~44</td>
<td>41.9</td>
</tr>
<tr>
<td>DEA-co-EA/80</td>
<td>$2.11 \times 10^4$</td>
<td>9.6</td>
<td>~55</td>
<td>55.5</td>
</tr>
</tbody>
</table>

Figure 5.1 Poly($N,N$-diethylacrylamide$\text{-co-N}$-ethylacrylamide)

Figure 5.2 Poly($N,N$-diethylacrylamide$\text{-co-N}$-dimethylacrylamide)
5.3 Results and Discussion

Figures 5.3 and 5.4 show that for each copolymer studied, the average aggregation number \( N_{\text{chain}} \) and average hydrodynamic radius \(<R>\) of the P(DEA-co-DMA/y) aggregates increase and then approach corresponding constants after a certain time, indicating the formation of stable aggregates.

![Figure 5.3](image)

**Figure 5.3** Time dependence of average aggregation number \( N_{\text{chain}} \) of P(DEA-co-DMA) mesoglobules formed under different heating rates.

It should be addressed that these aggregates were so stable that no change in the scattering intensity was observed over months. It is also helpful to note that the stabilization in water was reached without the addition of any ion or surfactant. The formation of such stable aggregates has been described as the mesoglobular phase in which a limited number of chains are associated together to form stable colloid particles existing between single-chain collapsed globules and macroscopic
The stabilization is due to the concentration of hydrophilic DMA segments on the periphery of the aggregates during microphase separation.

**Figure 5.4** Time dependence of average hydrodynamic radius ($<R_h>$) of P(DEA-co-DMA) mesoglobules formed under different heating rates.

**Figure 5.5** Typical hydrodynamic radius distributions ($f(R_h)$) of resultant P(DEA-co-DMA) mesoglobules formed under different heating rates.
The resultant stable mesoglobules are narrowly distributed with a relative width less than 0.05, as shown in Figure 5.5. This is understandable because the association is an average process. On the other hand, such narrow distributions imply that the association is less random and probably follows a special path. A combination of Figures 5.3 and 5.4 shows that for a given heating process, \( N_{\text{chain}} \) increases with the DMA content. On the other hand, for a given copolymer, the fast heating results in a much small \( N_{\text{chain}} \) with a slightly larger size, indicating that they must have a loose structure. It can be seen that at the very initial stage of the microphase transition, \( N_{\text{chain}} \) remains a constant, but \( <R_b> \) slightly decreases in the fast heating process, reflecting that intrachain contraction appears before interchain association. As expected, intrachain contraction must force the hydrophilic DMA segments to stay on the periphery to minimize the surface energy and slow down interchain association, resulting in a slower kinetics and smaller mesoglobules presumably consisting of many loosely packed small single- or pauci-chain collapsed globules. It is helpful to note (not straightforward) that intrachain contraction (‘folding’) can lead to a chain density lower than interchain aggregation (‘interpenetration’) because no polymer chain is infinitely flexible. This partially explains why the mesoglobules formed in the fast heating have a lower chain density. Such a lower chain density can also be viewed from the scaling between the scattered light intensity \( (I) \) and the scattering vector \( (q) \) for resultant stable mesoglobules formed in different heating process (Figure 5.6).

It has been known that the scaling exponent \( \alpha \) in \( I \propto q^{-\alpha} \) is the fractal dimension in the scaling between molar mass and size, i.e., \( M \propto R^2 \). The increase of \( \alpha \) from 1.8-1.9 to 2.2 indicates that the association changes from a diffusion-limited process to a reaction-limited process. In a reaction-limited process, many collisions only
results in a sticking (association), while in a diffusion-limited process, each collision leads to a sticking. Therefore, in a reaction-limited process, each coming particles or clusters has much higher chance to penetrate into the ‘fiords’ of the existing aggregates before they stick together,\(^{13}\) which results in a higher chain density. This explains why \(\alpha\) is higher for the mesoglobules formed in the slow heating.

\[\begin{align*}
\text{Slope} &= 1.8 \pm 0.1 \\
\text{Slope} &= 1.9 \pm 0.1 \\
\text{Slope} &= 2.2 \pm 0.1
\end{align*}\]

\(q / 10^{-3} \text{ (nm}^{-1}\text{)}\)

**Figure 5.6** Scattering vector \((q)\) dependence of scattered light intensity \((I)\) of resultant P(DEA-co-DMA) mesoglobules formed under different heating rates.

As for P(DEA-co-EA/y) copolymers, P(DEA-co-EA/40) with a lower EA content can form stable mesoglobules in both the fast and slow heating process, but P(DEA-co-EA/60) can only form stable mesoglobules in the fast heating process. In the fast heating, intrachain contraction related to the initial induction is more evident because both \(N_{\text{chain}}\) and \(<R_h>\) remain constants. The resultant stable mesoglobules have a larger \(<R_h>\), but much smaller \(N_{\text{chain}}\); namely, they are made of many loosely packed small single- or pauci-chain collapsed globules with a much lower chain density. On the other hand, in the slow heating, Figures 5.6 and 5.7 reveal an
unexpected initial sharp increase of both \(N_{\text{chain}}\) and \(<R_h>\), followed by a gradual decrease, indicating that interchain association formed in the initial stage is not stable.

**Figure 5.7** Time dependence of average aggregation number \((N_{\text{chain}})\) of P(DEA-co-EA) mesoglobules formed under different heating rates.

**Figure 5.8** Time dependence of average hydrodynamic radius \((<R_h>)\) of P(DEA-co-EA) mesoglobules formed under different heating rates.
One of the possible explanation for the unexpected decrease of $N_{\text{chain}}$ and $<R_h>$ is as follows. Poly($N$-ethylacrylamide) (PEA) homopolymer has a much higher lower critical solution temperature (−82 °C) than poly($N,N$-diethylacrylamide) (PDEA) homopolymer (−32 °C). Increasing the EA content makes P(DEA-co-EA) more hydrophilic so that its LCST becomes higher. For three P(DEA-co-EA) copolymers studied, the aggregation temperatures are well below 80 °C, at which the PDEA segments become hydrophobic, but the PEA segments remain hydrophilic. The copolymer chains with a higher EA content are so hydrophilic that short hydrophobic PDEA segments are not able to provide a sufficiently strong hydrophobic association. As soon as the temperature is raised higher than −32 °C, the association of short PDEA segments simultaneously leads to intrachain contraction and interchain association, resulting in an increase of $N_{\text{chain}}$ and $<R_h>$. During this process, long hydrophilic PEA segments are forced to stay on the periphery so that further interchain association are prevented. Inside each mesoglobule, the weak association and dissociation of short PEA segments should be in a dynamic equilibrium. Thermodynamically, when two chains are associated together, there is a penalty in both translational and conformational entropies, while for an intrachain association, there is no loss in translational entropy. Therefore, for a very weak interaction, intrachain association of a flexible chain should be more favored. In this way, interchain association inside each mesoglobule will eventually relax into intrachain association to reduce its free energy. This might explains the decreases of $N_{\text{chain}}$ and $<R_h>$. Such a relaxation from interchain to intrachain association can be better viewed for the association of P(DEA-co-EA/80) with a much higher EA content at 55.5 °C (Figure 5.9) because here the hydrophilic association is even weaker.
Figure 5.9 Time dependence of average aggregation number ($N_{agg}$) and average hydrodynamic radius ($<R_h>$) of P(DEA-co-EA/80) mesoglobules formed under different heating rates.

Besides thermodynamical consideration, the formation of stable mesoglobules can also be discussed from a kinetic and viscoelastic point of view. It can be visualized that as the solvent quality changes from good to poor, i.e., as the temperature increases, the degree of amphiphilicity of the copolymer chain increases; namely, the PDEA segments become hydrophobic, but the PDMA and PEA segments remain hydrophilic. The aggregation of insoluble PDEA segments leads to intrachain contraction and interchain association. It is known that for two particles to merge into one larger particle, they have to interact (contact) for a sufficient long time ($\tau_c$)
during which the copolymer chains inside each particle have to relax and diffuse into each other, which must take a certain time \( \tau_D \). If \( \tau_C \ll \tau_D \), each particle will act as an elastic glass ball so that two approaching particles will collid and bounds away. In this case, they are stabilized by the viscoelasticity.

As expected, intrachain contraction increases the copolymer concentration inside each mesoglobule, which slows down the chain relaxation so that \( \tau_D \) increases. On the other hand, the concentration of hydrophilic PDMA or PEA segments on the periphery reduces \( \tau_C \) to a great extent. This is why only amphiphilic copolymer chains can form stable mesoglobules in solution. Therefore, we need to reduce \( \tau_C \) and increase \( \tau_D \) in order to form smaller mesoglobules. The incorporation of more hydrophilic comonomers into a chain backbone is one way to decrease \( \tau_C \); while for a given composition, the segmentation of hydrophilic comonomer on the chain backbone is another way because they will have a less chance to be trapped inside during intrachain contraction and interchain association. Moreover, one can promote intrachain contraction and suppress interchain association by a quick change of the solvent quality or a dilution of the solution. It is helpful to note that for a given copolymer concentration \( (w/V) \), using long copolymer chains can also enhance intrachain contraction, resulting in smaller mesoglobules or even single-chain globules.\(^{14} \)

Neutral random copolymers, poly\((N,N\text{-diethylacrylamide-co-}N,N\text{-dimethylacrylamide})\) P(DEA-co-DMA) or poly\((N,N\text{-diethylacrylamide-co-}N\text{-ethylacrylamide})\) P(DEA-co-EA), are amphiphilic in water at temperatures higher than 32 °C because PDEA has a lower critical solution temperature. The association of such copolymers with a proper comonomer composition in the range 32 – 80 °C in which water is a selective solvent, can form stable mesoglobules existing between single-chain
collapsed globules and macroscopic precipitation. The formation and structure of such stable mesoglobules are essentially controlled by a competition between intrachain contraction and interchain association. The intrachain contraction forces hydrophilic DMA or EA segments to stay on the periphery, which stabilizes resultant mesoglobules. Increasing hydrophilic DMA or EA content generally leads to small mesoglobules. It is interesting to find that increasing the heating rate can change the association from a reaction-limited process to a diffusion-limited process. In the fast heating process, intrachain contraction is dominate and the collapsed chains have less chance to undergo further interchain association. Therefore, the mesoglobules formed in the fast heating consist of loosely packed small single- and pauci-chain globules. Our results reveal that for the copolymers with short hydrophobic segments, interchain association is too weak to form stable mesoglobules and there exists an interesting relaxation from interchain association to intrachain association as the time elapses.
5.4 References


