Cosolvent effect on morphogenic changes of self-assembled aggregates from biodegradable polylactones

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ABSTRACT

The solvent variation effect on the self-assembly phenomenon is discussed in this article. Two different biodegradable backbones of the same community (polycaprolactone and polylactide) were selected to visualize the morphological changes upon treatment of different solvents/cosolvents. The curiosity was to observe if the change occurred only with the solvents or it was assisted to the backbones also. Tetrahydrofuran, acetonitrile and 1,4 dioxane were used as polar aprotic solvent whereas, methanol was used as polar protic one with the common solvent water in each case. The macromolecular aggregates were formed using the solvent mixture and their morphological or conformational changes were monitored using different conventional techniques such as Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and cryo-Transmission Electron Microscopy (cryo-TEM). The spontaneously formed superstructures were thoroughly characterized and well documented here. The hierarchical aggregates disclosed here bears the potential of vast usage in the field of nanotechnology.

GRAPHICAL ABSTRACT

Super-aggregated nanostructures of biodegradable lactones derived thiobarbiturate homopolymers in different solvent mixtures

1. Introduction

Hierarchical self-assembly is the most promising aspect of macromolecular science in this new era.\cite{[1]} It is a spontaneous process through which the macromolecules reorganize themselves reversibly mainly by noncovalent interactions and at the same time, there is a tendency to minimize the surface free energy to be stabilized.\cite{[2,3]} The polymers can be self-assembled into interesting morphologies like micelle, vesicle, lamellae, rod, cylinder, worm-like and many more. These exciting morphologies can be regulated by internal as well as external parameters. The small changes of the internal parameters like polymer chain lengths, molecular weight, polydispersity index make significant differences in the sizes and shapes of self-aggregates.\cite{[4-6]} Similarly, external factors like pH, temperature, surface tension and solvent nature significantly affect the final architectures.\cite{[7,8]} Among these, solvents/cosolvents may have a great impact on the conformational changes of the supramolecular aggregates.\cite{[9-11]} The architectural changes are mainly controlled by the arrangements of the polymer chains.\cite{[12,13]} The contraction or expansion of polymer chains is governed by the interactions between the polymers and solvent molecules where the polarity, density, colloidal stability etc., are taken into consideration.\cite{[14,15]} Commonly organic solvents are used as cosolvents with water to form a binary solution, where one of them is a “good” solvent to the polymer chain and the other is considered as a “bad” solvent.\cite{[16]} Usually, the polymer chains dissolve in good solvent through chemical moiety-solvent interaction. Whereas, in bad solvents, the moieties are folded into each other to be self-organized.\cite{[16,17]} Preferably, miscible solvents with significant
polarity differences are chosen as cosolvents but sometimes, because of preferential adsorption, the polymer chain may collapse in miscible good solvents also. It implies that the solvent mixture may be strongly related to the disintegration of the hydrophobic domains.\textsuperscript{[18]} The effect of water content on the macromolecular architectonics is also noteworthy as

Figure 1. Structural representation of PCL-TBA and PLA-TBA.

Figure 2. Self-assembly study of PCL-TBA in acetonitrile/water solvent (a: TEM image; b: cryo-TEM image; c: DLS data, size: 500 nm, : 2.53).
the increasing water content transforms the spheres to rods and eventually to vesicles to be thermodynamically stable by reducing the interfacial energy.\cite{19}

Although, the cosolvent effect on block copolymers are well explored theoretically and experimentally but there are challenges too to control over the morphologies of amphiphilic homopolymers. Here, in this work, we have well explored the cosolvent effect on the previously synthesized (also reported by our research group)\cite{20} thiobarbiturate derived polycaprolactone (PCL-TBA) and polylactide (PLA-TBA) backbones (Figure 1) of near about the same molecular weights and thoroughly discussed the morphology transformations with changing solvents. For that purpose, we have used 1:1 solvent mixture keeping water as the common solvent with the miscible cosolvents: tetrahydrofuran (THF), methanol (MeOH), acetonitrile (ACN) and 1,4 dioxane (DX) with the different polarity index 4, 5.8, 5.1, and 4.8, respectively. Considering the excellent polarity of thiobarbiturate moiety, only polar solvents were selected to make the polymers readily soluble into the solvent mixtures.

2. Result and discussion

To obtain the excellent superstructures, four different organic solvents with distinct polarity differences were used. In the 1:1 solvent mixture, water was the common solvent and another component was the organic solvents. Here, THF, ACN and DX are polar aprotic solvents and MeOH was polar protic. In spite of a definite polarity difference, it was expected to follow a particular trend in the aggregate’s formation at least for the polar aprotic solvents as there should not be any additional hydrogen bonding interaction between the molecules as well as with water molecules. The polar protic solvent might behave differently due to the interaction with water molecules.

As stated earlier, in all solvent mixtures, water content was 50% in each case which was quite high to turn the morphologies toward the vesicle side as the polar moiety always tends to remain with the affinity of polar solvent and polymer backbones were inclined to coil to form bilayers. In this study, thiobarbiturate moiety played the role of a relatively hydrophilic entity whereas, the polymer chain was the hydrophobic counterpart of the amphiphilic nanoparticle.

Figure 3. Self-assembly study of PLA-TBA in acetonitrile/water solvent (a: TEM image; b: cryo-TEM image; c: DLS data, size: 150 nm, : 2.62).
Figure 4. Self-assembly study of PCL-TBA in dioxane/water solvent (a: TEM image; b: cryo-TEM image; c: DLS data, size: 700 nm, \(\cdot\) 2.23).

Figure 5. Self-assembly study of PLA-TBA in dioxane/water solvent (a: TEM image; b: cryo-TEM image; c: DLS data).
For acetonitrile/water and dioxane/water solvent mixtures, distinguishable spherical aggregates were observed with both the polymer backbones (PCL-TBA and PLA-TBA). In the case of acetonitrile/water, remarkable spheres with a definite outline were observed for polycaprolactone entity through TEM (Figure 2a) and SEM (Figure S1) images. Upon cryosection i.e., via cryo-TEM (Figure 2b), we observed higher order self-aggregates where small white spheres further aggregated to form superstructures that could be compared with the fruit called litchi. The size of the aggregates was also comparable with the hydrodynamic diameter of the self-aggregates observed from the DLS study (Figure 2c). Here, the polymer chains were expected to form the core of the aggregate by releasing the interfacial energy and the corona contains the thiobarbiturate functionality. It could also be supported by the dye encapsulation of the same moiety in the aforesaid solvent mixtures (Figures S10 and S11).

A quite similar type of aggregates was obtained from the TEM image (Figures 3a and S2) in the case of PLA-TBA also but the interior of the aggregates as observed from the cryo-TEM image (Figure 3b) was different than that of the PCL-TBA. A similar kind of size and shape of the superstructures were observed for the above polymers as noticed from TEM and SEM images but the DLS and cryo-TEM studies revealed different sizes and shapes of the nano-aggregates than that observed from TEM and SEM studies. This could be ascribed as the difference in the nature of backbone and characterization techniques. From TEM and SEM images, the morphologies and their sizes were obtained from the dry state whereas, in the DLS study, the size was obtained from the solution phase. For, cryo-TEM, interior morphologies were visualized where size was determined with the immediately frozen peripheral solvent molecules. It was the experimental technique that was responsible for different sizes of similar aggregates. We believed that there was no significant attraction or repulsion present between the acetonitrile molecule and the polymer chains. So, similar kind of morphologies was obtained from PCL-TBA as well as from PLA-TBA.

Moreover, the nature of the morphologies obtained from PCL-TBA (Figures 4 and S3) and PLA-TBA (Figures 5 and S4) with dioxane/water system was completely non-identical. This could be explained by the interaction between the solvent molecules and the polymer chains. When the polymer chains and solvent molecules repulsed each other, then the intramolecular miscibility within the polymer chains tended to form the core. The effect of this...
repulsion was inversely proportional to the aggregate size. Thus, with increasing the repulsive force, the size of the aggregates decreased.\cite{14}

The anomaly was observed in the case of polycaprolactone backbone where morphology conversion resulted in vesicular structure. We anticipated that the difference in the chemical structure of the two backbones was responsible for this type of observation. The presence of more polar groups (\(>\text{C}=\text{O}\)) in the PLA backbone allowed more repulsive force with dioxane molecules as compared to the PCL backbone (possessing a lesser number of \(>\text{C}=\text{O}\) groups) as dioxane contains more polar groups (i.e., \(-\text{O}-\) groups). Therefore, it was very much evident that the order of solvent-polymer chain repulsion was polylactide \(>\) polycaprolactone which also described the smaller spherical size of PLA-TBA than the vesicular structures of PCL-TBA in dioxane/water solvent mixture. The structural difference of the two backbones also resulted in dissimilar morphological patterns. Moreover, the aggregate size obtained from the TEM study was bigger than that of DLS and cryo-TEM for PLA backbone might be possible due to the overlapping of the aggregates with each other in the solvent mixture resulting in a higher dimension or we could assume that during the solvent evaporation method, higher-order self-assemblies were observed but in solution phase, the hierarchical self-aggregations could be prevented by the solvent layer in the vicinity of the self-aggregated species.\cite{21}

Next, the well-established and widely used solvent tetrahydrofuran was mixed with water to prepare the experimental solvent and all the morphological studies have been performed. The superstructure formation of polylactide in THF/water solvent (Figures 6 and S5) also supported the polymer chain-solvent repulsion theory. As the THF molecule contains an oxygen atom, it repulsed the oxygen atoms of PLA chains and made the system unstabilized. Additionally, the PLA moiety of PLA-TBA contains two methyl groups which acted as an auxiliary driving force to release the free energy and allowed the aggregates to get stabilized. In another way, the oxygen content was less in THF than that of dioxane which reduced the solvent particle-polymer chain repulsions in the case of THF/water-mediated self-assembly resulting in the bigger size super aggregates.

But for the polycaprolactone backbone in PCL-TBA, smaller spherical aggregates were observed in THF/water system (Figures 7 and S6). The carbonyl functionalities and the oxygen atoms were well spaced by five methylene groups in the PCL backbone. Hence the folding and packing were easy for this polymer due to the presence of flexible \(-\text{CH}_2\) groups (methylene chains) which helped the PCL-TBA to form aggregates. Here we hypothesized that the long-chain
length was the predominant factor over the solvent-polymer repulsion resulting in predominantly smaller aggregates as compared to that formed in the case of PLA-TBA.

Now the curiosity was directed toward the solvent dependent size variation. To explain this, the density, polarity, H-bond formation ability could be taken into consideration along with the differences in the chemical structures of the backbones. The order of superstructures’ sizes was observed in the order THF/water < ACN/water < DX/water. But if we consider the density and hydrogen formation ability then the order of the aggregates’ size should be ACN/water < THF/water < DX/water. In the case of ACN and DX, it followed the normal protocols for the different polymeric backbones. Dioxane possessed high density, H-bond formation ability and order of attraction as compared to acetonitrile. Hence, larger molecular aggregates were formed in dioxane/water than that of acetonitrile/water. But the molecular structures of the compounds also played a very crucial role to determine the morphology, so we proposed here, that the size, shape and type of morphology in THF/water were predominantly controlled by the polymer backbones over the solvent’s effects.

After a thorough investigation about the effect of different polar aprotic solvents on two different biodegradable backbones, our interest was driven toward the polar protic solvent. For that purpose, methanol was taken into consideration for its usefulness in the field of self-assembly study.

Spherical super aggregates were observed with the polycaprolactone backbone in PCL-TBA through the TEM (Figure 8a), cryo-TEM (Figure 8b) and SEM (Figure S7) studies. The formation of aggregates was also validated by the DLS data (Figure 8c).

Being a protic solvent, methanol was able to form enormous intra- or intermolecular hydrogen bonding with water molecules as well as with the thiobarbiturate moiety. The intramolecular hydrogen bonding was too strong to be considered. Though the driving force of self-assembly was strong enough to form the self-aggregates but the interference of the intra- or intermolecular hydrogen bonding might retards the nanoparticle to lower the interfacial energy to get the vesicular morphology. In this case, the extent of hydrogen bonding might become the governing factor over the presence of flexible methylene groups (-CH2-) in PCL-TBA.

On the other hand, in the case of polylactide backbone (PLA-TBA), the repeating unit was -CH3CHCOO- where the methyl group was always away from the plane. Hence packing of the chains was difficult due to rigidity. In addition to this structural rigidity, probably the intervention of the protic solvent’s hydrogen bonding ability inhibited the molecule to lower its energy. Because of these two opposite effects, the aggregation resulted in the worm-like morphology through TEM images (Figure 9a) and SEM (Figure S8) studies whereas in cryo-TEM images (Figure 9b) spherical morphology was obtained.

Figure 8. Self-assembly study of PCL-TBA in MeOH/water solvent (a: TEM image; b: cryo-TEM image; c: DLS data).
We would be able to elucidate the anomaly between the TEM and cryo-TEM images by elaborating the experimental procedure of these studies. For the TEM study, a copper grid was dipped into the aggregate’s solution and was dried under vacuum then images were obtained from the dried phase. But for cryo-TEM, a TEM grid was placed into the sample cryo plunge to drop cast the sample and after drop-casting it was plunged. Then the grid was dipped into an ethane pot. Next, it was kept in liquid nitrogen in the cryo holder. The cryo holder temperature should be $-170^\circ C$. Then instantly it was placed under the vacuum in the instrument. There should be a constant liquid nitrogen flow in the cryo holder so that the temperature does not increase from $-170^\circ C$ and the process should be very fast to prevent frost deposition. Here, the sample went through the high shear force along with the low relaxation time which sometimes resulted in the morphology transformation of worm-like structure to vesicular or micellar morphologies.\textsuperscript{[22]} Probably, due to this insufficient relaxation time we obtained spherical shape aggregates through cryo-TEM.

Figure 9. Self-assembly study of PLA-TBA in MeOH/water solvent (a: TEM image; b: cryo-TEM image; c: DLS data).

Figure 10. TEM images of PLA-TBA in MeOH/water solvent (transformation from spherical to worm-like structure).
experiment whereas, worm-like morphology was noticed in TEM and SEM images.

From these observations, we also proposed here that the thiobarbiturate derived poly lactide homopolymer (PLA-TBA) was aggregated into a spherical structure immediately in solution and eventually with continuous drying, it transformed into a worm-like shape (Figures 10 and 9).

Furthermore, dye encapsulation studies of each sample with both the backbones (PCL-TBA and PLA-TBA) depicted that solvent effect did not influence the self-assembly order i.e., no inversion of the molecular self-assembly occurred in each case (Figures S10 and S11).

3. Conclusion

Thiobarbiturate derived biodegradable compounds with different backbones (PCL-TBA and PLA-TBA) were made soluble in different solvent mixtures to understand the self-assembled pattern in detail. Polar protic and polar aprotic cosolvents were used to prepare a solvent mixture with water as the common solvent and the reason behind the purposeful selection of polar solvents was to make the thiobarbiturates easily soluble. The combined effect of different cosolvents on morphology as well as size changes of self-aggregates was thoroughly characterized and was well documented here. Also, the changes in the self-aggregation process related to the chemical nature of the backbones were investigated in detail. We envisaged that the intriguing solvent and backbone induced morphologies could be further explored in different fields of nanotechnologies in future.

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