Selective Preparation of Meso-porous Polyion Complexes and Evaluation of Their Properties and Functions

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Selective Preparation of Meso-porous Polyion Complexes and Evaluation of Their Properties and Functions

(ポリイオンコンプレックスメソ多孔体の創製とその物性・機能評価)

氏名 ウィボウォ アリー (Wibowo Arie)

Polymeric porous materials have been rapidly advancing in recent years due to their excellent properties derived from both high porosity and flexibility of polymers, such as high surface area and porosity, high processability, light-weight and tunable properties and functionalities. Owing to their remarkable properties, polymeric porous materials play a key role in numerous applications such as drug delivery, catalyst and catalyst support, filtration, and nanolithography. In previous examples, block copolymers are often used as a precursor for porous architecture that enables removal of a sacrificial block to create pores on utilizing their microphase-separated structures. Nevertheless, the method to precisely regulate nano-architectures in self-assembled structures of block copolymer is still a challenge, especially in aqueous medium even though there is a strong demand from ecological and biomedical viewpoints. Considering this point of view, polyion complexes (PICs) are very attractive materials because of their ability to form materials in aqueous medium from an oppositely-charged pair of polyelectrolytes without any cumbersome processes. In spite of potential utility of PICs, fabrication of porous PIC has not been found yet. Only limited examples of PIC nano-architectures have been reported e.g. micelles and unilamellar PIC vesicles termed by “PICsomes” prepared from a system containing PEG-based charged block copolymers. However, these structures may not yet represent as the complete set of all possible nano-architectures, since the self-consistent mean-field (SCMF) theory for AB diblock copolymer actually predicts the presence of other structures in between sphere and lamellar structures, such as bicontinuous structures presented as porous materials and cylindrical structures, which, to the best of my knowledge, have not been comprehensively studied for PIC system. Successful preparation of PICs with porous structures would be attractive in terms of simple, low-cost, and environment-friendly fabrication, because sacrificial components or templates to create pore might be not needed in PIC system. Therefore, this study seeks to: (1) establish the procedure to precisely control PICs morphology by tuning their thermodynamic parameter, (2) prepare porous PICs, and (3) obtain some insight about formation of porous PICs by following their transformation as a function of time.

Apparently, segregation between PEG and PIC phase drives the formation of these nano-architectures as in the case with microphase separation in AB block copolymers in selective solvents. Worth noting is that the transition from PIC micelles to PICsomes in these examples has a
strong correlation with the weight fraction of poly(ethylene glycol) (PEG) in the entire polymer system ($f_{\text{PEG}}$). Considering that we have already found polymeric micelles and unilamellar polymeric vesicles for the PIC system, other PIC nano-architectures, such as cylindrical and porous PICs, might be highly expected to be located in between these distinct micelles and lamellar assembly, which were probably achievable by a more careful fine-tuning of their $f_{\text{PEG}}$ composition. To verify this hypothesis, blending method of three types of polyelectrolytes (PEG-b-poly($\alpha,\beta$-aspartic acid)) (PEG-PAsp; $M_n$ of PEG = 2k, DP of PAsp = 85), homo poly($\alpha,\beta$-aspartic acid) (h-PAsp; DP of PAsp = 69), and PEG-b-poly([5-aminopentyl]-$\alpha,\beta$-aspartamide) (PEG-P(Asp-AP); $M_n$ of PEG = 2k, DP of P(Asp-AP) = 84) were used as the main strategy of this study because this method allowed precise control of $f_{\text{PEG}}$ by simply changing the blending ratio of PEG–PAsp and homo-PAsp ($b/h$ ratio). Afterward, a series of sample with polymer concentration 1 mg/ml were prepared at 25 °C by varying their $b/h$ ratio allowing for variation of $f_{\text{PEG}}$ from 6.5–12.1%.

At high $f_{\text{PEG}}$ composition ($f_{\text{PEG}}$ 12.1 and 11.1%), spherical micelles with diameter ~20 nm were found. In the case of $f_{\text{PEG}} = 10.0\%$, cylindrical micelles with a short axis of 12.4±1.7 nm and substantially distributed long axis length (239.2±91.0 nm) were mainly observed. Upon further lowering the $f_{\text{PEG}}$ ($\leq 9.4\%$), network architectures in the micrometer-sized PIC droplets were obtained. Detailed characterization using three dimensional (3D) tomography revealed that PICs at $f_{\text{PEG}} = 9.4\%$ and 8.8% consist of element looked like cylinders connecting each other, thus named here as “connected-cylinder PIC network”, while PICs at $f_{\text{PEG}} = 7.7\%$ and 6.5% consist of elements looked like plane continuously connecting each other, thus termed by “connected-plane PIC network”. TEM image analysis showed that the average PIC thickness of connected-cylinder PIC network was 12.4±1.3 nm, which is quite similar with short axis length of the cylindrical polymeric micelles observed at $f_{\text{PEG}} = 10.0\%$ (12.4±1.7 nm), while the average thickness of PIC plane (13.7±1.5 nm and 16.0±2.0 nm for $f_{\text{PEG}} = 7.7\%$ and 6.5%, respectively) are analogous to the thickness of PIC lamellar of previously reported unilamellar PIC vesicles (10–15 nm).

Sequence of PIC morphologies as a function of $f_{\text{PEG}}$ may present analogy of microphase segregation pattern modeled for AB diblock copolymer system, namely sphere-cylinder-bicontinuous-lamella, if compromising assumptions of the observed connected networks as bicontinuous phase, and absence of lamellar structure as the ultimate structure. Such change of microphase segregation pattern in AB diblock copolymer at constant temperature is described mainly from the volume balance between A- and B-segments connecting at the interface. In our system, A can be regarded as a PIC part, and B as hydrated PEG chains in aqueous phase due to highly hydrated nature of PEG. Spherical micelles were formed at PICs with $f_{\text{PEG}} 11.1\%$ and 12.1%, presumably relying on larger fraction of PEG part compared to that of PIC part. By decreasing $f_{\text{PEG}}$, or the replacement of PEG-PAsp with homo-PAsp, the number of PEG chains at the interface between PIC part and PEG part decrease, while relative volume for PIC part increase. Probably, this change in the balance resulted in the variation of PIC nano-structure from
spherical to cylindrical micelles, and then to grow for cylindrical or planar PIC network.

At room temperature, PEG strands tend to take their swollen state in water with high excluded volume due to its favorable interaction with water. It is interesting to note that PEG inherently exhibited dehydrated behavior by increasing in temperature, thus the volume balance between PEG part respect to PIC part can be modulated by temperature. In this context, PIC structure was examined at various preparation and incubation temperatures (25–70°C). Remarkably, substantial morphological change was observed by increasing incubation temperature for PICs prepared above $f_{\text{PEG}}$ of 8.8%, while at $f_{\text{PEG}}$ composition 7.7% and 6.5%, significant morphological change was not observed by increasing temperature, instead the network textures tend to take more densely packed network structures. Overall, the order of PIC structural change by increasing temperature presented similar trend with lowering $f_{\text{PEG}}$, but the transition seemed somewhat much more facilitated. This may be reasonable because increasing temperature led to decreasing PEG occupying volume in water, thus essentially resulting the same effect of decreased $f_{\text{PEG}}$. Concomitantly, this suggests the balance of PEG part and PIC part substantially contributed in determining microphase segregation pattern.

One of fascinating finding upon temperature modulation was the observation of “well-aligned lamellar structure” with PIC thickness of ~11 nm and spacing between PIC of ~12 nm at high temperature with $f_{\text{PEG}}$ composition 9.4% - 11.1% because the lamellar was the missing structure in the series of morphological change with $f_{\text{PEG}}$ at ambient temperature, and to the best of our knowledge, this is the first direct observation of clear alternating lamellar structure of PIC in water from block ionomers. Worthy to notice, the formation of well-aligned lamellar structure suggests presence of attraction force between each lamella for the association. Most likely, interaction between PEG segments and water decrease at higher temperature and hydrophobic attractive force between PEG layers may have developed because PEG chains shift to hydrophobic nature with elevating temperature. Alternatively, at room temperature, PEG is in fully hydrated state with large exclusion volume, thereby no significant attractive force develops to associate each PEG layer. Accordingly, excess water could penetrate into the connected cylinder network or connected plane network to form water channel, so that the mesoporous structures as typically seen in 3D-TEM images might be formed.

Another parameter that governed self-assembly process of block copolymers (BCPs) is degree of polymerizations $N$, because modulating degree of polymerization of one segment might also modify the volume balance between PEG part respects to PIC part. In this regards, two samples series of PICs were prepared by replacing PEG-PAsp (DP of PAsp = 85) with shorter (DP of PAsp = 50) and longer (DP of PAsp = 151) polypeptide chain of PEG-PAsp using the same b/h ratios to obtain some insight about the influence of polypeptide chain length to PICs morphology. At the same b/h ratio, decreasing chain length of polypeptide leads to increasing their $f_{\text{PEG}}$. However, trend of their structures transformation followed role of $f_{\text{PEG}}$ regardless their polymer chain length, implying that $f_{\text{PEG}}$ might be the main factor to control PIC structures. This result may be reasonable because increasing polypeptide chain of PEG-PAsp led to
increasing relative volume ratio of PICs respect to volume of PEG, thus induced transition of PIC nano-architectures to the more stable structure at lower $f_{\text{PEG}}$.

After finding PICs with network architectures, which is represent as mesoporous PICs, it is interesting to obtain further information about mesoporous PICs. Previously, mesoporous PICs were observed after overnight incubation with expectation that the obtained structures have been reached their equilibrium states. Considering that self-assembled process may consist of several steps during their formation, it is speculating that time-dependent structural transformation of PICs might also observe during formation of mesoporous PICs. In this regard, detail insight about formation of mesoporous PICs with network architectures, which is formed at $f_{\text{PEG}}$ 8.8%, were studied by following their transformation as a function of time to unveil their behavior during formation of mesoporous PICs with network architectures. Interestingly, time-dependent transformation of PIC structures was observed during formation process of mesoporous PICs with connected-cylinder network, and whole process can be initialized by vortex mixing. Firstly, unilamellar vesicles formed then various morphologies of PICs, namely multicompartiment PIC vesicles, blister-like PICs, and mesoporous PICs with connected-cylinder network were sequentially observed. Comparing with formation process of other self-assembled materials, formation process of mesoporous PICs with connected-cylinder network is very unique because time-dependent structural transitions were observed during the formation of mesoporous PICs with connected-cylinder network. This unique behavior of mesoporous PICs might offers great potential for the development of novel materials based on a polyion complex platform. Therefore, detail insight about self-assembly process and mechanism of this behavior were performed by changing their polymer concentration and repeating their process. Upon modulating their polymer concentration, I found that their formation rate of mesoporous PICs were depend on their polymer concentration. Upon repeating formation process of mesoporous PICs by vortex mixing initiation, same sequences of PIC morphological transformation regardless their cycle time, implying that this phenomenon was repeatable and can be initiated by vortex mixing. Understanding dynamic behavior of materials and their response toward small change in their environment are essential for the further development and rational design of new functional smart materials.

Considering their enormous amount of pores in mesoporous PICs, these materials present significant capacity in loading materials within their large inner space. Since their pore size are in mesoscopic range, mesoporous PICs might be favorable for accommodating nano objects, especially to facilitate the raising demand in biotechnology applications. Furthermore, precise control of mesoporous PICs at the physiological salt concentrations enhances their potential utilities toward biomedical applications, especially for the delivery of biologically active compounds without any loss of their activity, non-fouling property brought by PEGylated architecture, and environment-friendly material synthesis. Also, mesoporous PICs showed environment-sensitive properties, such as temperature and vortex mixing. These properties are quite useful for functionalization of mesoporous architecture or as smart materials.