Preparation of A Magnetically Attracted Poly(*N*-isopropylacrylamide)-Based Stimuli-Responsive Coacervate for Bioseparations

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Introduction: Coacervation has been widely used in biotechnology because "coacervate droplets" can contain some bioactive molecules without denaturation due to the fairly high water content. Poly(*N*-isopropylacrylamide) (PNIPAAm), that is a representative thermoresponsive polymer, is known to show a phase transition phenomenon accompanied by a drastic dehydration above a lower critical solution temperature (LCST). We have developed newly designed PNIPAAm-based functional polymers which have shown not only stimuli-responsive phase transition, but also coacervation (Maeda T. Biomacromolecules 2006;7:2230-2236). The smart coacervate droplets of a copolymer composed of NIPAAm, 2-carboxyisopropylacrylamide (CIPAAm) and 2-hydroxyisopropylacrylamide (HIPAAm) are easily formed only by changing external conditions (*e.g.*, temperature and pH etc.). Of particular interest is that the prepared droplets have concentrated target molecules by recognizing a slight difference in their molecular structures. Therefore, the smart coacervate should be very useful in bioseparation systems. In this study, magnetically controllable nanoparticles were integrated into the smart coacervate to allow an ease manipulation of the coacervate for capturing, concentrating, and collecting of target bioactive molecules (Figure 1).

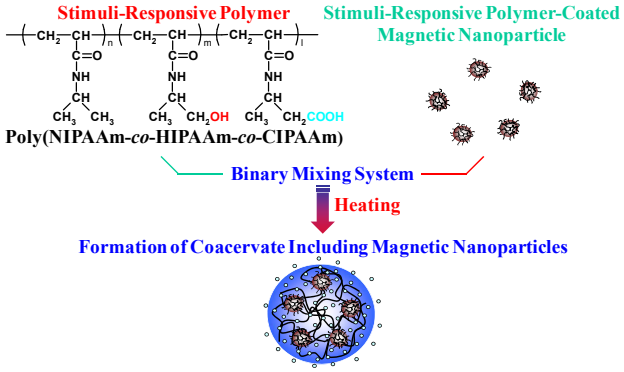

Figure 1. Conceptual diagram of a magnetically attracted smart coacervation system.

Methods: Poly(NIPAAm-*co*-HIPAAm-*co*-CIPAAm) was prepared by a free radical polymerization. Surfactant (oleic acid/oleylamine)-coated superparamagnetic iron oxide nanoparticle (SPION) was prepared by a previously reported method (Lattuada M. Langmuir 2007;23:2158-2168). The copolymer-coated SPION was prepared by a ligand exchange reaction. Citric acid-coated SPION was also prepared in the same procedure as a control. Coacervate was formed by heating a binary solution, consisting of the copolymer (0.50 mg/mL) and the polymer-coated SPION (100 μg/mL) in PBS containing salts (pH 7.4, 1.5 M). Magnetic attraction of the droplets was performed using a magnet of 500 mT. Gelation of the coacervate droplets was performed by adding a cross-linker, divinyl sulfone, to the droplets after changing a pH of the medium up to 10.

Results: The coacervate droplets were easily formed by a simple heating of the binary mixing solution including the copolymer and the copolymer-coated SPION above a LCST (33 °C). The obtained droplets had ca. 1.5 μm in diameter and were comparatively monodisperse (Figure 2; micrograph). In order to verify the presence of the SPION in the droplets, the droplets were cross-linked and the obtained microgels were observed using a TEM, indicating that the SPION certainly existed inside the gels (data not shown). In agreement with the result, the coacervate was attracted toward a magnet within 15 min (Figure 2). The velocity of the droplets was controlled by changing the polymer-coated SPION compositions. Citric acid-coated SPION, on the other hand, did not move toward a magnet. Thus, integrating smart coacervate and the polymer-coated SPION is crucial for the proposed magnetically attracted smart coacervation system.

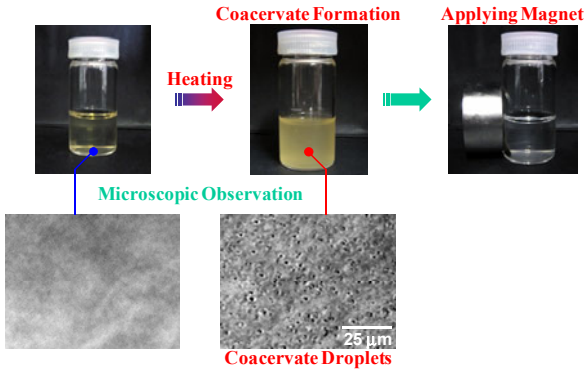

Figure 2. Photographs of a magnetically attracted stimuli-responsive coacervate and microscopic observation of the coacervate droplets.

Conclusions: We successfully prepared the magnetically attracted stimuli-responsive coacervate by properly designing both the copolymer and the SPION. Using this novel carrier, it should become possible not only to rapidly recover bioactive molecules but also to precisely deliver them to target sites using a magnet. Since the prepared copolymer possesses the reactive functional groups which bind a variety type of ligands for the target molecules, the proposed coacervation system would be very useful in biomedical fields such as bioseparations and drug delivery systems.