Thermosensitive N-isopropylacrylamide Nanoparticles Hydrogel Application on Biomolecules Identification

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Abstract. Intelligent hydrogel is hydrophilic polymer gel which is immiscible but significantly swells in water. Thermosensitive nanoparticles hydrogel is a kind of intelligent hydrogels, and its volume phase could change with the external temperature value resulting to its typical of low critical solution temperature. It has extraordinary nanometer characterization and sensitivity to the stimulus of the environmental conditions. In recent years, thermosensitive N-isopropylacrylamide nanoparticles hydrogel are one of the hotspots in hydrogels research. They have attracted much attention in the field of biomedicine due to their good biocompatibility and sensitive temperature response. Thermosensitive N-isopropylacrylamide nanoparticles hydrogel show good application prospects in drug delivery system, protein separation, medical diagnosis, biosensors, biomaterials and other fields. In this paper, the application research and development prospect of N-isopropylacrylamide thermosensitive nanoparticles hydrogel in macromolecule recognition was mainly summarized from protein to peptide. Moreover, the employ of nanoparticles in drug delivery and release, and capillary electrophoresis for DNA separation were also overviewed. Further promising applications of this biological material in macromolecule recognition are ever-accelerated. It is expected that thermosensitive N-isopropylacrylamide nanoparticles hydrogel will break through the limitations of biomedical field and be widely used in other fields.

1. Introduction
Nanoparticles hydrogel(NPs) is a kind of macromolecule polymer nanomaterials which are soluble in water. It has good biocompatibility, internal cross-linking network structure and strong affinity for biological molecules[1]. At the same time, it not only has nano/submicron structure, but also has unique nano-characteristics, such as permeability effect, interface effect, size effect, surface effect, volume effect and so on[2]. Intelligent NPs is a kind of gel system which can perceive the changes of external environment and generate volume phase transformation. According to the stimulation of external environment, it can be divided into thermosensitive[3], pH sensitive[4,5], magnetic susceptibility [6,7], photosensitive[8] and molecular sensitivity [9]. Some intelligent hydrogels can also respond to multiple stimuli, such as temperature and pH. This intelligent nanohydrogel is also called multiple stimulus responsive nanohydrogel[10]. Thermosensitive nanohydrogels are representative.

Temperature-sensitive NPs exhibit volume-phase transition (swelling or contraction) with the change of external temperature. This change is discontinuous. Near a certain critical temperature, the temperature changes slightly, but the volume changes suddenly. This phenomenon is called the phase transition of NPs. The temperature range that causes the phase transition is called lower critical solution temperature (LCST) or higher critical solution temperature (UCST). According to the phase
transition characteristics of temperature-sensitive nano hydrogels, it can be divided into positive temperature sensitive nano hydrogels and negative temperature sensitive nano hydrogels. Among the temperature-sensitive nano hydrogels currently studied, especially the negative temperature-sensitive nano-hydrogels are the most studied. Typically, nanohydrogels are formed from poly (N-isopropylacrylamide) (NIPAm) with cross-linking structure.

The temperature-sensitive monomer NIPAm is combined to provide a positive/negative charge and a hydrophilic/hydrophobic group, and a temperature-sensitive nano hydrogel polymer is prepared by precipitation polymerization[11]. NIPAm temperature-sensitive NPs undergo phase transitions at lower critical solution temperatures (LCST). When the solution temperature is lower than LCST, the hydrogen bonding between the hydrophilic part of the gel and the water molecules is dominant. The outer layer of the gel network will form a solvent shell which is mainly composed of hydrogen bonds and highly ordered, forming a relatively stable physical network structure with uniform mesh size. When the temperature rises, the hydrophobic action of the gel molecules is strengthened, and the formed hydrophobic layer destroys the peripheral hydrogen bonding and the solvent shell. When the temperature rises to the LCST, the water molecules are discharged from the gel, so a coal-globule transition occurs. As shown in Figure 1[12].

Fig.1 Schematic diagram of phase transition of NIPAm thermosensitive nanoparticles hydrogel[12]

In recent years, NIPAm temperature-sensitive NPs have been shown good application prospects in biological systems such as drug delivery systems[13], protein separation[14], medical diagnosis[15], biosensors[16,17], and biomaterials[18]. However, NIPAm temperature-sensitive NPs have not been well studied as imprinted polymers for the specific recognition of biological macromolecules such as proteins and peptides. This paper focuses on the application research and development prospects of NIPAm temperature-sensitive NPs specific recognition proteins and peptides. In addition, the research progress of NIPAm temperature-sensitive NPs in biological fields such as drug delivery and release and capillary electrophoresis for DNA separation are also briefly introduced.

2. Protein recognition

Similar to the imprinting effect, there are many interaction sites between biological macromolecules, and there are many interaction forces, including electrostatic interaction, hydrogen bonding, hydrophobic interaction, van der Waals force, etc. Therefore, polymers with corresponding sites of action can be designed for the surface groups of target polypeptide/protein [19-22]. By optimizing functional monomers, nanohydrogel particles having affinity and selectivity for the peptide or protein of interest can be synthesized.

Kenneth J. Shea et al[23] prepared a polymer nanohydrogel particle with a very high affinity for key vascular endothelial growth factor (VEGF_{165}), which inhibits the binding of this signalling protein to its receptor VEGFR-2 and Phosphorylation of the body and prevents migration of downstream VEGF_{165}-dependent endothelial cells and invasion of extracellular matrices. In addition, vivo experiments have shown that these polymer nano-hydrogel particles can inhibit the formation of new vessels mediated by vascular endothelial growth factor. The study found that this non-toxic polymer nano-hydrogel particles did not have any off-target effects, which means that it also recognizes the specificity of the target protein. The application prospects of this study are quite broad. By adjusting the monomer content and structure, the properties of these polymer nano-hydrogel particles have further room for optimization.
The preparation and isolation process of natural antibodies is complicated and expensive, and has certain limitations in the diagnosis and treatment of diseases. Therefore, the application of nano-hydrogels with good biocompatibility and diversified functions provides a new way for the treatment of diseases. Shea et al [24] synthesized the protoxin-imprinted nano-hydrogel particles (plastic antibody) by molecular imprinting technique, and used it as an artificial antibody to identify melittin, and studied it in living mice [25]. As shown in Figure 2 [26]. The study found that synthetic protoxin-imprinted nano-hydrogel particles, like natural antibodies, greatly improved the survival rate of mice injected with melittin.

![Fig.2 Schematic diagram of synthesis of plastic antibodies][26]

Shea et al [27] used the temperature sensitive monomer NIPAm as a structural monomer, and the electronegative acrylic acid (AAc) and the hydrophobic N-tert-butyl acrylamide (TBAm) as reaction monomers, N, N’-Methylenebisacrylamide (BIS) as a crosslinking agent, a series of nano-hydrogel polymers were synthesized by precipitation polymerization, and the polymer can achieve the effect of adsorption and desorption on lysozyme by changing the temperature. Hydride experiments [28] and affinity purification [29] were used to screen hydrogels with high affinity for different target proteins, and different target proteins and different monomers [30,31] and pH were studied [32]. And the influence of factors such as temperature [33] on the adsorption.

NIPAm temperature-sensitive NPs have many advantages, such as flexible structure, large specific surface area, multiple sites on the surface, strong stability and good biocompatibility, which can better combine with multiple sites on the protein surface. However, most of them are only in the laboratory stage. In the near future, with the deepening of research, NPs can meet the needs of medical clinics as artificial antibodies, breaking through the limitations of biomedicine and exerting its unique role.

### 3. Drug delivery and release

The application of NPs in drug delivery and controlled release has received extensive attention. The NIPAm temperature-sensitive NPs has an LCST that controls the loading and release of the drug by sensing the outside temperature, effectively over the biological barrier to deliver the drug to the human site of action. Therefore, the drug loading efficiency is increased, the bioavailability is increased, the drug concentration is controlled, and the side effects are reduced, and the problems of low bioavailability and frequent administration times in the conventional drug delivery mode are solved. Moreover, the treatment of specific sites can be achieved by active targeting with the change of external conditions. Among them, the release mode is that when the temperature is lower than the LCST, the hydrogel swells in the drug solution to adsorb the drug; when the temperature is higher than the LCST, it shrinks and releases the drug.

Hsiue et al [35] based on the temperature sensitivity of PNIPAm, its encapsulated drug was used as a controlled release eye drop for glaucoma treatment, and the depressurization time was 6 times longer than that of the common preparation. Zhang et al [36] loaded the model drug 5-fluorouracil into PNIPAm hydrogel at 25 °C, and then placed the drug-loaded hydrogel in a dialysis bag to form a novel thermosensitive drug delivery system. Studies have shown that drug release increases with increasing temperature. Snežana et al [37] prepared a temperature-sensitive hydrogel with NIPAm and 2-hydroxypropyl methacrylic acid (HPMet) as monomers, and studied by high performance liquid
4. Conclusions and prospects

As a kind of intelligent hydrogels, NIPAm thermosensitive NPs have great potential applications in protein recognition, drug delivery and release, and capillary electrophoresis for DNA separation. However, it has own defects, such as slow response speed and low mechanical strength, seriously affect its practical application in the field of biomedicine. In other areas, the application of NIPAm thermosensitive NPs is relatively small. In a word, with the development of science and technology, NPs can break through the limitations of biomedical field and be widely used in other fields.

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