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Thermally Sensitive Colloidal Particles: From Preparation to Biomedical Applications

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Abstract This short article is a condensed review of recent work devoted to thermally sensitive based polymer particles and their potential applications as biomolecules carriers in biomedical diagnostic. Firstly, several aspects related to synthesis of different thermally sensitive colloidal particles are presented. Secondly, the general colloidal properties of such particles are reported and illustrated. Finally, some fine applications of reactive, hydrophilic thermally sensitive particles in biomedical diagnostic are briefly presented.

Keywords Adsorption · Biomedical · Concentration · Core-shell · Desorption · Diagnostic · Microgel · Nucleic acid · Protein · Purification · Thermally sensitive

Introduction

Latex microspheres are largely studied according to their exhaustive and numerous applications in various fields: as a chromatography supports in analytical biochemistry, as a carriers in biomedical diagnostic, as a nano-capsules for drug delivery system and in academic studies as a colloidal model. Due to the versatility of the polymerization processes, various polymer latexes have been elaborated and used in numerous applications. Polystyrene-based particles for instance, have long been used as solid phase supports in diagnostic applications and principally in macroscopic agglutination assays [1]. However, in some in-vitro biomedical applications, hydrophilic particles are of great interest in order to avoid non-specific adsorption of proteins and their denaturation [2]. Then, various reactive and hydrophilic particles have been elaborated and evaluated in biomedical applications as a solid support of biomolecules. The hydrophilic character of the particles surface reduces drastically proteins adsorption [3–5] when both pH and salinity are simultaneously controlled. Stimuli-responsive biodegradable materials are reported to be of paramount importance in drug delivery. In biomedical diagnostic, such "smart materials" [6–9] are studied as new tools for biomolecules purifications: extraction, specific adsorption, and concentration enhancement. Various kinds of stimuli-responsive particles have been examined. Recently, pH, salinity- and thermally-sensitive colloidal particles have received an increasing attention as evidenced by the recent numerous papers published last decade [8–10]. In this direction, various thermally sensitive microgel particles have been elaborated and examined in biomedical diagnostic as a solid support of biomolecules [11–13].

Preparation

Various thermally sensitive materials (polymer solutions, gels and particles) have been prepared by controlling the recipe and the polymerization condition as shown in Fig. 1. To some extent, the physical aspect (gel, free polymer chains and particles) of the final polymer material depends: on the polymerization temperature (regarding the LCTS of the corresponding main homopolymer), on the crosslinker and finally, on the nature of used initiator. The gel formation is manly related to amount of used water-soluble crosslinker agent [14, 15]. Whereas, the water-soluble polymer is obtained when the polymerization is conducted in free crosslinker recipe [16]. To obtain particles, various as-

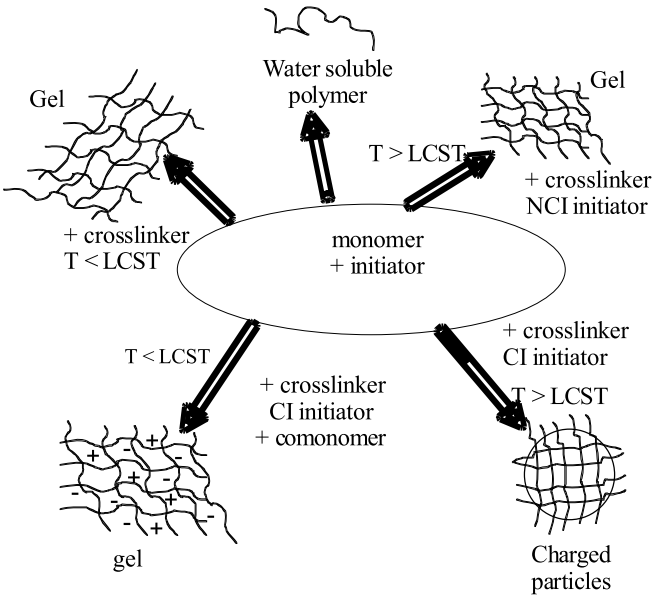

Fig. 1 Thermally sensitive materials. CI: Changed Initiator, NCI: Non Changed Initiator

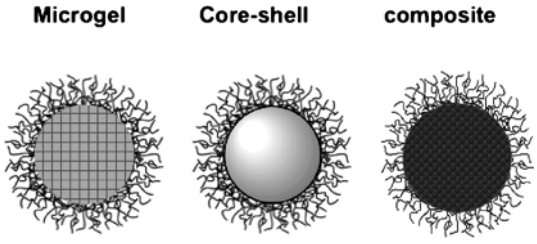

Fig. 2 Thermally sensitive microgel, core-shell and composite microspheres

pect should be controlled [11, 17, 18]: (i) the polymerization reaction temperature should be higher than the corresponding homopolymer low critical solution temperature (LCST), (ii) the colloidal stability should be induced by charge initiator (KPS, V-50 . . .) or the charged comonomer (i.e. acrylic acid) and (ii) the polymerization should be conducted in moderate solid content (preferably less than 5 w/w %) [19]. The preparation of colloidal particles is quite fastidious and delicate since various parameters affect the polymerization rate, the swelling ability of the particles, the amount of the formed water-soluble polymer and consequently, the final properties of the colloidal particles [20]. To prepare such particles, precipitation polymerization is used to obtain microgel particles and the combination of emulsion and precipitation polymerization leads to core-shell particles [12, 21].

Since the first works reported by Pelton et al. [11] and by Kawaguchi et al. [22], various reactive thermally sensitive microgel particles were developed such as the preparation of low charged thermally sensitive microgel using (NIPAM/MBA/cationic and anionic initiators) mixture, cyano-functionalized particles (NIPAM/MBA/Acrylonitrile/anionic initiators) [23], amino-containing poly(*N*-isopropylacrylamide) [19], polystyrene core-thermally sensitive shell latex particles [12], magnetic in nature [18, 24]. Nowadays, it is possible to obtain various thermally sensitive particles, from the relatively simple poly(*N*-isopropylacrylamide) microgel to much more complex polymers, polymer matrix and structures (i.e. core-shell, capsules) as shown in Fig. 2.

The optimization of polymerization process with a view to favour the particles formation should take into account the effect of each polymerization parameter (or reactants) on the particles conversion and water soluble-polymer formation (Fig. 3) by examining the amount of water-soluble polymer formation as a function of temperature, crosslinker agent, charged comonomer and charged initiator concentrations.

As expected, an increase of the charged initiator concentration, results in an increase of the formation of water-soluble polymers and a reduction of final particle size. The polymerization temperature must be higher than the LCST of the corresponding homopolymer, in order to promote the precipitation of oligomers formed in aqueous

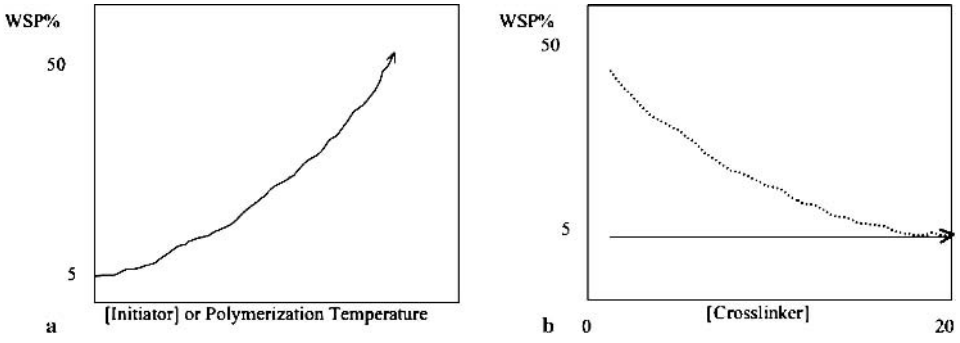

Fig. 3 **a** Influence of water-soluble initiator and temperature on the amount of water-soluble polymer formation. **b** Effect of cross-linking agent concentration on the water-soluble polymer formation

phase. As for the influence of charge initiator, increasing the temperature enhance the amount of water-soluble polymer formation. The concentration of water-soluble crosslinker used in the polymerization recipe should be appropriate in order to favour the crosslinking efficiency of the particles during the polymerization process. But, high crosslinker amount lead to low thermally sensitive particles. The charged comonomer contributes to water-soluble polymer formation and production of low particle size.

Physiochemical and Colloidal Properties

The colloidal characterization of thermally sensitive particles has been investigated by examining principally the effect of temperature on the colloidal properties of the particles. The swelling ability has been principally examined in detail.

Swelling Ability

The swelling ability of the microgels has been investigated as a function of temperature using dynamic light scattering (Fig. 4). Generally, significant reduction of hydrodynamic particle size is observed in the vicinity of the LCST of the corresponding polymer (i.e. 32 °C for *N*-isopropylacrylamide based particles) [25]. The swelling ability was found to be more significant for low crosslinked and low charged microgel particles. In the case of charged microgel particles, the influence of both salinity and pH has been investigated. It is important to notice that the volume phase transition of microgel particles occurs in a wider range of temperatures (5–10 °C) compared to the LCST of linear polymer (i.e. PNIPAM in aqueous phase). The observed swelling ability as a function of temperature is basically a consequence of the increase in the interaction parameter (χ) of the polymer. Interesting work has also been reported by Ballauff et al. [26] by investigating the volume phase transition temperature of thermally sensitive charged core-shell.

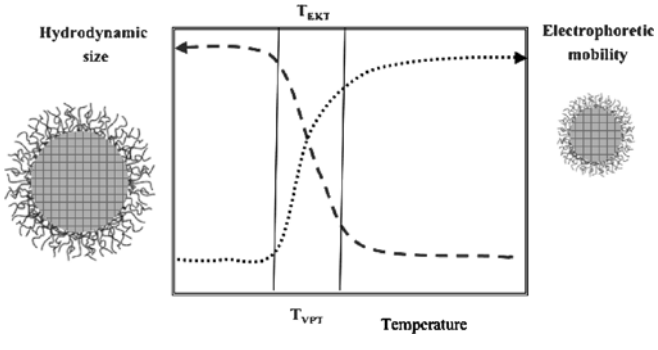

Fig. 4 The effect of temperature on the hydrodynamic size and electrophoretic mobility of PNIPAM based microgel particles. T_{VPT} is the volume phase transition temperature and T_{EKT} is the electrokinetic transition temperature

Electrokinetic Properties

The electrokinetic properties of thermally sensitive particles are examined by measuring the electrophoretic mobility as a function of temperature, pH and salinity. The general trend is the increase in the electrophoretic mobility (in absolute value) versus temperature. This phenomenon was attributed to the reduction of the particle size, which results in the increase of the interfacial charge density of the particles, and therefore the increase in electrophoretic mobility (at constant pH and salinity) as illustrated in Fig. 4. However, the investigation of electrophoretic mobility as a function of salinity, ions nature, solvent and pH as at constant temperature is questionable. In fact, only few works have been dedicated to such phenomena [27, 28].

Colloidal Stability

Nabzar et al. [28] has reported interesting research work in the colloidal stability of thermally sensitive particles by studying amino-containing *N*-isopropylacrylamide-styrene copolymer particles. The colloidal stability has been examined as a function of both temperature and salinity (at a constant pH). The emanate results from those studies revealed the high colloidal stability below the volume phase transition temperature due to electrosteric stabilization and hydration forces. Whereas, the colloidal stability was considerably reduced above the T_{VPT} as expected. In short, the increase in salt concentration reduces the T_{VPT} and consequently the colloidal stability. Then, the colloidal stability of such stimuli-responsive microgels should take into account both temperature and salinity concentration. To some extent, the colloidal stability of thermally sensitive particles is a reversible process as illustrated in Fig. 5.

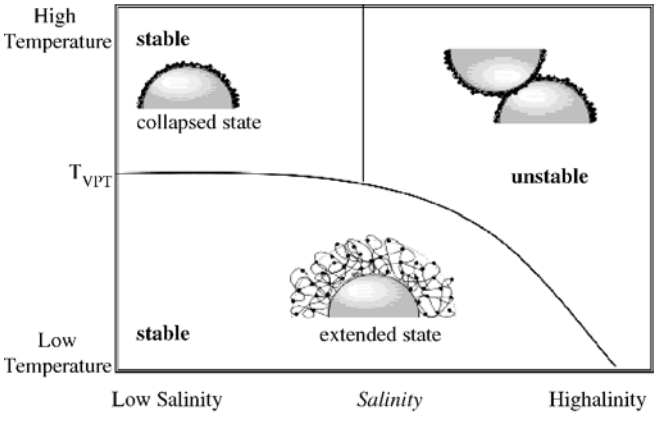

Fig. 5 Colloidal stability diagram of charged thermally sensitive particles as a function of temperature and salinity

Additional Properties

The internal and the interfacial microstructure of thermally sensitive microgel particles and core-shell latexes have been examined using the NMR technique [29] and fluorescence analysis [30] respectively. The NMR study of poly(NIPAM) microgel particles reveals that the water-soluble crossliner used is incorporated in a gradient composition (looser and looser from the core to the shell). This is in agreement with the high reactivity of such crosslinker. The Fluorescence analysis of the core-shell like particles (such as polystyrene-poly(NIPAM)) shows the complexity of the interface as a function of temperature and in some cases, the polystyrene core can be easily reached by the used hydrophobic probe. This point has been examined using fluorescent dye (pyrene).

Applications in Biomedical Diagnosis

Thermally sensitive particles exhibited outstanding capability for the immobilization of biomolecules such as proteins and nucleic acids. Various systematic studies have been reported by investigating the influence of physical chemistry parameters (i.e. pH, salinity, ions nature, temperature etc.) on the adsorption and desorption of biomolecules. Special attention has been focused on the adsorption and release of enzymes as model in drug delivery. The activity of entrapped and released enzyme molecules has been adequately examined. In this part, we will focus on some fine applications of thermally sensitive particles in biomedical diagnostic.

Proteins Purification and Concentration

The adsorption and the desorption of protein (or proteic materials) was found to be principally controlled by the incubation temperature as first reported by Fugimoto et al. [31] and then by Elaïssari et al. [32, 33]. The adsorbed amount of protein material onto thermally sensitive particles was examined in view of both electrostatic and hydrophobic interactions. As a general tendency, the proteins adsorption was principally controlled by the adsorption temperature (Fig. 6), which regulates the charge density and the hydration of the particles. The desorption of adsorbed proteins is then favoured by cooling the polymer particles bearing adsorbed proteins. Using such approach, protein concentration and purification are achieved by controlling the adsorption and the desorption, temperatures, the pH and the salinity during the incubation steps in order to enhance the desorption efficiency (below the volume phase transition temperature). The protein concentration is first reported to be possible by using anionic thermally sensitive core-shell magnetic particles [34]. The protein concentration step was examined by performing the desorption in a small volume.

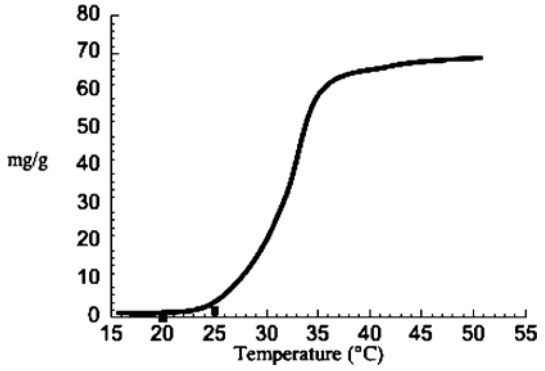

Fig. 6 Schematic illustration of proteins adsorption (in mg/g) onto thermally sensitive particles as a function of temperature [16, 18]

Nucleic Acids Extraction, Purification, Concentration and Amplification

Cationic thermally sensitive particles provide capacities that are potentially interesting for biomedical diagnosis and samples preparation (Fig. 7). The presence of cationic charges favours the attractive electrostatic interactions of nucleic acid molecules (ssDNA, DNA and RNA) negatively charged [32, 35]. It is interesting to notice that the adsorbed nucleic acids onto cationic poly(NIPAM) based particles can be desorbed by increasing both the pH and ionic strength. Whatever the incubation temperature, the quantities of nucleic acid molecules adsorbed decrease with increasing the pH, due to the reduction of cationic character of the particles. Then, to select the adsorption of nucleic acids rather than proteins, the adsorption should be performed below the T_{VPT}. To extract protein from any

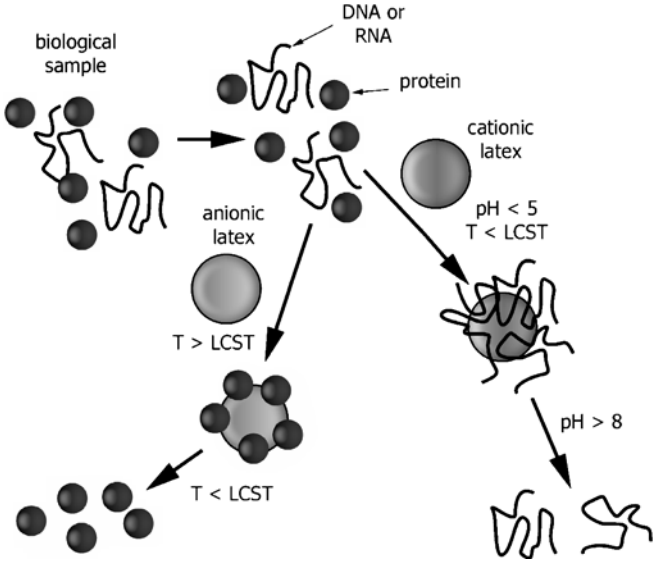

Fig. 7 Principle of separation and concentration of nucleic acids and proteins

biological sample containing nucleic acids, the use of negatively charged particles is strongly recommended.

Furthermore, amplification of captured nucleic acid molecules can be performed directly in the presence of such cationic hydrophilic particles without any release step. This is directly related the presence of hydrated shell and to total consumption of cationic charges on the particles. Consequently, for low particles amount, the PCR (Polymerase Chain Reaction) [17, 20] amplification of captured nucleic acids can be performed without removing the particles from the PCR medium, which reduces steps and then reduction in time consuming.

Conclusions

Although widespread, the use of hydrophilic monomers and functional comonomers in the precipitation polymerization often brings complex problems, some of them being far to be solved or even well understood principally in the case of combined polymerization processes. The distribution of water-soluble crosslinker in the polymer matrix is also questionable. In fact, the control of the crosslinker reactivity, will lead to well-defined microstructure particles.

The use of charged water-soluble comonomer leads to high amount of soluble oligomers and polymer chains. In such polymerization, special attention should be focussed on the reduction of water soluble-polymer formed generally after particles formation.

The colloidal characteristics of poly(*N*-alkylacrylamide) or poly(*N*-alkylmethacrylamide) based microgel particles depend on temperature, salinity and pH. The incubation temperature principally governs the swelling ability, the hydrodynamic size, the electrokinetic properties and the colloidal stability. The volume phase transition temperature is mainly related to the chemical composition of the polymer particles.

Such stimuli-responsive particles are explored in biomedical applications in order to improve the sensitivity via samples preparation (i.e. purification and concentration of targeted biomolecules). The cationic particles are explored as new tool for nucleic acids extraction, purification and concentration. Interestingly, such hydrophilic particles are compatible with various enzymatic nucleic acids amplifications. The use of negatively charged thermally sensitive particles was found to be useful for protein purification and concentration. This application has been extended to bacteria and viruses detection and found to be of great interest.

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