Reactive Polymer Based Colloids for Biomedical Applications

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Summary: This short review aimed to give to the reader brief applications of polymer colloids in biomedical area and principally in medical diagnostics. Colloidal polymer particles are mainly used as solid supports, as label for biomolecules reactions detection, and as a carrier. The elaboration of appropriate colloids is reached via numerous processes such as polymerization in dispersed media (dispersion, emulsion, precipitation, miniemulsion polymerizations) or as physico-chemical methodologies (i.e., self-assembly). After a short introduction, some particles elaborations are presented and their applications are briefly described and illustrated.

Keywords: latexes; nucleic acids; polymer colloids

Introduction

Polymer colloids have received an increasing interest as solid-phase supports in numerous applications, especially in the biomedical domain, due to the versatility of the heterophase elaboration processes (emulsion, dispersion, precipitation, physical processes) for making well-defined microspheres with appropriate particle sizes and surface reactive groups.[1,2] Then, the current researches are focused on the following points.

Synthesis of Reactive Colloidal Particles

This study is devoted not only to the synthesis of classical polymer particles such as hydrophobic ''polystyrene'' microspheres but also to the preparation of hydrophilic, smart (sensitive to the pH, salinity, and temperature), magnetic,[3] and labeled particles. The influence of each reagent involved in the recipe on the polymerizations kinetic (rate of polymerization, conversion, number of particles, etc.) and on the final dispersion properties is carefully examined. Then, various elaborations (i.e., polymerization) methodologies and processes are performed in order to prepare structured microgel and core-shell latex particles bearing shell with well-defined properties.[1]

Physico-Chemical and Colloidal Characterization of Latexes

After dispersions elaboration, many colloidal and surface properties are examined: particle size, particle size distribution, nature and concentration of surface groups (i.e., surface charge density), availability of reactive groups, morphology of the particles, hydrophilic/hydrophobic surface balance, electrokinetic properties, and colloidal stability of the particles.[4]

Interactions Between Biomolecules and Particles

The immobilization (adsorption and/or covalent grafting) of biomolecules is studied by taking into account the influence of physico-chemical parameters such as: pH, salinity, buffer nature, temperature, surface nature, and the presence of competitive adsorbing agents. The immobilized biomolecules onto latex particles are characterized with respect to their conformation and biological activity.[5,6]

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Applications of Polymer Particles-Biomolecules Conjugates in the Diagnostic Field

The obtained colloids and particles-biomolecules conjugates are evaluated in targeted biomedical applications such as immunology, specific capture of nucleic acid molecules, cell sorting and identification, bacteria isolation and detection, virus extraction, concentration and detection. Each application is examined by investigating the specificity, the stability, and the sensitivity of the prepared bare particles and/or particles bearing biomolecules such as antibody, single stranded DNA fragments (oligonucleotide noted, ODN).[2,7]

The specificity and the sensitivity of the targeted applications are directly related to the surface particles properties and to the accessibility of the immobilized biomolecules. The interactions between biomolecules and reactive particles are strongly dependent upon; on one hand the colloidal and surface properties of the dispersion, on the other hand the physico-chemical properties of the biomolecules.

Classical Polymer Particles

The objective of this part is to focus on some applications of classical polymer particles such as polystyrene latex particles in biomedical diagnostic. In this domain, polystyrene latexes have been used (as carrier for antigen and antibody reaction) in immuno-agglutination assay as first described in 1956 by Singer[8] and applied to rheumatoid factor detection. Such application is based on formation of macroscopic and visible cluster of polymer particles as a consequence of the capture of the target, which induces bridging flocculation.[9]

Experimentally, antibody-coated polystyrene particles dispersion in appropriate buffer solution, becomes unstable when the specific antigen molecules are in the solution and then recognized by the antibody. This antibody-antigen-antibody specific reaction led to bridging flocculation of the sensitive polymer particles (particles bearing immobilized antibody molecules). The flocculation of the sensitive particles is fast since only 2 min are needed to observe the aggregation nucleation process. In the absence of the specific antigen, the sensitive particles remained fully stable (Figure 1).

The immuno-agglutination assay is acceptably specific and sensitive but not quantitative. This method is used for quick examination before the investigation of more sensitive and quantitative test named enzyme linked immuno sorbent assay (ELISA)[10] as schematized below. It is

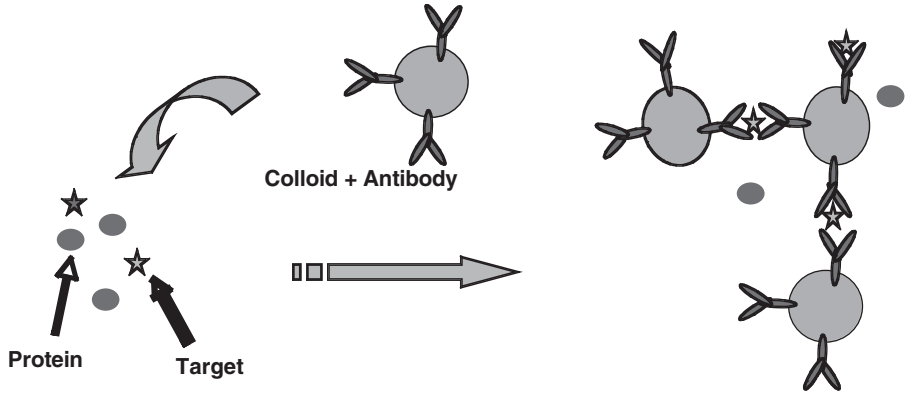


Figure 1.
Immuno-agglutination assay of antibody containing particles. The agglutination is induced by the specific capture of the targeted antigen molecules.

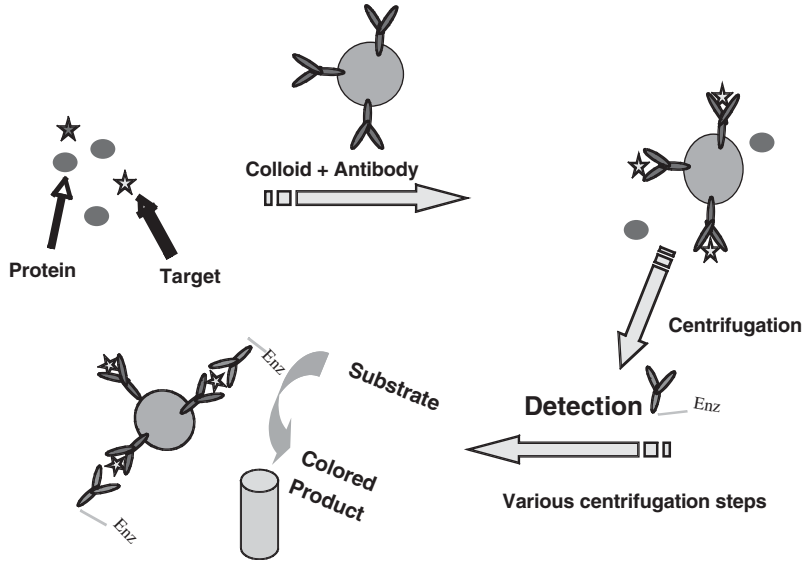


Figure 2.
Schematic illustration of immunoassay using labeled antibody (ELISA, enzyme-linked immuno sorbent assay). Enz = enzyme.

interesting to notice that, the immunoagglutination was also studied for viral particles capture as recently reported by Imbert-Laurenceau et al.[11] by using sensitive polystyrene particles (Figure 2).

Such application needs the use of sensitive latex particles (particles bearing immobilized antibody molecules). The capture of the target is achieved by adding a second antibody bearing an enzyme. The quantification is performed by adding subtract, which is sensitive to enzyme, and the supernatant becomes colored. The intensity analysis is directly related to the concentration of the captured target molecules.

The immobilization of antibody onto polymer particles used in such application is performed via physical adsorption[12] (mainly via hydrophobic interaction) or chemical grafting onto functional reactive groups as summarized in the Table 1.

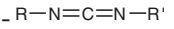
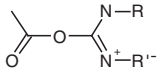
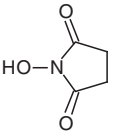
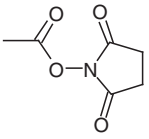
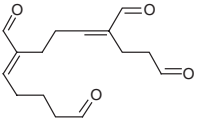
Polymer beads (large particle size) are also widely used in analytical immunoaffinity chromatography based on selective isolation of biomolecules. The target biomolecules are specifically captured onto polymer particles and released after purification step.[13,14]

Stimuli-Responsive Particles

Stimuli-responsive polymer particles are colloidal particles sensitive to pH, salinity, temperature, light, electrical field, etc. The most targeted applications are therapeutic approach in which such smart colloids are explored as a carrier. But, before any *in vivo* application, the particles should be biodegradable or bio-eliminable, which is far to be the case for the used stimuli-responsive polymer particles. Whereas, pH and thermally sensitive particles are studied in terms of preparation, physico-chemical characterization and their use studied in biomedical diagnostics.

Basically, the preparation of thermally sensitive charged and hydrophilic microgel particles is based on *N*-alkylacrylamide or *N*-alkylmethacrylamide monomers (i.e., *N*-isopropylacrylamide), methylene bis acrylamide (MBA), water soluble crosslinker, and water soluble radical initiator.[1] The polymerization should be conducted above the lower critical solubility temperature

Table 1.
Routine chemical grafting of biomolecules onto reactive colloidal particles.

Surface reactive compound	Reactive compound on biomolecule	Activating agent	Active derivative
	$-NH_2$	 carbodiimide	
	$-NH_2$	 *N*-hydroxysuccinimide	
$-NH_2$	$-NH_2$	 glutaraldehyde	
	$-NH_2$		
	$-NH_2$	-	-

(LCST). Using well appropriate monomer induces the functionalization of the final particles. Nowadays, various structured and stimuli-responsive submicron particles have been prepared using versatile comonomers, recipes, and methodologies in order to control the colloidal properties of the particles (particle size, swelling ability, surface charge density, electrokinetic properties, etc.). The colloidal properties of thermally sensitive and charged particles can be summarized as reported below. The hydrodynamic particle size decreases with increasing the incubation temperature, consequently, the surface charge density (i.e., zeta potential) increases. The volume phase transition temperature was found to be in the LCST range of the corresponding linear homopolymer (Figure 3).[15]

Due to hydrophilic character of such polymer particles, the adsorbed amount of protein was found to be negligible below the TVPT and high above.[16,17] Then, the first evaluation of these particles in biomedical diagnostic was in protein purification by performing the adsorption and the desorption above and below the TVPT, respectively. The second evaluation was in nucleic acids extraction and purification by performing the adsorption at acidic pH and below the TVPT of the used cationic thermally sensitive microgel particles.[18] The desorption of adsorbed nucleic acid molecules was favored at basic pH and moderate salinity as illustrated below (Figure 4).

Magnetic Carriers

Magnetic latexes are colloidal polymer particles containing magnetic materials (i.e., iron oxide nanoparticles). The presence of a magnetic material in the polymer particles endows the composite particles with magnetic properties.[3] Magnetic latexes are largely used in biomedical field as a solid support, in immunoassays, molecular biology, cell sorting, and bacteria and viruses isolation.[2] The magnetic property is principally used to facilitate separation via the use of single magnetic

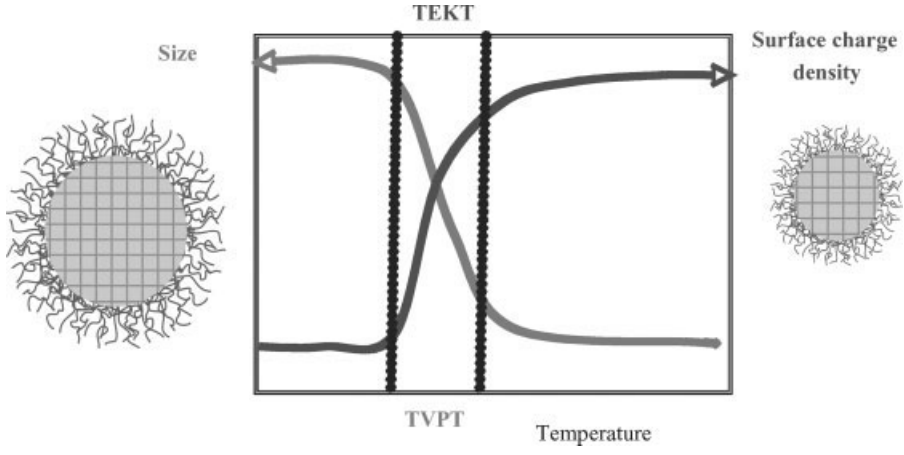


Figure 3.
Illustration of hydrodynamic particle size and surface charge density (i.e., electrophoretic mobility) of charged thermally sensitive particles as a function of temperature. TVPT is the volume phase transition temperature and TEKT is the electrokinetic transition temperature.

and particle guidance in microsystems. In addition, the magnetic property is also used to enhance the concentration of the targeted biomolecules and consequently the sensitivity of the biomedical diagnostic. Due to the possible elaboration of well-defined magnetic particles, various biomedical diagnostic tools are automated leading to rapid and high sensitivity analysis of biomedical samples.

In this section, first, the elaboration of reactive, submicron, highly magnetic latexes

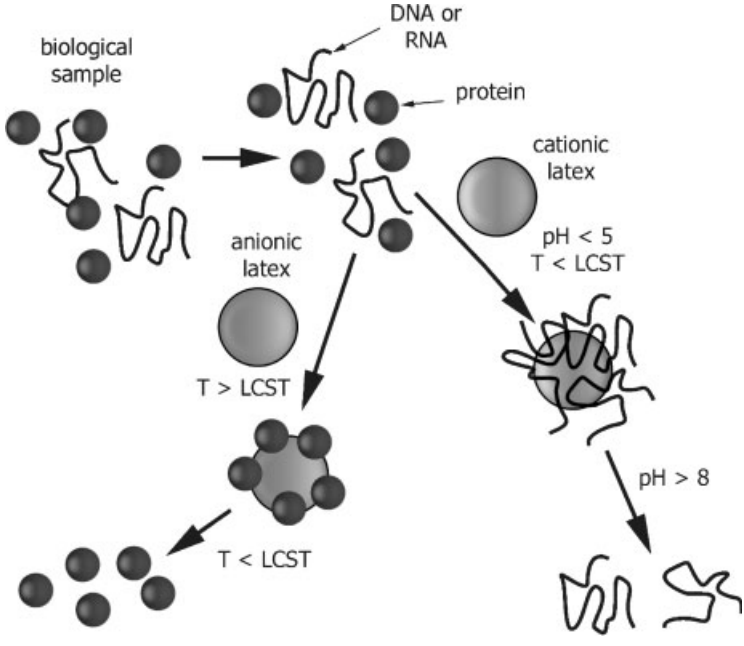


Figure 4.
Schematic illustration of proteins and nucleic acids extraction, purification, and concentration using thermally sensitive microgel particles.

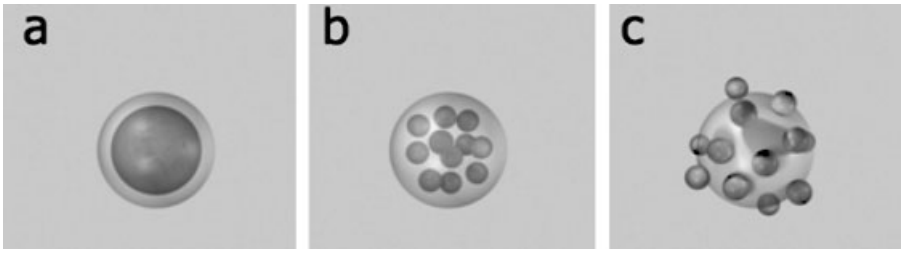


Figure 5.
Schematic illustration of magnetic latexes: (a) magnetic core-polymer shell particle, (b) iron oxide nanoparticles dispersed in polymer matrix, and (c) self-assembly of magnetic nanoparticles onto polymer seed.

is presented. Second, the pertinent applications are presented such as specific and non-specific biomolecules (nucleic acids, proteins, bacteria, and viruses) extraction, purification, concentration, and detection.

Elaboration of Magnetic Carriers

In recent years, increasing interest has been given to the preparation of magnetic latex particles for diagnostic applications. The pioneer work in this field was performed by Ugelstad et al.[19] who reported on the preparation of monosize magnetic microspheres of micrometer size and their utilization as a support for biomolecules.[20] Since this work, several methodologies on the preparation of magnetic latexes have been investigated as reported by (i) Kondo et al.[21] in the case of thermally sensitive poly(styrene/*N*-isopropylacrylamide/methacrylic acid) magnetic particles, (ii) batch emulsion polymerization of styrene in the presence of magnetic iron oxides by Charmot,[22] and (iii) investigation of the heterocoagulation concept and self-assembly by Furusawa et al.[23] and Sauzedde et al.[24] The possible magnetic latex morphologies are presented below (Figure 5).

Recently, new approach has been developed in our laboratory, which consists in the elaboration of functionalized magnetic latex particles from oil in water (o/w) magnetic emulsion[25] (i.e., ferrofluid droplets). The influence of the polymerization process and recipes was investigated as systematic studies. Consequently, submicron magnetic latex particles exhibiting asymmetric structure or homogeneous core-shell morphology were obtained as examined below by transmission electron microscopy (Figure 6).

Nucleic Acids Extraction, Concentration, and Detection

Due to the polyanion character of nucleic acid molecules (DNA and RNA), cationic colloidal particles are used for adsorbing

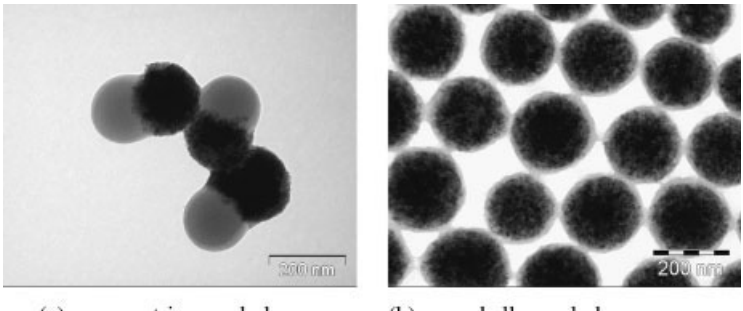


Figure 6.
TEM analysis of structured magnetic latexes as obtained from o/w magnetic emulsion transformation.[26]

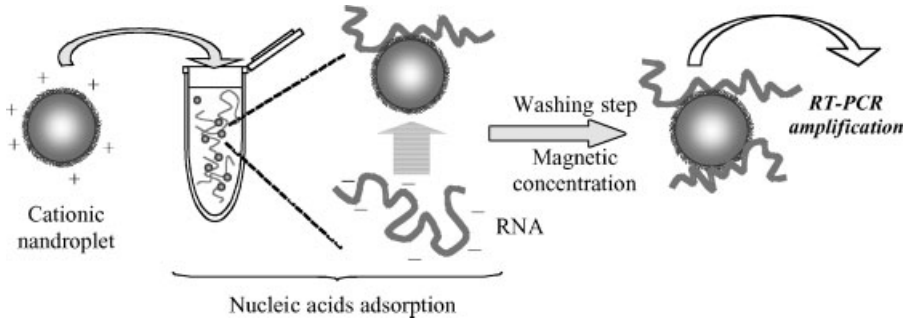


Figure 7.
Illustration of non-specific capture, purification, and concentration of nucleic acid molecules.

DNA and RNA via attractive electrostatic interactions. The adsorption is mainly controlled by the pH and the salinity of the incubation medium. The use of cationic magnetic latexes was explored in the adsorption of nucleic acids. The adsorption was reported to be rapid and high at low ionic strength and at acidic medium. After magnetic separation of nucleic acids containing magnetic particles, the supernatant is removed and the immobilized nucleic acids are then washed and desorbed in small volume to enhance their concentration. The desorption is principally governed by the pH (i.e., high desorption at basic pH) (Figure 7).[2]

The purified nucleic acid molecules are then used in specific biomedical diagnostic in order to identify the sequence of the target via specific capture of nucleic acid fragments. The purified nucleic acids are enzymatically amplified by PCR (in the case of DNA) and by RT-PCR in the case of RNA before any specific capture as presented below.

The specific capture of nucleic acids using magnetic particles is generally performed as follows. The capture probe of well-defined sequence is chemically immobilized on the magnetic latex particles. A given biological sample (or the above purified nucleic acids) is mixed with the magnetic particles-ODN conjugates. The target is then specifically captured via hybridization process (specific hydrogen binding). The detection is performed by adding the labeled detection probes (i.e., oligonucleotide labeled with enzyme). The addition of substrate is oxidized by the enzyme, which leads to colored supernatant as in immunoassay. This specific capture of nucleic acid molecules combined with well-optimized detection process leads to the enhancement of various diagnostics (Figures 8 and 9).

Conclusion

In this short and condensed review on polymer colloids in biomedical applications, various aspects related to reactive particles elaboration and some fine applications in biomedical diagnostic are presented.

The preparation of colloids should solve various questions related to the target application. In fact, colloidal particles bearing reactive groups, such as –COOH, $–NH_2$, –SH, etc., are suitable for the covalent binding of biomolecules in order to be used as a solid support for specific capture of targets. In this well-defined area, the immunodiagnostic and molecular biology used various kinds of colloidal particles such as polystyrene latexes, silica beads, and magnetic particles. The main problem remaining in this area is related to non-specific interactions between the targets and the solid support.

In addition to those applications, the magnetic colloidal particles are also explored in various sample preparations and found to be of great interest in reducing

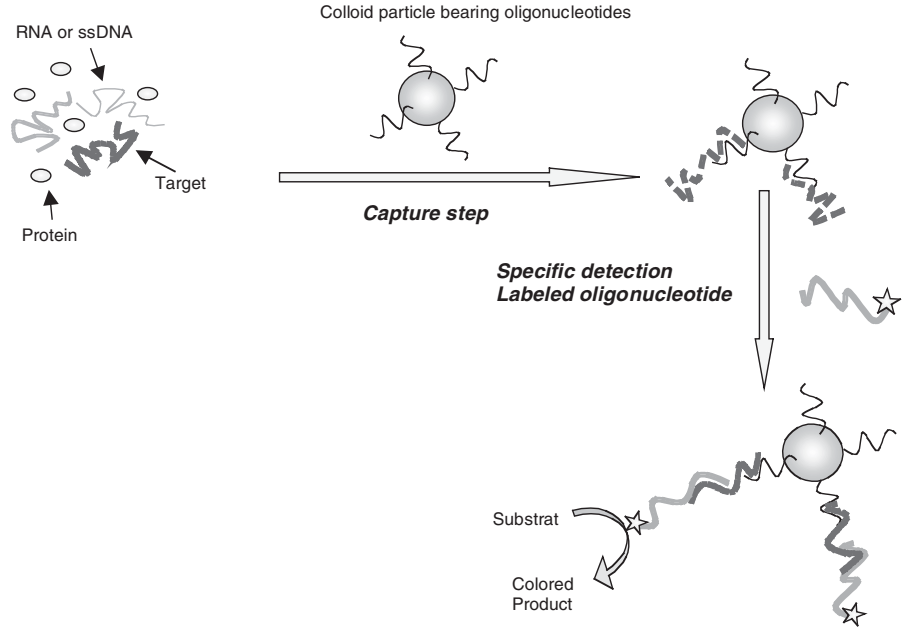


Figure 8.
Illustration of specific capture and detection of nucleic acids (ODN, oligonucleotide; ssDNA, single stranded DNA fragment). The intensity of the supernatant due to the presence of colored product was determined.

time-consuming and manipulation steps, and purification and concentration of captured biomolecules in small volume.

To target any biomedical diagnostic application, well appropriate colloidal particles need to be used. The elaboration of polymer-based particles can be performed using various polymerization processes in dispersed media. To prepare suitable solids support, the polymerization kinetics and the colloidal characterization are conducted as systematic studies. The characterization of the final particles is of great importance, since well-characterized particles help the investigation of biomolecules interactions with the colloidal support.

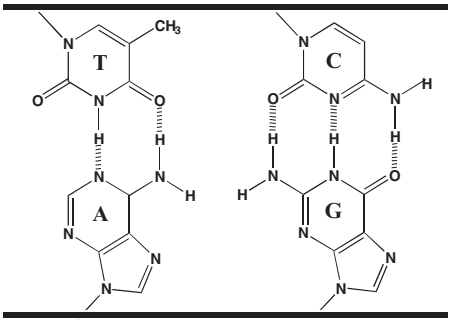


Figure 9.
Hybridization between complementary bases (thymine/adenine) and (cytosine/guanine).

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