Advances in the Biomedical Applications of Reactive Colloids

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This short review aimed to give to reader's brief applications of polymer colloids in biomedical area such as therapy and medical diagnosis as also developed in our laboratory. Indeed, the polymer particles and composite particles are commonly used in immunoassays as solid phase supports, for the encapsulation of active agents and for the immobilization of biomolecules such as oligonucleotides, proteins or antibodies. In the area of composite particles, magnetic particles bearing immobilized biomolecules are used in biomedical diagnosis such as immunoassay, specific nucleic acids concentration, cell labelling and separation and in numerous biotechnological applications.

Keywords: colloid, biomedical, in-vivo, in-vitro, biotechnology, polymer, latex, diagnostic, drug-delivery

1. INTRODUCTION

Polymer colloids have received an increasing interest as solid-phase supports in numerous applications, especially in the biomedical [1, 2], 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 [3, 4].

The inorganic colloidal nanoparticles are used in dipsticks as label in the detection step [2, 5]. In this field, gold nanoparticles [6, 8] are used in order to enhance and to facilitate the read of the results via the apparition of intense coloured line [9].

Polymer based colloids are elaborated using numerous processes [10]. The more examined particles are latexes and principally polystyrene based particles. The synthesis of those classical latexes mainly hydrophobic in nature is performed using radical polymerization in dispersed media such as: emulsion, miniemulsion, dispersion microemulsion etc... Recently special attention has been dedicated to the preparation of hydrophilic, smart (sensitive to the pH, salinity and temperature) [11] particles. Those particles are used as models for in vivo applications in drug delivery and also in in-vitro biomedical diagnostic area [12]. The magnetic particles [2] are specially designed in order to replace the heavy processes used during the separation of particles from the continuous phase (centrifugation and filtration). In fact, using any classical permanent magnet, the magnetic particles can be collected and concentrated in small volume. The elaboration of magnetic particles can be performed using different processes and now days, the market offer a variety of magnetic nanparticles, microspheres and beads bearing reactive groups [1, 13, 14].

Colloidal particles are used in both in-vivo and in-vitro biomedical applications. But before any real application, the particles are first conjugated with appropriate receptor or biomolecules in order to target the specificity of the application. Then, the obtained particles-biomolecules conjugates are evaluated in targeted biomedical applications such as local targeting15 in drug delivery system [16], in-vivo diagnostic [17], immunology [18], specific capture of nucleic acid [19] molecules, cell sorting [20] and identification, bacteria isolation [21] and detection, viruses [22] extraction, concentration and detection [1].

In this short and non-exhaustive revue, the aim is to give to the reads some information related to particles for in vivo applications and for in vitro biomedical diagnosis.

2. PARTICLES FOR IN-VIVO APPLICATIONS

Drug targeting is a novel approach in pharmaceutical technology receiving so much attention in medical research field with a great progress these last decades [16]. For such medical applications, nanoparticles (i.e. nanoliposomes, nanospheres and nanocapsules...) [16, 23, 24] should be biodegradable polymers particles in nature. They can be developed as a matrix incorporating a drug in the whole system (like nanospheres) or capsule-like system with a polymeric shell surrounding a core where the drug is encapsulated. These nanoparticles for drug delivery system offer many application possibilities such as in the field of medicine, biotechnology, cosmetic and also in both agriculture and the industry fields. For illustration, the schematic presentation of colloidal particles commonly used or studied in this field is below presented.

In addition to polymer-based particles, iron oxide containing polymer particles are also used. In fact, magnetic particles are found to be of great interest in in-vivo biomedical diagnosis in which they are used as contrast agent in order to perform resonance magnetic imaging (RMI) [25, 26].

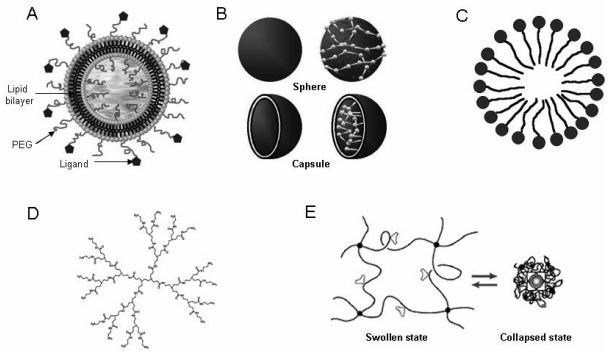

FIG. 1: Schematic presentation of: (A) liposomes, (B) polymer particles (spheres and capsules), (C) micelles, (D) dendrimer, (E) hydrogel.

3. PARTICLES FOR IN VITRO APPLICATIONS

3.1. Classical polymer particles

Non magnetic polystyrene latexes have been largely used (as carrier for antigen and antibody reaction) in immunoagglutination assay as described in 1956 by Singer [8] and first used for rheumatoid factor detection. A given antibody is chemically [27] or physically [28] immobilized onto polymer-based particles such as polystyrene latex particles. The immobilization performed using well-defined condition such as pH, salinity, temperature and antibody/particles ratio etc. The presence of any specific antigen in the biological sample reacts immediately with the antibody, which induces rapid flocculation of the latex particles via bridging flocculation mechanism [29, 30]. The formed clusters can be evidenced by naked eyes as illustrated in figure 2. The immunoagglutination [30] assay is acceptably specific and sensitive but not quantitative.

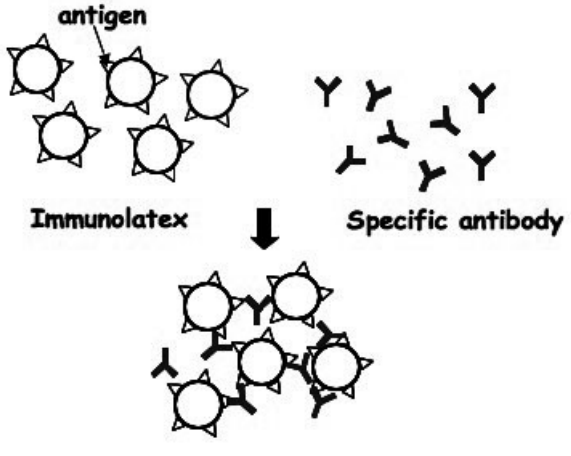

FIG. 2: Immuno-agglutination assay of antibody containing particles. The agglutination is induced by the specific capture of the targeted antigen molecules [31].

3.2. Magnetic carriers

Magnetic particles and magnetic latexes are widely used in bionanotechnology based applications and principally in biomedical diagnosis such as in immunoassays, molecular biology, cell sorting, and bacteria and viruses isolation [2]. In addition, the magnetic property is also used to enhance the concentration of the targeted biomolecules and consequently the sensitivity of the biomedical diagnostic.

Increasing interest has been dedicated to the preparation of magnetic particles and magnetic latex particles for diagnostic applications purpose. The pioneer works in this domain were reported by Ugelstad [32] by reporting not only on the preparation of magnetic latexes, but also their use in biomedical diagnosis. Other approaches were developed in order to prepare well-defined reactive magnetic particles such as; (i) thermally sensitive magnetic particles by Kondo et al. [33], (ii) batch emulsion polymerization of styrene in the presence of magnetic iron oxides by Charmot et al [34] and (iii) miniemulsion polymerization of styrene containing organic ferrofluid and more recently, (iv) transformation of oil in water magnetic droplet by Elaissari et al. [35] via seed emulsion polymerization process. It is interesting to notice, that only this last process leads to highly magnetic submicron latex particles.

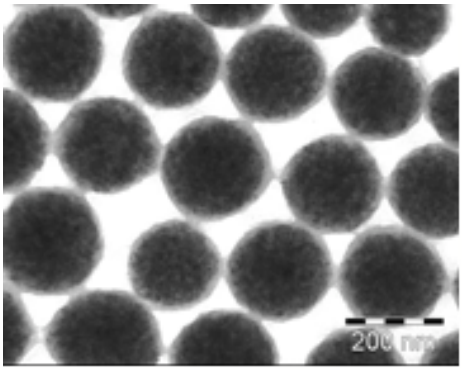

FIG. 3: Transmission Electron Microscopy analysis of structured core-shell morphology magnetic latexes as obtained from o/w magnetic emulsion transformation [26].

3.3. Nucleic acids extraction, concentration and detection

In in-vitro biomedical diagnosis, magnetic particles or beads are generally used in sample preparation and in some cases in order to separate easily the particles from the aqueous phase. In this area, two processes where used for nucleic acids extractions:

3.3.1. Non-specific capture of nucleic acids

The generic capture and purification of nucleic acids is performed using cationic magnetic beads. Then, by controlling the pH and the salinity of the medium it is possible to extract nucleic acids from any complex medium and to release

them in small volume which leads to purification and concentration processes by using cationic magnetic particles [2]. The extracted nucleic acid molecules can then be amplified on the magnetic beads or after desorption step and removal of the magnetic particles using PCR (polymerase chain reaction) [36] in the case of DNA molecule or RT-PCR (Reverse Transcriptase PCR) [37] in the case of RNA as below illustrated [38].

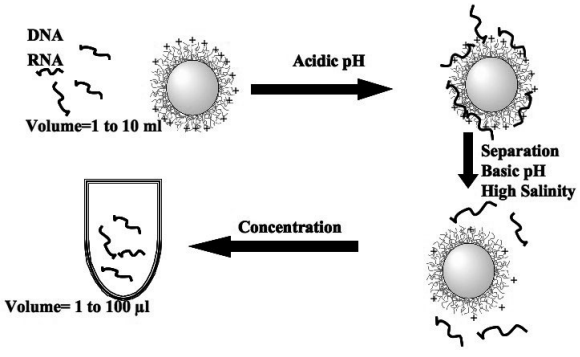

FIG. 4: Illustration of non-specific capture, purification and concentration of nucleic acid molecules. The adsorption of nucleic acid molecules is performed at acidic pH using cationic magnetic particles. After magnetic separation of magnetic particles and removal of the supernatant, the adsorbed nucleic acid molecules are desorbed using basic pH and high salinity medium. The released nucleic acids are then collected in small volume.

3.3.2. Specific capture of nucleic acids

The specific capture of nucleic acids [39, 40] 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 (ODN for oligonucleotide) conjugates. The target is then specifically captured via hybridization process (specific hydrogen binding). The detection is performed by adding the labelled detection probes (i.e. oligonucleotide labelled with enzyme) [41]. The addition of substrate is oxidized by the enzyme, which lead to coloured supernatant as in immunoassay. This specific capture of nucleic acid molecules combined with well-optimized detection process lead to the enhancement of this molecular biology based diagnosis [42].

4. CONCLUSION

The preparation of colloidal particles should solve specific questions related to the targeted applications. In fact, colloidal particles bearing reactive groups such as (−COOH, −NH2, −SH etc.…) are suitable for the covalent binding of biomolecules in order to be used as a solid support for specific capture of targets and also suitable for the encapsulation of active molecules (i.e. drug) or biomolecules (peptides, nucleic acids…).

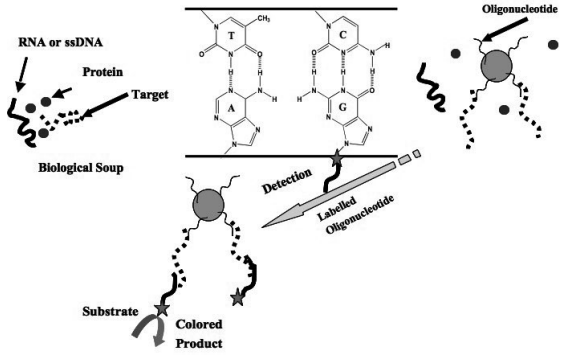

FIG. 5: Illustration of specific capture and detection of targeted nucleic acids. Particles bearing chemically grafted ODN (Oligonucleotide) are mixed with a given biological sample containing the target. After the specific capture of the target, the particles are extracted and washed before adding detection probe (ODN bearing enzyme). The evidence of the specific capture is performed by adding substrate which reacts with enzyme in order to lead to coloured medium [4].

To target any biomedical application (in-vivo or in-vitro use), well-appropriate colloidal particles need to be used. The elaboration of polymer-based particles can be performed using well established formulation recipes and polymerization processes. Before any application, the physical chemistry and the colloidal properties of the particles are of great interest. In fact, the examination of those properties is suitable in order to control the interaction between the particles and the used active agents, biomolecules etc…

To prepare suitable solids support, many criteria should be considered, the particles, the size distribution, the surface polarity of the particles, the surface charge density, the chemical composition of the particles, the compatibility (the degradability or the possible bio-elimination), the internal and external morphologies, the colloidal stability, the swelling ability and finally the intrinsic properties of the particles.

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 helps the investigation of biomolecules interactions with the colloidal support.

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