

Reactive Nanocolloids for Nanotechnologies and Microsystems

CHRISTIAN PICHOT

CNRS-bioMérieux, Ecole Normale Supérieure de Lyon 46, allée d'Italie, 69364 Lyon Cedex, France

1.1 INTRODUCTION

Nanoscience and nanotechnology continue to play a growing and tremendous interest, both on academic and industrial aspects. They have been applied in many systems such as in the development of nano and microdevices for nanodiagnostics, biotechnology, metrology, and molecular manufacturing. Such interest relies on the fact that it is now possible to manipulate nanometer-length atoms and molecules in order to create, according to a bottom-up technology, larger structures with outstanding properties. In biotechnology, many domains are concerned: diagnostics, microarrays, biological analysis, biochips, biominiaturization, drug delivery systems, and so forth. For instance, the development of lab-on-chips (the so-called microTAS (micro Total Analysis Systems) responds to the evolution of the bioassays toward miniaturization, which implies to deal with very small volumes of biological samples. Of course, such a drastic change raises many complex problems regarding the manipulation of fluids in confined micrometer channels integrated on a plan support (1–3).

It appeared obvious that the setup of these new systems needs appropriate tools as regards to transport, extraction, and detection. It has been found that colloidal particles, especially those having one dimension below 500 nm, proved to be very suitable and efficient tools due to their unique and versatile properties, and several examples of their use can be found in the literature (4,5). For a long time, the preparation of organic and inorganic colloids has benefited of a period of an active and fruitful research and development. A huge number of processes, more or less sophisticated, allow to carefully control the shape, particle size and size distribution, and structure and surface properties in relation to the field they have to be utilized. It

should be reminded that in these (nano)colloids, surface aspects become more and more predominant as dimension size is decreasing. Table 1.1 provides a nonexhaustive list of various submicronic-sized mineral, organic, and composite colloids, together with some examples in which they are applied. From this table, it is worthwhile to notice, first that nano-sized colloids (such as gold and quantum particles) play a very important role in the detection step of bioassays, especially in molecular diagnostics, and second that polymer dispersions offer a wide variety of organic-based colloids as such or as composites.

The major objective of this chapter aims at giving basic information regarding the main manufacturing methods of various types of (nano)colloids involved in the

TABLE 1.1 Some Examples of (Nano)colloids Used in Microsystems and Nanobiotechnologies.

Nature of particle	Size domain	Example of applications
<i>Inorganic particles</i>		
Gold particles	10–30 nm	Colorimetric detection of DNA sequences (6)
Other metallic and bimetallic particles (Pt, Pd, Ru)	2–10 nm	Labels for chip-based DNA detection (7)
Metal oxides (ferrofluids, superparamagnetic particles)	5–10 nm	Medical imaging (8)
Silica nanotubes	A few nanometers wide	Probes, biological sensing (9)
Semiconductor nanocrystals (quantum dots)	2–10 nm	Detection and quantification of biological molecules (10)
<i>Organic particles</i>		
Carbon nanotubes and fullerenes	A few nanometers	Templates, DNA targeting (11)
Dendrimers	10–50 nm	Reservoirs of drugs, DNA chips (12)
Polyelectrolyte complexes (natural and synthetic polymers)	50–200 nm	Drug targeting, vaccination (13,14)
Self-assemblies of polyethylene oxide block copolymers	50–200 nm	Stealth drug delivery systems (15)
Latexes	20–1000 nm	Solid-phase assays, vaccination, two-dimensional arrays (16)
<i>Organic/inorganic composite particles</i>		
Magnetic particles	100–1000 nm (and more)	Diagnostic (17), extraction of DNA, cells, virus (18)
Fluorescent nanoparticles	30–500 nm	Time-resolved fluorescence bioassay (19)
Silica-based nanoparticles	50–200 nm	Bioanalytical applications (20)
Polymer-metal nanocomposites (gold and polypyrrole)	10–30 nm	Bioassays (21)

development of nano and microsystems to be used in biotechnologies. After focusing on the special requirements that such nanoparticles should fulfill with regards to their colloidal and surface aspects, particularly their functionality, the main preparation methods will be reviewed and discussed depending on the nature of the organic or inorganic material.

1.2 WHAT CRITERIA FOR NANOCOLLOIDS IN NANO AND MICROSYSTEMS?

Due to their use in microsystems in which surface and volume effects are predominant, the design of nanocolloids needs to take into account a large number of variables with respect to molecular, surface, and colloidal properties of particles, such as those depicted in Table 1.2. It is obvious that for any application, nanocolloids should be preliminary characterized as completely as possible.

TABLE 1.2 Criteria and Related Properties to be Considered of for Nanocolloids Used in Microsystems.

Criteria	Property
Particle size and polydispersity	To determine surface area
Monodispersity	To get reproducible data
Colloidal stability	To keep nanoparticle stability against temperature, pH, shearing, and salinity
Surface charge density	To impart ionic charges at the interface
Density	To avoid sedimentation (large particles)
Cross-linking	To avoid solubilization in the solvents of organic colloids
Porosity	To favor the incorporation of dyes, small molecules, drugs, etc.
Specific functionality	
<ul style="list-style-type: none"> • Reactive surface groups 	Covalent grafting of nanoparticles on plane surfaces or with biomolecules
<ul style="list-style-type: none"> • Hydrophilicity 	Depletion of biomolecules, stealth effect
<ul style="list-style-type: none"> • Sensitivity to stimulus (T, pH, ionic strength, UV, light, electric, or magnetic fields) 	To change the nature and properties of colloidal particles
<ul style="list-style-type: none"> • Complexation (PEO, PMAA, metal chelates, etc.) 	Performing protein purification, oriented immobilization of proteins, antibodies
<ul style="list-style-type: none"> • Biological ligand (oligosaccharide, lipid, peptide, nucleic acid, antibody, protein) 	Recognition of antigens, specific cells, DNA, RNA, protein, lectins
<ul style="list-style-type: none"> • Magnetic 	Fast separation of colloids, imaging
<ul style="list-style-type: none"> • Color, fluorescence 	Detection by optical methods
<ul style="list-style-type: none"> • Biodegradability 	To be used for <i>in vivo</i> applications

PEO: polyethylene oxide; PMMA: polymethylmethacrylate.

1.2.1 Shape of Particles

Although, the spherical shape is the more thermodynamically stable form that many types of colloids usually adopt, it is worth mentioning that various and multiple other shapes can be obtained depending on the nature of material and process of preparation. This is particularly the case of inorganic colloids where ellipsoidal, rod-like, cubic, platelet, needle-like, and other shapes can be found. In the case of organic particles, it is also feasible to make colloids with nonspherical shape, but they are often in a thermodynamic metastable state, which leads, depending on the diffusion capability of the material (polymers), to a progressive evolution toward a spherical form.

1.2.2 Particle Size and Distribution

At first, the control of particle size and particle size distribution is a very important requirement since it defines the available surface area. As it will be discussed in more details in the next section, numerous appropriate preparation methods are now available, both for the synthesis of inorganic and organic nanocolloids in a large colloidal size domain (a few nanometers to 1000 nm). It is relatively easy now to produce colloids with very narrow size distribution, the so-called “monodisperse” colloids. This property will be discussed later on. The size monodispersity should be obeyed for several reasons: for the sake of reproducibility for immunoassays used in diagnostics; in drug delivery systems in which particle size should not overcome a limit; in transport in micrometer-sized channels, for the preparation of two or three-dimensional organization of particles on a surface or in a volume, and so forth.

1.2.3 Surface Charge Density and Colloidal Stability

In many cases, ionic surface charges must be imparted to the particles for different purposes. A major one is that efficient colloidal stability should be ensured to the particles for avoiding irreversible aggregation in the various steps of handling of such colloids: along their synthesis; during storage; their functionalization; and finally, in the numerous application domains, they are used: mixing either with other colloids or with biological fluids (usually exhibiting significant ionic strength) or under shearing. Since colloids, except in specific cases (microemulsions) are thermodynamically unstable, they can be made metastable for long-term periods provided an energy barrier is imparted by the presence of ionic charges (electrical stability) or of a polymer layer (steric stabilization).

The nature (anionic vs. cationic) and density of the surface charges must be taken into account for several reasons: surface properties of the device in which they could be immobilized or be transported; nature of other colloids with which they can be mixed; and physicochemical properties of the biological molecule to be fixed. Such ionic charges can be incorporated during the synthesis, especially by correctly adjusting the recipe: for instance, for polymerization in heterogeneous media

(nature and amount of the initiator usually bearing an ionic charge, addition of a surfactant, presence of ionic or ionogenic monomers or macromonomers, etc.) or by a chemical postreaction.

In general, for inorganic colloids such as metal oxides (silica, ferrite), hydroxyl groups are available at the surface and pH change can introduce anionic or cationic charges. As shown in the next section, surface modification of inorganic particles can be performed in order to incorporate organic species or various synthetic or natural macromolecules.

1.2.4 Interfacial Polarity

One major drawback when using nanoparticles as solid-phase supports (antibodies, proteins, nucleic probes, and enzymes) is that nonspecific adsorption could severely affect both the efficiency of the detecting device as well the conformation of the biomolecule and consequently its activity. In that purpose, the control of the hydrophobic–hydrophilic balance (HLB) at the particle interface is of a paramount importance to reduce this undesired adsorption. One common method is incorporating a hydrophilic layer like polyethylene oxide-based molecules.

1.2.5 Cross-Linking

In the case of organic-based nanoparticles, it is sometimes appropriate to deal with nonswellable or insoluble particles when they are handled in an organic solvent. This implies to incorporate a small amount of the so-called cross-linker able to develop a three-dimensional network. Such a network structure allows, provided colloidal stability is ensured, to make surface chemistry of the particles in organic solvents without the risk of coagulation or complete solubilization.

1.2.6 Functionality

In many applications, the use of particles offering one given or multiple functionalities is quite relevant, which requires to design the so-called *engineered particles* exhibiting physicochemical properties meeting the needs of a specific application.

One major strategy is to incorporate *reactive groups* that could be employed for many purposes: immobilization of biomolecules containing mainly carboxylic, amino, hydroxyl, or thiol groups; covalent binding of dyes; fluorescent labels or inorganic colloids (metal gold, ferrites, and quantum dots); surface binding onto plane surfaces. As it has been already extensively reviewed in many books (22,23), numerous and various reactive groups are available, depending on the chemical reaction selected to bind the molecule (which could often involve a preactivation step). In some cases, for highly reactive functions, it is necessary to keep the chemical group under protected form (aldehyde, amino, or thiol functions, for instance) and to recover them just before use. The biomolecule immobilization via molecular recognition, such as the streptavidin–biotin system, is also widely used, which implies to fix a

streptavidin molecule onto the nanoparticle surface. Other lock-and-key biomolecules can be used such as sugars moieties, antibodies, peptides, and so forth. For the sake of availability (confinement effect near the surface), the reactive function can be advantageously localized at the extremity of a spacer arm or within a hydrophilic polymer layer. Considerable amount of works has been achieved in order to identify and to quantify the amount of available reactive groups (24).

Many other functionalities can be conferred to the colloids depending on the application technique and on the type of detection (optical, electric, dielectric, and magnetism) involved for the analysis. In that respect, *magnetism* is a very important property that has been described in various review papers related to their manufacturing methodology, properties, and applications fields (25). A prerequisite when using such magnetic particles as a tool of separation is to keep the entire superparamagnetism property of the ferrite, meaning that they can be attracted to a magnetic field but do not retain remanent magnetism when the field is removed.

Fluorescent and colored colloidal particles have also attracted much interest for many years especially in the biological and biotechnological domains in which they are used for the detection and quantization of biomolecules and pathogen agents in biological samples (26). Various nanoparticles bearing conventional dyes or fluorescent probes are currently marketed in a broad range of size and surface functionalities. However, it appears that the use of these organic dyes presents drastic disadvantages mainly because of photobleaching problems.

Recently, alternated approaches were investigated so as to develop fluorescent nanoparticles with enhanced photostability such as *quantum dots (QDs)*, lanthanide oxides, and so forth (27,28). There is a challenge for making fluorescent and magnetic nanoparticles, which was indeed partially solved in performing an appropriate encapsulation process avoiding a close contact of iron oxide nanoparticles and dyes species (29).

In the last 10 years, a great deal of efforts have been focused on the design of stimuli-responsive particles, that is, particles that are able to change their structure and therefore their size and properties by the action of an external stimuli (temperature, pH, ionic strength, electric field, light, etc.). A considerable amount of studies have been devoted to polyacrylamide derivative colloids, which concern *in vitro* biological applications only (30).

At last, for applications in living systems, it is necessary to select degradable natural (polysaccharides) or synthetic (polyglycolic or lactic acids, silicones, and polycyanoacrylates) polymers exhibiting biocompatibility, bioresorbability, and nontoxicity.

1.3 MAIN PREPARATION METHODS

Numerous methods are now available for the preparation of nano and microparticles and the general approaches whether they are inorganic, organic, or composites can be classified as depicted in Table 1.3. Although, the production of fine particles can be envisaged by comminution methods of a bulk material, they will

TABLE 1.3 Preparation Methods of Inorganic or Organic Colloids to be Used in Nano and Microsystems.

Type of preparation method	Example	References
Condensation of small molecules		
• Precipitation process	Metal hydrous oxides	(31)
• Sol-gel method	Magnetites, ferrites	(32)
• Chemical reaction in aerosols	Metal oxides (TiO ₂ , SiO ₂)	(33)
• Ostwald ripening process	Silver halides	(34)
• Hydrolysis of silicon alkoxides	Silica	(35)
• Polyol process	Noble metals (Au, Pd, Ag, etc.)	(36)
• Dispersion polymerization	Latex particles	(37)
• Controlled hierarchical chemistry	Phosphorated dendrimers (cationic)	(38)
• Other techniques	Fullerens and nanotubes	(39)
Polymerization in heterogeneous media and related techniques		
• Emulsion	Polymer latexes	(40)
• Miniemulsion	Submicronic latexes, magnetic nanoparticles	(41)
• Direct and inverse microemulsion	Functionalized nanoparticles	(42,43)
Encapsulation of organic or inorganic particles		
• Association of preformed colloids		
- Heterocoagulation	Nanostructured composite latexes (anionic latex + cationic Fe ₃ O ₄ nanoparticles)	(29)
- Layer by layer	Composite nanoparticles (silica, Fe ₃ O ₄)	(44)
• Radical-initiated polymerization on the surface of inorganic nanoparticles	Composite latex + silica	(45)
• Polycondensation and mineral precipitation	Hollow nanoparticles (silica) cationic metal nanoparticles onto latex	(46)
• Simultaneous reaction of organic and inorganic precursors	Incorporation of macromolecules into organosilane networks	(47)
Formulation of colloids from preformed solutions of polymers		
• Organo-soluble polymers	Poly(lactic acid) and poly ε-caprolactone,	(48)
• Water-soluble polymers	Polysaccharides, alginates	(49)
Surface modification of preformed particles	Hydrophobic and hydrophilic latexes	(50,51)
Self-assembly processes of polyelectrolytes and architected polymers	PEO-based copolymers PEO-poly(lactic acid) Dextrane sulfate-chitosan	(52-54)

not be considered since at first they require high mechanical energy for making a fine dispersion and secondly, they are not at all suitable for preparing monodisperse colloidal particles.

1.3.1 General Remarks

It is worthwhile that several common features can be found in the different manufacturing methodologies used for the synthesis of the various types of colloids. They are reviewed below.

- Many preparation methods involve the condensation of small molecules, which could be metal atoms, metal salts or oxides, silicon alkoxides for inorganic colloids, or monomers for polymer-based colloids.
- The formation of the colloids from the initial molecules usually implies either a chemical reaction (hydrolysis, reduction, polymerization, or polycondensation) or a physical transformation (Ostwald ripening, spontaneous phase separation, or gelification).
- When starting from a homogeneous solution of small molecules, the formation of particles proceeds according to a similar mechanism as schematized in Fig. 1.1. It generally encompasses the following steps:
 - A chemical reaction: reduction of a metal (Au); hydrolysis of an alkoxide (silicon alkoxide); polymerization of a monomer
 - Formation of nuclei by precipitation (oligomers) or aggregation (metal atoms), which defines the “nucleation step”

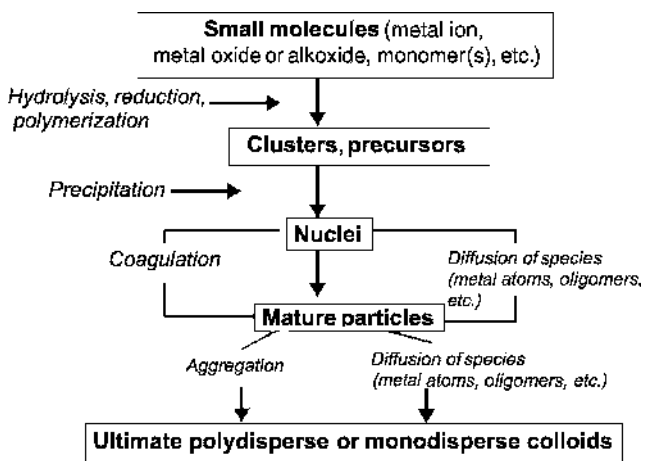


FIGURE 1.1 General schematic of the formation and growth of colloids by a precipitation process

- Growth of the nuclei to form particles by two main processes: diffusion of monomer, capture of oligomers, or metal atoms; coagulation of nuclei or clusters
- Stabilization of the final particles can be ensured by either existing surface charges, by adding hydrophilic polymer, or any surface-active agents.
- Under appropriated conditions, *monodispersed colloids* can be produced, a property that which has been formerly explained by LaMer (55), who also proposed a schematic diagram, indicating that the nucleation occurs when the species formed in solution reach a supersaturation concentration, the formed primary particles growing according to either a monomer addition or an aggregation model. Getting monodisperse colloids generally implies the control of the nucleation and growth steps; briefly, when the critical supersaturation level is reached, a fast nucleation step occurs followed by a progressive growth step. Homogeneous nucleation can be replaced by a heterogeneous nucleation process by introducing in the initial reaction medium foreign nuclei acting as a seed for capturing either elementary units or primary particles.
- The production of nanoparticles via self-assemblies of surface-active agents, especially micellar or microemulsion processes both in oil-in-water (O/W) or in water-in-oil (W/O) dispersions, has received much attention regardless of the type of colloids. Their development relies on considerable research works in the 1980, both on theoretical and practical aspects.
- The making of a microemulsion requires to use relatively high amount of an emulsifier and often with a cosurfactant (which could be an alcohol with a short alkyl chain such as *n*-pentanol, *n*-hexanol, etc.). The formulation of microemulsions is a spontaneous process, that is, it does not need mechanical shearing; they are thermodynamically stable (against coalescence and Ostwald ripening), optically transparent, and in the domain size 5–80 nm. A huge number of micelles are obtained allowing to solubilize large amounts of reactive species (metal oxides or alkoxydes, monomer, etc.) offering an important surface area (of the order of 100–300 m²/g of dispersed phase). As a result, reaction rates are considerably increased, and the synthesis of nanoparticles can elapse sometimes within a few minutes. The selection of the surfactant is obviously critical and must be adapted not only to the initial nature of the reacting system but also to the final nanoparticles (due to the modification of the interfacial and colloidal properties).

As an example, Figure 1.2 gives a schematic representation of a pseudoternary phase diagram (water–oil–surfactant) and showing that various mono and multiphase domains can be obtained: O/W and W/O microemulsions, lamellar, and bicontinuous phases. Most of studies and developments make use of globular microemulsions; however, reactions in other phases (bicontinuous phases, for instance) give rise to interesting microstructures, as referred in the next section.

- Characterization of the colloids during the synthesis and at the ultimate stage is a very important and necessary task for any application in which they are to be

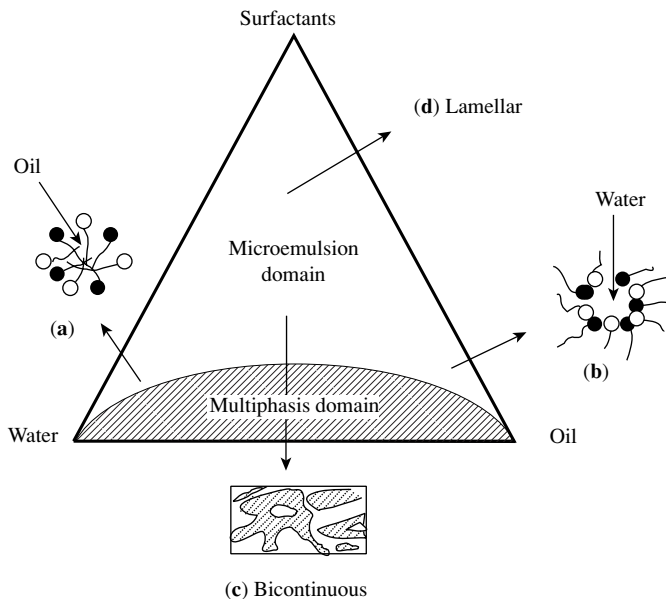


FIGURE 1.2 Phase diagram (water–oil surfactant) (adapted from (56)) (a) droplets oil-in-water; (b) droplets water-in-oil; (c) bicontinuous phase; (d) lamellar phase

involved. Many parameters should be determined among them: shape, particle size and polydispersity, surface chemistry, structure, composition. It is out of scope to detail all the techniques, which should be carried out for these analyses; the readers could consult the appropriate books and reviews on the subject.

1.3.2 Preparation of Inorganic Particles

In this section, we will describe only several important methods to produce inorganic colloids.

1.3.2.1 Precipitation Process The precipitation in homogeneous solutions of many inorganic salts has been long used for the production of various colloids in the domain size (10–100 nm). It was applied in the case of metal ions, metal (hydrus) oxides, silicon alkoxides, metal phosphates and sulfates, and so forth.

Case of Metal Ions Nanometer-sized *metal particles*, especially gold and silver, have received much increasing attention as regards to their application potentialities in various domains such as in biology for the detection of DNA or RNA (57,58). Colloidal dispersions of gold particles can be obtained by reduction of gold ions with various reactants: citrates, formaldehyde, hydrazine, and hydrogen peroxide. The mechanism of nanoparticle formation is a three-steps process following the scheme given in Fig. 1.3. After reduction of metal ions to metal atoms, aggregation of the atoms

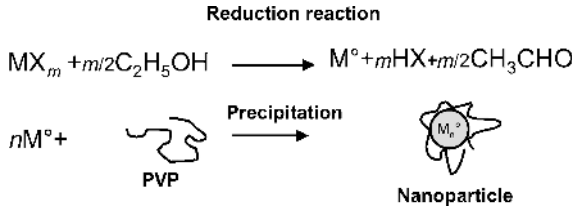


FIGURE 1.3 Formation of metal nanoparticle by reduction with an alcohol of metal salts in the presence of poly(*N*-vinyl-2-pyrrolidone) (PVP) as stabilizer

(or microclusters) leads to nuclei with an average diameter less than 1 nm. The growth of the formed nuclei proceeds by deposition of atoms or microclusters on the surface of nuclei. Stabilization is very critical in such metal nanoparticles and a steric stabilizer is usually added in the recipe. Poly(*N*-vinyl-2-pyrrolidone) (PVP) as water-soluble polymer is often employed in this purpose and showing different types of interactions with the surface metallic particle.

Semiconductor nanoparticles (the so-called quantum dots such as CdS) can be prepared by the precipitation of CdS in aqueous solution containing cadmium and sulfide ions provided stabilizer (polymer (gelatin or polyethylene glycol) or a ligand (thioacetamide) be also added to prevent the aggregation of the formed clusters. In the reaction, pH should be carefully controlled since it determines the particle size, a basic one allowing to stop the particle growth.

The hydrolysis of silicon alkoxides in homogeneous solutions also proceeds through a precipitation mechanism according to the widely described original Stöber process (59), which follows the reaction pathway, described in Fig. 1.4. It is worth mentioning as quoted by Brook (60) that silicon products and particularly silica show excellent compatibility with living systems, which explains their use in many biochemical, biological, and biotechnological applications. Obviously, silica particles have been developed not only as model systems but also as colloid supports as such or as composites with inorganic (ferric oxides) or organic polymers.

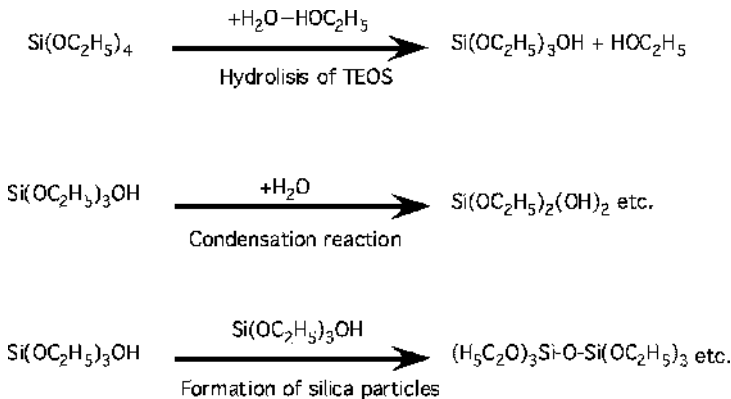


FIGURE 1.4 Schematic reaction steps leading to silica particles

Many fundamental works have been devoted to the understanding of this process owing to systematic studies investigating the influence of the nature of alkoxide and alcohol and the amount of water and ammonia on the resulting colloidal particles (in the size range from about 50 to 800 nm). Much work dealt with tetraethylorthosilicate (TEOS) upon studying the reaction rate (which encompasses both the hydrolysis and condensation reactions). Such a reaction was also examined as regards to its effect on the nucleation and growth mechanism with a view to control the particle size and monodispersity.

As it will be discussed later on, silica colloids as substrates can be surface modified. As illustrated in Fig. 1.5, extremely narrow size particles can be prepared by the Stöber process as well after surface modification to get amino-functionalized particles.

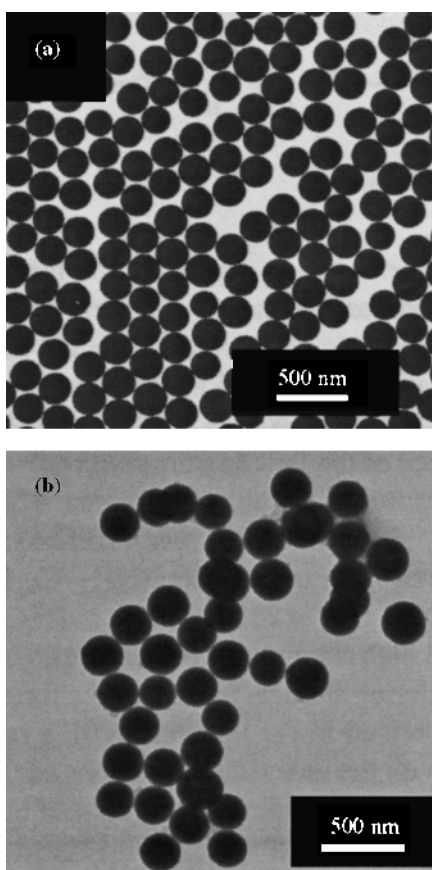


FIGURE 1.5 Transmission electron micrographs of silica particles: (a) native Stöber silica; (b) amino-silica from silanisation in acetone with a catalyst (from Reference (61))

Polyol Process (36) This process has been worked out with a view to produce metallic and bimetallic alloy particles with a controlled morphology in the micrometer and nanometer size. The preparation consists in a reduction reaction of metallic compounds dissolved in a solvent also acting as reducing agent. It has been applied to a large number of easily reducible noble metals such as Au, Ag, Ir, Pt, Os, Rd and less reducible metals such as Co, Ni, Cu (62). After dissolution of a given precursor (metal chloride or nitrate) in nonaqueous solvents like polyols such as α -diols ethylene, propylene, or tetraethylene glycols, the polyol acts to the reduction of species. The *in situ* formed metal particles proceeds, as already described, by a nucleation growth process leading to very fine nanoparticles. Experimental conditions have been well investigated so as to ensure the monodispersity of the final particles. Nanocolloids of gold, silver, and platinum were then synthesized in the size range from 5 to 30 nm.

1.3.2.2 “Gel-Sol” Method This method of preparation is based on the “gel-sol” physical process in which a highly viscous gel of a solid precursor is formed. The gel acts as a protective structure against coagulation of the solid and as reservoir of monomeric species (metal ions, for instance).

This process was applied in diluted and condensed systems to a variety of metal oxides, particularly for the preparation of magnetites and ferrites by partial oxidation of a ferrous hydroxide gel with nitrate (63). The particle formation also proceeds by a two-step nucleation/growth mechanism. Such a method leads to monodisperse and spherical particles in the colloidal size range; however, in many cases, nonspherical particles can be also produced.

1.3.2.3 Chemical Reaction in Aerosols The aerosol technique was developed as a pathway to produce valuable dispersed materials with predictable particle morphology. The main steps involved in the aerosol method are the following (64):

- generation of small droplets containing one or more reactive liquids
- use of evaporation and nucleation phenomena to favor narrow size distribution of the droplets
- exposure of the droplets to a coreactant vapor
- reaction of the liquids in the droplets with the surrounding vapor
- removal of the aerosol colloids

The process has been applied to the synthesis of various metal oxide particles (titania, silica, alumina, etc.) of size range (0.1–1.0 μm), micrometer-sized poly(*t*-butylstyrene) latex particles as well composite particle systems. It is interesting to notice that monodisperse colloids can be obtained in all cases.

1.3.2.4 Ostwald Ripening Ostwald ripening is a general process regarding the evolution of an emulsion resulting from interface energy. It includes a transfer of molecules contained in the dispersed phase through the continuous phase (aqueous one for example) provided the oil-like molecules be slightly soluble in this continuous

phase. This solubility allows the diffusion of hydrophobic molecules from the small droplets toward larger droplets with consequently a decrease in the overall interfacial area. Such a process concerns emulsions of tiny droplets about and below 100 nm, and the rate of diffusion increases with the molecular solubility of the lipophilic molecule.

We will see later on that this physical process has been utilized for making emulsions (the so-called miniemulsions) with stable submicronic sized-droplets by introducing an insoluble hydrophobic molecule (i.e., solvent) to prevent the Ostwald ripening effect.

This method was applied by Sugimoto (34) to produce monodisperse single-crystal silver halide particles by mixing tabular AgBr particles (410 nm) with fine spherical ones (42.5 nm). The growth of the large tabular grains was found to follow a spherical diffusion model proposed by the authors.

1.3.2.5 Micellar and Microemulsion Systems As already mentioned, self-assemblies of surfactants both in oil or in water continuous phases can serve as *microreactors* to induce the formation of nanocrystals. As an example, reverse micelles, obtained from the system Aerosol OT (1–4 bis-2-ethylsodium sulfosuccinate) as surfactant, isooctane as continuous phase; an aqueous solutions of cadmium and sulfide ions, were used to produce CdS and CdTe nanocrystals in the nanometer size range (about 4 nm) but with a relatively large polydispersity (65).

The microemulsion process has also been applied to the production of many metal oxide nanoparticles as reported in (66): metal oxides, silver halides, silicon oxides, and so forth. We will mostly concentrate on the case of silicon alkoxides. The hydrolysis of the alkoxide-based silica, tetraethoxysilane, takes place in micelles produced in O/W microemulsion, and many recipes can be found in the literature, a common one containing AOT as surfactant, an alkane (isooctane, cyclohexane) as continuous phase, and ammonia + water as reactant. The particle formation mechanism follows the sol-gel process with the TEOS present in the oil phase being hydrolyzed by water poles located at the boundaries of the aqueous droplets. Kinetics and mechanisms of silica nanoparticles formation have been described and a growth model was proposed (67).

1.3.2.6 Surface Modification of Preformed Particles Due to the hydrophilic nature of many inorganic colloids, it is often required to modify their functionality (mostly constituted of hydroxyl groups in silica, titania, and ferrite) by a chemical modification in order to improve the compatibility when they are combined with organic materials. The simplest modification is to bind an organic compound through the use of various coupling agents (such as those bearing a silane for silica particles), which converts the hydrophilicity of the inorganic surface into a hydrophobic character. However, for various purposes, the surface modification of inorganic colloids relies on the incorporation of a polymer phase. This can be performed according to quite a lot of methods as reported in Table 1.3. Surface polymerization can be radically initiated whether an initiating (azo or peroxy) or a polymerizable group be preliminary bound. More details related to the synthesis of organic–inorganic composite nanoparticles are given in the next section.

1.3.3 Organic Particles

The preparation of organic nanoparticles, mostly polymer based, can be performed according to three main approaches:

- polymerization in heterogeneous media
- modification of preformed particles
- formulation of colloidal dispersions from preformed polymers

1.3.3.1 Polymerization in Heterogeneous Media This technique, which has been developed for a long time, allows to produce colloidal dispersions of various nature, the so-called polymer latexes, applied in an increasing number of applications (68). Owing to the progresses at both the academic and industrial levels and due to the versatility of the process, such a technique has been more and more involved in the preparation of “high-tech” materials under dispersed form, especially for making nano and microparticles carefully controlled in terms of internal and surface structure. Figure 1.6 illustrates the range of particle sizes, which can be produced by using the main polymerization processes in heterogeneous media. Suspension polymerization, which proceeds via a bulk polymerization in initial monomer droplets (with several hundreds of micrometers size), is not a colloidal process and therefore not reported here; in addition, the obtained polymer particles are generally polydisperse in size.

On the contrary, for all other reported polymerization processes, the polymerization proceeds via a two-step mechanism involving at first the formation of the particles from (i) the continuous phase by the so-called *homogeneous nucleation* (as already mentioned) in soap-free emulsion, dispersion, and precipitation polymerization; (ii) out of the continuous phase, that is, through the presence of a

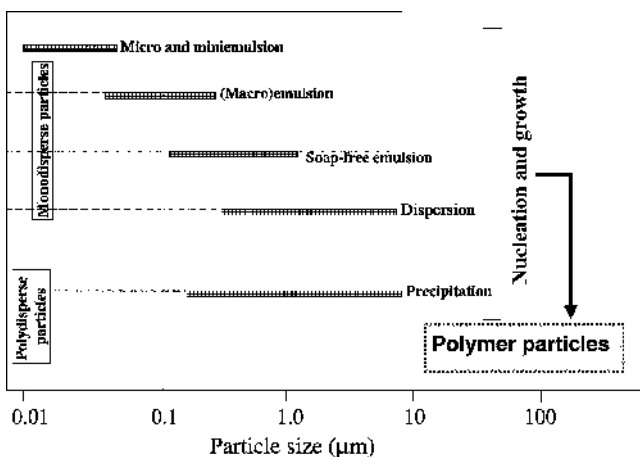


FIGURE 1.6 Nature of the heterogeneous polymerization process versus particle size and size monodispersity (adapted from Reference (15))

dispersed phase that could be micelles of surfactants (micellar nucleation), preexisting polymer, or mineral particles (*heterogeneous nucleation*).

Radical-initiated polymerizations are the more popular initiating systems; however, in the last decade, new processes have been explored based on different mechanisms than those of radical initiation, for example metathesis, or ionic polymerizations in aqueous media. It should also be emphasized on the recent introduction of controlled-radical polymerization methods, which appear highly suitable, especially for the design of latex particles with controlled surface and internal morphology (69).

Emulsion polymerization is the more developed heterogeneous polymerization process owing to the huge progresses accumulated at both the academic and industrial levels in the last 60 years. It has been widely used with numerous monomers (of various polarity) to produce latex particles applied in a continuously increasing range of domains (70). The versatility and flexibility of the process prove to be suitable for the synthesis of high-tech latex particles in the submicronic size range (down to 20 nm), to be used in biotechnology (calibration standards, diagnostic tests, solid-phase supports for biomolecules, medical imaging, synthetic vectors for drugs, biochips, etc.).

Briefly, conventional emulsion polymerization consists in dispersing a nonmiscible monomer in aqueous phase (mostly by using an emulsifier), then to initiate polymerization by a radical initiator leading to polymer particles of colloidal size much more smaller than the initial droplets. The various mechanisms involved in the formation and growth steps of the latex particles have been largely investigated, and the reader could report on many books and reviews devoted to the subject (24,40). Polymerization can be performed in the absence of surfactants, providing highly monodisperse particles but preferentially in a size range largely above 200 nm.

It has been well recognized that polymerization of two (or more) monomers, as named copolymerization, allows to produce polymer materials with physical and chemical properties more finely defined than with homopolymers. This is particularly the case of *copolymer latexes* in which surface and colloidal properties can be really tuned by taking advantage of the differences, in terms of reactivity and physicochemical properties (polarity, hydrophilicity, presence of ionic charges, etc.) of the two monomers involved in the polymerization process. In that purpose, considerable amount of work has been investigated to the kinetics and mechanisms of emulsion copolymerization in order to predict many features related to these copolymers, especially the control of the copolymer composition within the particle. In the frame of this review, it is worthwhile to focus on the potentialities of the copolymerization process to produce latex particles in which the internal morphology can be controlled (the so-called *structured latexes*) or those in which the interfacial functionality is tailored (*functionalized latexes*). It is obvious that both properties can be considered in a same latex particle.

In the case of functionalized latexes, as already mentioned in the previous section, numerous functionalities can be installed to particles. Emulsion polymerization techniques, especially those starting with a preformed batch of particles, the so-called *shot-growth* (i.e., inducing the polymerization of a functional monomer on batch particles at high conversion) and *seed* protocols (i.e., starting with a preformed population of particles), are particularly well adapted to carefully control the

incorporation of reactive groups (50). The following chemical groups can be listed containing either charges (sulfate, sulfonates, carboxylates, quaternary ammonium salts, phosphates, etc.) or not (carboxylic, aldehyde, chloromethyl, hydroxyl, amino, thiol, epoxide, acetal, activated ester, etc.) and more complex macromolecular structures (metal chelates, polyethylene oxide, polymethylmethacrylate, etc.). All these later chemical functions or macromolecules are quite useful:

- to control the particle size and monodispersity of the final latexes and especially in the case of charged monomers to allow the synthesis of stable nanoparticles (below 100 nm)
- to impart efficient steric stability (macromolecule)
- to induce subsequent reaction with biomolecules
- to incorporate a dye label, a specific ligand (oligosaccharide, lipid, peptide, nucleic acid, antibody, protein)
- to modify the surface of microsystems: two-dimensional assemblies of latex on silica wafers, microplates, biochemical devices, microfluidic channels.

Latex particles prepared with a hydrophilic layer (*hairy particles*) were found useful colloidal supports offering friendly environment when put in contact with biomolecules. Many routes can be explored to produce such particles based on different strategies: *layer-by-layer* method (LbL) (71); incorporation of a hydrophilic layer either covalently (surface polymerization of reactive surfactants (72)) or more simply by physical adsorption of amphiphilic block or graft copolymers (73). Coverage of latex particles by polyethylene oxide (PEO) proves to be quite suitable for biotechnological applications due to the immunogenicity, nontoxicity, and stealth effect of the PEO (74). The use of controlled radical polymerization methods (such as reversible addition–fragmentation transfer (RAFT), nitroxide-mediated polymerization (NMP), or atom-mediated transfer radical polymerization (ATRP)) are currently performed to tailor hairy particles with well-controlled polymer brushes.

Miniemulsion Polymerization In this process, the main difference with the emulsion process is that the monomer phase is more finely divided owing to the use of a mixture of a surfactant with a hydrophobic solvent (hexadecane, cetylic acid) together with a strong energy of agitation. In that case, the formed submicrometer-sized monomer droplets (which are prevented to coalesce by Ostwald ripening) can compete with existing micelles to capture aqueous-phase radicals. Final polymer particles have almost the same size than the initial monomer droplets and exhibit a long-term stabilization. In that process, the key points are the formation of the small droplets and the influence of the various parameters (nature of the emulsifier technique, amount of emulsifier, effect of the hydrophobic molecule, and nature of the initiator), which need to be taken into account, have been thoroughly investigated (75).

Polymerization can be radically initiated using either an organic or a water-soluble initiator. Controlled radical polymerization techniques have been successfully

performed with this process since the presence of the smaller initial droplets as active sites allow to avoid the nucleation step. Such a process has been applied for polymerizing many monomers provided they are not too soluble in the continuous phase whether they are hydrophobic (MMA, styrene) or hydrophilic (MMA–acrylamide mixture, acrylonitrile).

Nonradical-initiated polymerizations have also been explored: polycondensation (polyesters, polyurethanes) ionic polymerization (for instance, the anionic polymerization of cyanoacrylates).

This method has been recently used for the synthesis of nanosized magnetic nanoparticles by preparing separately a magnetite miniemulsion and a styrene miniemulsion and mixing them before polymerization (76).

Microemulsion Polymerization As already defined in the previous section, this process consists first in preparing a highly dispersed monomer phase. Many efforts have been directed to the formulation of microemulsions having the minimal amount of surfactant (less than 10 wt%) together with high solid contents (from 10% to 40%). Polymerization initiated (by photo or radical initiation) in the formed micelles leads to nanoparticles in the size range below 100 nm.

It was investigated both with O/W and W/O systems and mostly with globular microemulsions. Mechanisms of nucleation and growth have been elucidated with styrene as hydrophobic monomer and with acrylamide as water-soluble monomer (77). Due to the large amount of monomer-swollen micelles, polymerization rate is very fast and relatively narrow size distribution could be obtained at least with the acrylamide-based system.

Functionalized nanolatexes can be produced by incorporation of hydrophilic monomers (such as hydroxyethyl or dimethylaminoethyl methacrylates) with, however, a lack of stability in the final microlatexes (42,43).

Polymerizations have also been conducted in other structures than globular microemulsions, for instance, in bicontinuous phases. Under specific conditions, the bicontinuous morphology is retained after polymerization leading to polymer presenting a nanoporous structure of potential interest in biotechnology (nanovector, membrane separation) (78).

Dispersion Polymerization In that process, the initial monomer solution is homogeneous with the presence of steric stabilizing species (mostly hydrophilic polymers like poly-*N*-vinylpyrrolidone or better with block or graft copolymers with a well defined architecture). By introduction of an initiator, an insoluble polymer phase is produced, which is dispersed as colloidal particles through the already depicted homogeneous nucleation process. Particles can be obtained in a large size range (roughly between 200 and 20 μm) but the process is more adapted to produce monodisperse micrometer-sized latexes. It can be carried out both with many different monomers (hydrophobic and water soluble) in polar and nonpolar media with preferentially a radical initiation but also by other initiation processes such as ionic, metathesis, and ring opening) (37).

Precipitation Polymerization In this process, the monomer is soluble in the continuous phase and the formed polymer phase separated with no formation of a colloidal dispersed phase.

It was found that the polymerization of several acrylamide derivatives in aqueous phase in the presence of anionically charged initiator (potassium persulfate for instance) and a small amount of a cross-linker lead to highly monodisperse and electrosterically stable latexes. This comes from the property of polyNIPAM and of many other polyacrylamide derivatives to exhibit a low critical solubility temperature (LCST) in a broad range of temperatures, which means that a drastic change in the hydrophilic–hydrophobic balance (core-globule transition) occurs at the LCST. Consequently below LCST, polyNIPAM chains are fully expanded in the aqueous phase. A huge number of studies have been investigated in the case of *N*-isopropylacrylamide (the so-called NIPAM) showing that under adjusted experimental conditions (low dilution of reactants, monomer concentration, initiator, temperature, etc.), colloidal submicronic dispersions can be produced without the presence of any surface-active agent. On the colloidal point of view, thermally sensitive polymer latex particles display unique properties as regards to the dramatic change in the size and electrophoretic mobility of the particles. They were proposed as model systems of *thermally sensitive colloids* (Fig. 1.7) with quite a lot of potential applications, especially in biotechnologies (79).

Such a property has been widely extended to the synthesis of many other polymer microspheres being responsive to other external parameters such as pH, ionic strength, light, magnetic or electric field, and biological effect, offering the possibility to envision multiresponsive particles. In addition, various particle morphologies can be now designed depending upon the recipe (with preferentially the presence of a functional and/or charged comonomer) and the type of heterogeneous polymerization process (batch, shot-growth, seed). Hairy, core-shell and microgel particles can be currently produced in a large range of size (particularly between 100 and 1000 nm) and nature of polymer.

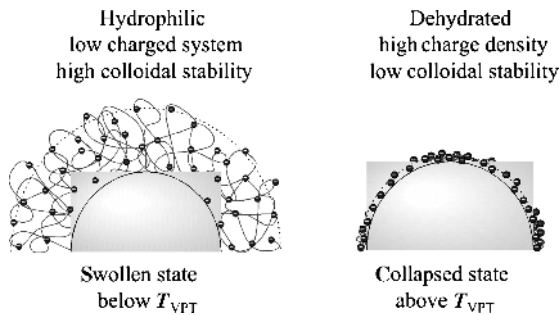


FIGURE 1.7 Illustration of thermally sensitive colloidal latex particles (polystyrene core–poly(NIPAM) shell) (T_{VPT} : volume phase transition temperature)

Artificial latexes

Principle: to start with a preformed polymer

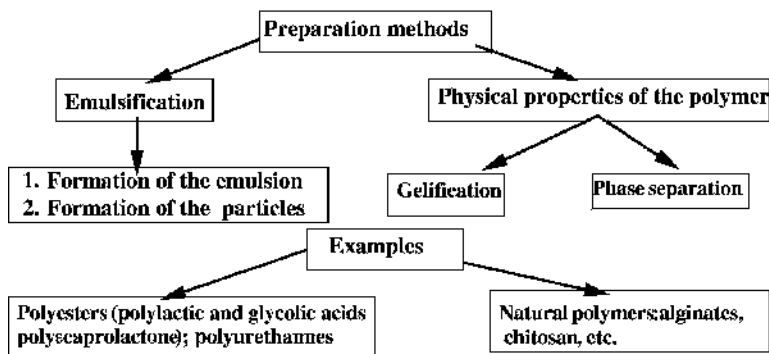


FIGURE 1.8 Main strategies followed for the preparation of polymer nanocolloids

1.3.3.2 Formulation of Polymer Colloids from Preformed Solution

Polymers Depending on the nature of the polymer (lipophile vs. water soluble), two main strategies can be followed to obtain *artificial latexes* as schematically depicted in Fig. 1.8. Such techniques are now well identified (80) and widely developed in the pharmaceutical and medicine fields where they are used for *in vivo* delivery of drugs, proteins, DNA, etc.

The first method relies on a two-steps technique: formation of an emulsion, that is, a finely dispersed phase containing the polymer solubilized in a solvent in an aqueous continuous phase containing a polymer stabilizer. The formation of a polymer emulsion of small droplets usually requires high-pressure homogenizer together with ultrasonic agitation providing submicrometer size particles. The second step consists in eliminating the solvent inside the droplets and this can be conducted by different methods, which have been well described: evaporation of the solvent or extraction by depleting the solvent of the dispersed phase by another solvent.

The second method is based on the properties of the polymers: the nanoprecipitation technique allows the formation of nanoparticles due to a phase separation process induced in the polymer solution by adding a nonsolvent of the polymer or by changing pH or salinity conditions. This process can be carried out with or without stabilizer but only under very diluted systems.

1.3.3.3 Others Techniques

Controlled Hierarchical Chemistry Among the many arborescent architectures that can be produced by such chemistry, *dendrimers* constitute a class of materials, which offer quite remarkable properties in relation with their potential use as highly “engineering” nanostructure: nearly perfect monodisperse polymer tree-like,

nanometer-size (down to 10 nm), high number of functional end groups available on the molecular surface.

The synthesis of such dendrimers can be obtained by an iterative sequence of reaction steps; the divergent synthesis starts from a central reactive core such as with the polyamidoamine (38).

Dendrimers prove to be very useful in biotechnologies such as a reservoir of drugs, transfection of DNA, and immobilization of water-soluble dendrimers onto functionalized surfaces (biochips) (12).

Allotropes of Carbon It is worth mentioning the increasing interest of allotropes of carbon, the so-called fullerenes and nanotubes. Both of them have a nanometer diameter size, the former being spherical in size and the second cylindrical with a length, which could reach up to millimeters. Due to their unique properties in the material science, especially in the nanotechnology domain for their applications in electronics and optics, carbon nanotubes have been widely studied in the last years with regards to their synthesis, properties, and applications (39,81). There are two main types of nanotubes the so-called single-walled nanotubes (SWNT) consisting of a single graphite sheet wrapped into a cylindrical tube and multiwalled nanotubes (MWNT), made of an array of nanotubes concentrically nested. Both of them can be obtained by carbon-arc discharge, laser ablation of carbon, or chemical vapor deposition. In the case of SWNT, the diameter of the nanotube is in the size range of 0.8–4 nm whereas the size is much larger for MWNT (from 1.4 to 100 nm). Impurities may be found in the final products, especially for the SWNT.

1.3.4 Composite Particles

Composite particles refer to colloidal dispersions in which organic and inorganic materials are intimately distributed within the particle and offering unique properties. One major effect is that at a nanometric level, surface properties predominate over volume properties. As detailed by Bourgeat-Lami in recent reviews, the synthesis of such composite systems can be classified in three main categories (82):

- assembly of organic and inorganic colloids
- synthesis by *in situ* polymerization
- simultaneous reaction in the presence of the inorganic or organic precursors

Nanocomposite particles are produced when using the two first methods, whereas the third one leads to *hybrid nanoparticles*.

1.3.4.1 Assembly of Organic and Inorganic Colloids There are many examples in the literature in which composite particles are obtained by controlling the organization of mineral core by an organic shell (polymer) or conversely. It was mainly explored with polymer latexes due to their extreme versatility as regards to nature of polymer, colloidal, and surface properties.

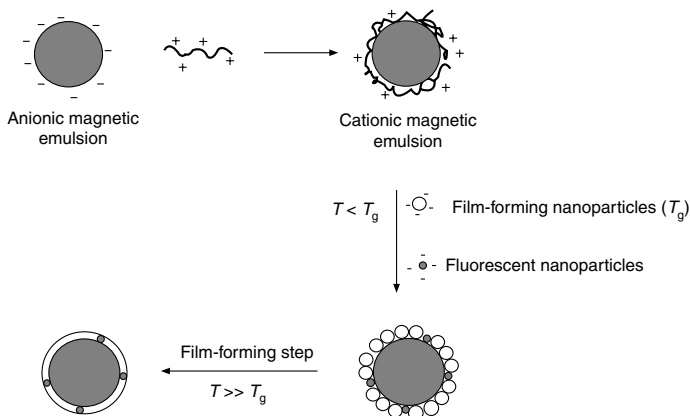


FIGURE 1.9 Stepwise heterocoagulation process used to prepare magnetic and fluorescent nanoparticles (29)

The heterocoagulation strategy (as shown in Fig. 1.9), based on the assembly of the two colloids by electrostatic interaction, has been investigated in many papers regarding the encapsulation of titania and silica. It was recently described for producing low-size (200–300 nm) magnetic nanoparticles (29). The strategy consists in inducing the flocculation of film-forming nanoparticles anionically charged (order of 40 nm) onto a preformed magnetic emulsions (containing iron oxide nanoparticles) being subsequently cationically charged by polyethylenimine. After heating the composite particles above the film formation temperature, stable magnetic nanoparticles were obtained with an average of 25% of magnetic material.

In that domain, the LbL assembly (71) has been proved quite powerful in view of preparing composite nanoparticles with outstanding properties. The process consists in adsorbing onto an organic particle a layer of inorganic nanoparticles and a polyelectrolyte of opposite sign. Figure 1.10 gives an illustration of such process in the elaboration of magnetic particles from oil in water magnetic droplets.

Such composite colloids dispersions were found to exhibit unexpected and specific properties (electric, magnetic, optic, and conductive), which are currently investigated in many places.

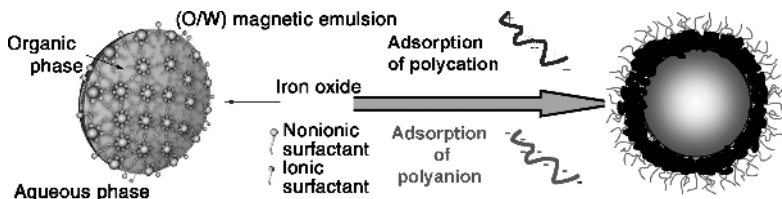


FIGURE 1.10 Schematic illustration of layer-by-layer encapsulation process of oil in water magnetic droplets

1.3.4.2 Synthesis by In Situ Polymerization Two types of particle morphology can be considered whether the inorganic phase be located in the core

or in the shell. In any cases, it is necessary to preliminary introduce functional groups at the surface of the organic or inorganic seeds.

In the case of mineral (core)-organic (shell) particles, two main strategies have been experienced:

- heterogeneous polymerization (emulsion, miniemulsion, dispersion) of various monomers in the presence of mineral colloids
- radical polymerization initiated from the inorganic particles surface

In the case of organic(core)-mineral (shell) composite particles, their synthesis relies on either the mineral polycondensation by sol-gel process or precipitation of metal salts on latex particles. Hydrolytic polycondensation via sol-gel process has been performed mostly with metal alkoxides of structure $(M(OR)_4)$ using various substrates as templates, latex particles, for example. It is then possible to prepare composite latex particles with an organic layer of silica or titania, the latex particles, if correctly functionalized, are able to capture the oligomers formed in the continuous phase as polycondensation proceeds. As illustrated in Fig. 1.11, such composite particles can be transformed in hollow particles upon thermal decomposition or solubilization of the organic core.

It can be interesting to focus on a recent strategy for making low size magnetic particles containing a large amount of magnetic material. It consists in inducing styrene polymerization inside submicronic droplets of a stable and relatively monodisperse magnetic emulsion in the presence of small amount of a cross-linker and an amphiphilic polymer. The final magnetic latexes were found to exhibit a narrow size and to contain up to 60% in weight iron oxide. As shown in Fig. 1.12, a perfect core-shell morphology is observed proving the efficient encapsulation of the ferrites, which makes such particles quite adapted for diagnostic and extraction of various biomolecules (83).

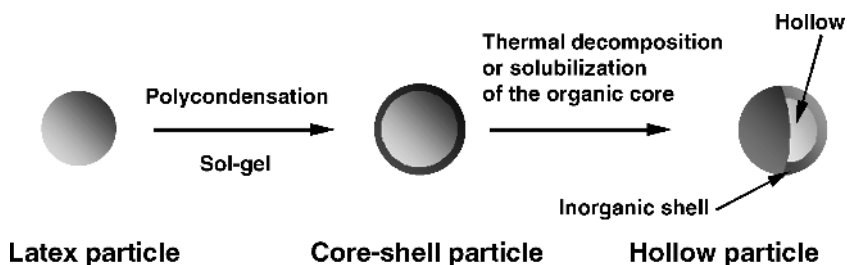


FIGURE 1.11 Principle of the preparation of hollow particles from a latex particle with an organic core and a mineral shell

1.3.4.3 Simultaneous Reaction in the Presence of the Inorganic or Organic Precursors Hybrid nanomaterials have received much interest in the last few years, and their preparation under colloid state was envisioned resulting in an intimate mixing of mineral and organic phases at a molecular level. A recent paper, for instance, reported the synthesis of such hybrid colloids by emulsion polymerization of

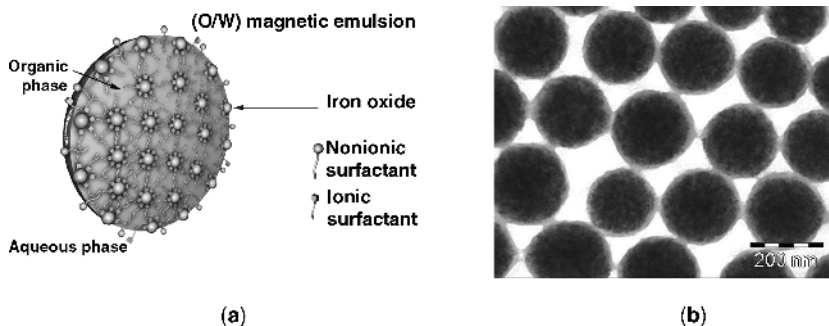


FIGURE 1.12 Transformation of oil in water magnetic emulsion (a) into submicronic magnetic latex (b) obtained by emulsion polymerization of styrene and divinylbenzene onto a ferrofluid emulsion stabilized by polyacrylic acid based amphiphilic surfactant and initiated by potassium persulfate (83)

a monomer mixture containing a comonomer, which is an inorganic precursor (an organosilane bearing a vinyl or methacrylate group). This leads to the formation of a fine dispersion of silica clusters inside the particles (49).

Finally, it should be also focused on the possibility to synthesize inorganic nanoparticles bearing a layer of stimuli-responsive polymer chains with chemical reactive end groups. For instance, the preparation of core-shell magnetic nanoparticles with a thermally sensitive biodegradable shell (dextran grafted with a poly[NIPAM]-based polymer) was recently disclosed providing *multifunctional colloids* with potential applications in drug-targeting delivery and magnetic imaging resonance (84). In the recent years, many research studies have been devoted to the synthesis of composite nanoparticles constituted of a gold or semiconductor (QDs) core surrounded by a stimuli-responsive layer that could be a synthetic (co)polymer, a biomolecule (peptide) or a polysaccharide with obvious applications in bioassays and drug delivery systems (85). In that purpose, self-assemblies of block copolymers with the polymer or the biomolecule as hydrophilic block can serve as useful templates for the encapsulation of the inorganic material.

Radical and preferentially controlled radical polymerization techniques initiated at the surface of inorganic colloids proved to be suitable to make stimuli-sensitive hairy composite nanoparticles.

1.4 CONCLUSION AND PROSPECTS

The preparation of well-characterized inorganic and organic colloids in the submicronic size (down to several nanometers in the case of metals) benefits of a considerable amount of works both on academic and practical aspects. It took advantage of the tremendous progresses accomplished in the physicochemistry of dispersed systems in aqueous media as well as in the organic and mineral chemistry (precipitation and sol-gel processes, reactions in self-assemblies, new types of heterogeneous polymerizations, controlled radical polymerization methods). All

these manufacturing techniques allow to carefully control many relevant parameters such as shape, particle size, polydispersity, morphology, and surface functionality of the particle, all of them being of importance in the design of nanobiotechnologies.

As recently quoted (86), it should be noticed the attractive interest of gold, quantum dots, and magnetic nanoparticles used as tags or labels in many applications such as in biochips, nanobiosensors with the purpose to detect DNA, or proteins in small volumes within a reduced time.

Due to the very active research in this domain, it may be anticipated that more complex materials in the nanocolloidal range are being elaborated, offering new potentialities in diagnostics and drug deliveries. It is worth mentioning several approaches dedicated to the synthesis of new kinds of nanoparticles (asymmetric, hollow, nanostructured, nanoporous). In the last few years, many studies evidenced the important role of various polymeric systems or nanotubes as colloid-size templates for the synthesis of nanostructures and superstructures. In the case of polymeric systems, self-assemblies of amphiphilic block copolymer surfactants (frozen micelles), dendrimers, vesicles, liposomes, emulsions, microemulsions, and latex particles were found appropriate (87). Polymerization can be carried out inside or at the surface of the selected template allowing to synthesize inorganic, inorganic/organic composites with tunable properties. A careful control of the association of mineral and organic material is also a challenge for creating new multifunctionalized and/or adaptive composite and hybrid nanocolloids. Due to their unique properties, stimuli-responsive nanoparticles, as smart material, should continue to offer outstanding potentialities for innovative applications.

It also appears that microfluidic reactors can be adequately designed for producing various kinds of nanocolloids (gold, nanocomposites, semiconductors, etc.) as well as to investigate particle interactions. In the domain of superstructures, there is also an attractive research to encourage the development of two or three-dimensional assemblies of nanoparticles on various surfaces or on living systems.

The development of nanosized colloids in life science activities, regardless of their nature, should not avoid systematic and independent research studies concerning their control in terms of dissemination, potential pollution, and toxic effects. This is an indispensable safety caution for people manipulating or in contact with such dispersed materials as well as for the environment.

It is obvious that multidisciplinary research on these nanocolloids would be really creative and productive, at first for elaborating new engineered materials at nanometer level and secondly for evaluating their chemical, physical, and biological properties together with their performances in nanobiotechnologies.

REFERENCES

1. Manz A, Becker H. *Microsystem Technology in Chemistry and Life Sciences*. New York: Springer; 1999. p. 263.
2. Jähnisch K, Hessel V, Löwe H, Baerns M. *Chemistry in microstructured reactors*. *Angew Chem* 2004;43:406–446.

3. Terray A, Oakey J, Marr DWM. Microfluidic control using colloidal devices. *Science* 2002;296:1841–1844.
4. Guyon S, Bílková Z, Minc N, Fütterer C, Viovy JL. Microfluidic proteolytic microreactors based on magnetic nanoparticles for proteomics. *Nanobiotechnologies II*. 22–24 April 2003, Grenoble.
5. Yoshinaga K. Surface modification of inorganic particles. In: Sugimoto T, editor. *Fine particles, Synthesis, Characterization and mechanism of growth*. Surfactant Science Series 92, New York: Marcel Dekker; 2000. pp. 626–646.
6. Park S-J, Taton TA, Mirkin CA. Array-based electrical detection of DNA with nanoparticle probes. *Science* 2002;295:1503–1506.
7. Fritzsche W, Taton TA. Metal nanoparticles as labels for heterogeneous, chip-based DNA detection. *Nanotechnology* 2003;14:63–73.
8. Tanimoto A. Magnetic resonance imaging with supermagnetic nanocapsules. In: Arshady R, editor. *Microspheres Microcapsules & Liposomes*. Volume, 3 Radiolabelled and magnetic particles particulates in medicine and biology. Londres: Citus Books; 2001. pp. 525–556.
9. Wang J-X, Wen L-X, Wang Z-H, Wang M, Shao L, Chen J-F. Facile synthesis of hollow silica nanotubes and their application as supports for immobilization of silver nanoparticles. *Scripta Materialia* 2004;51(11):1035–1039.
10. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Iyer G, Weiss S. Peptide-coated semiconductor nanocrystals for biomedical applications. *Proc SPIE* 2005;5704: 57–68.
11. Singh R, Pantarotto D, McCarthy D, Chaloin O, Hoebeke J, Partidos CD, Briand J-P, Prato M, Brianco A, Kostarelos K. Binding and condensation of plasmid DNA onto functionalized carbon nanotubes: toward the construction of nanotube-based gene delivery vectors. *J Am Chem Soc* 2005;127(12):9.
12. Miska B, Slomkowski S, Chemimi MM, Delamar M, Majoral J-P, Caminade AM. *Colloid Polym Sci* 1999;277:58.
13. Buchhammer HM, Mende M, Oelmann M. *Colloid Surf A* 2003;218:151.
14. Cui Z, Mumper RJ. *J Control Rel* 2001;27:1039.
15. Kissel T, Li Y, Unger F. ABA-triblock copolymers from biodegradable polyester A-blocks and hydrophilic poly (ethylene oxide) B-blocks as a candidate for in situ forming hydrogel. *Adv. Drug Delivery Rew*, 2002; 54:99–134.
16. Pichot C, Delair T. Speciality applications of latex polymers. In: van Herk AM, editor. *Chemistry and Technology of Emulsion Polymerization*, Blackwell Publishing, Oxford; 2005. pp. 257–277.
17. Meza M. Application of magnetic particles in immunoassays. In: Häfeli U, Schütt W, Teller J, Zborowski M, editors. *Scientific and Clinical Applications of Magnetic Carriers*. New York: Plenum Press; 1997. pp. 303–309.
18. Elaïssari A, Veyret R, Mandrand B, Chatterjee J. Biomedical applications for magnetic latexes. In: Elaïssari A, editor. *Colloidal Biomolecules, Biomaterials*. Volume 116, Surfactant Science series, 116, M Dekker; 2004. pp. 1–29.
19. Härma H. Particle Technologies. *Technol Rev* 2002;126:1–30.
20. Drake TJ, Zhao XJ, Tan W. Bioconjugated silica nanoparticles for bioanalytical applications. In: Niemeyer CM, Mirkin CA, editors. *Nanobiotechnology*.

21. Wenguang L, Jiab QX, Wanga H-L. Facile synthesis of metal nanoparticles using conducting polymer colloids. *Polym Commun* 2006;47:23–26.
22. Pichot C. Surface-functionalized latexes for biotechnological applications. *Curr Opin Colloid Int Sci* 2004;9:213–221.
23. Ito K, Cao J, Kawaguchi S. Functional polymer colloids from functional monomers and macromonomers. In: Arshady R, Guyot A, editors. *Functional Polymer colloids & microparticles*. Volume IV, London: Citus; 2002. pp. 109–135.
24. Fitch RM. In: Fitch RM, editor. *Polymer Colloids: A Comprehensive Introduction*. Academic Press; 1998. pp. 145–172.
25. Arshady R, editor. *Radiolabelled and magnetic particles particulates in medicine and biology. Microspheres Microcapsules & Liposomes*. Volume 3, Londres: Citus Books; 2001. pp. 429–582.
26. Nickkova M, Dosev D, Gee SJ, Hammock BD, Kennedy IM. *Anal Chem* 2004;77: 684.
27. Murphy CJ. *Anal Chem* 2002;74:520A.
28. Dosev D, Nickkova M, Liu M, Guo B, Liu G-yXia Y, Hammock BD, Kennedy IM. Application of Eu:Gd03 nanoparticles to the visualisation of protein micropatterns. *Proc SPIE* 2005;5699:473–481.
29. Lansalot M, Sabor M, Elaïssari A, Pichot C. Elaboration of fluorescent and highly submicronic polymer particles via a stepwise heterocoagulation process. *Colloid Polym Sci* 2005;283:1267–1277.
30. Kawaguchi H. Temperature hydrogels microspheres. In: Arshady R, editor. *Microspheres, Microcapsules, Vol 1: Preparation and Chemical Applications*. Citus Books; 1999. pp. 237–251.
31. Matijevec E, Sapieszko RS, Forced hydrolysis in homogeneous solutions. In: Sugimoto T, editor. *Fine Particles, Synthesis, Characterization and Mechanism of Growth*. Surfactant Science Series 92, New York: Marcel Dekker; 2000. pp. 2–34.
32. Sugimoto T. Phase transformation from solid precursors. In: Sugimoto T, editor. *Fine Particles, Synthesis, Characterization and Mechanism of Growth*. Surfactant Science Series 92, New York: Marcel Dekker; 2000.
33. Kudas TT, Hampden-Smith MJ. *Aeorol Processing of Materials*. New York: Wiley-VCH; 1999.
34. Sugimoto T. *Adv Colloid Interface* 1987;28:65.
35. Giesche H. Hydrolysis of silicon alkoxides in homogeneous solutions. In: Sugimoto T, editor. *Fine Particles, Synthesis, Characterization and Mechanism of Growth*. Surfactant Science Series 92, New York: Marcel Dekker; 2000. pp. 126–146.
36. Silvert P-Y, Tekaia K. *Solid State Ionics* 1995;82:53.
37. Sudol ED. Dispersion polymerisation. In: Asua JM, editor. *Polymeric Dispersions: Principle and Applications*. NATO ASI Series E 335. Dordrecht: Kluwer Academic; 1997. pp. 141–153.
38. Caminade A-M, Majoral J-P. Nanomaterials based on Phosphorus dendrimers. *Acc Chem Res* 2004;37:381.
39. Murr LE, Bang JJ, Esquivel EV, Guerrero PA, Lopez DA. Carbon nanotubes, nanocrystal forms, and complex nanoparticle aggregates in common fuel-gas combustion sources and the ambient air. *J Nanoparticle Res* 2004;6:241–251.

40. Lowell PA, El-Aasser MS. *Emulsion Polymerization and Emulsion Polymers*. Chichester: John Wiley & Sons, Ltd.; 1997.
41. Tang PL, Sudol ED, Adams ME, Silibi CA, El-Aasser MS. Miniemulsion polymerization. In: Daniels ES, Sudol ED, El-Aasser MS, editors. *Polymer Latexes: Preparation, Characterization and Applications*. ACS Symposium Series, 492, Washington DC: 1992. pp. 72–98.
42. Antonietti M, Basten R, Lohmann S. Polymerization in microemulsion—a new approach to ultrafine, highly functionalized polymer dispersions. *Macromol Chem Phys* 1995;196:441–466.
43. Larpent C, Bernard E, Richard J, Vaslin S. Polymerization in microemulsions with polymerizable cosurfactants; a route to highly functionalized nanoparticles. *Macromolecules* 1997;30:354–362.
44. Caruso F, Mövwald H. Preparation and characterization of ordered nanoparticles and polymer composite multilayers on colloids. *Langmuir* 1999;15:8276–8281.
45. Janssen EAWG, van Herk AM, German AL. Encapsulation of inorganic filler particles by emulsion polymerization. *ACS Div Polym Chem Polym Prep* 1993;34:532–533.
46. Bourgeat-Lami E. Hollow particles: synthetic pathways and potential applications. In: Elaïssari A, editor. *Colloidal Polymers*. Surfactant Science Series, 115. New York: Marcel Dekker; 2003. pp. 189–223.
47. Vitry S, Mezzino A, Gauthier C, Cavaillé J-Y, Lefebvre F, Bourgeat-Lami E. Hybrid copolymer latexes cross-linked with methacryloxy propyl trimethoxy silane. Film formation and mechanical properties. *CR Acad Sci II C* 2003;6:1285–1293.
48. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int J Pharm* 1989;55:R1–R4.
49. De S, Robinson D. Polymer relationships during preparation of chitosan–alginate and poly-L-lysine–alginate nanospheres. *J Control Release* 2003;89:101–112.
50. Pichot C, Delair T. Functional nanospheres by emulsion polymerization. In: Arshady R, editor. *Microspheres Microcapsules & Liposomes*. Volume 1. Londres: Citus Books; 1999. pp. 125–163.
51. Kawaguchi K. Functional polymer microspheres. *Prog Polym Sci* 2000;25:1171–1210.
52. Otsuka H, Nagasaki Y, Kataoka K. Self-assembly of polyethylene glycol-based block copolymers for biomedical applications. *Curr Opin Colloid Interface Sci* 2001;3–10.
53. Gref R, Couvreur P, Barratt G, Mysiakine E. Surface-engineered nanoparticles for multiple ligand coupling. *Biomaterials* 2003;24:4529.
54. Schatz C, Lucas JM, Viton C, Domard A, Pichot C, Delair T. Formation and properties of positively charged colloids based on polyelectrolyte complexes of biopolymers. *Langmuir* 2004;20 (18):7766–7778.
55. LaMer VK. *Ind Eng Chem* 1952;44:1270.
56. Candau F. Polymerization in Microemulsions. In: Kumar P, Mittal KL, editors. *Handbook of Microemulsion Science and Technology*. New-York: Marcel Dekker; 1999. pp. 679–712.
57. Li H, Rothberg. Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *PNAS* 2004;101 (39):14036–14039.
58. Sato K, Hosokawa K, Maeda M. Non-crosslinking gold nanoparticles aggregation as a detection method for single-base substitutions. *Nucleic Acid Res* 2005;33 (1):1–5.
59. Stöber W, Fink A, Bohn E. *J Colloid Interface Sci* 1968;26:62–69.

60. Brook MA. Silicon in a biological environment. In: *Silicon in Organic Organometallic and Polymer Chemistry*. John Wiley & Sons, Inc.; New York: 2000. pp. 459–473.
61. Pacard E, Brook MA, Ragheb AM, Pichot C, Chaix C. Elaboration of silica colloid / polymer hybrid support for oligonucleotide synthesis *Colloids Surf B Biointerface* 2006;47:176–188.
62. Fiévet F, Figlarz M, Lagier J-P. Patents: Europe 0,113,281. 1987; USA 4,539,041. 1985; Finland 74,0416. 1998; Canada 1123, 5910. 1988; Norway 163887. 1988, Japan 04,024402. 1992.
63. Sugimoto T, Matijevic E. *J Colloid Interface Sci* 1980;74:227.
64. Matijevic E, Partch RE. Synthesis of monodispersed colloids by chemical reactions in aerosols. In: Sugimoto T, editor. *Fine particles, Synthesis, Characterization and Mechanism of Growth*. Surfactant Science Series, 92. New York: Marcel Dekker; pp. 147–188.
65. Gobe M, Kon-no K, Kandori K, Kitahara A. *J Colloid Interface Sci* 1983;93:291.
66. Pileni MP, Motte L, Petit C. *Chem Mater* 1992;4:338.
67. Osseo-Asare K. Hydrolysis of silicon alkoxides in microemulsions. In: Sugimoto T, editor. *Fine Particles, Synthesis, Characterization and Mechanism of Growth: Surfactant Science Series 92*, New York: Marcel Dekker; 2000. pp. 147–188.
68. Antonietti M, Tauer K. 90 years of polymer latexes and heterophase polymerization: more vital than ever. *Macromol Chem Phys* 2003;204:207–219.
69. Qiu J, Charleux B, Matyjaszewski K. Controlled/living radical polymerization in aqueous media: homogeneous and heterogeneous systems. *Prog Polym Sci* 2001;26:2083–2134.
70. van Herk AM, editor. *Chemistry and Technology of Emulsion Polymerization*. Oxford: Blackwell Publishing; 2005.
71. Caruso F, Lichtenfeld H, Giersig M, Möhwald H. Electrostatic self-assembly of silica nanoparticle-polyelectrolyte multilayers on polystyrene latex spheres. *J Am Chem Soc* 1998;120:8523–8524.
72. Guyot A, Tauer K. Polymerizable and polymeric surfactants. In: Texter J., editor. *Reactions and Synthesis in Surfactants Systems*. Applied Surface Series, 100, (XXVIII). New York: Marcel Dekker; 2001. pp. 547–576.
73. Riess G. Micellisation of block copolymers. *Prog Polym Sci* 2003;28:1107–1170.
74. Millton-Harris J. Introduction to biomedical and biotechnical applications of polyethylene glycol. *ACS Polym Preprints (Div Polym Chem)* 1997; 38:520–521.
75. Landfester K. Recent developments in miniemulsions—Formation and stability mechanisms. *Macromol Symp* 2000;150:171–178.
76. Ramirez LP, Landfester K. Magnetic polystyrene nanoparticles with high magnetic content obtained by miniemulsion process. *Macromol Chem Phys* 2003;204:22–31.
77. Candau F. Microemulsion polymerization. In: Asua JM, editor *Polymeric Dispersions: Principles and Applications*. NATO ASI Series E, 335. Dordrecht: Kluwer Academic; 1997. pp. 127–140.
78. Gan LM, Ti TD, Chew CH, Teo WK, Gan LH. Microporous polymer materials from microemulsions of zwitterionic microemulsions. *Langmuir* 1995;11:3316–3320.
79. Pelton R. Temperature-sensitive microgels. *Adv Colloid Interface Sci* 2000;85 (1):1–33.
80. Vauthier C, Fattal E, Labarre D. From polymer chemistry and physicochemistry to nanoparticulate drug carrier design and applications. In: Yaszemski MJ, Trantolo DJ,

- Lewandrowski KU, Hasirci V, Altobelli DE, Wise DL. *Biomaterial Handbook—Advanced Applications of Basic Sciences and Bioengineering*. New York: Marcel Dekker; 2004. pp. 563–598.
81. Baughman Ray H, Zakhidov Anvar A, de Heer Walt A. Carbon nanotubes—the route toward applications. *Science* 2002;297:787–792.
 82. Bourgeat-Lami E. Organic/inorganic nanocomposites. In: Arshady R, Guyot A, editors. *Dendrimers, Assemblies and Nanocomposites*. MML Series 5 London: Citus Books; 2002. pp. 149–194.
 83. Montagne F, Mondain-Monval O, Pichot C, Elaïssari A. Highly magnetic latexes from submicrometer oil in water ferrofluid emulsions. *J Polym Sci A Polym Chem* 2006;44:2642–2656.
 84. Zhang L, Srivastava RS, Misra RDK. Core-shell magnetite nanoparticles surface encapsulated with smart stimuli-responsive polymer: synthesis, characterization, and LCST of viable drug-targeting delivery system. *Langmuir* 2007;23:6342–6351.
 85. Kim J.-H., Lee TR. Discrete thermally responsive hydrogel-coated gold nanoparticles for use as drug-delivery vehicles. *Nanobiotechnology* 2006;67(1):61–69.
 86. Jain KK. Nanotechnology in clinical laboratory diagnostics. *Clin Chim Acta* 2005;358: 37–54.
 87. Lui T, Burger C, Chu B. Nanofabrication in polymer matrices. *Prog Polym Sci* 2003;28: 5–26.x