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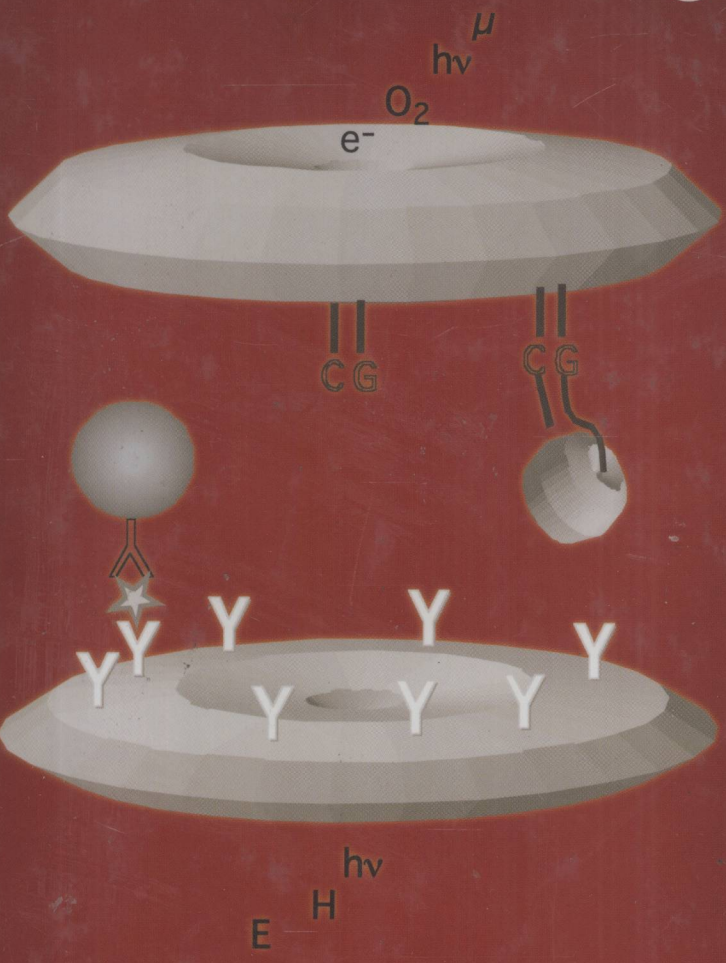

COLLOIDAL NANOPARTICLES IN BIOTECHNOLOGY

ABDELHAMID ELAISSARI

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Edited by

Abdelhamid Elaissari
LAGEP Laboratory
Claude Bernard University
Villeurbanne, France

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ABOUT THE EDITOR

Dr. Abdelhamid Elaissari is the Director of Research at LAGEP Laboratory, Claude Bernard University, Villeurbanne, France. He received his undergraduate education from Agadir University, Morocco in 1988. In 1991, he moved to the Institute Charles Sadron at Louis Pasteur University in Strasbourg, France from where he got his PhD in chemical physics of polymers and colloids. In the same year, he got a permanent position at the Centre National de la Recherche Scientifique (CNRS), in Lyon and then joined CNRS-bioMérieux Laboratory (a semiacademic laboratory in Lyon, France) until its closing in 2007. During this period, Dr. Elaissari has developed various techniques related to colloids from synthesis to biomedical diagnostic applications. He has now moved to the Engineering Processes and Automatic Laboratory (LAGEP), which is a three-member collaboration between CNRS, Claude Bernard University of Lyon, and CPE-Engineering School. In this well-known academic laboratory, Dr. Elaissari conducts fundamental research with applications of reactive and stimuli-responsive colloids for biomedical, environmental, and bionano-technological applications.

Dr. Elaissari has been an adviser for some 30 advanced student projects, about 15 PhD projects, and various postdocs. He is the author and the co-author of some 120 papers, more than 10 chapters, and approximately 15 patents. He has also edited two books prior to *Colloidal Nanoparticles in Biotechnology.*

PREFACE

In the past decades, considerable attention has been paid to the combination of colloidal particles, biomolecules, microfluidics, multidetection and automated microsystems in order to perform powerful tools for biomedical diagnostics, food analysis, and in general way, environmental analysis. The traditional biomedical diagnostic based the capture of target biomolecules via various manual steps is now shifted to more sophisticated technologies in order to reduce time consuming, sensitivity enhancement, reduction of number of steps, analysis of small volumes (few microliter or nanoliter), and finally one use devices in integrated microsystems.

In this direction, colloidal particles are largely used in biomedical applications (diagnostic and therapy) in which they are principally used as solid-phase supports of biomolecules, nanoreators of active molecules (drug), or basically as carriers in various technological aspects. Nowadays, the colloidal particles are used not only as solid support but also as detection tools in various biomedical applications as first established in *in vivo* biomedical diagnostic in cancer diseases detection for instance. To answer the appropriate schedule of conditions for a given application, well reactive particles should be considered. The needed reactive particles relative to the target application are elaborated using many heterophase processes such as emulsion, dispersion, precipitation, self-assembly, and physical processes. In this direction various polymer-based colloids have been prepared and explored for *in vitro* biomedical applications and recently for possible integration in automated systems, nanobiotechnologies, and microsystems.

The colloidal particles are "designed" by considering various criteria related to the targeted applications such as particle size, size distribution, surface polarity, surface reactive groups, hydrophilic-hydrophobic balance of the surface, and also intrinsic properties for instance (nonexhaustive list). Consequently, the particles synthesis process should be well adapted in order to prepare structured particles latex particles bearing shell and core with well-defined properties. In fact, low charged polystyrene latex particles for instance are used in rapid diagnostic tests based on agglutination process. Magnetic particles are first used in immunoassays and in molecular biology for specific capture of single stranded DNA fragments. Magnetic colloids are then used as a carrier to make easy and possible biomolecules extraction, concentration, and purification as pointed out using cationic magnetic latex particles for nucleic acids extraction, purification, concentration, and amplification and more recently in viruses isolation. Labeled colloidal particles are generally used as detection tools as well studied and performed in cell sorting application and now in lab-on-chip for CCD camera detection.

The specificity and the sensitivity of the targeted biomedical application efficiency are directly related to the surface particles properties, to the intrinsic characteristics of the used materials, to the accessibility of the immobilized biomolecules and to the affinity between targeted biomolecules and the particles surface. The interaction between biomolecules and the particles surface is a complex domain, which contains various physical aspects such as the affinity, the interfacial diffusion phenomena, the immobilized biomolecules conformation, the possible exchange processes, and various anthers physicochemical properties of both biomolecules and particles surface.

The main object of this book is to report on new studies of colloids and nanoparticles in bio-nano-technologies for biomedical and environmental diagnostic. The state of the art in the elaborations and the properties of nanocolloids is presented and illustrated. Special attention is focused on new stimuli-responsive particles and reactive nanoparticles bearing intrinsic properties. The integration of reactive colloidal particles in microfluidic-based technology is a challenging field and needs to consider. In this direction, various aspects are considered and discussed in this book by considering the compatibility of reactive colloidal particles with the microfluidic based Microsystems and biosensors. The intrinsic properties of colloidal particles were also considered and special attention was focused on both magnetic particles and quantum dots fluorescent nanocrystals. Consequently, this book is prearranged in order to show to the readers the use of colloidal particles in biotechnologies

PROF. HATEM FESSI

Director,
LAGEP Laboratory

CONTRIBUTORS

Matt Aldissi, Fractal Systems, Inc., 200 9th Avenue N., Suite 100, Safety Harbor, FL 34695, USA

Mikhail Artemyev, Institute of Physico-Chemical Problems, Belarussian State University, Minsk, Belarus

Teresa Basinska, Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland

Olivier Carion, Institut de Biologie de Lille, UMR CNRS 8161, 1 rue du Professeur Calmette, 59021 Lille Cedex, France

Yann Chevolot, AMPERE and INL, Ecole Centrale de Lyon, 69134 Ecully Cedex, France

Jean-Pierre Cloarec, AMPERE and INL, Ecole Centrale de Lyon, 69134 Ecully Cedex, France

Cecile Cottin-Bizonne, Université de Lyon, Université Lyon 1, CNRS UMR 5586, Laboratoire PMCN, F-69622 Villeurbanne, France

Jean-Olivier Durand, Institut Charles Gerhardt, UMR 5253 cc 1701, Université Montpellier 2, place Eugène Bataillon, 34095 Montpellier Cedex 05, France

Abdelhamid Elaissari, Claude Bernard University, LAGEP Laboratory, 43 Boulevard du 11 novembre 1918, Bât. CPE-308G 69622 Villeurbanne Cedex, France

Ouafâa El-Mahdi, Institut de Biologie de Lille, UMR CNRS 8161, 1 rue du Professeur Calmette, 59021 Lille Cedex, France

Rosaria Ferrigno, Institut des Nanotechnologies de Lyon, INL, CNRS UMR 5270, Université de Lyon, Lyon, F-69003, France, and Université Lyon 1, Villeurbanne, F-69622, France

Jean-Michel Garcia, Institut de Biologie de Lille, UMR CNRS 8161, 1 rue du Professeur Calmette, 59021 Lille Cedex, France

Céline Genty, Claude Bernard University, LAGEP Laboratory, 43 Boulevard du 11 novembre 1918, Bât. CPE-308G 69622 Villeurbanne Cedex, France, and Université Bordeaux 1, UPR 8641, Centre de Recherche Paul Pascal – CNRS, Avenue A. Schweitzer, 33 600 Pessac, France

Nicole Jaffrezic-Renault, LSA, Claude Bernard University Lyon 1, 69622 Villeurbanne Cedex, France

Haruma Kawaguchi, Graduate school of Science and Technology, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan

Anne Le Nel, Laboratoire PCC, Institut Curie (UMR CNRS/IC 168), Paris Cedex 05, France, and JE 2495 Protéines et Nanotechnologies en Sciences Séparatives, Université Paris XI, Châtenay-Malabry, France

Céline Maillet, Institut de Biologie de Lille, UMR CNRS 8161, 1 rue du Professeur Calmette, 59021 Lille Cedex, France

Lionel Marcon, Institut d'Electronique de Microélectronique et de Nanotechnologie, UMR CNRS 8520, Department of ISEN, 41 Bd Vauban, 59046 Lille Cedex, France, and Institut de Biologie de Lille, UMR CNRS 8161 (CNRS, Universités de Lille 1 et 2, Institut Pasteur de Lille), 1 rue du Professeur Calmette, 59021 Lille Cedex, France

Claude Martelet, AMPERE and INL, Ecole Centrale de Lyon, 69134 Ecully Cedex, France

Oleg Melnyk, Institut de Biologie de Lille, UMR CNRS 8161, 1 rue du Professeur Calmette, 59021 Lille Cedex, France

Olivier Mondain-Monval, Université Bordeaux 1, UPR 8641, Centre de Recherche Paul Pascal – CNRS, Avenue A. Schweitzer, 33 600 Pessac, France

Igor Nabiev, EA n°3798 Détection et Approches Thérapeutiques Nanotechnologiques dans les Mécanismes Biologiques de Défense, Université de Reims Champagne-Ardenne, 51 rue Cognacq Jay, 51100 Reims, France

Peihong Ni, College of Chemistry and Chemical Engineering, Soochow University, Suzhou 215123, China

Vladimir Oleinikov, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia

Christophe Olivier, Institut de Biologie de Lille, UMR CNRS 8161, 1 rue du Professeur Calmette, 59021 Lille Cedex, France

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

Duangporn Polpanich, Department of Chemistry, Faculty of Science, Mahidol University, Rama VI Rd, Bangkok 10400, Thailand, and National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Thailand Science Park, Klong Luang, Pathumthani 10120, Thailand

Manoj K. Ram, Fractal Systems, Inc., 200 9th Avenue N., Suite 100, Safety Harbor, FL 34695, USA

CONTENTS

CHAPTER 1

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

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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
Inorganic particles		
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)
Organic particles		
Carbon nanotubes and fullerens	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)
Organic/inorganic composite particles		
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	
• Reactive surface groups	Covalent grafting of nanoparticles on plane surfaces or with biomolecules
• Hydrophilicity	Depletion of biomolecules, stealth effect
• Sensitivity to stimulus (T, pH, ionic strength, UV, light, electric, or magnetic fields)	To change the nature and properties of colloidal particles
• Complexation (PEO, PMAA, metal chelates, etc.)	Performing protein purification, oriented immobilization of proteins, antibodies
• Biological ligand (oligosaccharide, lipid, peptide, nucleic acid, antibody, protein)	Recognition of antigens, specific cells, DNA, RNA, protein, lectins
• Magnetic	Fast separation of colloids, imaging
• Color, fluorescence	Detection by optical methods
• Biodegradability	To be used for *in vivo* 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 supeparamagnetism 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