NANOPARTICLES: AN OVERVIEW OF PREPARATION METHODOLOGIES, CHARACTERIZATION & APPLICATIONS

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Abstract

Nanoparticles have attracted the attention of many research groups and have been utilized in quite a number of areas during the last few years. In general, two main strategies are used for their preparation: the dispersion of preformed polymers and the polymerization of monomers. Different techniques may be used to prepare the polymer nanoparticles, such as solvent evaporation, salting-out, supercritical fluid technology, micro-emulsion and mini-emulsion. The choice of technique is based on a number of factors, for example, particle size, particle size distribution, area of application, etc. This review attempts to provide the general description of the preparation, characterization and applications of nanoparticles.

Keywords: Nanoparticles, biodegradable polymers, particle size.

1. Introduction

Usually, the efficacy and bioavailability of any drug depend on its route or mode of administration. Thus, almost all the pharmaceutical companies focus on the development of various novel drug delivery systems to deliver the appropriate dose of the drug of a particular therapeutic (small molecules, proteins, or nucleic acids) to a specific target site. As this cannot be generally achieved, drugs have to be administered in much higher doses, thus leading to toxic side effects. The concept of site-specific delivery of the drug arises from this particular drawback of the conventional dosage form. Nanoparticles have a huge potential in addressing this drawback. They offer site targeting properties, which leads to reduced toxicity and ultimately better patient compliance.

Nanoparticles can be defined as particulate dispersions or solid particles having a size range of 10-1000nm. The drug can be entrapped, dissolved, encapsulated or attached to the matrix of the particle. The primary objectives in fabricating Nanoparticles as delivery system are to control the particle size, surface properties and release of the drug in order to achieve the site-specific action of the drug at therapeutically optimum rate and dose regimen. Nanoparticles are made from biocompatible and biodegradable materials such as

polymers, either natural (e.g., gelatin, albumin) or synthetic (e.g., polylactides, polyalkylcyanoacrylates), or solid lipids. In the body, the drug loaded in nanoparticles is usually released from the matrix by diffusion, swelling, erosion, or degradation. Although liposome have been used as carriers with specific benefits including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are quite limited due to their inherent problems like low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. But on the other hand, the Nanoparticles provide some unique advantages over the liposome. For example, they help to increase the stability of drugs/proteins and have useful controlled release properties. [1,2,3]

Some of the benefits of using Nanoparticles as a drug delivery system are as follows:

1. Site-specific target may be achieved incorporating targeting ligands to the surface of particles or by use of magnetic guidance.
2. The dosage can be administered by various routes such as oral, nasal, parenteral, intra-ocular etc.
3. Their particle size and surface characteristics can be manipulated to achieve both passive and active drug targeting after parenteral administration.
4. They control and sustain the release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug in order to achieve increase in drug therapeutic efficacy and reduction in side effects.
5. The rate of drug and also the particle degradation characteristics can by regulated of the choice of matrix constituents.

Despite of these advantages, nanoparticles also have some limitations. For instance, the small size and greater surface area may cause particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. Besides this, the small particle size and large surface area readily result in limited drug loading and burst release. The other disadvantages involves use of harsh toxic solvent in the preparation process and higher manufacturing costs [4,5,6].

2. Preparation techniques of nanoparticles

Nanoparticles can be prepared either from pre-formed polymers or by polymerization of monomers using chemical reactions [7]. Various strategies like solvent evaporation, salting out, dialysis and supercritical fluid technology can be applied for the preparation of nanoparticles from preformed polymers. In contrast, the particles are directly prepared from monomers using various polymerization methods like microemulsion, miniemulsion, surfactant-free emulsion and interfacial polymerization. The method of preparation is selected on the basis of a variety of

factors like size requirement, type of polymeric system, area of application, etc.

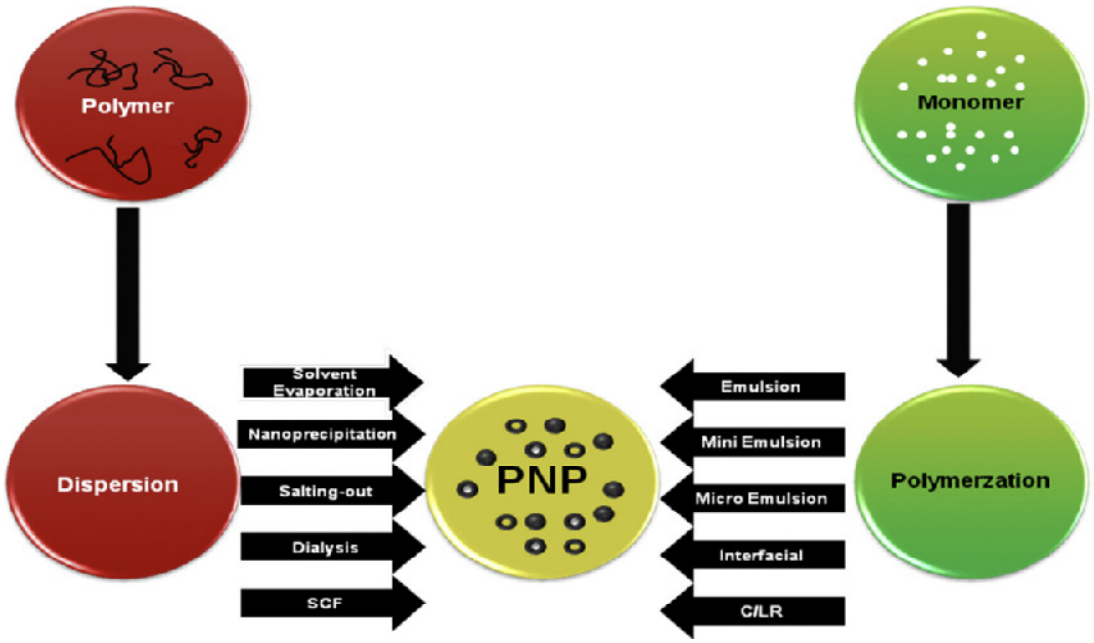

Figure 1: various techniques for the preparation of polymer nanoparticles. SCF: supercritical fluid technology, C/LR: controlled/living radical

These are some of the many factors which have to be considered while choosing a particular technique for nanoparticles preparation.

2.1 Dispersion of preformed polymers

Many methods developed and successfully utilized to prepare nanoparticles by dispersing preformed polymers are discussed in brief in the following sections.

2.1.1 Solvent evaporation

Here, polymer solutions are prepared in a volatile solvent and emulsion is formulated. The emulsion is converted into a nanoparticles suspension on evaporation of the solvent for the polymer, which is allowed to diffuse across the continuous phase. [8,9] In the conventional methods, two strategies are used for formation of emulsion: the preparation of single emulsions(e.g. o/w) and preparation of double emulsions (e.g. w/o/w). These techniques utilize high-speed homogenization or ultra-sonication or combination of both, followed by evaporation of solvent. Then the solidified nanoparticles are collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized to obtain free-flowing powder. Normally, a polymer is dissolved in an organic phase containing the surfactant/ stabilizer forms the water phase.

Although the solvent evaporation technique is a simple method for the preparation of nanoparticles, it is time-consuming and possible coalescence of the nano-droplets during the evaporation process may affect the final size and morphology of the particle.

2.1.2 Salting out

This process is a modified version of emulsion process which involves a salting-out process, which avoids surfactants and chlorinated solvents. The emulsion is formulated with a polymer solvent which completely miscible with water and emulsification of the polymer solution in the aqueous phase is achieved, without utilization of any high sheer forces [10], by dissolving high concentration of salt or sucrose chosen for a strong salting-out effect in the aqueous phase. Magnesium chloride, calcium chloride and magnesium acetate are commonly used suitable electrolytes [11-18]. The miscibility properties of water with other solvents are modified as these components dissolve in the water. A reverse salting out effect, obtained by dilution of the emulsion with an excess amount of water, results in the precipitation of the polymer dissolved in the droplets of the emulsion. In fact, after dilution, migration of the solvent for the polymer from the emulsion droplets is induced due to the reduction of the salt concentration in the continuous phase of the emulsion.

2.1.3 Nanoprecipitation

Also known as the solvent displacement method, the basic principle of this method is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution. Rapid diffusion of the solvent into non-solvent phase results in the decrease of interfacial tension between the two phases, which increases the surface area and leads to the formation of small droplets of organic solvent [19,20]. Nanoprecipitation system comprises of three basic components: the polymer (synthetic, semi synthetic or natural), the polymer solvent and the non-solvent of the polymer. Organic solvent (i.e., ethanol, acetone, hexane, or dioxane) which is miscible in water and easy to remove by evaporation is selected as the polymer solvent. Due to this reason, acetone is the most commonly employed polymer solvent in this method [19,21,22]. Sometimes, it consists of binary solvent blends, acetone with small amount of water [23], blends of acetone with ethanol [24-25] and methanol [26]. On the other hand, the non-solvent phase consisting of a non-solvent or a mixture of non-solvents is supplemented with one or more naturally occurring or synthetic surfactants. Nanoprecipitation is an easy, fast and reproducible method which is widely used for the preparation of both nanospheres and nanocapsules. Although low polymer surfactant concentrations are being used, challenges pertaining to low polymer concentration in the organic phase need to be addressed.

2.1.4 Supercritical Fluid Technology

As may be noted, the methods in the preceding subsections involve organic solvents, and the need to develop environmentally safer methods for the production of PNP has motivated research on the utility of supercritical fluids as more environmental friendly solvents, with the potential to produce PNPs with high purity and without any trace of organic solvent [27,28]. Supercritical fluid and dense gas technology are expected to offer an interesting and effective technique of particle production, avoiding most of the drawbacks of the traditional methods. Here, the solute is dissolved in a supercritical fluid to form a solution, followed by the rapid expansion of the solution across an orifice or a capillary nozzle into ambient air. The high degree of supersaturation, accompanied by the rapid pressure reduction in the expansion, results in homogenous nucleation and, thereby, the formation of well-dispersed particles.

2.2 Polymerization of monomers

The techniques mentioned previously did not involve any polymerization process. To attain the desired properties for a particular application, suitable polymer nanoparticles must be designed, which can be done during the polymerization of monomers. Processes for the production of PNPs through the polymerization of monomers are discussed, focusing principally on mini-, micro-, and emulsion polymerization techniques as the three major techniques currently in use.

2.2.1 Emulsion polymerization

Emulsion polymerization is the most frequent method used for the preparation of a variety of specialty polymers. The use of water as the dispersion medium is environmentally friendly and also allows excellent heat dissipation during the process of the polymerization. The ingredients used in the process comprise of water, a monomer of low water solubility, water-soluble initiator and a surfactant. Initiation occurs when a monomer molecule dissolved in the continuous phase collides with an initiator molecule that may be an ion or a free radical. Phase separation and formation of solid particles can take place before or after the termination of the polymerization reaction [29]. Polystyrene (PS) [30–37], poly(methylmethacrylate) (PMMA) [38,39], poly(ethylcyanoacrylate) (PECA) and poly(butylcyanoacrylate) [40] nanoparticles were produced by dispersion via surfactants into solvents, such as cyclohexane, n-pentane, and toluene.

2.2.2 Mini-emulsion polymerization

A typical formulation used in mini-emulsion polymerization consists of water, monomer mixture, co-stabilizer, surfactant, and initiator. The key difference between emulsion polymerization and mini-emulsion polymerization is the

utilization of a low molecular mass compound as the co-stabilizer and also the use of a high-shear device (ultrasound, etc.). Mini-emulsions are critically stabilized, require a high-shear to reach a steady state and have an interfacial tension much greater than zero. Polyacrylic acid nanoparticles were synthesized by Kriwet et al. [41] using a co-emulsifier system consisting of a mixture of Span 80 and Tween 80. The polymerization was initiated by free radicals, and the particle size was dependent on the type of radical initiator used.

2.2.3 Micro-emulsion polymerization

Micro-emulsion polymerization is a new and effective approach for preparing nano-sized polymer particles and has attracted significant attention. Although emulsion and micro-emulsion polymerization appear similar because both methods can produce colloidal polymer particles of high molar mass, they are entirely different when compared kinetically. In micro-emulsion polymerization, an initiator, typically water-soluble, is added to the aqueous phase of a thermodynamically stable micro-emulsion containing swollen micelles. The polymerization starts from this thermodynamically stable, spontaneously formed state and relies on high quantities of surfactant systems, which possess an interfacial tension at the oil/water interface close to zero. Furthermore, the particles are completely covered with surfactant because of the utilization of a high amount of surfactant. Initially, polymer chains are formed only in some droplets, as the initiation cannot be attained simultaneously in all micro-droplets. Later, the osmotic and elastic influence of the chains destabilize the fragile micro-emulsions and typically lead to an increase in the particle size, the formation of empty micelles, and secondary nucleation [42].

3. Characterization of nanoparticles and its effect on drug delivery

3.1 Particle Size

Particle size and size distribution are the most essential characteristics of the nanoparticles. They determine the in vivo distribution, biological fate, toxicity and the targeting capability of nanoparticle systems. Additionally, they have an influence on the entrapment efficiency, release kinetics and stability of nanoparticles. Currently, the quickest and most routine method of examining the particle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy needs the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties [43]. The report obtained by photon-correlation spectroscopy is usually confirmed by the scanning or transmission electron microscopy.

3.2 Surface Properties

The zeta potential of a nanoparticle is generally used to characterize the surface charge of the nanoparticles [44]. It exhibits the electrical potential of particles and is influenced by the

composition of the particles and the medium in which it is being dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents the aggregation of the particles. The zeta potential may also be utilized to determine whether a charged active material is encapsulated within the core of the nanocapsule or adsorbed on the surface.

3.3 Drug Loading

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading may be done by two ways: (a) Incorporating at the time of nanoparticles preparation (b) Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution.

3.4 Drug Release

Various methods which can be used to study the in vitro release of the drug are: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion technique; (3) reverse dialysis bag technique; (4) agitation followed by ultracentrifugation/centrifugation; (5) Ultra-filtration or centrifugal ultra-filtration techniques. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.

4. Applications of nanoparticle based drug delivery system

Some of the applications of nanoparticles in the field of medicine are described below.

4.1 In tumor targeting

The rationale of using nanoparticles for tumor targeting is based on 1) nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles; 2) nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ. Verdun et al showed that in mice treated with doxorubicin loaded into poly (isohexylcyanoacrylate) nanospheres that greater concentrations of doxorubicin manifested in the liver, spleen and lungs than in mice treated with free doxorubicin [45].

4.2 Long circulating nanoparticles

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a lot of work has been devoted to developing so-called “stealth” particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes[46]. A major

breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamers, poloxamines, and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the mononuclear phagocytic system MPS [47]. These coatings provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface which repel plasma proteins [48,49].

4.3 Nanoparticles for oral delivery of peptides and proteins

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration [50].

4.4 For gene delivery

Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment [51]. Hedley et al. [52] reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein.

4.5 Nanoparticles for drug delivery into the brain

Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cellpenetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis [53-56].

5. Conclusion

The foregoing research shows that nanoparticulate systems have great capabilities, being able to alter poorly soluble, poorly absorbed and labile drugs into promising deliverable drugs. The core of this

system can enclose a variety of drugs, proteins, genes and is characterized by a long circulation time due to the hydrophilic shell which prevents recognition by the reticular-endothelial system. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required.

References

1. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release 2002; 78: 15-24.
2. Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R)): PLGA nanoparticles containing vitamin E TPGS. J Control Release 2003; 86: 33-48.
3. Bala I, Hariharan S, Kumar MN. PLGA nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst 2004; 21:387–422.
4. 12.R. M. Mainardes, M. C. Urban, P. O. Cinto, N. M. Khalil, M. V. Chaud, R. C. Evangelista and M. P. Gremiao. Colloidal carriers for ophthalmic drug delivery, Curr Drug Targets 6 (2005), no. 3, 363-371.
5. W. H. De Jong and P. J. Borm. Drug delivery and nanoparticles: Applications and hazards. Int J Nanomedicine 3 (2008); 2: 133-149.
6. N. K. Varde and D. W. Pack. Microspheres for controlled release drug delivery. Expert Opin Biol Ther 4 (2004); 1: 35-51.
7. Geckeler KE, Stirn J. Polyreaktionen – Mechanismen, Systematik, Relevanz. Naturwissenschaften 1993; 80:487–500
8. Allemann E, Gurny R, Doelker E. Drug loaded nanoparticles preparation, methods and drug targeting issues. Eur J Pharm Biopharm 1993; 39:173–91.
9. Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates—a review. J Control Release 2008; 128:185–99.
10. Ganachaud F, Katz JL. Nanoparticles and nanocapsules created using the ouzo effect: spontaneous emulsification as an alternative to ultrasonic and high-shear devices. ChemPhysChem 2005; 6:209–16.
11. Allemann E, Gurny R, Doelker E. Preparation of aqueous polymeric nanodispersions by a reversible salting-out process: influence of process parameters on particle size. Int J Pharm 1992; 87:247–53.
12. De Jaeghere F, Allemann E, Leroux JC, Stevels W, Feijen J, Doelker E, Gurny R. Formulation and lyoprotection of poly(lactic acid-coethylene oxide) nanoparticles: influence on physical stability and in vitro cell uptake. Pharm Res 1999; 16:859–66.
13. Nguyen CA, Allemann E, Schwach G, Doelker E, Gurny R. Synthesis of a novel fluorescent poly(d,l-lactide) end-capped with

1-pyrenebutanol used for the preparation of nanoparticles. Eur J Pharm Sci 2003; 20:217–22.

14. Zweers MLT, Engbers GHM, Grijpma DW, Feijen J. In vitro degradation of nanoparticles prepared from polymers based on dl-lactide, glycolide and poly(ethylene oxide). J Control Release 2004; 100:347–56.
15. Galindo-Rodriguez SA, Puel F, Briancon S, Allemann E, Doelker E, Fessi H. Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles. Eur J Pharm Sci 2005; 25:357–67.
16. Zweers MLT, Engbers GHM, Grijpma DW, Feijen J. Release of anti-restenosis drugs from poly(ethylene oxide)-poly(dl-lactic-coglycolic acid) nanoparticles. J Control Release 2006; 114:317–24.
17. Fan YF, Wang YN, Fan YG, Ma JB. Preparation of insulin nanoparticles and their encapsulation with biodegradable polyelectrolytes via the layer-by-layer adsorption. Int J Pharm 2006; 324:158–67.
18. De Jaeghere F, Allemann E, Feijen J, Kissel T, Doelker E, Gurny R. Cellular uptake of PEO surface-modified nanoparticles: evaluation of nanoparticles made of PLA:PEO diblock and triblock copolymers. J Drug Target 2000; 8:143–53.
19. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 1989; 55:R1–4.
20. Mishra B, Patel BB, Tiwari S. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine: NBM 2010; 6:9–24.
21. Dalpiaz A, Vighi E, Pavan B, Leo E. Fabrication via a nonaqueous nanoprecipitation method, characterization and in vitro biological behavior of N6-cyclopentyladenosine-loaded nanoparticles. J Pharm Sci 2009; 98:4272–84.
22. Fong-Yu C, Saprina Ping-Hsien W, Chio-Hao S, Tsung-Liu T, Ping- Ching W, Dar-Bin S, Jyh-Horng C, Patrick Ching-Ho H, Chen-Sheng Y. Stabilizer-free poly(lactide-co-glycolide) nanoparticles for multimodal biomedical probes. Biomaterials 2008; 29:2104–12.
23. Thioune O, Fessi H, Devissaguet JP, Puisieux F. Preparation of pseudolatex by nanoprecipitation: influence of the solvent nature on intrinsic viscosity and interaction constant. Int J Pharm 1997; 146:233–8.
24. Murakami H, Kobayashi M, Takeuchi H, Kawashima Y. Preparation of poly(dl-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. Int J Pharm 1999; 187:143–52.
25. Chang J, Jallouli Y, Kroubi M, Xu-bo Y, Feng W, Chun-sheng K, Peiyu P, Betbeder D. Characterization of endocytosis of

transferrincoated PLGA nanoparticles by the blood–brain barrier. Int J Pharm 2009; 379:285–92.

26. Assis DND, Mosqueira VCF, Vilela JMC, Andrade MS, Cardoso VN. Release profiles and morphological characterization by atomic force microscopic and photon correlation spectroscopy of 99mTechnetium-fluconazole nanocapsules. Int J Pharm 2008; 349:152–60.
27. York P. Strategies for particle design using supercritical fluid technologies. Pharm Sci Technol Today 1999; 2:430–40.
28. KawashimaY. Nanoparticulate systems for improved drug delivery. Adv Drug Deliv Rev 2001; 47:1–2.
29. Kreuter J. The mechanism of termination in heterogeneous polymerization. J Polym Sci Polym Lett Ed 1982; 20:543–5.
30. Munoz-Bonilla A, Herk AMV, Heuts JPA. Preparation of hairy particles and antifouling films using brush-type amphiphilic block copolymer surfactants in emulsion polymerization. Macromolecules 2010; 43:2721–31.
31. Garay-Jimenez JC, Gergeresa D, Young A, Lim DV, Turos E. Physical properties and biological activity of poly(butyl acrylate–styrene) nanoparticle emulsions prepared with conventional and polymerizable surfactants. Nanomedicine: NBM 2009; 5:443–51.
32. Lu S, Qu R, Forcada J. Preparation of magnetic polymeric composite nanoparticles by seeded emulsion polymerization. Mater Lett 2009; 63:770–2.
33. Gao J,WuC. Modified structural model for predicting particle size in the microemulsion and emulsion polymerization of styrene under microwave irradiation. Langmuir 2005; 21:782–5.
34. Zhang J, Cao Y, He Y. Ultrasonically irradiated emulsion polymerization of styrene in the presence of a polymeric surfactant. J Appl Polym Sci 2004; 94:763–8.
35. Yih-Her C, Yu-Der L, Karlsson OJ, Sundberg DC. Particle nucleation mechanism for the emulsion polymerization of styrene with a novel polyester emulsifier. J Appl Polym Sci 2001; 82:1061–70.
36. Thickett SC, Gaborieau M, Gilbert RG. Extended mechanistic description of particle growth in electrosterically stabilized emulsion polymerization systems. Macromolecules 2007; 40: 4710–20.
37. Mock EB, Bruyn HD, Hawkett BS, Gilbert RG, Zukoski CF. Synthesis of anisotropic nanoparticles by seeded emulsion polymerization. Langmuir 2006; 22:4037–43.
38. Costa C, Santos AF, Fortuny M, Araujo PHH, Sayer C. Kinetic advantages of using microwaves in the emulsion polymerization ofMMA. Mater Sci Eng C 2009; 29:415–9.
39. Cheng X, Chen M, Zhou S, Wu L. Preparation of SiO2/PMMA composite particles via conventional emulsion

polymerization. J Polym Sci Part A Polym Chem 2006; 44:3807–16.

40. Su-Jung Y, Chun H, Mi-Sun L, Kim N. Preparation of poly(Nvinylcarbazole) (PVK) nanoparticles by emulsion polymerization and PVK hollow particles. Synth Met 2009; 159:518–22.
41. Kriwet B, Walter E, Kissel T. Synthesis of bioadhesive poly(acrylic acid) nano- and microparticles using an inverse emulsion polymerization method for the entrapment of hydrophilic drug candidates. J Control Release 1998; 56:149–58.
42. Puig JE. Microemulsion polymerization (oil-in water). In: Salamone JC, editor. Polymeric materials encyclopedia, vol. 6. Boca Raton, FL: CRC Press; 1996. pp. 4333–41.
43. Swarbrick J, Boylan J. Encyclopedia of pharmaceutical technology. 2nd ed.; Marcel Dekker: New York, 2002.
44. Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: a review. Crit Rev Ther Drug Carrier Syst 2002; 19: 99-134.
45. Verdun C, Brasseur F, Vranckx H, Couvreur P, Roland M. Tissue distribution of doxorubicin associated with polyhexylcyanoacrylate nanoparticles. Cancer Chemother. Pharmacol 1990; 26: 13-18.
46. Storm G, Belliot S, Daemen T, Lasic D. Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. Adv. Drug Deliv. Rev. 1995; 17: 31-48.
47. Torchilin V, Trubetskoy V. Which polymer can make nanoparticulate drug carriers long circulating? Adv. Drug Deliv. Rev. 1995; 16: 141-155.
48. Jeon SI, Lee JH, Andrade JD, De Gennes PG. Protein--surface interactions in the presence of polyethylene oxide : I. Simplified theory. J. Colloid Interface Sci. 1991; 142: 149-158.
49. Jeon SI, Andrade JD. Protein--surface interactions in the presence of polyethylene oxide : II. Effect of protein size. J. Colloid Interface Sci. 1991; 142:159-166.
50. Damge C, Michel C, Aprahamian M, Couvreur P, Devissaguet JP. Nanocapsules as carriers for oral peptide delivery. J. Control. Release 1990; 13: 233-239.
51. Panyam J, Zhou WZ, Prabha S, Sahoo SK, Labhasetwar V. Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. Faseb J2002; 16: 1217-26.
52. Hedley M, Curley J, Urban R. Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cell responses. Nat Med 1998; 4: 365-368.
53. Kreuter J. Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. J Nanosci Nanotechnol 2004; 4: 484-8.

54. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov 2002; 1: 131-9.
55. Ji B, Maeda J, Higuchi M, Inoue K, Akita H, Harashima H, Suhara T. Pharmacokinetics and brain uptake of lactoferrin in rats. Life Sciences 2006; 78: 851-855.
56. Scherrmann JM, Temsamani J. The use of Pep: Trans vectors for the delivery of drugs into the central nervous system. International Congress Series 2005; 1277: 199-211.