23 Nanoparticles for Drug Delivery

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23.1 INTRODUCTION

Polymer nanoparticles are particles of less than 1 µm diameter that are prepared from natural or synthetic polymers. Nanoparticles have become an important area of research in the field of drug delivery because they have the ability to deliver a wide range of drugs to different areas of the body for sustained periods of time. The small size of nanoparticles is integral for systemic circulation. Natural polymers (i.e., proteins or polysaccharides) have not been widely used for this purpose since they vary in purity, and often require crosslinking that could denature the embedded drug. Consequently, synthetic polymers have received significantly more attention in this area. The most widely used polymers for nanoparticles have been poly-ε-caprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their co-polymers, poly(lactide-co-glycolide) (PLGA) [1–3]. In addition, block co-polymers of PLA and poly(ethylene glycol) (PEG) and poly(amino acids) have been used to make nanoparticles and micelle-like structures [4,5]. These polymers are known for both their biocompatibility and resorbability through natural pathways. Additionally, the degradation rate and accordingly the drug release rate can be manipulated by varying the ratio of PLA or PCL, increased hydrophobicity, to PGA, and increased hydrophilicity.

During the 1980s and 1990s, several drug delivery systems were developed to improve the efficiency of drugs and minimize toxic side effects [6]. The early nano- and microparticles were mainly formulated from poly(alkylcyanoacrylate) [6]. Initial promise of microparticles was dampened by the fact that there was a size limit for the particles to cross the intestinal lumen into the lymphatic system following oral delivery. Likewise, the therapeutic effect of drug-loaded nanoparticles was relatively poor due to rapid clearance of the particles by phagocytosis postintravenous administration. In recent years, headway has been made in solving this problem by the addition of surface modifications to nanoparticles. Nanoparticles, such as liposomes, micelles, worm-like micelles, polymersomes, and vesicles have also been proposed recently in the literature as promising drug delivery vehicles because of their small size and hydrophilic outer shell.

In recent years, significant research has been done on nanoparticles as oral drug delivery vehicles. For this application, the major interest is in lymphatic uptake of the nanoparticles by the Peyer's patches in the gut associated lymphoid tissue (GALT). There have been many reports on the optimum size for Peyer's patch uptake ranging from <1 to <5 µm [7,8]. It has been shown that microparticles remain in the Peyer's patches while nanoparticles are disseminated systemically [9].

Nanoparticles have a further advantage over larger microparticles because they are better suited for intravenous (IV) delivery. The smallest capillaries in the body are 5 to 6 µm in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 µm, and should not form aggregates, to ensure that the particles do not form an embolism.

Clearly, a wide variety of drugs can be delivered using nanoparticulate carriers via a number of routes. Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, biological macromolecules, etc. [10–13]. They can be formulated for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver, spleen, or made for long-term systemic circulation. Therefore, numerous protocols exist for synthesizing nanoparticles based on the type of drug used and the desired delivery route. Once a protocol is chosen, the parameters must be tailored to create the best possible characteristics of the nanoparticles. Four of the most important characteristics of nanoparticles are their size, encapsulation efficiency, zeta potential (surface charge), and release characteristics. In this chapter, we intend to summarize many of the types of nanoparticles available for drug delivery, the techniques used for preparing polymeric nanoparticles, including the types of polymers and stabilizers used, and how these techniques affect the structure and properties of the nanoparticles. Additionally, we will discuss advances in surface modifications, targeted drug delivery applications, and release mechanisms and characteristics.

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23.2 SYNTHESIS OF SOLID NANOPARTICLES

As stated previously, there are several different methods for preparing nanoparticles. Additionally, numerous methods exist for incorporating drugs into the particles. For example, drugs can be entrapped in the polymer matrix, encapsulated in a nanoparticle core, surrounded by a shell-like polymer membrane, chemically conjugated to the polymer, or bound to the particle's surface by adsorption. Many of the previously mentioned nanoscale carriers such as micelles and polymersomes are synthesized via self-assembly mechanisms. The following section will deal primarily with the production of solid nanoparticles. A summary of these methods including the types of polymer, solvent, stabilizer, and drugs used is given in Tables 23.1 and 23.2.

The most common method used for the preparation of solid, polymeric nanoparticles is the emulsification–solvent evaporation technique. This technique has been successful for encapsulating hydrophobic drugs, but has had poor results in incorporating bioactive agents of a hydrophilic nature. Briefly, solvent evaporation is carried out by dissolving the polymer and the compound in an organic solvent. Frequently, dichloromethane is used for PLGA copolymers. The emulsion is prepared by adding water and a surfactant to the polymer solution. In many cases, nano-sized polymer droplets are induced by sonication or homogenization. The organic solvent is then evaporated and the nanoparticles are usually collected by centrifugation and lyophilization [7,14–17].

A modification on this procedure has led to the protocol favored for encapsulating hydrophilic compounds and proteins, the double or multiple emulsion technique. First, a hydrophilic drug and a stabilizer are dissolved in water. The primary emulsion is prepared by dispersing the aqueous phase into an organic solvent containing a dissolved polymer. This is then re-emulsified in an outer aqueous phase also containing a stabilizer [8,9,15,18–20]. From here, the procedure for obtaining the nanoparticles is similar to the single emulsion technique for solvent removal. The main problem with trying to encapsulate a hydrophilic molecule like a protein or a peptide drug is the rapid diffusion of the molecule into the outer aqueous phase during the emulsification. This can result in poor encapsulation efficiency, i.e., drug loading. Therefore, it is critical to have an immediate formation of a polymer membrane during the first water-in-oil emulsion. Song et al. [15] were able to accomplish this by dissolving a high concentration of high-molecular-weight PLGA in the oil phase consisting of 80/20 wt% dichloromethane/acetone solution. Additionally, the viscosity of the inner aqueous phase was increased by increasing the concentration of the stabilizer, bovine serum albumin (BSA). The primary emulsion was then emulsified with Pluronic F68 resulting in drug-loaded particles of approximately 100 nm [15].

Another method that has been used to encapsulate insulin for oral delivery is phase inversion nanoencapsulation (PIN) [15,21]. In one example, Zn–insulin is dissolved in Tris-HCl and a portion of that is recrystallized by the addition of 10% $ZnSO_4$. The precipitate is added to a polymer solution of PLGA in methylene chloride. This mixture is emulsified and dispersed in 1 L of petroleum ether, which results in the spontaneous formation of nanoparticles [21].

All of the techniques mentioned previously use toxic, chlorinated solvents that could degrade certain drugs and proteins if they come into their contact during the process. Consequently, an effort has been made to develop other techniques in order to increase drug stability during the synthesis. One such technique is the emulsification–diffusion method. This method uses a partially water-soluble solvent like acetone or propylene carbonate. The polymer and bioactive compound are dissolved in the solvent and emulsified in the aqueous phase containing the stabilizer. The stabilizer prevents the aggregation of emulsion droplets by adsorbing the surface of the droplets. Water is added to the emulsion to allow the diffusion of the solvent into the water. The solution is stirred leading to the nanoprecipitation of the particles. They can then be collected by centrifugation or the solvent can be removed by dialysis [22,23].

One problem with this technique is that water-soluble drugs tend to leak out of the polymer phase during the solvent diffusion step. To improve this process for water-soluble drugs, Takeuchi

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TABLE 23.1
Comparison of Methods for Nanoparticle Preparation

Method	Polymer	Solvent	Stabilizer	Size	References
Solvent diffusion	PLGA	Acetone	Pluronic F-127	200 nm	[88]
	PLGA	Acetone/DCM	PVA	200–300 nm	[58]
	PLA-PEG	MC	PVA/PVP	~130 nm	[114]
	PHDCA	THF	—	150 nm	[98]
	PLGA	Acetone	Sodium cholate	161 nm	[96]
	PLGA	Propylene carbonate	PVA or DMAB	~100 nm	[53]
Solvent displacement	PLA	Acetone/MC	Pluronic F68	123 ± 23 nm	[106]
	SB-PVA-g-PLGA	Actone/Ethyl acetate	Poloxamer 188	~110 nm	[87]
Nanoprecipitation	PLGA/ PLA/ PCL	Acetone	Pluronic F68	110–208 nm	[59]
	PLGA	Acetonitrile	—	157.1 ± 1.9 nm	[57]
Solvent evaporation	PLA-PEG-PLA	DCM	—	193–335 nm	[129]
	PLGA	DCM	PVA	800 nm	[47]
	PLGA	DCM	TPGS	>300 nm	[67]
Multiple emulsion	PLGA	Ethyl acetate	—	>200 nm	[97]
	PEG-PLGA	DCM	PVA	~300 nm	[93]
	PLGA	Ethyl acetate/MC	PVA	335–743 nm	[92]
	PLGA-mPEG	DCM	—	133.5±3.7–163.3±3.6	[130]
	PLGA	DCM	PVA	213.8 ± 10.9 nm	[51]
	PLGA	DCM/Acetone	PVA	100 nm	[46]
	PLGA	Ethyl acetate	PVA	192±12 nm	[49]
	PLGA	Ethyl acetate	PVA	300–350 nm	[55]
	PLGA	DCM	PVA	380±40–1720±110 nm	[50]
Salting out	PLA	Acetone	PVA	300–700 nm	[60]
Ionic gelation	Chitosan	TPP	—	278±03 nm	[106]
Interfacial deposition	PLGA	Acetone	—	135 nm	[103]
Phase inversion nanoencapsulation	PLGA	MC	—	>5 µm	[52]
Polymerization	CS-PAA	—	—	206±22 nm	[64]
	PECA	—	Pluronic F68	320±12 nm	[66,85]
	PE-2-CA	—	—	380±120 nm	[84]
Modified microemulsion	PolyoxStyl 20-stearyl ether	—	Emulsifying wax	~67 nm	[106]

DCM, dicloromethane; MC, methylene chloride; PVP, polyvinylpyrrolidone; PHDCA, poly(hexadecylcyanoacrylate); THF, tetrahydrofuran; SB-PVA-g-PLGA, sulfobutylated PVA-graft-PLGA; PCL, poly(epsilon-caprolactone); TPP, sodium tripolyphosphate; PAA, poly(acrylic acid); PECA, polyethylcyanoacrylate; PE-2-CA, polyethyl-2-cyanoacrylate.

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TABLE 23.2
Comparison of Nanoparticle Size for Different Drug-Loaded Particles

Polymer	Drug	Size	References
PLGA	Doxorubicin	200 nm	[88]
PLGA/PLA/PCL	Isradipine	110–208 nm	[59]
PLGA	U-86983	144 ± 37–88 ± 41 nm	[46]
PLGA	Rose Bengal	150 nm	[103]
PLGA	Triptorelin	335–743 nm	[92]
PLGA	Procaine hydrochloride	164 ± 1.1–209.5 ± 2.7 nm	[57]
PLGA-mPEG	Cisplatin	133.5 ± 3.7–163.3 ± 3.6 nm	[130]
PLGA	Insulin	>1 μm	[52]
PLGA	Hemagglutinin	~250 nm	[6]
PLGA	Haloperidol	800 nm	[47]
PLGA	Estrogen	~100 nm	[53]
PEO-PLGA	Paclitaxel	150 ± 25 nm	[45]
PLA	Tetnus toxoid	>200 nm	[97]
PLA	Savoxepine	~300–700 nm	[60]
PLA	PDGFRβ tyrphostin inhibitor	123 ± 23 nm	[106]
PLA-PEG-PLA	Progesterone	193–335 nm	[129]
PECA	Amocicillin	320 ± 12 nm	[85]
Poly(butyl cyanoacrylate)	Dalargin	250 nm	[83]
Chitosan	Cyclosporin A	283 ± 24–281 ± 05 nm	[119]
PLGA	Paclitaxel	>300 nm	[67]
PLGA	Paclitaxel	< 200 nm	[94]
PLA	N^6-cyclopentyladenosine	210 ± 50–390 ± 90 nm	[91]

et al. [23] changed the dispersing medium from an aqueous solution to a medium chain triglyceride and added a surfactant, Span® 80, to the polymer phase. The nanoparticles were collected from the oily suspension by centrifugation. A double emulsification solvent diffusion technique has also been demonstrated to increase encapsulation efficiency of water-soluble drugs and maintenance of protein activity [24]. Protein activity during the fabrication process is a delicate balance between energy input (mechanical stirring, homogenization, and sonication) and particle size. A synergistic effect between mechanical stirring and ultrasound was shown to produce nanoparticles of 300 nm in diameter, while maintaining 85% of the starting activity of the cystatin protein [10]. Table 23.3 shows the changes in particle size with varied stirring rates. The addition of protein protectants, such as BSA or sugars, was also found to be essential in maintaining the biologically active, three-dimensional structure of cystatin [10]. These protectants may serve to shield proteins from interfaces during nanoparticle formation and lyophilization.

Several parameters can also be changed to benefit the encapsulation of hydrophilic molecules. Govender et al. [2] found that increasing the aqueous phase pH to 9.3 and incorporating pH-responsive excipients, such as poly(methyl methacrylate-co-methacrylic acid) (PMMA-MAA) and lauric and caprylic acid, increased hydrophilic drug encapsulation without affecting the particle size, morphology, or yield. Murakami et al. [25] effectively modified the solvent diffusion technique by using two water-miscible solvents, one with more affinity for PLGA and the other with more affinity for the stabilizer, PVA, such as acetone and ethanol.

Nanoparticles can also be synthesized by the nanoprecipitation method. Briefly, the polymer and drug are dissolved in acetone and added to an aqueous solution containing a surfactant/stabilizer. The acetone is evaporated under reduced pressure and the nanoparticles remain in the suspension resulting in particles from 110 to 208 nm [26]. The salting-out process is another method, which does not require the use of chlorinated solvents. Using this technique, a water-in-oil emulsion is formed

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TABLE 23.3
The Effect of Combination of Stirring and Bath Sonication on Size of Particles Made from Polymer RG® 503H (Mean ± S.D., n=3)

Organic Solvent	Particle Size (nm)		
	10,000 rpm[a]	7500 rpm[a]	5000 rpm[a]
Ethyl acetate	254±16	254±30	331±25
Dichloromethane/acetone	235±19	318±14	314±28

[a] Stirring rate.
Source: Reprinted from Cegnar, M. et al., *Eur. J. Pharm. Sci.*, 22, 357–364, 2004.

containing polymer, acetone, magnesium acetate tetrahydrate, stabilizer, and the active compound. Subsequently, water is added until the volume is sufficient to allow for diffusion of acetone into water, which results in the formation of nanoparticles. This suspension is purified by cross-flow filtration and lyophilization [27]. However, one disadvantage to this procedure is that it uses salts that may be incompatible with many bioactive compounds.

In most published techniques, nanoparticles are synthesized from biocompatible polymers. However, it is possible to make biodegradable nanoparticles from monomers or macromonomers by polycondensation reactions [28,29]. These processes also result in sizes ranging from 200 to 300 nm. Nanoparticles can also be made from hydrophilic polysaccharides like chitosan (CS). CS nanoparticles can be formed by the spontaneous ionic gelatin process [18,30]. CS-poly(acrylic acid) nanoparticles have also been made by polymerization of acrylic acid and the "dropping method" [31]. The resulting nanoparticles have small sizes and positive surface potentials. This technique is promising as the particles can be prepared under mild conditions without using harmful organic solvents.

23.3 PROCESSING PARAMETERS

The method of producing polymeric nanoparticles has several independent variables. Consequently, total drug loading, nanoparticle stability, and release characteristics may vary with slight changes in processing parameters. First, one must consider the selection of the components used in the nanoparticle production, including the polymer, molecular weight of the polymer, the surfactant, the drug, and the solvent [32]. For example, different surfactants may produce particles of different sizes [33]. An increase in polymer molecular weight will cause an impact on the release rate from the particles causing slower pore formation within the particles and therefore slower release [9,34]. Other processing variables include the time of emulsification, the amount of energy input, and the volume of the sample being emulsified. As energy input into an emulsion increases, the resulting particle size decreases [35]. In addition, there are four separate concentrations that can be altered: the polymer, drug, surfactant, and solvent. Often, a low concentration of surfactants will result in a high degree of polydispersity and aggregation [36]. Finally, the recovery of the particles can be changed depending on the method of lyophilization or centrifugation.

23.3.1 SURFACTANT/STABILIZER

One key parameter is the type of surfactant/stabilizer to be used. A wide range of synthetic and natural molecules with varying properties has been proposed to prepare nanoparticles. Feng and Huang [17] have investigated the use of phospholipids as natural emulsifiers. In their study, dipalmitoylphosphatidylcholine (DPPC) improved the flow and phagocytal properties due to a denser packing of DPPC molecules on the surface of the nanoparticles leading to a smoother surface than particles made with the synthetic polymer, poly(vinyl alcohol) (PVA). DPPC also improved the encapsulation

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efficiency compared to PVA using the emulsification–solvent evaporation method. In a different study conducted by Kwon et al. [22], PLGA nanoparticles prepared using didodecyl dimethyl ammonium bromide (DMAB) were smaller than particles prepared with PVA. Lemoine and Preat [8] found that the presence of PVA in the inner aqueous phase produced smaller particles than Span® 40 [7]. When Pluronic is used as a stabilizer, the grade used can have a distinct effect on the size of the nanoparticles. For example, particles prepared with Pluronic F68 were smaller than particles prepared with Pluronic F108 [37].

A promising stabilizer for nanoparticles is the amphiphile D-α-tocopheryl polyethylene glycol 1000 succinate vitamin E (TPGS). TPGS has high emulsification efficiency, can increase incorporation efficiency when used as a matrix component, and can be used as a cellular adhesion enhancer. TPGS can be used at a concentration as low as 0.015% (w/v); in fact a lower concentration decreases particle size and polydispersity [38]. Figure 23.1 shows scanning electron micrographs of PLGA particles made with PVA and TPGS. Moreover, the addition of TPGS dramatically reduced the release rate of paclitaxel from PLGA nanoparticles compared to those made with PVA (Figure 23.2). Using TPGS as an emulsifier, uptake by Caco-2 cells was greater than that of PVA-coated particles [33]. In addition, only TPGS-coated particles were found to be taken up in the nucleus [33].

The amount of stabilizer used will also have an effect on the properties of the nanoparticles. Most importantly, if the concentration of the stabilizer is too low, aggregation of the polymer droplets will occur and little if any nanoparticles will be recovered. Alternatively, if too much of the stabilizer is used, the drug incorporation could be reduced due to interaction between the drug and the stabilizer. However, when the stabilizer concentration is between the "limits," adjusting the concentration can be a means of controlling nanoparticle size. For example, in using the solvent evaporation technique, increasing the PVA concentration will decrease the particle size [8,15]. However, when using the emulsification–diffusion method, Kwon et al. [22] found that a PVA concentration from 2 to 4% was ideal for creating smaller nanoparticles, ~100 nm in diameter.

23.3.2 Type of Polymer

Biodegradable polymers retain their properties for a limited period of time *in vivo* and then gradually degrade into materials that can become soluble or are metabolized and excreted from the body. In order to be used for *in vivo* applications, the polymers used for such systems must have favorable properties for biocompatibility, processability, sterilization capability, and shelf life. In the past, polystyrene or gold nanoparticles were used to investigate particle distribution and uptake. However, biodegradable polymer particles have several properties, such as hydrophobicity, surface charge, particle size distribution, density, or protein adsorption, which are different from polystyrene and gold

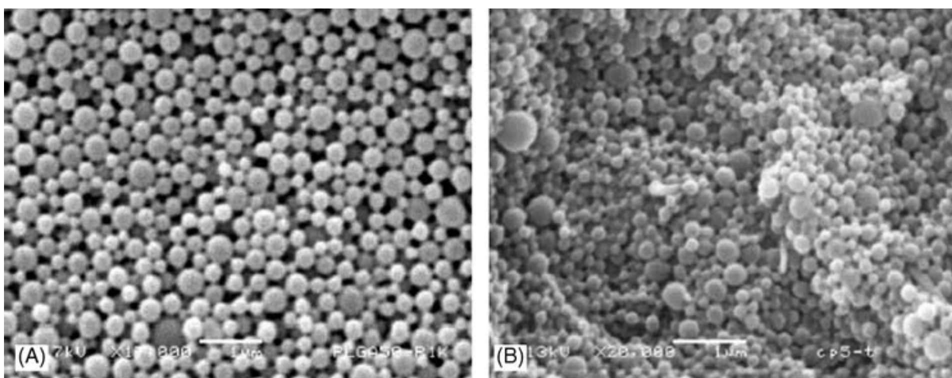

FIGURE 23.1 SEM images of coumarin 6-loaded PLGA particles coated with PVA (A) and vitamin E TPGS (B) (scale bar = 1 μm). (Reprinted from Yin Win, K. and Feng, S. S., *Biomaterials*, 26, 2713–2722, 2005.)

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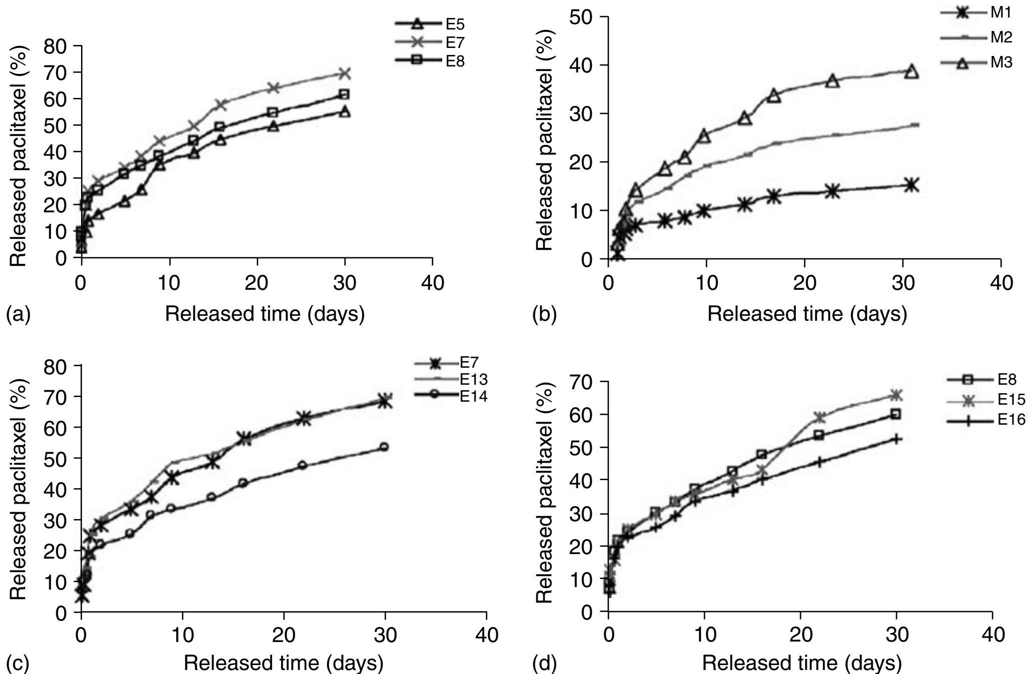

FIGURE 23.2 *In vitro* release curves of paclitaxel-loaded nanoparticles prepared under various experiment parameters (a) E5: PLA, E7: PLGA (75:25), E8: PLGA (50:50); (b) ratio for PLGA–TPGS: M1 (2:1), M2 (1:1), M3 (1:2); (c) PLGA (75:25) concentration — E7: 0.125, E13: 0.188, E14: 0.25; (d) PLGA (50:50) concentration — E8: 0.125, E15: 0.188, E16: 0.25. (Reprinted from Mu, L. and Feng, S. S., *J. Control. Release*, 86, 33–48, 2003.)

that might have an impact on results of these studies [39]. Figure 23.3 and Figure 23.4 show examples of physical internalization of PLGA nanoparticles by vascular smooth muscle cells (VSMCs).

23.3.2.1 Poly(lactide-co-glycolide)

Many biodegradable systems rely on the random co-polymers of PLGA. These classes of polymers are highly biocompatible and have good mechanical properties for drug delivery applications [40]. In addition, PLA and PLGA have been approved by the FDA for numerous clinical applications, such as sutures, bone plates, abdominal mesh, and extended-release pharmaceuticals. PLGA degrades chemically by hydrolytic cleavage of the ester bonds in the polymer backbone. Its degradation products, lactic acid and glycolic acid, are water-soluble, nontoxic products of normal metabolism that are either excreted or further metabolized to carbon dioxide and water in the Krebs cycle [41,42]. The composition of the PLGA random co-polymer, that is the relative amount of lactic acid and glycolic acid monomeric units, determines the degradation rate [34,43]. Since PGA is more hydrophilic than PLA a higher proportion of PGA incorporated into the co-polymer will increase the degradation rate by allowing more biological fluids to penetrate and swell the polymer matrix.

23.3.2.2 Poly(lactic acid)

Poly(lactic acid) occurs naturally as the pure enantiomeric poly(L-lactic acid) (LPLA) with a semi-crystalline structure. However, most types of PLA used for biological applications exist in the racemic D,L form (DLPLA) and are amorphous polymers. PLGA is also an amorphous polymer; both DLPLA and PLGA have glass transition temperatures above body temperature. The biomedical uses

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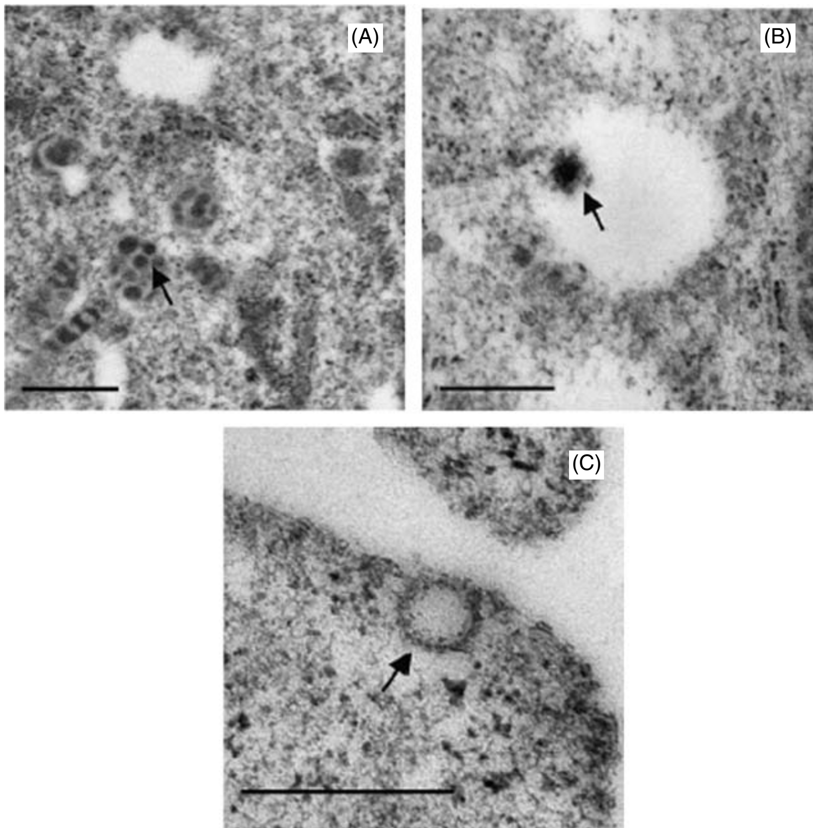

FIGURE 23.3 TEM images showing (A) nanoparticles (indicated by arrow) present in cytoplasm (×16,900), (B) a nanoparticle (indicated by arrow) interacting with vesicular membrane (×21,000), and (C) control vascular smooth muscle cells (VSMCs) (untreated cells) with nanoparticle-like vesicles of approximately 100 nm (indicated by arrow) (×38,000). Scale bars, 500 nm. (Reprinted from Panyam, J. et al., *Int. J. Pharm.*, 262, 1–11, 2003.)

of PLA have been reported since the 1960s [44]. Numerous systems already utilize PLGA and PLA, including several micro- and nanoparticle systems as well as devices to control thyrotropin-releasing hormone in controlling metabolism [42], L-dopa to treat Parkinson's disease [45], and naltrexone in treating narcotic addiction [46] to successfully achieve long-term delivery. Several intraocular systems, including Vitrasert® (Bausch and Lomb), offer biocompatible delivery systems with controlled release drug therapy for periods ranging from several days up to 1 year [47].

23.3.2.3 Poly-ε-caprolactone

Poly-ε-caprolactone is another biodegradable and nontoxic polyester. PCL is polymerized similarly to PLA and PLGA, by ring-opening polymerization [48,49]. PCL is a semicrystalline polymer owing to its regular structure. The melting temperature of PCL is above body temperature, but its T_g is −60°C; so in the body, the semicrystalline structure of PCL results in high toughness, because the amorphous domains are in the rubbery state [50]. Hydrolysis of PCL yields 6-hydroxycaproic acid, which enters the citric acid cycle and is metabolized. Degradation of PCL occurs at a slower rate than PLA. PCL has also been used in blends and co-polymers with other biodegradable polymers [51]. Combinations of polymers allow the user to tailor mechanical properties and degradation kinetics, among other characteristics, to suit the needs of a specific application.

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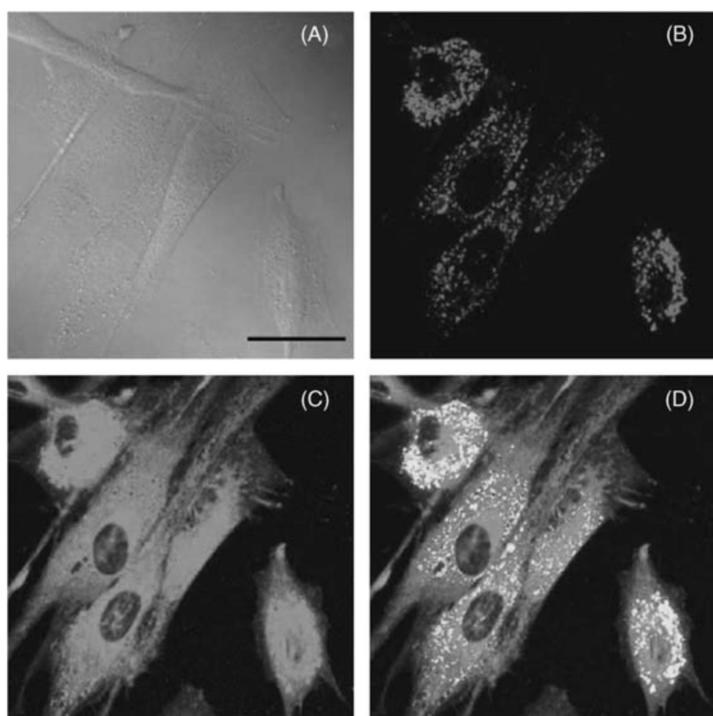

FIGURE 23.4 Confocal microscopic images demonstrating the intracellular distribution of 6-coumarin-loaded nanoparticles in VSMCs. (A) Differential interference contrast image showing the outline of the cells. (B) Cells stained with LysoTracker® Red and visualized using RITC filter. (C) Uptake of green fluorescent 6-coumarin-loaded nanoparticles in VSMCs visualized using FITC filter. (D) Overlay of (B, C) showing the co-localization of nanoparticles with endolysosomes. Scale bar, 25 μm. (Reprinted from Panyam, J. et al., *Int. J. Pharm.*, 262, 1–11, 2003.)

The amorphous regions of a semicrystalline polymer degrade prior to the crystalline domains [52]. This can lead to a change in the release profile. Thus, polymers that have a higher percent crystallinity are more impervious to water and therefore degrade at a slower rate. The drug entrapped in the amorphous region is released first and at a faster rate than the drug entrapped by the crystalline domains. The percent crystallinity in a polymer depends on the type of polymer used in the application, the polymer's composition, and the processing conditions for the polymer system. PCL typically has the highest percent crystallinity and the slowest degradation rate. This is evident by comparing PCL and LPLA versus PGA and PEG.

23.3.3 Polymer Choice

The polymer chosen to formulate the nanoparticles will strongly affect the structure, properties, and applications of the particles. As stated previously, PLGA has been the most common polymer used to make biodegradable nanoparticles, however, these are clearly not the optimal carrier for all drug delivery applications. For each application and drug, one must evaluate the properties of the system (drug and particle) and determine whether or not it is the optimal formulation for a given drug delivery application. For example, poly(butyl cyanoacrylate) nanoparticles have been successful in delivering drugs to the brain [53]. Other cyanoacrylate-based nanoparticles, such as polyalkylcyanoacrylate (PACA) and polyethylcyanoacrylate (PECA), have also been prepared. They are considered to be promising drug delivery systems due to their muco-adhesive properties and ability to entrap a variety of biologically active compounds. These polymers are biodegradable, biocompatible, as well as compatible

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with a wide range of compatible drugs [37,54]. Furthermore, these polymers have a faster degradation rate than PLGA, which in some cases may be more desirable. PECA nanoparticles have been prepared by emulsion polymerization in the presence and absence of different molecular weights PEGs, using Pluronic F68 as the stabilizer [55].

pH-sensitive nanoparticles made from a poly(methylacrylic acid and methacyrlate) co-polymer can increase the oral bioavailability of drugs like cyclosporin A by releasing their load at a specific pH within the gastrointestinal tract. The pH sensitivity allows this to happen as close as possible to the drug's absorption window through the Peyer's patches [56].

Other groups have successfully prepared nanoparticles from functionalized PLGA polymers. In one study, Jung et al. [57] synthesized nanoparticles made of a branched, biodegradable polymer, poly(2-sulfobutyl-vinyl alcohol)-g-PLGA. The purpose of using sulfobutyl groups attached to the hydrophilic backbone was to provide a higher affinity to proteins by electrostatic interactions that would favor adsorptive protein loading. Adjustments can be made to the characteristic nanoparticles by differing degrees of substitution of sulfobutyl groups. In another case, a carboxylic end group of PLGA was conjugated to a hydroxyl group of doxorubicin and formulated into nanoparticles [58]. This modification produced a sustained release of the drug that was approximately six times longer than the unconjugated drug [59]. The presence of the carboxylic end group on PLGA may also help in preserving cystatin activity in PLGA particles [10]. However, the carboxylic acid end group may also increase the overall release rate of drug from particles, so in some cases, a methyl-capped PLGA carboxylic acid end group can be utilized.

23.3.4 Polymer Molecular Weight

Polymer molecular weight, being an important determinant of mechanical strength, is also a key factor in determining the degradation rate of biodegradable polymers. Low-molecular-weight polymers degrade faster than high-molecular-weight polymers thereby losing their structural integrity more quickly. As chain scission occurs over time, the small polymer chains that result become more soluble in the aqueous environment of the body. This introduces "holes" into the polymer matrix. Consequently, lower molecular weight polymers release drug molecules more quickly [9,15]. This can be used to further engineer a system to control the release rate. A combination of molecular weights might be used to tailor a system to meet the demands of specific release profiles.

23.3.5 Collection Method

Another factor that can affect the properties of nanoparticles is the final freeze-drying process. It has been reported that additives such as saccharides are necessary for cryoprotection of nanoparticles in the freeze-drying process [60]. These saccharides may act as a spacing matrix to prevent particle aggregation. Because of the possibility of aggregation, freeze-drying procedure can affect the "effective" nanoparticle size, and consequently, their release behavior and accordingly the drug pharmacokinetics [61].

Nanoparticles can also be collected by dialysis, ultracentrifugation, and gel filtration. While gel filtration has shown decreased encapsulation efficiency and total drug incorporation, it is thought that this collection method may remove drug adsorbed onto the particle surface, which may cause a dangerous release of drug immediately upon immersion in body fluid [62]. Figure 23.5 shows the difference in particle size and morphology in particles collected by gel filtration and ultracentrifugation. This burst effect will be discussed later in more detail.

23.4 CHARACTERIZATION

23.4.1 Size and Encapsulation Efficiency

When considering a particular polymeric nanoparticle for a given drug delivery application, particle size and encapsulation efficiency are two of the most important characteristics. It is necessary

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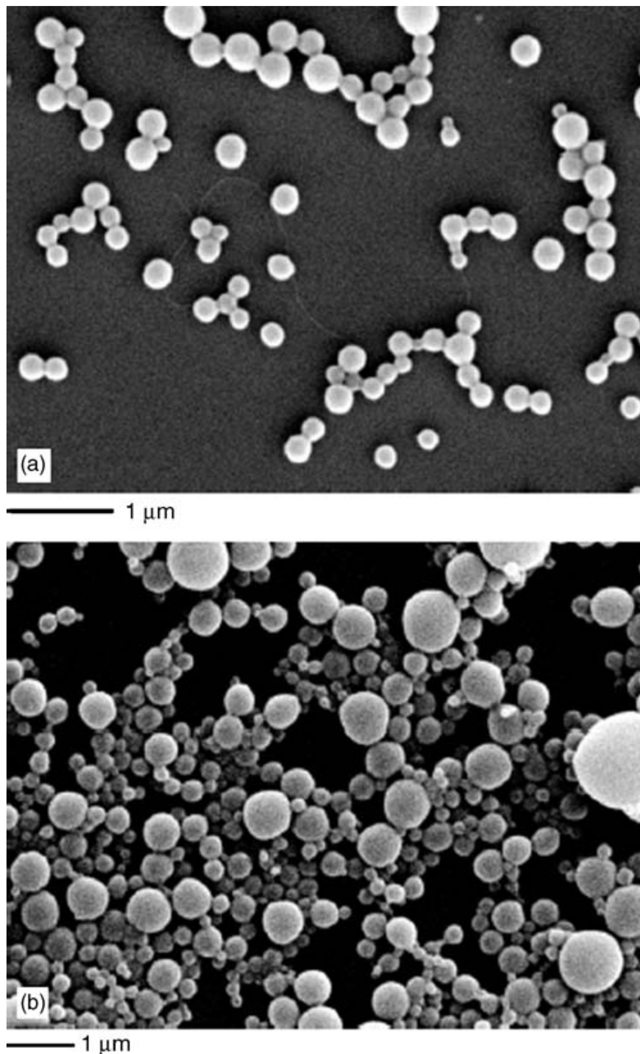

FIGURE 23.5 SEM of Oct-CPA-loaded nanospheres prepared by nanoprecipitation method: (a) II-Oct-CPA sample recovered by gel filtration and (b) IV-Oct-CPA sample recovered by ultracentrifugation. (Reprinted from Dalpiaz, A. et al., *Biomaterials*, 26, 1299–1306, 2005.)

to determine first what the goal of the nanoparticle delivery system is before determining the size desired. For example, if the goal is rapid dissolution in the body or arterial uptake, then the size of the nanoparticles should be ~100 nm or less. If prolonged dissolution is required, or targeting the mononuclear phagocytic system (MPS), larger particles around 800 nm, or particles engineered to have a stealth quality would be preferable. A comparison of various drugs encapsulated and the resulting sizes of the particles are summarized in Table 23.2. From examination of these data, it appears that the encapsulation efficiency increases with the diameter of the nanoparticles. In one study, the encapsulation efficiency was maximized in the double-emulsion solvent evaporation technique when the pH of the internal and the external aqueous phases were brought to the isoelectric point of the peptide being encapsulated, methylene chloride was used as a solvent, and the PLGA was rich in free carboxylic end groups [63]. It has also been found that by adding a freeze-thaw step during the primary emulsion of a double-emulsion process, there is an increase in overall protein incorporation by inducing the polymer phase to precipitate around the primary emulsion [35].

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The molecular weight of the polymer has opposite effects on nanoparticle size and encapsulation efficiency. Smaller size nanoparticles, ~100 nm, can be prepared with lower molecular weight polymer however, at the expense of reduced drug encapsulation efficiency. On the other hand, an increase in polymer concentration increases encapsulation efficiency and the size of the nanoparticles [9,15,22].

The synthesis method can also have a profound effect on the encapsulation efficiency. In loading paclitaxel into PLGA nanoparticles using the nanoprecipitation method, when the drug and polymer were mixed first and then solubilized in the organic solvent prior to fabrication, encapsulation efficiency was 15% [3]. However, when a solution of drug was used to dissolve the polymer prior to fabrication, nearly 100% encapsulation efficiency was achieved.

23.4.2 Zeta Potential

Another characteristic of polymeric nanoparticles that is of interest is zeta potential. The zeta potential is a measure of the charge of the particle, the relation being that the larger the absolute value of the zeta potential, the larger the amount of charge of the surface. In a sense, the zeta potential represents an index for particle stability. In the case of charged particles, as the zeta potential increases, the repulsive interactions will be larger leading to the formation of more stable particles with a more uniform size distribution. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of ± 30 mV [64]. This stability is important in preventing aggregation. When a surface modification like PEG is added, the negative zeta potential is lowered, increasing the nanoparticles stability [18].

23.4.3 Surface Modification

Before deciding which of the techniques to be used for synthesizing nanoparticles, one must consider what is the nature of the drug as well as the means and duration desired for the delivery. That will determine not only how the particles are synthesized, but also what the nature of the particles should be. In particular, the body recognizes hydrophobic particles as foreign and thus they are rapidly taken up by the MPS. However, if sustained systemic circulation is required then the surface of the hydrophobic nanoparticles must be modified in order to prevent phagocytosis [65].

Following intravenous administration, hydrophobic nanoparticles are rapidly cleared from the systemic circulation by the MPS, ending in the liver or the spleen [27]. If the goal is to treat a condition in the liver, then the proper choice for the application would be a hydrophobic nanoparticle. While it would appear that the hydrophobic nature of most biodegradable particles would limit the applicability of these carriers in many drug delivery applications, one may overcome concerns of clearance by the MPS through surface modification techniques. The goal of these modification techniques is to produce a particle that is not recognized by the MPS due to the hydrophilic nature of the surface [65,66].

Several types of surface-modified nanoparticles that have been described in recent literature are summarized in Table 23.4. The most common moiety used for surface modification is PEG [65,66]. PEG is a hydrophilic, non-ionic polymer that has been shown to exhibit excellent biocompatibility. PEG molecules can be added to the particles via a number of different routes including covalent bonding, mixing in during nanoparticle preparation, or surface adsorption [4,20,65–68]. The presence of a PEG brush on the surface of nanoparticles can serve other functions besides increasing residence time in the systemic circulation. For one, PEG tethers on the particle surface can reduce protein and enzyme adsorption on the surface, which for PLGA-based particles will retard degradation [68]. The degree of protein adsorption can be minimized by altering the density and molecular weight of PEG on the surface [65]. The stability of PLA particles has been shown to increase in simulated gastric fluid (SGF) with the addition of PEG on the particle surface. After 4 h in SGF, 9% of the PLA nanoparticles converted into lactate versus 3% conversion for PEG–PLA particles [68]. PEG is also believed to facilitate transport through the Peyer's patches of the GALT [18].

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TABLE 23.4
Nanoparticle Sizes after Surface Modifications

Polymer	Surface Modification	Size (nm)	References
PLGA	Poloxamine 908	~160	[103]
PLGA	Poloxamer 407	~160	[103]
PLGA	Chitosan	500± 29	[49]
PLGA-mPEG	mPEG	133.5 ± 3.7–163.3 ± 3.6	[130]
PLGA-mPEG	mPEG	113.5 ± 14.3	[100]
PLGA-PEG	PEG	198.1 ± 11.1	[51]
PLA	PEG	164–270	[96]
PLA	PEG 6000	295	[60]
PLA-PEG	PEG	>200	[97]
PLA-PEG	PEG	~130	[114]
PHDCA	PEG	~150	[98]
PECA	PEG	220 ± 10–280 ± 8	[85]
PBCA	Polysorbate 80	250	[83]
PEG-PLGA	PEG	~300	[93]
Polyoxyl 20-stearyl ether	Thiamine	67	[105]
PEG-PACA	Transferrin	101.4 ± 7.2	[108]
PLA	Polysorbate 80	~160	[104]
PEI-b-PLGA	PEG	~100	[24]
PLA	Neutravidin™	~270	[111]
Glycoprotein-liposomes	Lectin	100	[19]

As stated previously, the primary reason for interest in preparing PEG-functionalized particles is to improve the long-term systemic circulation of the nanoparticles [65,66]. The PEG-functionalized particles are not seen as a foreign body and in combination with the nanoscale size of the particles, are not taken up by the body, allowing them to circulate longer providing for a sustained systemic drug release. Because of their behavior, these PEG-functionalized nanoparticles are often called "stealth nanoparticles" [66]. Furthermore, it has been determined that PEG MW is important with respect to MPS uptake. For example, Leroux et al. [27] showed that an increase in PEG molecular weight in PLGA nanoparticles was associated with less interaction with the MPS and longer systemic circulation. Also, PEG-containing PLGA nanoparticles synthesized by Li et al. [20] were able to extend the half-life of BSA in a rat model from 13.6 min to 4.5 h [69]. Another study compared the dosages of PLGA nanoparticles versus PEG–PLGA nanoparticles. The PLGA nanoparticle pharmacokinetics seemed to depend on MPS saturation. However, the pharmacokinetics of PEG–PLGA dosages did not exhibit the same dependence on dosage/MPS saturation due to their stealth nature [70]. PEG may also benefit nanoparticle interaction with blood constituents. PLGA nanoparticles were shown to cause damage to red blood cells; PEGylated nanoparticles caused less damage [71]. It should be noted that the red blood cell damage was also concentration dependent.

Another way to prevent nanoparticles from becoming sequestered and eliminated by the spleen is by noncovalent adhesion of particles on to erythrocyte membranes [72]. This adhesion is controlled by van der Waals, electrostatic, hydrophobic, and hydrogen-bonding forces. The adhesion did not change the morphology of the red blood cells, and the particles were retained on their membranes for 24 h and beyond. This technology improved the circulation time of nanoparticles 10-fold.

Poloxamer and poloxamines have also been shown to reduce capture by macrophages and increase the time for systemic circulation. Similarly, PLGA particles coated with poloxamer 407 and poloxamine 908 extended the half-life of rose bengal, a hydrophilic model drug, with ~30% left in the bloodstream after 1 h postnanoparticle administration, as opposed to 8% present after 5 min postfree drug administration [73].

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Another polymer used for surface modification is CS. The addition of CS to the surface of PLGA nanoparticles resulted in increased penetration of macromolecules in mucosal surfaces [30]. CS-coated PLGA particles were able to increase the positive zeta potential of the particles and increase the efficiency of tetanus toxoid protein encapsulation. Radiolabeled tetanus toxoid was used to show enhanced transport across nasal and intestinal epithelium using CS-coated particles versus uncoated particles, with a higher percentage of ^{125}I present in the lymph nodes for CS-coated particles [18].

23.5 NANOPARTICULATE DELIVERY SYSTEMS

23.5.1 LIPOSOMES

Lipid bilayers occur throughout science and nature — cells use them to regulate chemical species within and outside of cells. Liposomes serve as excellent mimics of naturally occurring cell membranes [74]. Investigations with liposomes mimicking natural cell functions, especially those involving chemical transport, has led to their use as vehicles for drug delivery [75–78]. A primary advantage of liposomes is their high level of biocompatibility. Liposomes now constitute a mainstream technology for drug delivery; clinical approval has been given to liposomal formulations of anticancer drugs, doxorubicin (Doxil®/Caelyx® and Myocet®) and daunorubicin (Daunosome®) [79]. Another advantage of liposomes is their ability to transport a wide diversity of drugs that can be hydrophilic, lipophilic, or amphiphilic. This relates to the amphiphilic nature of phospholipid molecules themselves, which self-assemble in water to form bilayers that enclose an aqueous interior. Hydrophilic drugs can therefore be entrapped within the aqueous core, whereas hydrophobic drugs partition into the hydrocarbon-rich region of the bilayer. Loading techniques, such as the ammonium sulfate method [80] and the pH gradient method [81], can be used to place amphiphilic drugs (e.g., doxorubicin and vincristine, respectively) at the inner-phospholipid-monolayer/water interface [79].

A major disadvantage of liposomes as drug delivery vehicles is their rapid clearance from blood via the MPS, or reticuloendothelial system. This limitation was overcome by the advancement of 'PEGylated Stealth®' liposomes, which avoid protein adsorption, and hence subsequent recognition and uptake, via a surface coating of PEG [80–82]. Incorporation of PEG into ordinary liposomes increases their circulation half-life from minutes to hours [79,83]. In addition to conveying this stealth quality, PEG also increases the susceptibility of liposomes to ultrasound-induced leakage [84]. This quality might prove useful in the development of a targeted, localized drug delivery system using external ultrasound as a remote mechanical stimulus to trigger drug release. Liposomes can also be decorated with glycoproteins and sugar chains to target specific cells [85].

23.5.2 POLYMERIC MICELLES

Polymeric micelles can be assembled from block co-polymers composed of hydrophilic and hydrophobic segments. The hydrophobic segment creates the inner core of the micelle, while the hydrophilic segment creates the outer shell in an aqueous media [86]. Polymeric micelles can be used as a drug delivery device by either physically entrapping the drug in the core (i.e., hydrophobic drugs can be trapped inside the micelle by hydrophobic interactions), or by chemically conjugating the drug to the hydrophobic block prior to micelle formation [87,88]. Drugs either physically or chemically trapped in the hydrophobic core are protected from chemical degradation, causing unwanted side effects [87,88]. Micelles have several benefits as drug delivery vehicles. Their hydrophilic outer shell and small size (<100 nm) renders these particles nearly invisible to the reticuloendothelial system, allowing for long-term circulation in the bloodstream. Furthermore, micelle stability is relatively high due to the fact that, unlike other aggregates for drug delivery such as vesicles, they are a thermodynamically equilibrium aggregate. Micelles spontaneously form when the concentration of

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the amphiphile composing them (lipids, surfactants, or block copolymers) is higher than a critical concentration called the critical micelle concentration (CMC). The CMC is significantly lower for higher molecular weight polymers as opposed to surfactants or lipids. Micelle stability is further enhanced by crystallization or rigidity in the polymer core, which prevents rapid dissolution of block co-polymer micelles *in vivo*.

Triblock copolymers such as poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) (PEO-b-PPO-b-PEO) or Pluronics® have frequently been studied to create block co-polymer micelles. Pluronics® conjugated to brain-specific antibodies or insulin have been used to solubilize haloperidol and other small compounds, such as FITC [89].

Polyethylenimine (PEI)-block-PLGA have been used to form micelles for cellular uptake [90]. Figure 23.6 shows size, polydispersity, and morphology of these micelles using transmission electron microscopy. Using confocal laser scanning microscopy (CLSM), PEI–PLGA micelles were shown to be absorbed onto human keratinocyte cell surfaces and translocated into the cytoplasm.These particles were not cytotoxic to the cells for up to 2 days at a concentration of 50 μg/mL. By comparison there was limited cellular internalization of PLGA particles [90].

Poly(ethylene oxide)-Poly(amino acid) (PEO-PAA) block co-polymers have received a great deal of attention, because the biodegradable amino acid core has free functional groups for chemical modification [88]. To achieve this, PEO-PBLA (Poly(β-benzyl L-aspartate)) is synthesized from β-benzyl *N*-carboxy L-aspartate anhydride (BLA-NCA) and α-methyl-ω-aminopoly(oxyethylene). PEO-P(Asp) is then prepared by debenzylation under alkaline conditions of the PEO-PBLA. Following this step, ADR is conjugated via amide bond formation [91]. Micelles prepared from drug conjugates have the added advantage that extended drug release will still be possible in the

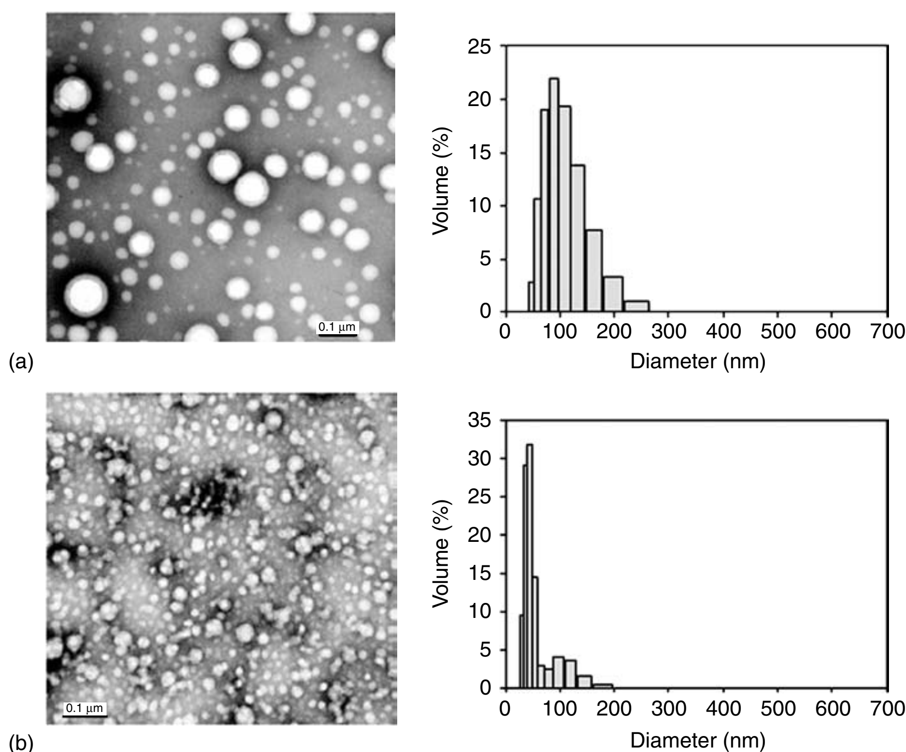

FIGURE 23.6 TEM pictures and size distributions of PEI–PLGA aggregates formed in (a) pure water, (b) 30 m*M* HCl (aqueous solution) (scale bar = 100 nm).(Reprinted from Nam, Y.S. et al., *Biomaterials*, 24, 2053–2059, 2003.)

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event of dissociation of the micelle as the drug must still be cleaved from the polymer chain. Stability of these PEO-P(Asp(ADR)) micelles has been shown to be dependent on a longer chain of PEO in comparison to the chain length of P(Asp) and ADR content. PEO-PBLA could also be synthesized with free hydroxyl groups on the outer core of the micelle for further conjugation to a targeting moiety (i.e., antibody, glucose) [87].

23.5.3 Worm-Like Micelles

Cylindrical worm micelles [92] are a promising new class of supermolecular drug/dye carriers [93] to explore for a number of reasons. First, even if microns long, they can "worm" through small pores, including gels, and circulate for perhaps weeks. Second, targeted worms can cooperatively zip up — binding with high affinity — to surfaces or cells that bear suitable receptors. And third, once bound, internalization by the cell leads to delivery of a relatively large amount of drug all at once [94].

It is of central importance that several system characteristics be elucidated, including polymer molecular weight versus worm diameter, worm stability, and flexibility [93]. The first issue has already been addressed with vesicles [95]. Nanoscale worm micelles can be very stable; they appear similar to filamentous phages that have been used with great success *in vivo* for phage display of targeting ligands (including tumors) [96]. Unlike phages that carry nucleic acids, worm micelles can carry lipophilic drugs.

23.5.4 Polymersomes

Recently, interest has been focused on polymeric vesicles, composed of hydrophobic–hydrophilic diblock co-polymers, as drug delivery vehicles [95,97–100]. The advantages of these polymersomes, as compared to liposomes, include enhanced mechanical stability and greater flexibility to tailor bi-layer characteristics, such as thickness and chemical composition [98,101–104]. Moreover, it has been speculated that protein interactions with the polymeric bilayers will greatly differ from their interactions with lipid ones, thereby affecting drug delivery characteristics, such as circulation time *in vivo*. Indeed, Photos et al. [105] have shown that the circulation time of polymersomes *in vivo* increases with the bilayer thickness. Pata and Dan [106] have found that the characteristics of the polymeric bi-layer, when compared to the lipid bi-layer, are quite different and can be used for tailoring the carrier properties. Recently, Meng et al. [107] have synthesized biodegradable polymersomes from block co-polymers of PEG-PLA.

Finally, polymeric vesicles have been synthesized from multiblock co-polymers of Pluronic F27 with a PLA block on either end [108]. The vesicle structure is characterized by a hydrophilic core with hydrophobic layers, and can form several conformations such as bilayer or onion-like vesicles. The PLA-F27-PLA co-polymers exhibit a decrease in T_g and T_m values, which indicate an increased permeability and chain mobility in aqueous solutions. This may explain the high burst release from these vesicles, but does not eliminate this class of particles as a drug delivery system [108].

23.6 TARGETED DRUG DELIVERY USING NANOPARTICLES

23.6.1 Oral Delivery

Oral delivery of nanoparticles has focused on uptake via the Peyer's patches in the GALT. Peyer's patches are characterized by M cells that overlie the lymphoid tissue and are specialized for endocytosis and transport into intraepithelial spaces and adjacent lymphoid tissue. There have been several differing opinions as to the ease of nanoparticle transport through the M cells, and the method by which this occurs [109,110]. One theory is that nanoparticles bind the apical membrane of the M cells, followed by a rapid internalization and a "shuttling" to the lymphocytes [110,111]. The size and surface charge of the nanoparticles are crucial for their uptake. However, there have only been two published phase 1 clinical trials examining the oral uptake of PLGA nanoparticles encapsulating

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Escherichia coli antigens, with no clear benefit determined [109]. There is some promise in identifying M cell receptors and targeting them on the surface of nanoparticles. The carbohydrate epitope, sialytated Lewis antigen A (SLAA), has been identified on human M cells [109]. This application of targeted nanoparticles in oral delivery holds tremendous promise for the development of oral vaccines and in cancer therapy.

23.6.2 Brain Delivery

Another exciting application of surface-modified particles is targeted drug delivery to cells or organs. Kreuter et al. [53] were able to deliver several drugs successfully through the blood–brain barrier using polysorbate 80-coated poly(butylcyanoacrylate) nanoparticles. It is thought that after administration of the polysorbate 80-coated particles, apolipoprotein E (ApoE) adsorbs onto the surface coating. The ApoE protein mimics low-density lipoprotein (LDL) causing the particles to be transported via the LDL receptors into the brain. The effects of polysorbate-80 on endocytosis by the blood–brain barrier were confirmed by Sun et al. [112] with PLA nanoparticles. Nanoparticles made from emulsifying wax and polyoxyl 20-stearyl ether linked to a thiamine surface ligand, fabricated using microemulsion precursors and had an average final diameter of 67 nm [113]. These particles are able to associate with the blood–brain barrier thiamine transporters and thereby increase the unidirectional transfer coefficient for the particles into the brain.

23.6.3 Arterial Delivery

There are other specific areas, where nanoparticle administration may have an advantage over microparticulate-based drug delivery systems. One area that has been of recent interest is in prevention of restenosis [15,114]. Restenosis is a major postoperative concern following arterial surgery. In order to inhibit VSMC proliferation, drugs must be delivered at a high concentration over a long period of time. Nanoparticles offer an advantage, because the medication would not have to be delivered systemically as they are small enough for cellular internalization and connective tissue permeation. Several types of drugs including antiproliferative agents have been used to test this method of delivery. PLA nanoparticles were loaded with platelet-derived growth factor receptor β tyrphostin inhibitor and delivered intra-lumenally to an injured rat carotid artery [115]. The drug had the desired effect of preventing restenosis, but of significance was the absence of drug in other areas of the arteries and systemic circulation. Song et al. [15] found that specific additives after nanoparticles formation, such as heparin, DMAB, or fibrinogen, could enhance arterial retention of the particles. Suh et al. [14] created PEO–PLGA nanoparticles, which had an initial burst release of 40% of the antiproliferative drug in the first 3 days. However, a total of 85% of the drug was released after 4 weeks. This shows that nanoparticles have a great potential for long-term arterial drug delivery.

23.6.4 Tumor Therapy

One of the most common targeting applications for nanoparticles is of chemotheraputic agents against cancerous cells and tumor sites. Figure 23.7 shows the cellular internalization of PLGA nanoparticles into MCF-10A neoT cells. Oral delivery of chemotherapy is of high interest, because chemotheraputic agents are eliminated by the first-pass effect with cytochrome p450 [33]. Another reason is that it has attracted attention is due to the phenomena known as the enhanced permeation and retention (EPR) effect. The vasculature around tumor sites is inherently leaky due to the rapid vascularization necessary to serve fast-growing tumors [116]. Much attention has also been given to lymphatic targeting using nanoparticles. In addition, poor lymphatic drainage at the site prevents elimination of the particles from the tumor tissue. Another benefit of tumor targeting is the fact that many cancer cells over-express specific antigens. Coating or binding the ligands on nanoparticulate surfaces can exploit this property. One example is covalently attaching sugar chains to nanoparticles.

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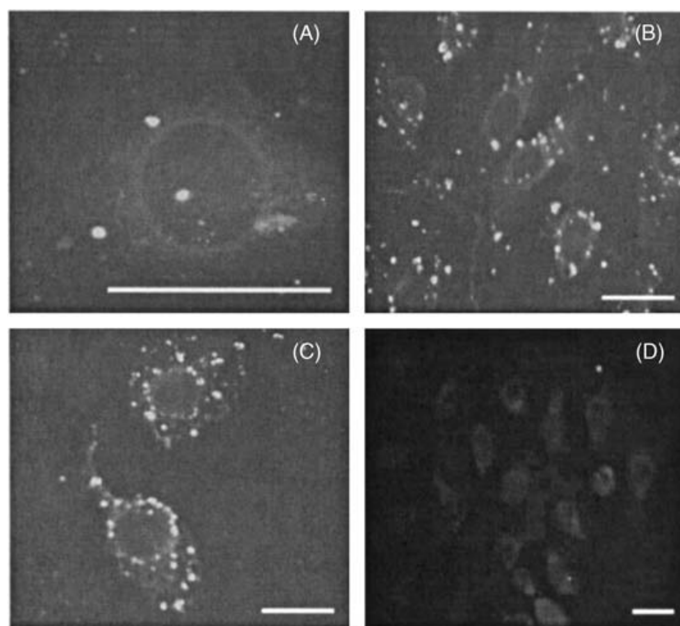

FIGURE 23.7 Internalization of NPs loaded with Alexa Fluor 488-labeled cystatin into MCF-10A neoT cells. (A) 10 min incubation, concentration = 100 μg NP/mL; (B) 30 min incubation, concentration = 100 μg NP/mL; (C) 45 min incubation, concentration = 100 μg NP/mL; (D) free Alexa Fluor 488-labeled cystatin, 45 min incubation, concentration = 0.1 μ*M*. Scale bar, 20 μm. All the images are representative of at least two independent experiments. (Reprinted from Cegnar, M. et al., *Exp. Cell Res.*, 301, 223–231, 2004.)

Yamazaki et al. [85] used sugar chain remodeled glycoprotein–liposome conjugates for binding to E-selectin. These particles showed uptake by solid tumor tissue. Cancer cells overexpress transferrin, which is normally used for iron uptake. Paclitaxel-loaded PACA nanoparticles with a PEG linker chain conjugated to transferrin were shown to have a decreased burst release over nontargeted particles and were able to increase the lifespan in tumor-bearing mice with a decreased weight loss compared to mice given conventional paclitaxel [117]. The decreased burst may be due to the removal of surface-associated drug during the activation process and the decreased weight loss may point to a reduction in paclitaxel-associated side effects.

Yokoyama et al. [91,118–121] have conducted numerous studies on the chemical conjugation as well as physical entrapment of adriamycin (ADR, also known as doxorubicin), an anticancer drug, in PEO–Poly(aspartic acid) micelles (PEO–P(Asp)). It was found that polymer micelles with conjugated ADR alone or physically entrapped ADR alone did not have anti-tumor activity. However, high amounts of both chemically conjugated and physically entrapped ADR were necessary for antitumor activity [121]. Other anticancer drugs have been used in block co-polymer micelles. Cisplatin has been bound to P(Asp) using the same block co-polymer PEO-P(Asp) [122]. Methotrexate (Mtx) esters of PEO-block-poly(2-hydroxyethyl-L-aspartimide) (PEO-PHEA) were found to form stable micelles with a sustained release profile, dependent on the amount of Mtx substitution [123].

One method for targeting that is attracting attention is by exploiting the high noncovalent binding of biotin and avidin. Each avidin molecule binds four biotin molecules. Using the multifunctionality of this technology, drugs and homing molecules for cancer cells can be combined [124]. By using biotinylated PEG–PCL and associating that with avidin-bound lectin, the amount of nanoparticles associated with Caco-2 cells is increased dramatically [1]. NeutrAvidin^{TM} has been covalently attached to the surface of PLA nanoparticles via sulfhydrl groups [125]. These thiol

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groups are bound via a carbodiimide reaction with cystamine. Using this technology, biotinylated antibodies or ligands can be attached to the particle surface. Biotinylated worm-like micelles have also been found to be stable in an aqueous solution for at least a month, and have the potential for the delivery of large quantities of hydrophobic drugs or dyes [94].

23.6.5 Lymphatic System and Vaccines

The lymphatic absorption of a drug via the GALT has an advantage over a portal blood route since it avoids any liver presystemic metabolism, known as the first-pass effect. This could be beneficial for anticancer treatment, mucosal immunity, as well as having the potential for staining the lymph nodes prior to surgery [126]. Nanoparticles can also be used to carry antisense oligonucleotides and plasmid DNA that can be used to treat some forms of cancer and viral infections, as well as a new vaccination approach. Antisense oligionucleotides normally have poor stability and cannot easily penetrate cells, but are easily encapsulated in nanoparticles [13,127]. High doses of antibiotics and antiparasitics are given to treat gastrointestinal bacteria and parasites because only 10 to 15% of the drug administered is absorbed [64]. The increased muco-adhesivity of nanoparticles could be effective in treating these pathogens with lower doses of drugs.

Nanoparticles have also had some success as a new delivery vehicle for vaccines. CS nanoparticles have been successful as a nasal vaccine in some animal studies producing significant IgG serum responses and superior IgA secretory responses when used in influenza, pertussis, and diphtheria vaccines [128]. Another option for vaccine delivery is the delivery of PLGA nanoparticles to dendritic cells [12]. Dendritic cells initiate an antigen-specific immune response. The co-delivery of antigens and immunomodulators in the same particles helps to overcome peripheral tolerance against self-antigens. This technology can potentially be used for cancer vaccines or hepatitis antigens.

23.6.6 Pulmonary Delivery

The lungs owing to their large surface area, good mucosal permeation, well-developed vascular system, thin alveolar walls, and low activity of drug-metabolizing enzymes are another target area for nanoparticles. Direct administration to the lungs is beneficial because of the decreased systemic side effects, the availability for increased dose levels at the site, and the lack of first-pass metabolism. The tracheo-bronchial region is protected by a mucosal barrier, which can be cleared by nanoparticle technology. One obstacle for the inhalation of nanoparticles is their inappropriate mass median aerodynamic diameter. They do not have sufficient size for sedimentation or impaction deposition mechanisms and would be exhaled postinhalation [129]. Carrier particles have been proposed, which after deposition in the carrier matrix dissolve, while the nanoparticles are released.

When CS-coated nanoparticles were administered via the lungs, there were detectable blood levels of the drug 24 h after administration, as opposed to 8 h for the noncoated particles [23]. PACA nanoparticles have been found to be cytotoxic to airway epithelial cells, but gelatin and human serum albumin nanoparticles of ~200 nm in diameter were taken up by bronchial epithelial cells with no evidence of cytotoxicity or inflammation [130].

Nanoparticles have been used to target other mucosal surfaces. Long-term extra-ocular (cornea and conjunctiva) drug delivery with nanoparticles provides an improvement in conventional drug delivery in this region. It was possible to deliver drug-loaded CS nanoparticles to the extra-ocular structures over the course of 48 h at higher levels than with free drug, without exposing the inner ocular structures (iris and aqueous humour) to the drug [131].

Nanoparticles may be used for skin delivery to prolong the residence time of sunscreen agents in the stratum corneum or to deliver vitamin A to the upper layers of the skin [132]. CLSM demonstrates nanoparticles accumulating in the follicular openings, with higher percentages of smaller diameter nanoparticles accumulating.

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23.7 DRUG RELEASE

23.7.1 MECHANISMS

Biodegradable polymers release drug in one of the two ways: erosion and diffusion. Release from biodegradable polymers *in vivo* is governed by a combination of both mechanisms, which depends on the relative rates of erosion and diffusion. Erosion is defined as the physical dissolution of a polymer as a result of its degradation [133]. Most biodegradable polymers used for drug delivery are degraded by hydrolysis. Hydrolysis is a reaction between water molecules and bonds in the polymer backbone, typically ester bonds, which repeatedly cuts the polymer chain until it is returned to monomers. Other biodegradable polymers are enzymatically degradable, which is also a type of chain scission. As water molecules break chemical bonds along the polymer chain, the physical integrity of the polymer degrades and allows drug to be released.

There are two possible mechanisms of erosion. When water is confined to the surface of the matrix, as in the case of a hydrophobic polymer, chain scission will occur only on the surface and the drug will be released as the surface of the polymer matrix erodes. If the water penetrates the polymer matrix faster than it hydrolyzes the bonds on the surface, then erosion will occur throughout the entire material — this is also called bulk erosion. In many cases, the erosion of a polymer matrix *in vivo* is some combination of these mechanisms. Degradation by surface erosion alone may be preferred, because the degradation rate can be controlled through the surface area of the matrix [134].

In the case of diffusion-controlled release, the drug's concentration gradient in the polymer matrix is the driving force for the molecules to diffuse into the surrounding medium. The diffusion of a drug molecule through the polymer matrix is dependent upon the solubility of the drug in the polymer matrix and the surrounding medium, the diffusion coefficient of the drug molecule, the molecular weight of the drug, its concentration throughout the polymer matrix, and the distance necessary for diffusion. Drugs can be either distributed evenly throughout the matrix or encapsulated as a reservoir [135]. The release rate for the reservoir system also factors in the membrane thickness and area. Practically, reservoir systems often have a lag period after placement *in vivo*, as opposed to the burst release present for most other systems. However, these systems need to be carefully engineered to prevent premature membrane rupture that might release a toxic amount of drug into the body.

When a drug is dissolved in the matrix and the mechanism for delivery is diffusion, then the driving force for release is the concentration gradient and release predictions can be made based on Fick's laws of diffusion [134]. Cumulative release from diffusion-controlled matrix devices is inversely proportional to the square root of time [136]. This presents an engineering challenge because surface area becomes smaller due to degradation, with a resulting decrement in the release rate.

Frequently, diffusion-controlled release is important in the early stages of drug release. For many of the polymeric delivery systems, there is some concentration of drug molecules entrapped near the surface of the matrix and adsorbed onto the surface of the matrix. Upon immersion into a medium, the release of these drug molecules is controlled by the rate of diffusion of the drug into the surrounding environment. This can cause a problem referred to as the "burst effect," which can potentially release a toxic amount of drug in some geometries (frequently 50% or more of the incorporated drug) into the body within the first 24 h [137]. This burst release is part of what is frequently referred to as a biphasic release profile [3]. During the first phase, the burst release, the structural integrity of the nanoparticles is maintained. The second phase, or the linear release, is characterized by pore formation, particle deformation, and fusion [138]. One method for eliminating the burst release is by tailoring the collection of the nanoparticles to remove the drug attached to the surface. By collecting PLA nanoparticles through gel filtration, the antiischemic drug N^6-cyclopentyladenosine near the surface was removed, which decreased the overall encapsulation efficiency, and eliminated the burst release from the particles [62].

The specific chemical and biological characteristics of the drug and the polymer are crucial in designing a polymeric delivery system. For example, drugs with greater hydrophilicity can increase the overall release rate by promoting polymer swelling and degradation which in turn increases drug

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diffusion, and certain drug molecules may potentially react with the polymer matrix [139]. The drug's molecular weight, solubility in biological fluids as well as its miscibility in the polymer matrix will influence the drug's diffusivity from the system and the concentration profile of the drug throughout the matrix. Since polymeric delivery systems are rarely homogenous throughout the entire matrix, the drug's diffusivity, and therefore release rate, can change with the local polymer composition and structure. One example of this phenomenon showing the effect of drug polymer interaction is the release of the prodrug, 3-methoxyxanthone and its active form xanthone, from PLGA nanocapsules [140]. Incorporation into nanocapsules was significantly higher than that of solid nanoparticles. The release profile of these drugs suggest a physical interaction of the drug with the polymer when given the nanocapsule structure [140].

23.7.2 Release Characteristics

The release characteristics of polymeric nanoparticles are among the most important features of the drug/polymer formulations because of the possible applications in sustained drug delivery. There are several factors that affect the release rate of the entrapped drug. Larger particles have a smaller initial burst release and longer sustained release than smaller particles. In addition, the greater the drug loading, the greater the burst and faster the release rate. For example, PLA nanoparticles containing 16.7% savoxepine released 90% of their drug load in 24 h, as opposed to particles containing 7.1% savoxepine, which released their content over 3 weeks [27]. The initial burst release is thought to be caused by poorly entrapped drug, or drug adsorbed onto the outside of the particles. When using polymers, which interact with a drug, like PLGA with a free COOH group and proteins, the burst release is lower and in some cases absent, and drug release is prolonged [9,63].

The addition of other polymers to PLA-based polymers can also be used to control drug release. For example, PEG has been polymerized into a PLA homopolymer creating a PLA-PEG-PLA co-polymer [141]. The amount of the drug (in this case progesterone) released increased with the PEG content and the molecular weight of the co-polymers. The drug release continued to increase as the total molecular weight of the co-polymers decreased. The initial burst was decreased in the absence of lower molecular weight polymers. The content of PEG in the co-polymer affected the size of the particles as well as the degradation of the polymers. Similar effects were seen with PLGA-mPEG nanoparticles loaded with cisplatin [142]. Consequently, it would be possible to alter the release rate of the drug by changing the amount of PEG in the co-polymer as well as the molecular weights of the polymers.

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