Chapter

Aliphatic Polyester Nanoparticles for Drug Delivery Systems

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Abstract

Drug delivery systems using aliphatic polyester nanoparticles are usually prepared via an emulsion process. These nanoparticles can control drug release and improve pharmacokinetics. Aliphatic polyesters are linear polymers containing ester linkages, showing sensitivity to hydrolytic degradation. The byproducts then promote autocatalytic degradation. These byproducts could enter the Krebs cycle and be eliminated from the body, resulting in the high biocompatibility of these nanoparticles. The properties of these polyesters are linked to the drug release rate due to biodegradation, i.e., polymer crystallinity, glass transition temperature, polymer hydrophobicity, and molecular weight (MW), all of which relatively influence hydrolysis. Mathematical equations have been used to study the factors and mechanisms that affect drug dissolution compared to experimental release data. The equations used as models for predicting the kinetics of drug release include the *zero-order*, *first-order*, *Higuchi*, *Hixson-Crowell*, and *Korsmeyer-Peppas* equations. Aliphatic polyester-based controlled drug delivery has surrounded much of the current activity in the estimation parameters of nanoparticles and stimulated additional research. Polymeric nanoparticles have potential in a wide range of applications, such as in biotechnology, vaccine systems, and the pharmaceutical industry. The main goal of this chapter is to discuss aliphatic polyester nanoparticles as drug carrier systems.

Keywords: aliphatic polyester, nanoparticles, emulsion, drug release kinetics, drug delivery systems

1. Introduction

Our inspiration for the examples contained within this chapter comes from our direct experience. Chumnanvej et al. retrospectively investigated the clinical outcomes of patients treated after ruptured cerebral arteriovenous malformation (AVM) admitted to Ramathibodi Hospital, Thailand. The results showed that approximately 50% and 7% of cases had symptoms of intracranial hemorrhage and hemorrhage associated with aneurysms, respectively [1]. A subarachnoid hemorrhage (SAH) is a hemorrhage that occurs in the subarachnoid space and leads to rupture. After cerebral surgery, these patients carry the risk of developing vasospasms within 4–14 days after surgery, with the peak occurring at 7 days [2]. Vasospasms can result from a reduction in the rapid release of blood escape from the cerebrum. Nicardipine hydrochloride (NCH) is a calcium channel blocker that

is used to treat this vascular condition; nevertheless, the half-life of NCH is approximately 8 h and provides an insufficient dose to treat vasospasms.

From a biomedical engineering point of view, polymers can be employed, particularly in neurosurgery, as surgical sutures, vascular grafts, stents, implants, tissue substitutes, or drug delivery systems. Additionally, these polymers could be biopolymers. Polymers must have the required physical properties and also be biocompatible for their intended purpose. Biopolymers are biocompatible polymeric materials that could be appropriate for treating certain neurosurgical complications. Biopolymers can respond to both the physiological and biological environments, and the kind of response is the main characteristic that determines whether the biopolymer should be used to construct biomedical devices or be applied in advanced neurosurgical applications. To our knowledge, there are three classifications of biopolymer medical-associated applications: (i) synthetic polymers, (ii) tissue engineering, and (iii) controlled-release agents. For cerebrovascular surgery applications, biopolymers have been applied to create new types of responsive delivery systems. This kind of polymer is needed to treat certain complications during cerebrovascular surgery, such as cerebrovascular spasms. When polymers are fabricated as controlled-release agents, they must have the appropriate mechanical characteristics that are suitable for use as drug delivery agents to treat vasospasms [3–5].

Due to the short biological half-life of NCH, we were able to modify the NCH pharmacokinetics via carboxymethyl cellulose/poly(D,L-lactide-co-glycolide) (NCH-CMC/PLGA) nanoparticles using a contemporary emulsion process [6]. PLGA is an aliphatic polyester with hydrophobic properties that is highly biocompatible and biodegradable. The insolubility of PLGA was improved by the addition of CMC to increase the hydrophilicity of these nanoparticles to enhance the therapeutic index. These nanoparticles gave rise to a new drug delivery system that was capable of solving some of the major bottlenecks of the NCH-PLGA microparticles prepared by the double emulsion process in our previous research [7]. The NCH-CMC/PLGA nanoparticles were flexibly designed to protect the developed delivery system against the drug degradation observed in the absence of a delivery system and improve the insufficient drug release during the first 6 days of microparticle treatment. These nanoparticles had a rate of drug release and a daily dose that was sufficient to treat vasoconstriction during the 4–14 days that this condition could occur. The results demonstrated that the designed aliphatic polyester nanoparticles had wide application prospects in drug delivery systems. These polymeric nanoparticles could release drugs on demand and are easily customized depending on the desired application. Potentially, these same nanoparticles could be extended to many fields and fulfill the different purposes of delivery systems; for example, in the food, pharmaceutical, cosmetics, biotechnology, and sustainable agriculture industries. Consequently, this chapter focuses mainly on describing the core characteristics of aliphatic polyester nanoparticles fabricated using an emulsion process.

2. Aliphatic polyesters

Currently, biodegradable polymers are utilized as a component of drug delivery systems. There are a large number of literature reports that have studied aliphatic polyesters, and their high biocompatibility and biodegradability potential has been demonstrated. Most of these polyesters have been approved for use as drug delivery devices by the Food and Drug Administration (FDA) [8]. Among these studies, aliphatic polyesters have been used to circumvent several major challenges that occur during drug delivery applications. In the 1960s, these polymers were initially

used with drug delivery systems to control drug release [9]. They can improve pharmacokinetics by maintaining the concentration of the drug at a therapeutic level and minimizing side effects.

Aliphatic polyesters are linear polymers with repeating units formed through ester linkages (R_1–COO–R_2). They are classified into two types according to the structure of their repeating units, poly(alkylene dicarboxylate)s and poly(hydroxy acid)s, as summarized in **Figure 1**. Poly(alkylene dicarboxylate)s consists of two types of repeating units between succinic acid (HOOC–$(CH_2)_2$–COOH) and alkanediol (HO–R–OH) and are prepared by esterification [10, 11]. Examples include poly(eth ylene succinate) (PES), poly(butylene succinate) (PBS), and poly(butylene succinate-co-butylene adipate) (PBSA). Additionally, poly(hydroxy acid)s are aliphatic polyesters that have hydroxy acid (HO–R–COOH) repeating units that consist of carboxylic acid (–COOH) and hydroxyl (–OH) groups. These polymers can be further classified according to the bonding position of the hydroxyl group (α, β, or ω) in the polymer chain. As a result, these hydroxy acids containing hydroxyl groups can be classified as poly(α-hydroxy acid)s, poly (β-hydroxyalkanoate)s, and poly(ω-hydroxyalkanoate)s.

Poly(α-hydroxy acid)s have a hydroxyl group attached to the α-carbon (or first carbon) atom bonded to the carboxylic acid. Poly(lactide) (PLA) and poly(glycolide) (PGA) are members of the poly(α-hydroxy acid) family with repeating lactic acid and glycolic acid units, respectively. PLA has three stereoisomers: poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(D,L-lactide) (PDLLA). Furthermore, poly(D,L-lactide-co-glycolide) (PLGA), another member of this family, is formed from a block copolymer containing both PDLLA and PGA [10, 12, 13]. Poly(β-hydroxyalkanoate) has a hydroxyl group bonded to the β-carbon of the carboxylic acid, which is the carbon atom next to the α-carbon.

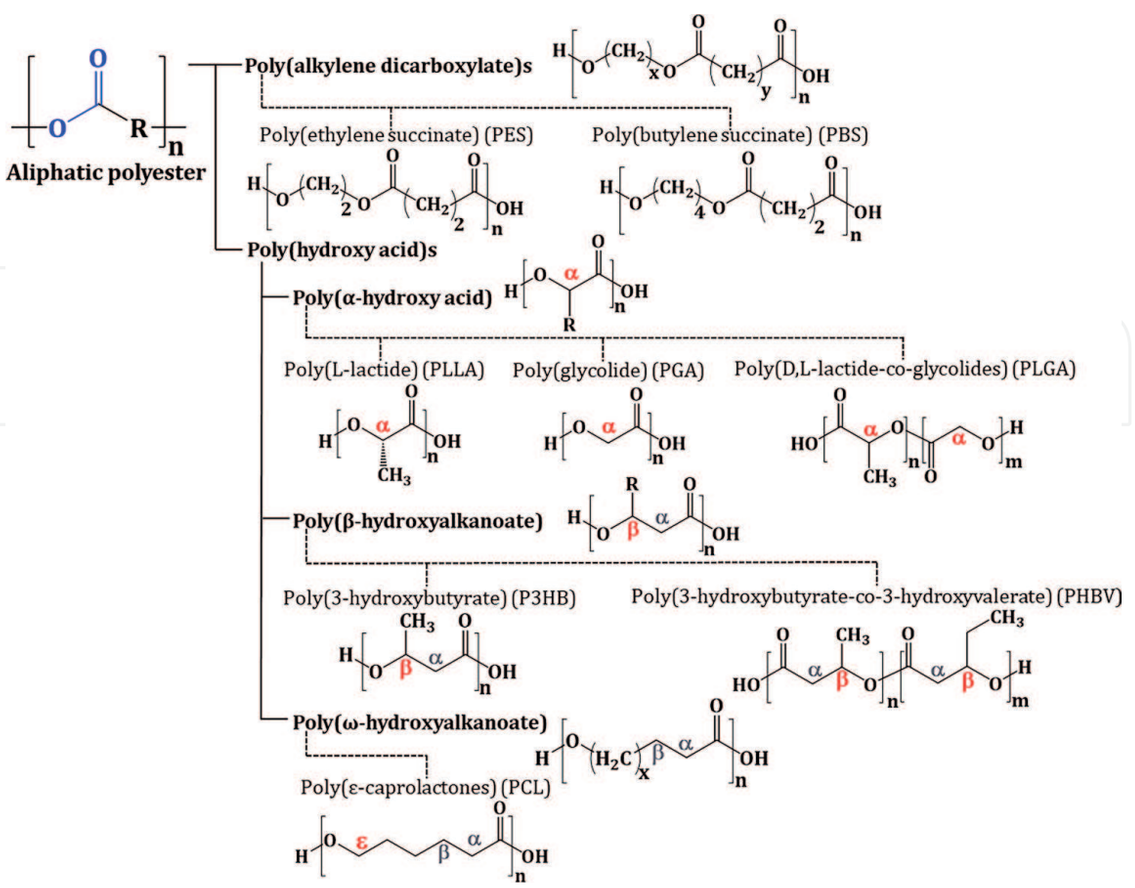

Figure 1.
Classification and examples of aliphatic polyesters [10].

This family includes poly(hydroxyalkanoate), i.e., poly(3-hydroxybutyrate) (P3HB), poly(3-hydroxyvalerate) (P3HV) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), etc. Additionally, hydroxyl groups attached to a carbon that is three or more carbon atoms away from the carbonyl are poly(ω-hydroxyalkanoate)s, such as poly(ε-caprolactones) (PCL) [10, 13]. The ester linkages of these polymers can be degraded under physiological conditions, and their degradation products are nontoxic to human connective tissue.

2.1 Biodegradation and biocompatibility of aliphatic polyesters

The ester linkages of aliphatic polyesters are sensitive to hydrolytic attacks in aqueous media. The water molecules break down the long polymer chains into small molecules with increased water solubility. Alternatively, aliphatic polyesters degraded via enzymatic hydrolysis have been reported. Esterase and lipase are important hydrolytic enzymes that can cleave the internal ester bonds of these polyesters. In addition to both of these enzymes, proteases can degrade the members of the poly(α-hydroxy acid) family as a result of the α-ester linkages in their backbone [14]. These degradation products (i.e., PLA, PGA, and PLGA) can enter the Krebs cycle and be eliminated from the body as carbon dioxide (CO_2) and water, resulting in the high biocompatibility of aliphatic polyesters [12]. **Figure 2** shows the biodegradation mechanism in which these polyesters are converted into carbon dioxide and water.

The hydrolysis of polyesters happens in three stages. During the first stage, water is absorbed onto the exterior surface and matrix swelling and polymer chain transfer occur. This absorption results from the hydrophobic properties of aliphatic polyesters and leads to surface erosion. In the next stage, an amorphous region becomes swollen followed by stress relaxation of the polymer chain [6]. Next, the matrix can absorb additional water molecules via a diffusion mechanism that leads to the hydrolysis of the polymeric chains in the matrix, creating matrix porosity. After the final stage, the hydrolysis products are acid derivatives (R–COOH), which are catalyzed by acids that trigger polyester hydrolysis. The parent derivatives can cause dissociation of the carboxyl end groups into their conjugate carboxylate anions ($R–COO^-$) also forming the acidic proton (H^+) byproducts. The acid produced catalyzes the hydrolytic reaction, generating additional acid. Continuous mass loss occurs via the formation of these fresh acids, resulting in the autocatalytic degradation of the polyester. Moreover, the addition of acidic compounds (i.e., drugs or additives) and environmental pH can modify the rate of polymer degradation for catalytic hydrolysis [15, 16].

An example of the above process is that of PLGA degradation. Synthesized PLGA has two different structures, as it can be acid-terminated or ester-terminated. Acid-terminated PLGA and ester-terminated PLGA have end-group structures of carboxylic acids (PLGA–COOH) and esters ($PLGA–COOCH_3$), respectively. End group functionalization causes differences in PLGA biodegradation.

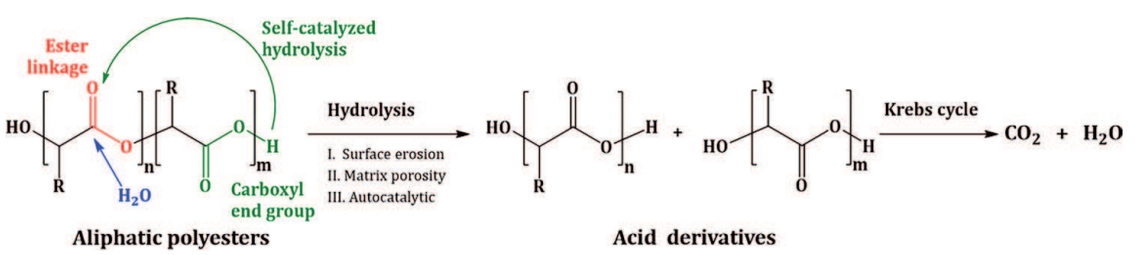

Figure 2.
Hydrolytic degradation of aliphatic polyesters.

Acid-terminated PLGA is degraded rapidly in comparison with the more hydrophobic ester-terminated PLGA. Carboxylic end-capped PLGA can be used as an acid to initiate autocatalytic degradation; thus, these acids have been applied to catalyze acid hydrolysis and promote autocatalytic degradation. Therefore, acid-terminated PLGA hydrolyzes the ester linkages more easily than ester-terminated PLGA as a result of the carboxylic end groups that are capable of producing acidic byproducts, as shown in **Figure 3** [17].

Furthermore, the rate of degradation of PLGA is dependent on the PDLLA:PGA ratio as determined by the weight percentages of PDLLA and PGA, such as 50:50, 65:35, 75:25, and 95:5. The decelerated degradation of PLGA occurs at a high amount of PDLLA, whereas a higher amount of PGA indicates rapid degradation. PDLLA, with methyl groups in its structure, has reduced chain mobility (chain configuration), leading to more hydrophobicity than PGA, which is devoid of these methyl groups (**Figure 1**). PGA is a highly hydrophilic polymer that can absorb more water molecules [6, 13]. Thus, PGA hydrolysis is more complimentary than PDLLA hydrolysis. As a rule, the drug release of PLGA is affected by the biodegradation of PDLLA and PGA. A 50:50 ratio of PDLLA:PGA has been shown to prompt degradation and produce higher rates of drug release [18, 19].

2.2 The properties of aliphatic polyesters properties in delivery systems

Following an earlier example, these differences in chemical structure illustrate how the changes in the physicochemical properties of the polymers are linked to the drug release rate via aliphatic polyester biodegradation. The biodegradation characteristics are controlled by polymer crystallinity, glass transition temperature (T_g), polymer hydrophobicity, and molecular weight (MW), all of which influence hydrolysis.

2.2.1 Polymer crystallinity

The crystalline regions of polymers consist of regular and orderly arranged polymeric chains that result in nanoparticles with high drug-loading abilities. Highly crystalline polymers present difficult mass transfer of both water and drug molecules. Water molecules are strongly absorbed and restricted within the

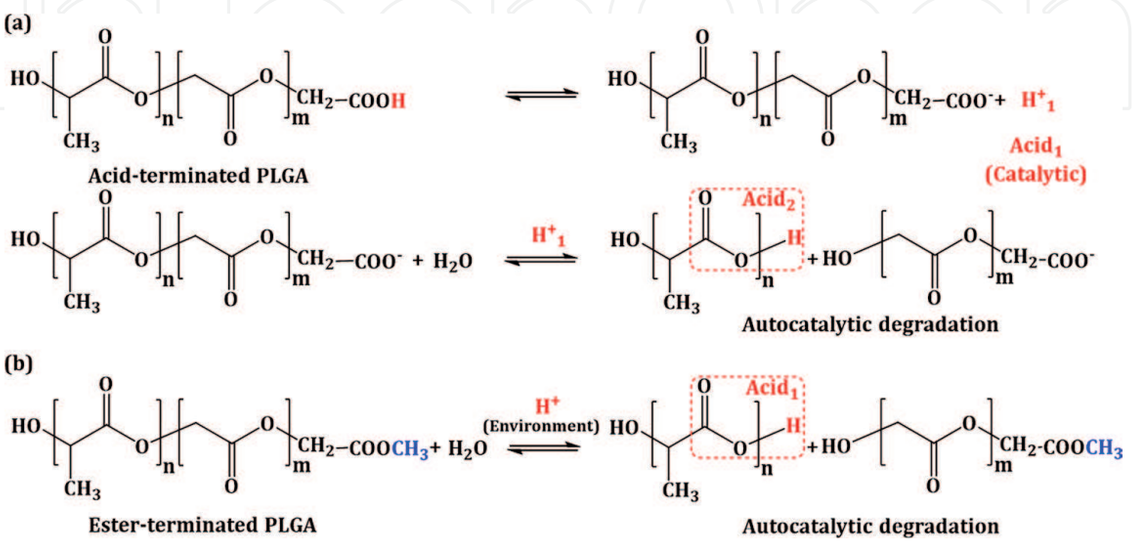

Figure 3.
Proposed hydrolysis reactions of acid-terminated PLGA (a) and ester-terminated PLGA (b) by autocatalytic degradation.

orderly polymeric chains of the crystalline regions, leading to a lower swelling rate. Crystalline polymer regions are resistant to hydrolysis compared to amorphous regions [16, 20]. As a result, the crystalline regions display delayed degradation. The byproducts of later hydrolysis still contain a very large number of high MW compounds in the polymer in the matrix, resulting in a high degree of chain entanglement. Polymer chain entanglement has an important effect on the drug release rate. High amounts of entanglement cause the drug release rate to be slower than that of highly disordered amorphous polymer materials.

2.2.2 T_g

The T_g value may impact the kinetics of drug release from delivery systems. For instance, PLGA micro/nanoparticles were prepared via the emulsion solvent evaporation method [6, 7, 21–23]. The residual solvent was removed via evaporation to harden the nanoparticles. Solvent evaporation promotes the transition to the glassy state from the rubbery state, as observed by hardening. As a result, the removal of solvents is comparable to the glass transition caused by reducing the temperature below T_g. Additionally, drug loading in micro/nanoparticles can decrease T_g through plasticizing effects. Increased drug loading leads to drug dispersion throughout the polymeric matrix, which increases the distance between polymeric chains. This causes the free volume to increase and reduces the T_g of the polymer [23]. The formation of glassy PLGA affects delivery systems by, for example, lowering the elastic modulus and transfer rates of water and the drug molecules and slowing the degradation and drug release rates. In comparison, the formation of the rubbery state facilitates higher mass transfer rates, and a higher elastic modulus leads to faster degradation and drug release rates.

2.2.3 Polymer hydrophobicity

The hydrophobicity of aliphatic polyesters is key to their hydrolytic degradation. When aliphatic polyesters are more hydrophobic, they are less water-sensitive and less susceptible to ester linkage hydrolysis than more hydrophilic polyesters. This causes a decrease in nanoparticle swelling, which results from the low water uptake. These polymers have high hydrolytic resistance and a slower degradation rate. Additionally, a large number of entanglements causes a slower release rate.

2.2.4 Polymer MW

The MW of a polymer is the molecular mass of its polymeric chains and affects the delivery system properties. High MW polymers have a large amount of chain entanglement and low chain mobility. Compared with low MW polymers, high MW polymers show the benefits of lower mass transfer rates, a lower swelling rate, a lower elastic modulus, and reduced solubility. Low MW polymers have a reduced number of polymeric chain entanglements, leading to greater molecular mobility. Water molecules can be comfortably absorbed in the polymeric matrix of these low MW polymers, resulting in faster hydrolytic degradation and a faster drug release rate.

3. Polymeric nanoparticle drug delivery systems

Nanoparticles in drug delivery systems normally have sizes ranging from 1 to 1000 nm. In essence, nanoparticles with sizes in this range have a high surface

area to volume ratio and their behavior in the body could cause increases in the absorption potential and cytotoxicity as the expanded surface is proportional to cytotoxicity [24]; thus, these nanoparticles could potentially lead to an increase in cytotoxicity. Nevertheless, nanoparticles prepared from aliphatic polyesters could possibly lead to a reduction in cytotoxicity, as they are highly biocompatible and biodegradable. Aliphatic polyester nanoparticles play key roles in improving the pharmacokinetics, drug bioavailability, specific delivery at the site of action, and stability in the bloodstream. Their high surface area can adsorb drug molecules and encapsulate a large number of drugs in their matrix, effectively carrying drugs, proteins, DNA, and organs. Therefore, the development of aliphatic polyester nanoparticles is one of the most successful ideas for drug delivery innovation.

3.1 Polymeric nanoparticles

Polymeric nanoparticle delivery systems refer to both nanospheres and nanocapsules. Their classification is based on the positional characteristics of the drugs and polymers, as shown schematically in **Figure 4a** and **b.** Nanospheres contain drug molecules within a uniformly dispersed polymeric matrix, while nanocapsules entrap the drug molecules inside their core, a cavity surrounded by a polymeric membrane that acts as a shell [25].

Regarding the molecular orientation surface, we propose a possible schematic of the polyester chain on the nanoparticle surface as shown in **Figure 4**. In the case of PLGA nanospheres, the polymeric chains ignore water molecules, which is consistent with computational simulation results that demonstrated that PLGA is strictly hydrophobic [26]. At both the surface and interface, the carbon backbones clump together rather than being distributed in the aqueous solution. This causes the hydrophobic molecules to have minimal contact with hydrophilic molecules. On the other hand, the oxygen atoms of the hydroxyl groups (–OH), carboxylic acids (–COOH), and ester groups (–COO–) interact with the water molecules residing near the water-PLGA interface, and the hydrogen atoms in the polymer chain resemble the oxygen atoms. Hydrophobic drugs display the tendency to accumulate

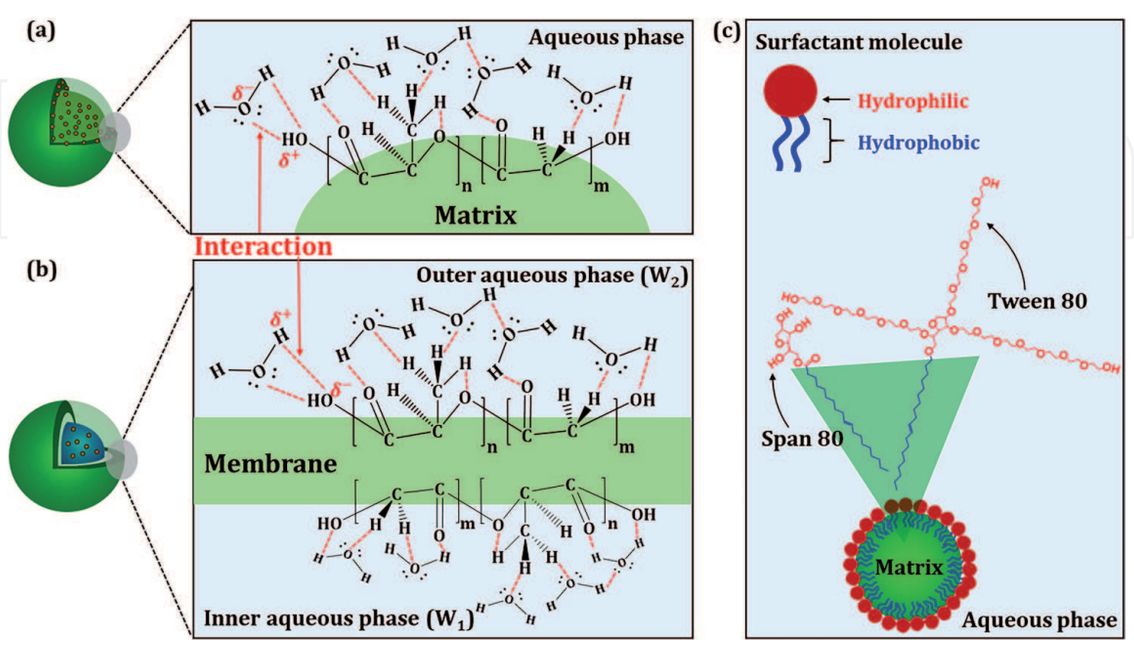

Figure 4.
Schematic representation of the structures and molecular orientations of PLGA nanospheres (a), PLGA nanocapsules (b), and the packing of emulsifiers (Span 80 and Tween 80) at the interface of the dispersed polymer droplets (c).

on the surface, with a few drug molecules migrating to the core, leading to the uniform distribution of drug molecules within the polymeric matrix, especially in the vicinity of the nanosphere surface (**Figure 4a**). The nanocapsules have contact with both the inner and outer aqueous solutions. Thus, the molecular orientation of aliphatic polyesters on the inner and outer membrane surfaces corresponded to nanospheres. Nevertheless, the hydrophilic drugs tend to be entrapped inside the core of a cavity surrounded by the polymeric membrane due to the insolubility between the drug and the polymer (**Figure 4b**).

3.2 Polymeric nanoparticle preparation

The preparation of nanospheres and nanocapsules follows an uncomplicated basic principle, the emulsification solvent evaporation method. The guiding principles are that the drug and polymer dissolve in each other during nanosphere preparation. As a result, the drug molecules are uniformly distributed in the polymeric matrix. Examples of hydrophobic drugs with hydrophobic polymers were previously discussed in the section on nanosphere molecular orientation. However, nanocapsules are prepared from drugs and polymers that are essentially insoluble in each other. This property promotes the formation of core-shell structures to load drugs.

The preparation of both types of nanoparticles depends upon drug solubility and polymer solubility. Therefore, drug solubility is a strict limitation in the use of aliphatic polyesters, as drug solubility affects the type of nanoparticles that can be prepared because polyesters are hydrophobic polymers. This indicates that the use of water-soluble drugs could produce nanocapsules, but insoluble drugs yield nanospheres. In addition to drug solubility, nanoparticles of aliphatic polyesters can be easily prepared. This section describes the preparation of nanoparticles via emulsification methods for these polyester-controlled drug delivery systems.

3.2.1 Single emulsion method

Oil-in-water emulsification (O/W emulsion) can be used to prepare nanospheres that are ideal for entrapping hydrophobic drugs (i.e., steroids) [19] or water-soluble drugs entrapped on nanosphere surfaces [27]. Examples include amoxicillin-loaded PCL micro/nanospheres modified surface by chitosan for antibacterial applications. The amino groups (**–**NH_3^+) of chitosan interact with PCL via carboxylate anions (**–**COO^-) on the micro/nanosphere surface. Amoxicillin is encapsulated by the electrostatic interactions between the negative charges of the drug and the residual positive charge on the surface of chitosan, as observed by the zeta potential values of −20.03 mV and +53.93 mV, respectively. This study varied the ratios of PCL:chitosan to be 1:1, 1:3, and 1:5, and the results showed that increasing the ratio improved the encapsulation efficiency (~73–83%) and controlled drug release (~5.76–6.56 mg) over 7 days compared to amoxicillin-loaded PCL micro/nanospheres, which showed rapid drug release within 12 h due to the low encapsulation efficiency (~5.4%). Additionally, the amoxicillin-loaded PCL micro/nanosphere modification inhibited microbial growth, but the chitosan-free micro/nanospheres were less effective [27].

3.2.2 Double emulsion method

Water-in-oil-in-water ($W_1/O/W_2$) double emulsions could be used to prepare nanocapsules for the loading of water-soluble molecules, such as hydrophilic drugs, proteins, peptides, and vaccines [19]. This system is an extraordinarily complex

dispersion system of liquid membranes. In addition, the contemporary $W_1/O/W_2$ double emulsion method led to the preparation of PLGA nanocapsules. This emulsion method can enhance the solubility of poorly soluble drugs in the medium. As mentioned previously in the introduction, we prepared NCH-CMC/PLGA nanocapsules via a contemporary emulsion process. The composition of phase W_1 included NCH and CMC. Notably, NCH dissolves in citric acid solution, but CMC is insoluble in acidic solutions, as citric acid is a crosslinking agent of CMC. This method fabricated two systems, citric acid crosslinking droplets and CMC droplets. To generate the crosslinking droplets, solid NCH was first dissolved in citric acid mixed poly(vinyl alcohol) (PVA) solution (W_1). This solution was then added to an organic solution of PLGA with stirring (O). Similarly, the CMC solution (W_1) was added to PLGA solution with stirring, forming CMC droplets. Both droplets were added together and ultrasonicated to form crosslinked droplets (W_1/O single emulsion). This single emulsion was again added and ultrasonicated, forming the $W_1/O/W_2$ double emulsion. This unique synthesis enhanced the drug loading of the nanoparticles for controlled drug release for up to 16 days. As a result, NCH-CMC/PLGA nanoparticles containing calcium channel blockers were created. This method paved the way to generate a treatment for the prevention of vasospasm complications after surgery for ruptured intracranial aneurysms. There are several applications for calcium channel blocker prolonged-release implants. This application involves the binding of calcium channel blockers in composite polymer formulations for slow release at a rate that is effective to treat cerebral vasospasm in animal models. This drug delivery system is objectively locally positioned at the surgical site after the intracranial aneurysm is secured. These nanoparticles are located around the inflamed vessels and can avoid being washed away. The strategy for controlled release is based on polymer degradation at a rate that is effective for the treatment of cerebral vasospasms for 4–14 days. However, a clinical scenario needs to be investigated in the future [6].

3.3 Polymeric nanoparticle stabilization

Stabilizing the produced nanoparticles is a key factor in the delivery systems because the nanoparticles need to protect the encapsulated drugs from degradation. Agglomeration and flocculation are the destruction methods of nanoparticles and can produce macroscopic lumps that lead to the diffusion of drug molecules and their degradation in the residence environment. To stabilize these nanoparticles, an emulsion stabilizer is required, and this can be efficiently accomplished with amphiphilic molecules. These molecules contain two components, a hydrophilic part, and a lipophilic side. Typical examples of amphiphilic molecules include Span 80 and Tween 80 as nonionic emulsifiers. Additionally, anionic emulsifiers, cationic emulsifiers, and zwitterionic emulsifiers provide structural diversity.

The most commonly used rule for emulsifier selection is the hydrophilic-lipophilic balance (HLB) scale (HLB scale ranges from 0 to 20). This scale indicates the relative fraction of hydrophilic to lipophilic parts within the emulsifier molecule. High HLB values (8.0–18.0) are more suitable for use in O/W emulsions because they have a higher degree of hydrophilicity. In this case, O/W emulsions with a large amount of water are required to allow the formation of polymer dispersion droplets. As a result, these emulsifiers can widely expand the applications of the hydrophilic molecule for nanoparticle isolation, which then leads to interrupted agglomeration. Emulsifiers that have HLB values between 3.5 and 6.0 are most commonly used in W/O emulsions because they are more lipophilic [28, 29]. **Figure 4c** illustrates the expansion of the hydrophilic molecules of Span 80 (HLB = 4.3) and Tween 80 (HLB = 15.0) [7] at the interface of dispersed polymer droplets.

Furthermore, polymers have been used as emulsion stabilizers during emulsifier preparation methods, i.e., PVA, chitosan, and alginate [22, 30]. High MW polymers (>10 kDa) show driving forces that are equivalent to or greater than van der Waals-London (VDWL) forces. These VDWL forces are the primary source of attraction between nanoparticle surfaces and cause them to clump together. Through the addition of polymers, the polymeric chains can stabilize the nanoparticles via electrostatic, steric, and depletion forces. Electrostatic stabilization can be applied to charged polymers based on the simple principle that similar electric charges repel each other. Nanoparticle surfaces with zeta potential values greater than +30 mV or less than −30 mV are normally considered stable, as presented in **Figure 5a**. Steric stabilization is achieved on the surfaces of nanoparticles via adsorbed or chemically attached nonionic/ionic polymers. These uncharged polymers have a steric barrier due to a long chain that prevents the nanoparticles from sticking together (**Figure 5b**). Moreover, the addition of a high concentration of polymer for stabilization could lead to a high amount of free polymer in the dispersion medium. These free polymers establish repulsive forces between the nanoparticles to prevent nanoparticle aggregation and act as depletion stabilizers (**Figure 5c**) [31].

For example, PVA is an uncharged polymer that is commonly used as an emulsion stabilizer for the preparation of PLGA nanoparticles. The hydroxyl groups on the PVA chains are adsorbed at the interface with the PLGA chains via the hydroxyl, carboxylic acid, and ester groups. The interface between PLGA and PVA is an area with strong interactions, resulting in high nanoparticle stabilization [32, 33]. This interaction corresponds to the molecular orientation of PLGA nanospheres and PLGA nanocapsules (**Figure 4**). **Figure 5d** proposes a schematic representation of the molecular orientation of PVA at the surface of PLGA nanoparticles.

The use of polymers as stabilizers affects the characteristics of the nanoparticles to a greater extent than the use of other emulsifiers; as a result, these nanoparticles have a high MW. As a result, the use of low MW polymers or polymers at a low concentration results in reduced stability, lower drug loading, a rapid release rate, and a smaller size compared with high MW polymers or polymers present in high concentrations [6]. In addition, the charged polymers affect aliphatic polyester

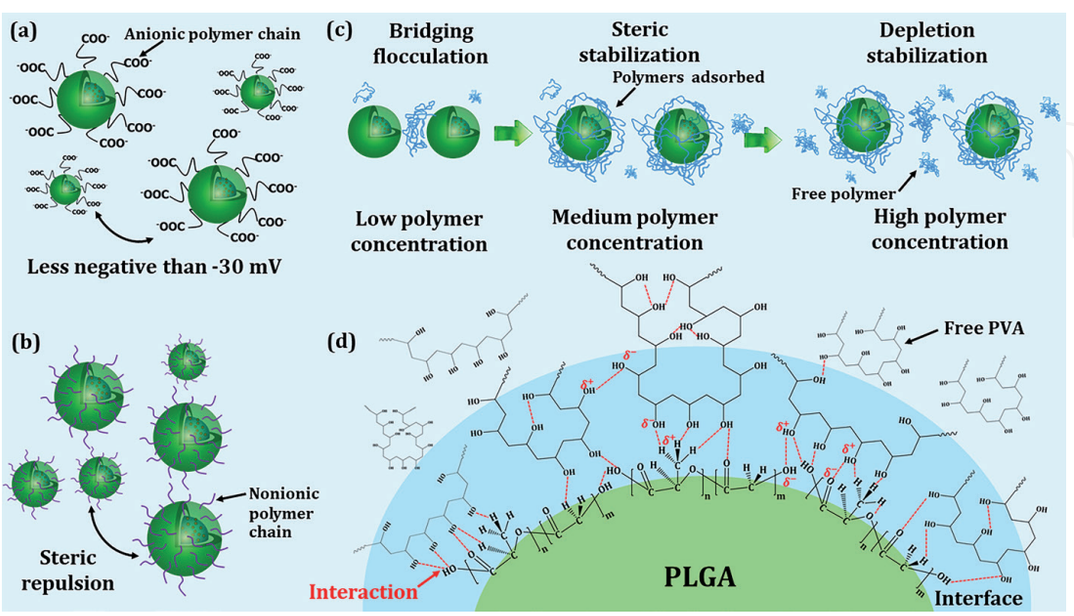

Figure 5.
Schematic representation of the molecular interactions of electrostatic stabilization (a), steric stabilization (b), depletion stabilization (c), and PVA at the surface of PLGA nanoparticles (d), based on information from a review article [31–33].

nanoparticles. Alginate is an anionic polymer and contains carboxylate anions ($-COO^-$) on its polymer chains. These anions repel the anions of the aliphatic polyesters, resulting in a larger size and permitting an increase in the retention of cationic drugs. However, cationic polymers (i.e., chitosan with a $-NH_3^+$ groups) interact with the carboxylate anions of the stabilizing polymers, leading to reduced particle size and increased retention of anionic drugs [30].

3.4 Mathematical models of drug release

Mathematical knowledge could assist in clarifying the drug release mechanism from polymer-based nanoparticles for controlled delivery. The exact mass transport and the relationship between drug release as a function of time can be described through various mathematical models. These models are able to predict analytics via data obtained from the drug release experiments to explain drug release kinetics. The coefficient of determination (R^2) and adjusted coefficient of determination (adj.-R^2) are investigated by statistical analysis to determine the relationship that the equation explains. The highest values of R^2 and adj.-R^2 from curve fitting involving describing the optimal relations (maximum accuracy) as a function of release time can be predicted with these Equations [6, 21]. This chapter will discuss some of the most commonly used equations to predict the kinetics of release from polymeric nanoparticles, including the *zero-order*, *first-order*, *Higuchi*, *Hixson-Crowell*, and *Korsmeyer-Peppas* equations, as summarized in **Table 1**.

When predicting drug release rates, the *zero-order model* represents controlled drug release that occurs at a constant rate depending on only the time and is independent of the amount of polymer. In the *first-order model*, the amount of drug released tends to depend on the polymer concentration, which affects the swelling and porosity of the nanoparticles. The *Hixson-Crowell model* describes the rate of drug release according to the cube root of its volume, in which matrix dissolution occurs. As a result, the diameter and surface area of the nanoparticles decreases proportionally over time according to the cube root of the weight at that particular time [34]. The *Higuchi model* predicts the release rate via diffusion control based on Fick's first law, which is square root time-dependent ($t^{1/2}$) [35].

Generally, the release mechanism can be predicted by the *Korsmeyer-Peppas model*. This model determines the exponential relationship between the rate of drug release and time. It is based on polymeric matrices with different geometries, following the released exponential (n). The exponential n-value indicates three types

Model	Equation	Graphical representation
Zero-order	$Q_t = K_0 t$	Cumulative release vs time
First-order	$\log M_t = \log M_0 + \frac{K_1 t}{2.303}$	log cumulative remaining vs time
Hixson-Crowell	$M_0^{1/3} - M_t^{1/3} = K_{HX} t$	Cumulative remaining$^{1/3}$ vs time
Higuchi	$Q_t = K_H t^{1/2}$	Cumulative release vs time$^{1/2}$
Korsmeyer-Peppas	$\log Q_t = n \log t + \log K_{KP}$	log cumulative release vs log time

Q_t is the cumulative amount of drug release at time (t), Q_0 is the initial amount of drug release at (t_0), M_t is the cumulative amount of drug remaining at time (t), M_0 is the initial amount of drug remaining at (t_0), t is time, n is the released exponent of Korsmeyer-Peppas, and K_0, K_1, K_{HX}, K_H, and K_{KP} are the zero-order, first-order, Hixson-Crowell, Higuchi, and Korsmeyer-Peppas constants, respectively.

Table 1.
Mathematical models of drug release [6].

of release mechanisms (**Figure 6**). The first type is the mechanism of Fickian diffusion, where the rate of diffusion of drug release is considerably greater than the rate of polymeric chain relaxation. The second mechanism is anomalous transportation via diffusion and swelling/erosion via slow rearrangement of polymeric chains. The final type is only the swelling/erosion mechanism. The release mechanisms of swelling and erosion are affected by an expansion in porosity and polymeric chain cleavage, respectively. Thus, the anomalous transportation and swelling/erosion mechanisms are both non-Fickian diffusion [36].

Using equations to predict release mechanisms can be confusing. Therefore, we proposed the flowchart sequence of use steps for these equations to describe drug release from PLGA nanoparticles [6]. The initial step investigates whether the rate of drug release is independent or dependent on the various concentrations by comparing R^2 values between the *zero-order* and *first-order* models. Next, the *Hixson-Crowell equation* and *Higuchi equation* were compared to determine whether the release rate depends on the probability of matrix dissolution or is diffusion controlled. Finally, the mechanism of drug release was investigated via the *Korsmeyer-Peppas equation* to determine the exponential n-value, as proposed in **Figure 6.**

3.5 Estimation of the formulation parameters for the polymeric nanoparticle system

Nanoparticles are one of the best choices of novel delivery systems because of their ease of preparation and long shelf life. Nanoparticle systems have fascinating physical properties that have been recognized over a wide range of utilizations. The characterization of different formulation parameters of nanoparticles is considered in the following sections.

3.5.1 Physical appearance

The physical appearance of nanoparticles can be examined visually for optical clarity, homogeneity, and fluidity, and also microscopically using a scanning electron microscope [37]. **Figure 7** illustrates digital photographs of PLGA nanoparticles [6] compared to PLGA microparticles [7] prepared via an emulsion process. Due to the optical transparency, the nanoparticles appeared as more transparent dispersions than the microparticles, which appeared white.

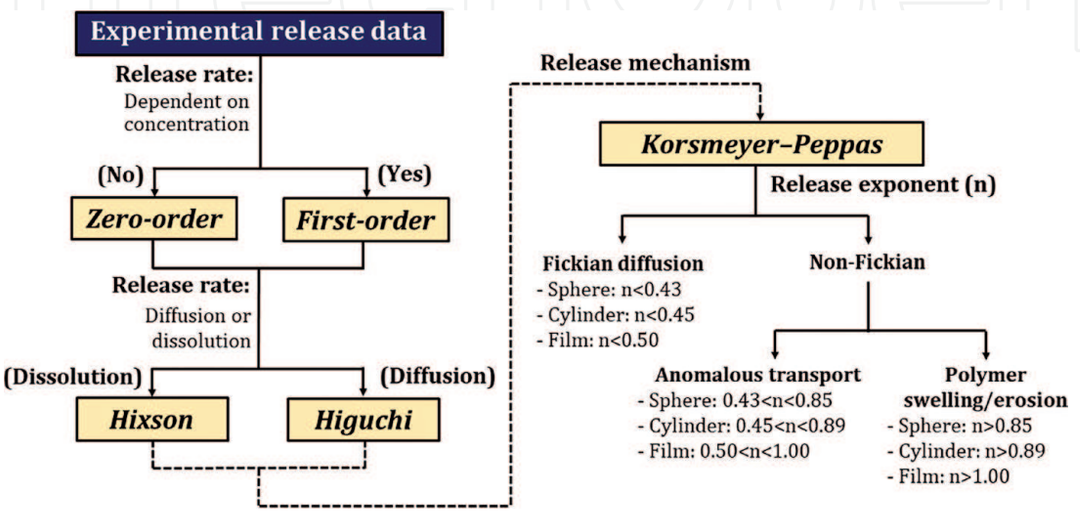

Figure 6.
Flowchart sequence of the steps used for the equations that predict drug release.

3.5.2 Rheology

Nanoparticles have high surface energy, resulting in agglomeration. In particular, aliphatic polyester-based nanoparticles rapidly agglomerate due to the strong interactions between the hydroxyl, carboxylic acid, and ester groups of the polymeric structures interacting with other nanoparticles. This behavior promotes the nanoparticles to be dispersed in media, affecting the rheological properties, such as viscosity and yield stress, of the suspension. Rheological characteristics can be examined with a Brookfield viscometer. The rheological properties show the nanoparticle region and its separation from other regions and play a principal role in stability [37, 38].

3.5.3 Particle size, polydispersity, and zeta potential

Dynamic light scattering is used to investigate particle size, zeta potential, and fluctuations in the intensities of scattering of particles due to Brownian motion. This technique also analyzes the polydispersity index to measure the wideness of the size distribution and quality of the dispersion [39, 40].

3.5.4 Drug stability

The stability of nanoparticles can be measured via visual inspection in closed tubes at both room temperature and elevated temperatures. Each month, the nanoparticles can be evaluated based on their phase separation, % transmittance, and globule size [7, 41]. Examples include PLGA nanoparticle dispersions [6] compared to PLGA microparticle dispersions [7] in phosphate-buffered saline (pH 7.4 at 37°C) to mimic the physiological environment. The nanoparticle suspension presented no phase separation, suggesting that these nanoparticles did not precipitate (**Figure 7a**). The microparticles could have a higher gravitational force relative to the nanoparticles, leading to their easy precipitation, which can be observed from the phase separation of the microparticle solution (**Figure 7b**). Therefore, drug administration of these microparticles needs to carry a "*shake well before use*" label [22].

3.5.5 Drug solubility

Excess drug was added to the nanoparticles to optimize the formulation, similar to the optimization of the other ingredients. The nanoparticles were continuously stirred

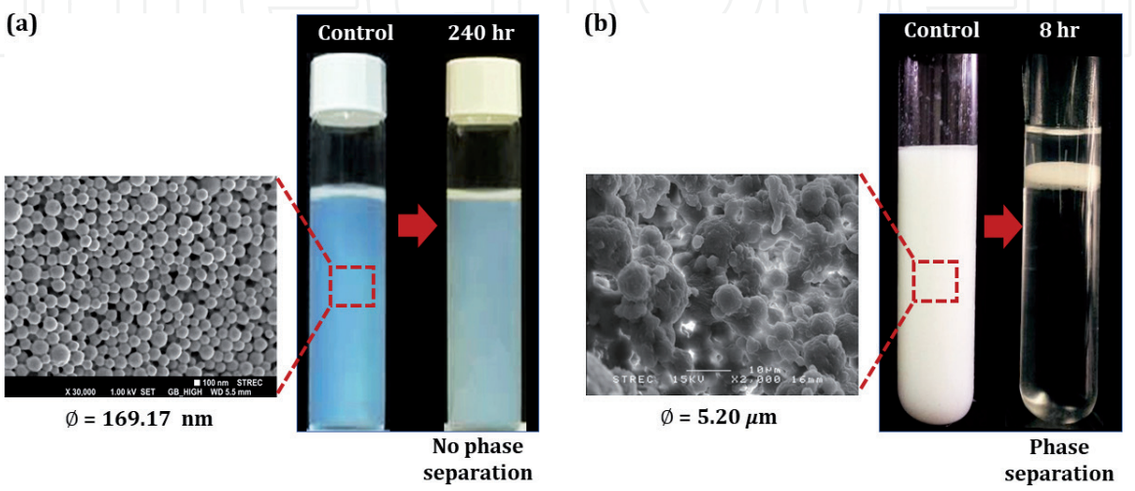

Figure 7.
SEM micrographs and digital photographs of PLGA nanoparticles (a) and PLGA microparticles (b) prepared via an emulsion process [6, 7].

for 24 h at room temperature, and samples were removed and centrifuged at 600 rpm for 10 min at predetermined time points. The quantity of soluble drugs in the optimized nanoparticles was determined by calculating the amount of drug present in the residue and comparing it with the total amount of added drug. The individual ingredients were used for comparison of the solubility of the drug in the nanoparticles [7, 42].

3.5.6 In vitro*,* ex vivo*, and* in vivo *drug release*

In vitro and *in vivo* studies provide extrapolated data to reflect the bioavailability of drug formulations. The *in vitro* drug release profile of nanoparticles can be determined with a Franz diffusion cell. The donor compartment has a cellophane membrane as a barrier, comprising the encapsulated drug in the nanoparticles. The receptor compartment is filled with buffer solution, usually phosphate-buffered saline (pH 7.4), and the system is stirred with a magnetic stirrer at 100 rpm and 37°C. At predetermined time intervals, samples of the dispersion are withdrawn from the medium of the receptor compartment and an equal amount of medium is returned to the system at this same time. The withdrawn sample is filtered using a 0.22–50 μm filter (e.g., Millipore, USA) and analyzed for drug release using UV-visible spectrophotometry at the specific wavelength that shows peak absorption of the drug [39, 43, 44].

An *ex vivo* drug release study profile of nanoparticles can be performed with a diffusion cell. Suitable skin and the underlying cartilage removed from ears can be cut up and placed on the diffusion cell filled with receptor solution. Vesicular process samples are placed on the dorsal surface of the skin. At predetermined time intervals, samples of the dispersion are withdrawn from the medium of the receptor compartment and an equivalent amount of the medium is returned at the same time. The withdrawn sample is then analyzed for the amount of drug released using high-performance liquid chromatography (HPLC) or UV-visible spectrophotometry at a specific wavelength [44].

In vivo, drug release study profiles of nanoparticles can be carried out by administering the nanoparticles to living animals. At predetermined time intervals, blood samples are withdrawn, followed by centrifugation and analysis of drug release using HPLC.

3.6 Applications of polymeric nanoparticle systems

Nanoparticle systems have been used as delivery vehicles in a variety of drug delivery systems for controlled release because of their potential to solubilize active agents. Aliphatic polyester nanoparticles have various goals, which are accomplished in different ways, as outlined in the following sections.

3.6.1 Oral delivery

Nanoparticle formulations have many benefits over conventional oral delivery methods, including increased absorption, minimized drug toxicity, enhanced oral bioavailability, and excellent clinical potency. Hence, nanoparticle systems have been modified for use in drug delivery systems for compounds such as oral drugs, steroids, hormones, diuretics, and antibiotics [40, 45, 46].

3.6.2 Parenteral delivery

Parenteral delivery via the intravenous route has been used for controlled drug delivery and drug targeting to particular sites. Aliphatic polyester nanoparticles

significant in the parenteral delivery system since they can improve drug solubility, long-term stability in the body, biocompatibility, and ease of development for delivery to a targeted site. Nanoparticle formulations have prominent advantages over other systems. Additionally, fine nanoparticles are cleared more slowly than coarse particles [47, 48].

3.6.3 Topical delivery

Hydrophilic and lipophilic drugs poorly penetrate the skin due to the limitations of their solubility in both the lipid phase and aqueous solution. The topical delivery of drugs via nanoparticle formulations has several advantages over other conventional topical methods for the treatment of skin disorders while showing minimal side effects. Nanoparticles can incorporate both lipophilic and hydrophilic drugs. Additionally, drug degradation, salivary degradation, and hepatic first-pass metabolism in the stomach can be avoided, while benefiting from sustained drug delivery, long-term stability, enhanced permeability, and reduced toxicity [49, 50].

3.6.4 Pulmonary drug delivery

The treatment of lower respiratory infections is difficult due to the presence of microbes deep in the respiratory system. Nanoparticles as a targeted drug delivery system to the site of infection have been actively applied to enhance antimicrobial drug resistance, sustain drug release, and increase bioavailability. Importantly, nanoparticle systems that contain multiple antimicrobial substances can be used [51, 52].

3.6.5 Vaccine delivery

Recently, there have been many research topics related to nanoparticles as carriers for vaccines. The main idea is to use the nanoparticle as an effective carrier to deliver an inactivated organism to the membrane to stimulate the immune system. One study found that it is possible to generate genital mucosa immunity from vaccines that are added to the nasal mucosa. The synthesized proteins passed into the mucosal membrane surface by using nanoparticles to absorb the antigen using adjuvant facilitation [53–55].

There are several mechanisms to load antigens into the nanoparticles, such as encapsulation, encapsulation with coating, encapsulation with targeting, physical adsorption, chemical conjugation, and conjugation with a targeting mechanism. Hydrophobic interactions are known to be effective ways to load antigens into nanoparticles by physical adsorption processes, which apply weak interactions to dissociate antigens and nanoparticles in the body. During protein synthesis, the encapsulated antigens are mixed with nanoparticle precursors, which is a consequence of the release of the encapsulated antigen when the nanoparticle degrades *in vivo*.

The first clinical trial application received was for the use of nanoparticles for influenza and human immunodeficiency virus (HIV) proteins. A recombinant HIV glycoprotein 120 (HIV-gp120) antigen mix in nanoparticles was studied in mice and guinea pigs by intranasal immunization and demonstrated a robust serum anti-gp120 immunoglobulin G (IgG) response [53]. The constituent of Prepandrix® has been approved for the use of the flu vaccine AS03 (an adjuvant system containing α-tocopherol and squalene in an oil-in-water emulsion) during the influenza pandemic. AS03 has been used as a reference for other vaccines, such as Arepanrix®, to control influenza infection caused by H1N1 and H5N1 [54].

The balance between immunogenic benefits and safety needs to be considered and is achieved by selecting the correct adjuvant, antigen, and emulsion composition. These factors are considered the main factors of concern during vaccine development, as they directly affect the benefit-risk balance. Vaccine efficiency is vital, and this factor is the major element controlling pandemics through the selection of the correct oil composition, adjuvant, surfactant, and antigen for the specific disease.

Aliphatic polyester-based nanoparticles could be delivered as adjuvants for vaccines during the coronavirus disease 2019 (COVID-19) pandemic [56, 57]. Polymers are the main material for vaccine delivery. For example, the most popular aliphatic polyesters have been used in vaccine adjuvants for lymph nodes targeting HIV, *Bacillus anthracis*, *Chlamydia trachomatis*, and malaria [58]. Moreover, the addition of a poly(ethylene glycol) (PEG) coating around PLA (PEG-PLA) nanoparticles has been found to facilitate the transport of the antigen of tetanus toxoid to the rat nasal epithelium for nasal vaccine delivery [59].

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References

[1] Chumnanvej S, Sarnvivad P. Retrospective 5-year clinical outcome of interdisciplinary treatment for ruptured cerebral arteriovenous malformation: The lesson learned. Archives of Medicine. 2015;**7**(44):1-7

[2] Alahamd Y, Swehli H, Rahhal A, Sardar S, Elhassan M, Alsamel A, et al. Calcium channel blockers. In: Ambrosi P, Ahmad R, Abdullahi A, Agrawal A, editors. New Insight into Cerebrovascular Diseases—An Updated Comprehensive Review. London: IntechOpen; 2020. pp. 216-233

[3] Kasuya H. Development of nicardipine prolonged-release implants after clipping for preventing cerebral vasospasm: From laboratory to clinical trial. Acta Neurochirurgica Supplement. 2013;**115**:41-44

[4] Barth M, Pena P, Seiz M, Thomé C, Muench E, Weidauer S, et al. Feasibility of intraventricular nicardipine prolonged release implants in patients following aneurysmal subarachnoid haemorrhage. British Journal of Neurosurgery. 2011;**25**(6):677-683

[5] Thomé C, Seiz M, Schubert GA, Barth M, Vajkoczy P, Kasuya H, et al. Nicardipine pellets for the prevention of cerebral vasospasm. Acta Neuro chirurgica Supplement. 2011;**110**(Pt 2): 209-211

[6] Soomherun N, Kreua-ongarjnukool N, Niyomthai ST, Chumnanvej S. kinetics of drug release via nicardipine hydrochloride-loaded carboxymethyl cellulose/poly(D,L-lactic-co-glycolic acid) nanocarriers using a contemporary emulsion process. ChemNanoMat. 2020;**6**(12):1754-1769

[7] Soomherun N, Kreua-ongarjnukool N, Chumnanvej S, Thumsing S. Encapsulation of nicardipine hydrochloride and release from biodegradable poly(D,L-lactic-co-glycolic acid) microparticles by double emulsion process: Effect of emulsion stability and different parameters on drug entrapment. International Journal of Biomaterials. 2017;**2017**:1743765

[8] Urbánek T, Jäger E, Jäger A, Hrubý M. Selectively biodegradable polyesters: Nature-inspired construction materials for future biomedical applications. Polymers. 2019;**11**(6): 1061

[9] Silvers AL, Chang C-C, Parrish B, Emrick T. Strategies in aliphatic polyester synthesis for biomaterial and drug delivery applications. In: Degradable Polymers and Materials: Principles and Practice. 2nd ed. Washington, DC: American Chemical Society; 2012. pp. 237-254

[10] Mochizuki M, Hirami M. Structural effects on the biodegradation of aliphatic polyesters. Polymers for Advanced Technologies. 1997;**8**(4): 203-209

[11] Jacquel N, Freyermouth F, Fenouillot F, Rousseau A, Pascault J-P, Fuertes P, et al. Synthesis and properties of poly(butylene succinate): Efficiency of different transesterification catalysts. Journal of Polymer Science Part A Polymer Chemistry. 2011;**49**:5301

[12] Ginjupalli K, Shavi GV, Averineni RK, Bhat M, Udupa N, Nagaraja UP. Poly(α-hydroxy acid) based polymers: A review on material and degradation aspects. Polymer Degradation and Stability. 2017;**144**:520-535

[13] Burg K. Chapter 6—Poly(α-ester)s. In: Kumbar SG, Laurencin CT, Deng M, editors. Natural and Synthetic Biomedical Polymers. Oxford: Elsevier; 2014. pp. 115-121

[14] Panyachanakul T, Sorachart B, Lumyong S, Lorliam W,

Kitpreechavanich V, Krajangsang S. Development of biodegradation process for poly(DL-lactic acid) degradation by crude enzyme produced by *Actinomadura keratinilytica* strain T16-1. Electronic Journal of Biotechnology. 2019;**40**:52-57

[15] Netti PA, Biondi M, Frigione M. Experimental studies and modeling of the degradation process of poly(lactic-co-glycolic acid) microspheres for sustained protein release. Polymers (Basel). 2020;**12**(9):2042

[16] Casalini T. 3—Bioresorbability of polymers: Chemistry, mechanisms, and modeling. In: Perale G, Hilborn J, editors. Bioresorbable Polymers for Biomedical Applications. Cambridge: Woodhead Publishing; 2017. pp. 65-83

[17] Ali M, Walboomers XF, Jansen JA, Yang F. Influence of formulation parameters on encapsulation of doxycycline in PLGA microspheres prepared by double emulsion technique for the treatment of periodontitis. Journal of Drug Delivery Science and Technology. 2019;**52**:263-271

[18] Vey E, Rodger C, Booth J, Claybourn M, Miller AF, Saiani A. Degradation kinetics of poly(lactic-co-glycolic) acid block copolymer cast films in phosphate buffer solution as revealed by infrared and Raman spectroscopies. Polymer Degradation and Stability. 2011;**96**(10):1882-1889

[19] Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel). 2011;**3**(3): 1377-1397

[20] Gorrasi G, Pantani R. Hydrolysis and biodegradation of poly(lactic acid). In: Di Lorenzo ML, Androsch R, editors. Synthesis, Structure and Properties of Poly(Lactic Acid). Cham: Springer International Publishing; 2018. p. 119-151.

[21] Soonklang C, Tassanarangsan C, Soomherun N, Kreua-ongarjnukool N, Niyomthai ST. Study kinetics models of clindamycin hydrochloride from poly(D,L-lactic-co-glycolic acid) particles. International Journal of Pharma Medicine and Biological Sciences. 2021;**10**(2):68-74

[22] Soomherun N, Kreua-ongarjnukool N, Niyomthai ST, Chumnanvej S. Enhancement vehicles of cardene loading poly(D,L-lactic-co-glycolic acid) nanoparticles in vitro controlled release for biomedical application. International Journal of Pharma Medicine and Biological Sciences. 2021;**10**(1):1-7

[23] Park K, Otte A, Sharifi F, Garner J, Skidmore S, Park H, et al. Potential roles of the glass transition temperature of PLGA microparticles in drug release kinetics. Molecular Pharmaceutics. 2021;**18**(1):18-32

[24] De Jong WH, Borm PJA. Drug delivery and nanoparticles: Applications and hazards. International Journal of Nanomedicine. 2008;**3**(2):133-149

[25] Garg A, Garg S, Swarnakar NK. Chapter 11—Nanoparticles and prostate cancer. In: Yadav AK, Gupta U, Sharma R, editors. Nano Drug Delivery Strategies for the Treatment of Cancers. Cambridge: Academic Press; 2021. pp. 275-318

[26] Wilkosz N, Łazarski G, Kovacik L, Gargas P, Nowakowska M, Jamróz D, et al. Molecular insight into drug-loading capacity of PEG–PLGA nanoparticles for itraconazole. The Journal of Physical Chemistry B. 2018;**122**(28):7080-7090

[27] Metheeparakornchai C, Kreua-ongarjnukool N, Thumsing S, Pavasant P, Limjeerajarus C. Development of amoxicillin-loaded modified polycaprolactone microparticles in medical application.

International Journal of Pharma Medicine and Biological Sciences. 2021;**10**(2):88-93

[28] Zheng Y, Zheng M, Ma Z, Xin B, Guo R, Xu X. 8—Sugar fatty acid esters. In: Ahmad MU, Xu X, editors. Polar Lipids. Cambridge: Academic Press and AOCS Press; 2015. pp. 215-243

[29] Björkegren S, Karimi RF, Martinelli A, Jayakumar NS, Hashim MA. A new emulsion liquid membrane based on a palm oil for the extraction of heavy metals. Membranes. 2015;**5**(2):168-179

[30] Rescignano N, Fortunati E, Armentano I, Hernandez R, Mijangos C, Pasquino R, et al. Use of alginate, chitosan and cellulose nanocrystals as emulsion stabilizers in the synthesis of biodegradable polymeric nanoparticles. Journal of Colloid and Interface Science. 2015;**445**:31-39

[31] Shi J. Steric Stabilization, Literature Review, Center for Industrial Sensors and Measurements. Department of Materials science & Engineering Group Inorganic Materials Science. 2002:1-42

[32] Murakami H, Kobayashi M, Takeuchi H, Kawashima Y. Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. International Journal of Pharmaceutics. 1999;**187**(2):143-152

[33] Tham CY, Abdul Hamid Z, Ismail H, Ahmad Z. Poly(vinyl alcohol) in fabrication of PLA micro- and nanoparticles using emulsion and solvent evaporation technique. Advanced Materials Research. 2014; **1024**:296-299

[34] Hixson AW, Crowell JH. Dependence of reaction velocity upon surface and agitation. Industrial & Engineering Chemistry. 1931;**23**(8): 923-931

[35] Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. Journal of Pharmaceutical Sciences. 1963;**52**(12): 1145-1149

[36] Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. International Journal of Pharmaceutics. 1983;**15**(1):25-35

[37] Basheer HS, Noordin MI, Ghareeb M. Characterization of microemulsions prepared using isopropyl palmitate with various surfactants and cosurfactants. Tropical Journal of Pharmaceutical Research. 2013;**12**(3):305-310

[38] Thakker KD, Chern WH. Development and validation of in vitro release tests for semisolid dosage forms-case study. Dissolution Technologies. 2003;**10**(2):10-16

[39] Thumsing S, Israsena N, Boonkrai C, Supaphol P. Preparation of bioactive glycosylated glial cell-line derived neurotrophic factor-loaded microspheres for medical applications. Journal of Applied Polymer Science. 2014;**131**(8):40168

[40] Sonthirak P, Kreua-ongarjnukool N, Thumsing S. Fabrication of PLGA/ GELATIN micro particles for control release bio-substances. International Journal of Advances in Science, Engineering and Technology. 2018;**6**(4): 81-85

[41] Jadhav K, Shetye S, Kadam V. Design and evaluation of microemulsion based drug delivery system. International Journal of Advances in Pharmacological Science. 2010;**1**:156-166

[42] Tomšič M, Podlogar F, Gašperlin M, Bešter-Rogač M, Jamnik A. Water–Tween 40®/Imwitor 308®–isopropyl

myristate microemulsions as delivery systems for ketoprofen: Small-angle X-ray scattering study. International Journal of Pharmaceutics. 2006;**327**(1): 170-177

[43] Bendas ER, Tadros MI. Enhanced transdermal delivery of salbutamol sulfate via ethosomes. AAPS PharmSciTech. 2007;**8**(4):214-220

[44] Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of Controlled Release. 2000;**65**(3):403-418

[45] Metheeparakornchai C, Kreua-ongarjnukool N, Thumsing S, editors. Encapsulation of biological substance in polycaprolactone-graft-chitosan microparticles by layer-by-layer technique in dental application. In: Proceedings of the Pure and Applied Chemistry International Conference (PACCON'19). 2019: p. PO47-PO52. 7-8 February 2019. Bangkok, Thailand: Mahidol University and Chemical Society of Thailand.

[46] Limjeerajarus CN, Sonntana S, Pajaree L, Kansurang C, Pitt S, Saowapa T, et al. Prolonged release of iloprost enhances pulpal blood flow and dentin bridge formation in a rat model of mechanical tooth pulp exposure. Journal of Oral Science. 2019;**61**(1): 73-81

[47] Araújo FA, Kelmann RG, Araújo BV, Finatto RB, Teixeira HF, Koester LS. Development and characterization of parenteral nanoemulsions containing thalidomide. European Journal of Pharmaceutical Sciences. 2011;**42**(3): 238-245

[48] Patel R, Patel K. Advances in novel parentral drug delivery systems. Asian Journal of Pharmaceutics. 2010;**4**: 193-199

[49] Aggarwal N, Goindi S, Khurana R. Formulation, characterization and evaluation of an optimized microemulsion formulation of griseofulvin for topical application. Colloids and Surfaces B: Biointerfaces. 2013;**105**:158-166

[50] Bakshi P, Jiang Y, Nakata T, Akaki J, Matsuoka N, Banga A. Formulation development and characterization of nanoemulsion-based formulation for topical delivery of heparinoid. Journal of Pharmaceutical Sciences. 2018; **107**:2883-2890

[51] Mansour HM, Rhee Y-S, Wu X. Nanomedicine in pulmonary delivery. International Journal of Nanomedicine. 2009;**4**:299-319

[52] Bivas-Benita M, Oudshoorn M, Romeijn S, van Meijgaarden K, Koerten H, van der Meulen H, et al. Cationic submicron emulsions for pulmonary DNA immunization. Journal of Controlled Release. 2004;**100**(1): 145-155

[53] Zhao L, Seth A, Wibowo N, Zhao C-X, Mitter N, Yu C, et al. Nanoparticle vaccines. Vaccine. 2014;**32**(3):327-337

[54] Reed SG, Bertholet S, Coler RN, Friede M. New horizons in adjuvants for vaccine development. Trends in Immunology. 2009;**30**(1):23-32

[55] Akhter S, Jain G, Ahmad F, Khar R, Jain N, Iqbal Z, et al. Investigation of nanoemulsion system for transdermal delivery of domperidone: ex-vivo and in vivo studies. Current Nanoscience. 2008;**4**:381-390

[56] Gadhave RV, Vineeth SK, Gadekar PT. Polymers and polymeric materials in COVID-19 pandemic: A review. Open Journal of Polymer Chemistry. 2020;**10**:66-75

[57] Stojanowski J, Gołębiowski T. Focus on COVID-19: Antiviral polymers in

drugs and vaccines. Polymers in Medicine. 2020;**50**(2):75-78

[58] Gutjahr A, Phelip C, Coolen A-L, Monge C, Boisgard A-S, Paul S, et al. Biodegradable polymeric nanoparticles-based vaccine adjuvants for lymph nodes targeting. Vaccines. 2016;**4**(4):34

[59] Vila A, Evora C, Soriano I, Jato JL, Alonso M. PEG-PLA nanoparticles as carriers for nasal vaccine delivery. Journal of Aerosol Medicine. 2004; **17**:174-185