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Research Article

# Polymeric Microparticles as an Efficient Carrier for the delivery of Proteins and Peptides

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**ABSTRACT**

Peptides and proteins are increasingly being used to treat a variety of ailments. These biologically generated drugs are relatively safe and non-toxic. Given their structural complexity, they provide highly selective therapy for the intended target region. Peptides and proteins, on the other hand, are unstable and quickly metabolized in the biological milieu, making their distribution difficult. Thus, for efficient delivery of these drugs and to protect their integrity, polymeric microparticles are emerged as potential carrier. In this short review, we discussed several aspects related to the design of microparticles loaded with proteins or peptides.

## INTRODUCTION

Polymers are large molecules that can be found naturally or produced synthetically. The polymers are the chain of repeating components called monomers. The term "polymer" originates from the Greek word poly and meros, which mean "many" and "parts," respectively. In general, the molecular weight of a polymer chain increases with its length. The polymerization degree, or degree of polymerization (DP), measures the length of the chain, where small polymers with a low DP are known as oligomers. A polymer's fundamental structure explains the atomic structure and configuration of the polymer chain. The secondary structure of the polymer explains the chain's conformation based on chain interactions, and the tertiary structure of the polymer expresses the folding or shape of the polymer, whereas the quaternary structure showed the shape of a collection of tertiary polymers.

Several polymers are of interest in materials technology, including fibers, plastics, rubbers, and elastomers, while polymers from natural resources include life's building elements, such as proteins, nucleic acids, and polysaccharides. As shown in Figure 1A, polyesters are commonly used polymers that are made by esterifying monomer units, which means reacting an acid and an alcohol, to produce polymer as main product and the water as a byproduct of the reaction. Polyesters and other polymers produced by condensation are hydrolyzed in an aqueous environment[1].

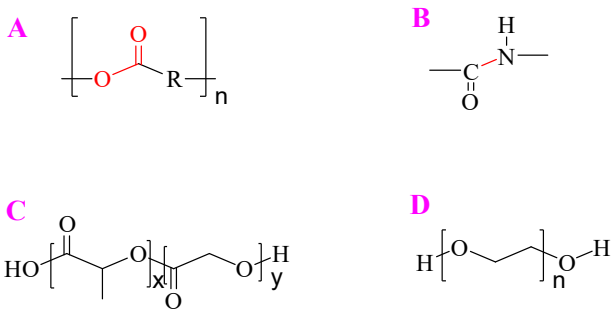


**Fig. 1.** The molecular structure of (A) polyester, (B) peptide bond, (C) poly (lactic-co-glycolic acid), PLGA and (D) poly (ethylene glycol), PEG.

Proteins, also known as polypeptides, are chains of chiral amino acids joined together by peptide bonds (Figure 1 B). Proteins are normally made up of amino acid chains that are longer than 100 units, whereas peptides are made up of amino acid chains shorter than 100 units, similar to oligomers. Peptides with 50–60 residues in length can be synthesized utilizing the solid-phase approach[2]. A single amino acid in a protein or peptide can be acidic, basic, or neutral, while units of tertiary proteins can be positively or negatively charged. Protein`s tertiary structures can be divided into three categories: fibers, membranes, and globular structures. Protein denaturation refers to a permanent alteration in the protein's structure, typically induced by factors such as high temperatures or the introduction of potent chemicals.

## PROPERTIES OF PROTEINS AND PEPTIDES

Proteins and peptides play significant roles in a wide range of biological activities. They help to speed up and move other molecules, regulate gene activity along with immune system functions[3] and can create receptors to support their structures inside (intracellular receptor) and outside (extracellular receptor) of cells[4]. Biologically generated therapies, such as peptides and proteins, are thus becoming popular in the pharmaceutical industry for treating a wide range of disorders, including AIDS and cancer[5]. Research on therapeutic peptide has expanded since the commercialization of the peptide insulin in 1920s, and the number of peptides accepted for clinical usage is continually increasing[6]. Peptides, or proteins, are the main ingredients in many of the new biotechnologically medications that the FDA is testing on humans or looking for treatment of diseases like cancer, autoimmune diseases, and infectious diseases[7]. At the moment, there is a lot of interest in designing protein-based vaccine via oral delivery as well as orally deliverable insulin[8].

Peptides and proteins are safer than other non-biologic medications since therapeutic benefits can be demonstrated at low doses[7]. Peptides are an intriguing class of drug because they have the ability to reach and cover target locations with specificity and selectivity[9]. They have a variable shape and can produce peptide-protein interactions in the cellular milieu, which can then change disease-associated protein interactions[6]. Compared to other smaller molecules, peptide metabolites are rarely hazardous, and the metabolism process is predictable, making them appealing options for the treatment of many disorders[9].

Proteins are extensively utilized as growth factors; however, the formulation of protein delivery systems frequently compromises their bioactivity. Proteins are more likely to lose bioactivity during formulation preparation and processing than smaller-molecular peptides. Peptides can also be utilized to replicate a protein's active area and produce similar therapeutic benefits while being smaller[10]. The synthesis of peptide for medicinal purpose is simple yet potentially expensive[9].

It is important to check the physiochemical and molecular properties of peptides and proteins before using them as medicines, since their shape, molecular weight, stability, and polarity are all very different[8]. The use of peptides and proteins as drugs has several obstacles, including 1) size, 2) hydrophilicity and poor absorption through cellular membranes, and 3) chemical and physical instability.

A protein's molecular weight and three-dimensional structure determine its dimensions. Depending on environmental variables such as pH, temperature, and ionic strength, a protein can exist as a single molecule, a cluster of proteins, or a complex aggregate of proteins, resulting in changes in diameter. When thinking to design a delivery systems for peptides or proteins, it is critical to understand their dimensions[9], since their stability is dependent on conformation[8]. Additionally, a peptide or protein's secondary

and tertiary structures are primarily determine its biological activity[11]. Peptides and proteins are difficult to absorb through cell membranes due to their size and charge[9]. The quantity of anionic and cationic groups on the surface, such as -COO- and -NH3+, as well as the pH of the surrounding environment, determine the electrical properties. The polarity of a protein or peptide influences its solubility and interaction with other molecules. The balance of polar and non-polar groups on the protein's outer surfaces dictates its hydrophilicity or hydrophobicity as well as surface activity. In general, majority of proteins are hydrophilic, or water-soluble[8].

Enzymes in the body are capable of breaking down chemicals like peptides and proteins[8,9]. The peptide chain can be denatured or unfolded by cutting it in half or unfolding it. This process is called hydrolysis. They also tend to agglomerate due to dampness, which can impair their structure[11]. Conformational changes in proteins and peptides occur above the protein denaturation temperature (Tm). The acidity or basicity of the environment, as well as interactions with other chemicals, also have an impact on the stability of peptides and proteins for example, certain salts can reduce protein function[8].

Different storage factors, such as light, temperature, and/or humidity, also play a crucial role in stability or degradation of proteins and peptides. According to Fine et al. [12] one of the most common types of storage instability in peptides used as drug is conformational instability, which can lead to unwanted immunogenic effects in the biological environment and lowers the drug's bioavailability. Apart from the conformational instability, peptides can break down through hydrolysis, oxidation, or deamidation, which can lead to deactivation during storage. The amino acid content determines the breakdown route. To avoid this, peptides should be stored in an airtight container as lyophilized form at neutral pH.

Current research in the field of peptide/protein-based drug delivery focuses on designing peptide delivery systems that are not only secure but also biodegradable, biocompatible, customizable, and cost-effective[13]. Proteins and peptides are unable to pass through cell membranes and are subject to enzymatic degradation, which decreases their bioavailability and effectiveness, especially when taken orally [9]. Applications that allow for prolonged release of a protein or peptide are being developed to eliminate fluctuations in their concentrations after administration[11]. Because of the poor cellular membrane permeability and the access to therapeutic targets inside the cell are limited, most peptide delivery research has focused on targeting outside of the cell[6].

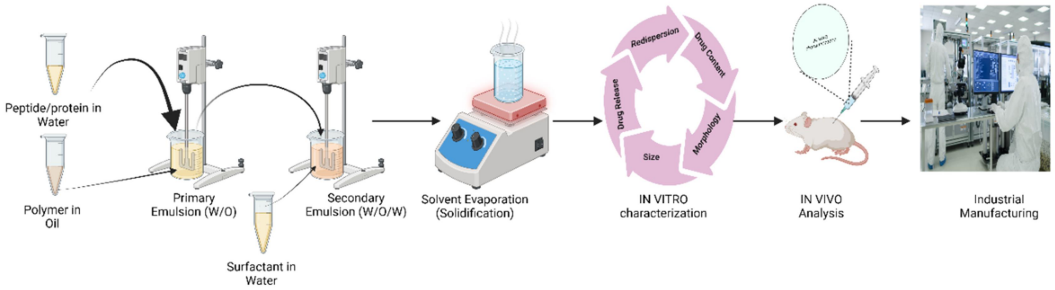


**Fig. 2.** Overview of the preparation of microparticles loaded with proteins/peptide.

## TRANSPORTING PEPTIDES AND PROTEINS USING POLYMERIC MICROPARTICLES

### Micro Particles Properties For The Delivery Of Peptides And Proteins

When designing a carrier for peptides and proteins delivery, the type of desired therapy must be evaluated initially to determine the needs[10]. Delivery systems, especially those based on particles, require certain fundamental features. These include the capacity to be scaled up for commercial production[5], cost-effective manufacturing[8], non-toxicity to cells[14], and sufficient stability during storage. Developing carriers containing biologically produced pharmaceuticals and scaling up for commercialization can be expensive[5]. To keep

the proteins and peptide bioactivity and to protect them from outside factors like enzymatic degradation[10], the carrier should be able to load high quantity of drug and release the loaded cargo in sustained manner at desired location for the therapeutic effect.

The loading capacity (LC) of a carrier for peptide or protein distribution is the highest amount that can be incorporated into a given amount of the carrier. This can be compared to the intended dosage to decide the necessary particles quantities. Encapsulation efficiency (EE) determines the fraction of medicine loaded as well as the fraction lost during formulation preparation. While encapsulation efficiency is vital for drug administration, the amount of wasted drug must also be considered while designing the economic elements of the manufacturing. Loading capacity is determined by comparing the mass of the encapsulated drug to the total combined mass of the carrier and the drug, as illustrated in equation (1). Encapsulation efficiency is established by comparing the mass of the encapsulated drug to the total mass of the drug used in preparation, as illustrated in equation (2)[15].

$$LC = \frac{m_{encapsulated\ drug}}{m_{carrier+drug,total}} \quad (1)$$

$$EE = \frac{m_{encapsulated\ drug}}{m_{drug,total}} \quad (2)$$

When designing a carrier for protein or peptide delivery, it is crucial to carefully choose the loading technique, such as whether the protein or peptide should be loaded before or after fabrication. Additionally, the molecular weight of the drug and the characteristics of the individual amino acids should be taken into account. The dimensions, electrical charge, and water-repellent properties of the peptide or protein drug, along with its interaction with the carrier, can greatly affect the rate at which it is released. Moreover, the release and subsequent functionality of the drug are influenced by whether it is in its free form or conjugated to the carrier[10,16].

In biomedical applications, natural and synthetic polymers such as polyesters, polyanhydrides, polysaccharides, and polyethers are most frequently utilized polymers[17]. Hyaluronic acid and gelatin, both natural polymers are of interest because they are both water-loving, biodegradable materials that can be used to prepare hydrogel carriers.Out of various synthetic polymer poly(lactic-co-glycolic acid), sometimes known as PLGA[5] is a common polymer utilized in microparticles preparation[10]. The use of biodegradable polymers is advantageous due to its enzymes or water based hydrolysis in the biological environment [5]. Biodegradable polymers are most often biocompatible found naturally or synthesized in laboratories. Properties that requires in a polymer for fabrication of degradable micro particles are that it is stable, breaks down slowly in biological environments, dissolves easily in organic solvents, has a low melting point, and has the right mechanical properties like being flexible and tough[17].

Polymeric micro particle can be administered as an injectable formulations but, the process of creating long-acting injectable polymeric carriers is challenging and expensive. FDA granted approval in 1986 for the first injectable carrier composed of degradable PLGA, which was designed for delivering the peptide triptorelin acetate. Subsequently, several comparable formulations have received approval[18]. For syringe injection, biodegradable polymer microparticles should have a diameter of less than 125 μm[5].

Microparticles are defined by their diameter, which ranges from 1-1000 μm[19], rather than their internal or exterior structure. The final size of the micro particle in injectable formulations is a compromise between the ability to inject, which is better with smaller particles and the ability to encapsulate, which is often worse with smaller particles, and the ability to release, which is faster with smaller particles[5]. Microspheres and microcapsules are distinct forms of microparticles. Microspheres are characterized by their spherical shape, whereas microcapsules possess a core that can be either solid or non-solid, which distinguishes them from the substance that makes up the surface. Unless otherwise specified, a microparticle is generally assumed to possess a uniform/homogenous internal composition consisting of a polymer and a pharmaceutical compound. Figure 3 illustrates the characteristic structures of microparticles loaded with drugs.

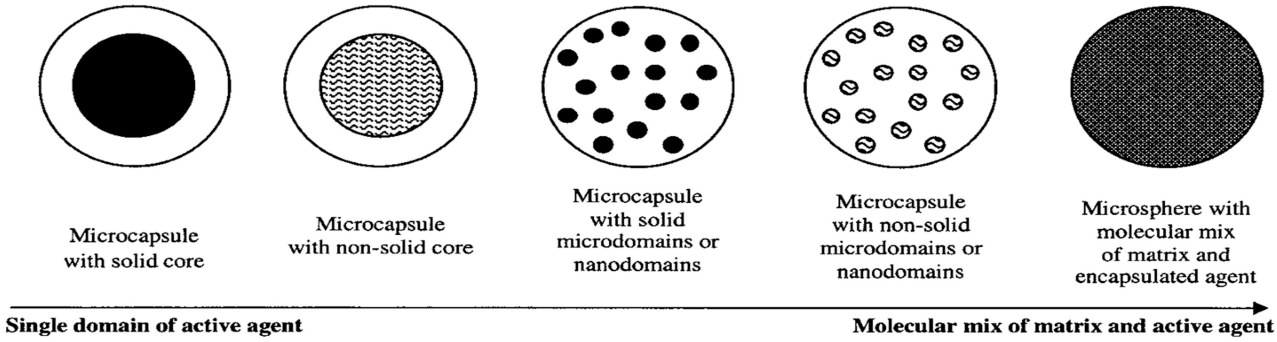


**Fig. 3.** Typical structures of drug-loaded microparticles [19].

**Effects of Different Parameters on Microparticle Preparation**

Microparticles can be synthesized using several methods, such as double emulsion and spray drying. This article focuses on the double emulsion technique, which is particularly well suited for encapsulating hydrophilic medications, such as peptides and proteins, within microparticles. The double emulsion approach is a frequently employed technique in clinical settings to produce PLGA microparticles loaded with peptides[11] and this method is commonly used to produce drug-loaded particles spanning a range of diameters, from a few micrometers to several hundred micrometers.

The double emulsion procedure is often used to make water-in-oil-in-water (w/o/w) emulsion. This procedure starts with creating a primary water-in-oil emulsion and then solidifying its particles in a secondary aqueous phase[11]. The therapeutic compound is dissolved in a water phase, and then the polymer is dissolved in an oily phase, like dichloromethane. A primary emulsion is made when the continuous phase is mixed, sonicated, or homogenized to make droplets of the dispersed phase. Surfactants are substances that are employed to keep the formulation stable and hinder the preliminary droplets from aggregation. When the primary emulsion is emulsified with a secondary water phase, micrometer-sized droplets form. A biocompatible, non-immunogenic stabilizing ingredient that dissolves easily in water should be used in the secondary emulsion. Polyvinyl alcohol (PVA) is one of that example. Greater shear forces result in smaller particles. When the oil phase is removed or evaporated, the final polymeric microparticles become solid and then they are taken out of the solution, cleaned, and freeze-dried (lyophilized) for long term storage. Organic solvents used to prepare microparticles with hydrophobic polymer mixtures can affect the activity of sensitive peptides or proteins[5]. Figure 4 depicts the double emulsion process used to create a water-in-oil-in-water emulsion.

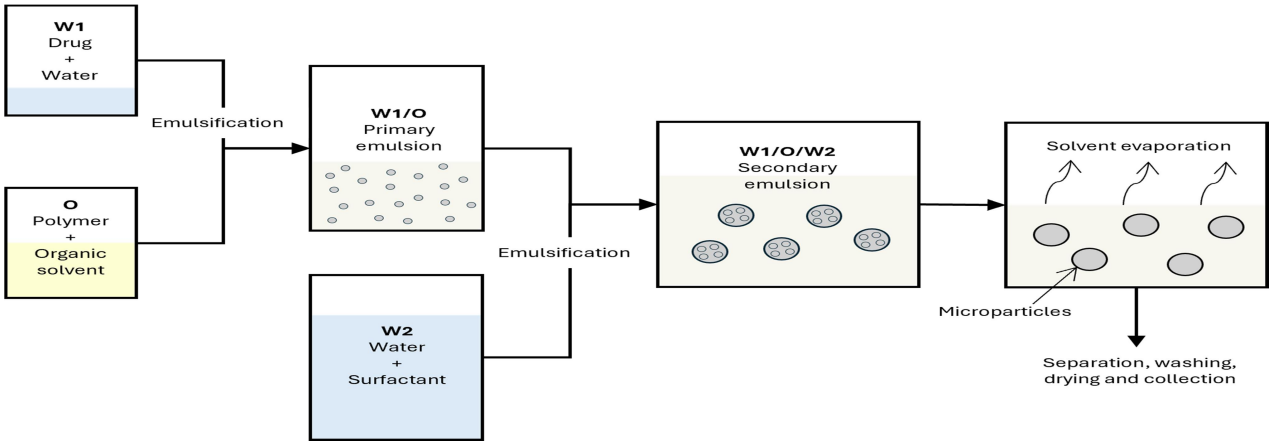


**Fig. 4.** Scheme of the double emulsion solvent evaporation method, where W1 = the first aqueous phase, O = the oil phase and W2 = the second aqueous phase[20].

Organic solvent commonly removed via evaporation, stabilizes their interior porous structure. The modes and rate of evaporation can have a significant effect on the final particle structure and, consequently, releasing properties. Slow drying rates have been demonstrated to produce coherent particle architectures while decreasing encapsulation efficiency. Faster drying rates can be achieved, for example, by raising the temperature of the solution, which weakens the overall release later, or by utilizing a more water-miscible organic solvent or a combination of organic solvents. During quick drying, some solvent residue may remain in the microparticle core, posing a safety risk.

Making a double emulsion instead of a single emulsion complicates the process and its stability, and hence affects particle creation. Furthermore, the stability of the double emulsion is highly correlated with drug loading, encapsulation efficiency, and, as a result, the drug release profile. Even though a high concentration of the drug makes loading better, but the solubility and stability of the peptide or protein, limit the concentration in the preliminary aqueous phase because they clump together at too high a concentration. Excipients, such as sugars or polyethylene glycol (PEG), can be utilized to improve peptide and protein stability[5].

In the double emulsion method, the amount of peptide or protein encapsulated is depend on how stable the primary emulsion is, how quickly the organic solvent evaporates, and how well the different phases in the colloidal system are balanced[11]. Surfactants have been found to reduce encapsulation capacity in certain cases. However, adding a surfactant like PVA to the preliminary emulsion has been shown to increase the time of peptide and protein releases by creating smaller primary droplets[5].

Finally, the viscosity of the phases influences encapsulation performance[11]. The polymer content in the oil phase influences the stability of the primary droplets, and a diluted oil phase has a reduced viscosity. Due to this, the primary droplets are highly movable [5]. Additionally, if there is too low viscosity in the organic phase, the peptide in the preliminary emulsion may leak into the secondary aqueous phase[11]. At low polymer concentrations, the final microparticles solidify more slowly as the organic phase is removed; the particles get smaller as the secondary droplets solidify; and the thin polymer phases that form in the final carrier often fail to control drug release[20]. The elements that contribute negatively to emulsion stability can be avoided by optimizing the microparticle preparation parameters[11].

According to optical microscope observation, microparticles in dual emulsions form within a variety of morphologies, including plain microparticle structures and capsules. When the primary aqueous phase droplets inside the secondary emulsion droplets mix as the solvent evaporates, honeycomb-like inner structures can also form. It has been demonstrated that the morphology is altered by the stirring speed during secondary emulsification. Honeycomb structures are most common at low swirling speeds; capsules become more abundant as the stirring speed increases; and plain particle formation is most prevalent at high stirring rates. The honeycomb-like structure appears to be the best for effective drug encapsulation[21].

**Mechanisms of Protein and Peptide Loading and Release**

Because most bioactive proteins are hydrophilic, they require polar groups, such as hydroxyl, carboxyl, sulfate, or phosphate, on the microparticle carrier in order to load efficiently. On the other hand, the carrier can make electrostatic contacts with groups on the protein that have opposite charges, as well as hydrophobic interaction between the non-polar groups on the protein and the carrier. The quantity of anionic -COO- and cationic -NH3+ groups is important because the electrical characteristics of peptides and proteins determine their encapsulation and release properties. This fluctuates according to the surrounding pH. Peptides may be broken down faster by enzymes than proteins, and the attachment of a peptide to a polymeric carrier may depend more on ionic and hydrophobic interactions to ensure the best release rates and good loading. Furthermore, once entrapped, peptides may be more challenging to keep in the carrier due to their smaller size than proteins.

The peptide and protein can be released through diffusion, degradation, or by both. [10], depending on the type of used polymer. Diffusion from particles is the most straightforward method. Other mechanisms include changes in pore size, chemical interactions, or particle network disintegration (dissociation). This can occur as a result of changes in the immediate environment's temperature, pH, enzyme activity,

or ionic strength, which impair the electrostatic contacts within the carrier and the peptide and protein. As an example, at low pH levels, anionic polymers usually attract proteins and peptides; however, at elevated pH values, they normally oppose one another due to the charge of peptides tending to shift from positive to negative as pH increases. The release of loaded peptide and proteins can be controlled by adjusting such variables while tailoring the administration accordingly. However, proteins frequently contain both anionic and cationic surface groups[18].

In non-degradable carriers, the peptide or protein is released when water enters the polymeric matrix and dissolves the drug. The peptide or protein then moves through the porous network of the hydrophobic polymer phase to the outside water phase. While non-degradable carriers release drug without polymer degradation, degradable polymeric transporters often release at the same rate as polymer degrade. Degradable polymeric particles release biological drugs or peptide or protein more rapidly than non-degradable particles because they are shorter and have a bigger surface area within a given volume[5]. Some synthetic degradable polymers, like PLGA, produce an acidic waste when they break down, which can harm the surrounding cellular biological system[10].

Biological drugs released from microparticles made using the double emulsion method usually start with a burst release[11] before the polymer starts to break down significantly. This is followed by a period of dormancy, during which the polymer starts to break down, and finally a period of sustained, slower, and continuous release[12]. The first burst release is often linked to the peptide or protein dissolution at the surface of the microparticles or the release of a drug trapped in the surface layers of the microparticles instead of an encapsulated peptide or protein [11] but it's not just a surface event. Pagels and Prud'homme[5] report that subsequent bursts may also be noticed. Moreover, fissures in the precipitating polymer wall may develop when the first droplets' solvent is removed, which could have an impact on the release[21]. The closure of surface pores, which happens when the microparticles are heated above the glass transition temperature (Tg) of the polymer, prevents surface release. To achieve the longest feasible sustained release profile for long-term protein or peptide treatment, burst release should be minimized.

The initial burst release causes an elevated regional drug concentration, which can produce local toxicity, whereas proper burst release allows the drug's first therapeutic benefits to occur. By changing the shape or composition of the microparticle, coating it with a drug-free material, or extracting it for a longer time, the burst release can be slowed down or stopped. This will lead to a more continuous release profile.

The polymer breakdown or degradation rate often determines the release following the burst release[11]. For example, the breakdown rate of PLGA has been found to be associated with the release rate of a peptide or protein, but polymer breakdown is not required for release[5]. The release during the sustained or continuous phase depends on the carrier structure's integrity (for example, the amount of van der Waals forces it generates) and the ratio of its parts. During continuous release, the adsorption and desorption of the protein from the polymer also change the rate at which the protein is released. To ensure full release, protein adsorption should be avoided. The overall release characteristics remaining stationary after the burst release and can be attributed to a polymer breakdown rate that is excessively slow in the given time period[11].

**Commercially Available Polymers for the Delivery of Peptides and Proteins**

Polyester poly(lactic-co-glycolic acid), or PLGA (Figure 1C), is the most common degradable hydrophobic polymer used for peptide or protein delivery because it is biocompatible, breaks down predictably in living tissue[5], isn't toxic, and has the appropriate mechanical properties[22]. PLGA microparticles have been found to be effective in delivering peptide or protein over time. Since the 1980s, PLGA-based microparticles have been clinically used for long-term peptide administration.

PLGA is a lactic acid and glycolic acid copolymer that is readily available in a variety of formulations[22]. The homopolymer poly(lactic acid) (PLA) is one of the most commonly used polymers in pharmaceutical applications due to its biocompatibility, biodegradability, and bio absorbability, as well as its tiny carbon footprint. However, PLA's hydrophobic surface makes it less effective for encapsulation of the drugs[17]. It also breaks down more

slowly than the copolymer PLGA[22], which limits its use in drug delivery[17].

PLGA is soluble in a number of solvents, including ethyl acetate, dichloromethane, acetone, chloroform, and tetrahydrofuran. Direct polycondensation of glycolic acid and lactic acid or ring-opening polymerization of lactide and glycolide can be used to synthesize it. The latter usually leads to polymer with higher molecular weights than the former [22]. In water, PLGA breaks down through bulk erosion[5] , and ester linkage hydrolysis[22]. Changes in the amounts of lactic acid and glycolic acid, as well as the ester-terminated versus carboxylic-acid-terminated PLGA, can affect how well it breaks down, how much drug it can release, and how quickly it releases. PLGA is a polymer that can be tailored for peptide-based drug delivery because of its higher molecular weight and greater lactic acid to glycolic acid ratio, which both make the chain more hydrophobic and slower degradation [5].

Peptides could be prepared for one to six month release duration using PLGA[11]. Three commercially available peptides delivered by PLGA-based systems include octreotide acetate, leuprolide acetate, and exenatide acetate[23]. However, the use of PLGA microparticles to deliver peptide or protein is still required optimization. For instance, PLGA microparticles increase the initial burst of peptide-based therapeutics, and the polyester breakdown products are acidic, which lowers the pH of the surrounding environment. As a result, adverse therapeutic effects and the degradation of peptide can happen[11].

When PLGA degrades, carboxyl and hydroxyl end groups are produced. The enhanced hydrophilicity of PLGA with free carboxylic acid end groups, or PLGA-COOH, results in increased water absorbance and hydrolysis and a higher degradation rate [22]. However, conjugation of cationic peptides to PLGA with COOH end groups via an acylation reaction in an enthalpically driven way are feasible such as cationic peptide octreotide's with N-terminal primary amines could interact with two COOH groups.

In addition to acylation, cationic octreotide and leuprolide have been shown to be efficiently absorbed by PLGA-COOH via so-called remote loading, which does not require complex microparticle formation processes or the presence of an organic solvent, but result in high drug encapsulation[24]. Cationic peptides can bind to the surface and move into the polymer network when PLGA-COOH has enough chain mobility[25]. There is some disagreement about how peptides are encapsulated within PLGA-COOH. One concept is that the peptide forms a salt pair or ionic bond with carboxylic anions (-COO-) on the surface of the polymer. For the deprotonation of the -COOH group a basic environment is required [23].In conclusion, interactions between a cationic peptide and the -COOH functionality of PLGA can improve peptide and protein loading[24] and the interaction between peptide, PLGA, and COOH is important for making new and standard peptide-PLGA formulations[23].

Also, the polymer polyethylene glycol, or PEG (Figure 1D), has been used in peptide and protein delivery to keep the drugs safe from degradation by enzymes, improve water solubility and avoid protein/petide agglomeration. PEG is non-toxic and has a higher hydrophilicity than other polymers of comparable size. However, peptide or protein delivered by attaching to the PEG may face steric hindrances[3]. This is because there aren't enough acceptable functional groups, and PEG doesn't degrade which limits its use [14].

PEG is frequently mixed with a water-repellent polymer to produce an amphiphilic polymer that has both hydrophilic and hydrophobic components and this amphiphilic polymer can be used as an emulsifier to make stable double emulsions for drug loading in the colloidal system. At the right concentration, PEG acts like an amphiphile. The oil-to-water ratio determines how well PEG stabilizes an emulsion. Similarly, removal of PEG has been demonstrated to result in a separated emulsion in a specific formulation that benefited from the presence of PEG [26]. Overall, amphiphilic polymeric surfactants have gained popularity because their composition may be adjusted, allowing for greater control over emulsion morphologies, for example.

## MICROPARTICLES CHARACTERISATION METHODS

### Laser Diffraction

The most popular analytical technique for determining particle size distributions is laser diffraction. Although it works best with sizes larger than 4 µm, laser diffraction can assess dry or wet particles with diameters ranging from 0.01

to 3500 µm. The system is based on the phenomenon of laser light scattering from the studied particles. Since larger particles scatter light at higher intensities and lower angles, while smaller particles scatter light at lower intensities and greater angles, the light intensity and angle of scattering provide information about particle size. The Dv10, Dv50, and Dv90 percentiles, which denote that ten, fifty, or ninety percent respectively, of the particles are smaller than the given diameter, are commonly used to describe the particle size distribution. Considering that most microparticles are not spherical in shape but the laser diffraction analyses the samples believing the particles to be spherical, which might sometimes provide false results [27].

**Optical Microscopy**

Optical microscopy, sometimes referred to as light microscopy, is an inexpensive analytical method for observing, quantifying, and examining objects and living things that are too small for the naked eye. It is becoming more and better applied in biomedical research[28]. The morphology of the microparticles must be understood to calculate the ideal drug dosages and release rates and to ensure product quality. Laser diffraction is sometimes used in conjunction with optical microscopy studies to offer a more comprehensive understanding of the stability and performance of the microparticles [27].

**UV-Vis Spectroscopy**

Ultraviolet-visible (UV-Vis) analysis is a popular scientific approach for identifying, characterizing, and quantifying analytes in a wide range of applications, including drug development technology. It is based on the phenomenon where photon energy causes distinctive electronic transformations in an analyte. The sample analyte absorbs light from a monochromatized light lamp using lenses and an optical component capable of diffracting light to a certain wavelength. Following that, a UV-Vis detector processes the signal output and looks for any transmitted light that the sample in the cuvette has not yet absorbed. The sample is typically in liquid form and must interact with UV-Vis radiation. Each substance has a unique range of absorbed wavelengths based on its chemical structure. The substance being examined is frequently colored in order to absorb visible light. If the sample is not colored, it absorbs ultraviolet light rather than visible light.

When examining data, UV-Vis absorption is characterized using Beer-Lambert's law. It states that the absorbance “A” is proportional to the concentration “c” of the absorbing material at a constant analytical route “b”, or the length of a sample cuvette. Less light passes through the sample with higher “A” levels. In quantitative analysis, the wavelength at which the maximum absorbance is detected is used, and calibration curves for the analytes at fixed concentrations are made. Although a calibration curve should ideally be linear with a zero y-intercept, in practice they diverge because particles in a sample become less independent as absorbance increases. Moreover, the refractive index of the sample varies with concentration, impacting absorptivity; at low concentrations, the refractive index can be considered constant, resulting in a linear calibration curve. Therefore, it is often recommended to use an absorbance range of $0.3 < A < 1.0$. Dilute the sample appropriately to prevent absorbances greater than 1.0 a.u.[29]. The amount of loaded peptide or proteins in the microparticles are often determined by using this technique.

**SEM**

A scanning electron microscope, or SEM, works on the principle of focusing a beam of electrons onto a sample, causing the emission of various signals that can be used to produce an image. This high-resolution imaging technique is commonly used to study the surface morphology of microparticles. By SEM, the microparticles can be studied in powder and in solution form. The surface morphology of microparticles provides valuable information about their physical and chemical properties, aiding in various research and industrial applications. Additionally, SEM can also be used to determine the size, shape, and distribution of particles in a sample, making it a versatile tool for the characterization of microparticles[30].

**HPLC**

High performance liquid chromatography is an advanced analytical technique compared to UV-vis spectroscopy. Basically, the instrument is equipped with an autosampler, high-pressure pump, column, detector, and data analysis software. Utilizing high-pressure pumps, a mobile phase is

pumped through a column (stationary phase). The compound gives a peak based on its interaction with the stationary phase, allowing for the separation and identification of components in a mixture. HPLC is commonly used in the pharmaceutical, environmental, and food industries for quality control and research purposes due to its ability to detect trace amounts of compounds in a sample[31].

In polymeric microparticles, HPLC is used to precisely measure the active ingredients, to find any degraded products, and to check the purity of the active ingredients[32]. HPLC is essential for quality control because it gives more reliable results than UV spectroscopy[33].

## IN VITRO DRUG RELEASE

The term drug release defines the process by which a drug is released from its polymeric microparticle and becomes available for absorption in the body[34]. Understanding drug release is crucial for optimizing the effectiveness and safety of the drug. For oral dosage forms, the "dissolution" test is usually called a performance test because the drug is supposed to dissolve quickly in the test medium. On the other hand, for non-oral dosage forms like injectable microparticles and transdermal delivery systems, the test is usually called a drug release or in vitro release. For injectable microparticle formulation, in vitro drug release methods are divided into three main categories: (1) sample and separate; (2) dialysis membrane; and (3) continuous flow techniques. In the dialysis membrane method, the pre-dispersed or dissolved microparticle can be placed in the dialysis membrane, shaken in releasing media, and then samples are to be withdrawn at a specific time, measured, and plotted to understand the drug release pattern[35].

## CONCLUSION

Polymeric microparticles offer a viable and flexible method for delivering peptides and proteins, effectively addressing various issues involved with administering these drugs. The microparticle delivery system has numerous advantages; including preventing the drug from degradation, providing controlled and sustained release, and the possibility of targeted delivery. Various polymers, both natural and synthetic, have been explored, each contributing unique properties that can be tailored to specific applications. Various techniques, such as emulsification, spray drying, and solvent evaporation, have been optimized to prepare microparticles. However, a double emulsification technique has been mostly employed to prepare microparticles with desired characteristics, ensuring efficient encapsulation and release profiles. Despite some of the commercially available polymers, there is a need to develop new polymers with enhanced properties for the preparation of polymeric microparticle.

**Author Contribution:**

Conceptualization, K.K.B; data collection and resources, I.K.; writing—original draft preparation, I.K and V.K; writing—review and editing, K.K.B. and C-E.W; supervision, K.K.B and C-E.W All authors have read and agreed to the published version of the manuscript.

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