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Review of Development of Ganciclovir as Potential Ophthalmic Drug Delivery Systems

Lidya Agriningsih Haruna [1], Rathapon Assutjarit [1], La Ode Muhammad Julian Purnama [1], Muhammad Hajrul Malaka [1], Sewan Theeramunkong [1], Wirhamsah Al Ramadan [2], Adryan Fristiohady [1,2,*]

[1] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Thammasat University Research Unit in Drug, Health Product Development and Application (DHP-DA), Thammasat University, Khlong Luang, 12120, Pathum Thani, Thailand; Lidyaagriningsih38468@gmail.com (L.A.); rathapona@hotmail.com (R.A.); laodemuhammadjulianpurnama@gmail.com (L.O.M.J.P.); mhajrulmalaka@gmail.com (M.H.M.); sewan@tu.ac.th (S.); adryanfristiohady@uho.ac.id (A.F.);
[2] Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Halu Oleo, Sulawesi Tenggara, 93232, Indonesia; wirhamsahtkj.2001@gmail.com (W.A.-R);
* Correspondence: adryanfristiohady@uho.ac.id (A.F.);

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Abstract: Ganciclovir (GCV) plays a vital role in the treatment and management of ocular viral infections such as Herpes simplex virus (HSV) and cytomegalovirus (CMV) retinitis. However, GCV has low corneal penetration, is poorly permeable across the membrane, and has poor drug bioavailability, which poses a challenge in treating eye diseases. Besides that, conventional topical eye formulations, such as eye drops, gels, and ointments, have limitations such as poor tear turnover, poor residence time of the drug, frequent dosing intervals, wastage of dosage, and excessive systemic absorption leading to poor ocular bioavailability. Many strategies have been investigated to improve corneal permeation and ocular bioavailability of GCV. The journal review was written using the library study method from 2001-2023, which contained information about the Ganciclovir Formulation for Ophthalmic Drug Delivery System. The journal review discussed some approaches to achieving therapeutic goals for GCV. The results of this review showed that some of these approaches, including liposome, microemulsion, nanoparticle microsphere, polymeric nanoparticles, and gold nanoparticles, can improve the limitation of conventional formulation of GCV by increasing penetration, permeability, as well as bioavailability GCV in the ocular.

Keywords: ganciclovir; nanoparticle; ocular; liposome; microsphere.

1. Introduction

Herpes simplex virus (HSV) and cytomegalovirus (CMV) retinitis have been the most common viral infections observed worldwide. Following a primary ocular herpetic infection, latency of the virus occurs, followed by recurrent and relapse of ocular viral infections, which can lead to corneal perforation and structural damage of the cornea, resulting in blindness [1-3]. Ganciclovir (9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine) (GCV) (molecular weight 255.23), a synthetic acyclic nucleoside analog of guanine is a broad-spectrum antiviral drug exhibits antiviral activity against HSV and CMV [1, 3-6].

GCV is vital in treating and managing ocular viral infection to prevent viral DNA replication, but it does not eliminate the virus from the tissue. Hence, long-term therapy is necessary to control the disease [1, 7]. GCV competitively inhibits viral replication mainly through two mechanisms: (i) GCV-5′-triphosphate inhibits viral deoxyribonucleic acid (DNA)

polymerase by impairing the viral DNA synthesis, the formation of deoxyguanosine triphosphate, and hence the replication of the virus. (ii) GCV triphosphate is directly combined with DNA chains and prevents further viral replication [3, 5, 8].

GCV is a hydrophilic molecule (water solubility 4.3mg/mL at 25°C) with a low partition coefficient (logP=-1.66), low corneal penetration, and poorly permeable across the membrane, belonging to class III Biopharmaceutics Classification System (BCS) cause the ocular bioavailability of GCV to be poor [8-10]. Poor drug bioavailability poses a challenge in treating diseases affecting the eye [11].

Various formulations are available in the ocular drug delivery system but are less efficient in treating the disorders of the anterior and posterior eye segments [8]. The conventional topical eye formulations, such as eye drops, gels, and ointments, have limitations such as poor tear turnover, poor residence time of the drug, frequent dosing intervals, wastage of dosage, excessive systemic absorption leading to poor ocular bioavailability (only 5% for eye drops) [12]. The poor bioavailability of conventional topical eye formulations is due to a lack of corneal permeability, nasolacrimal drainage, and metabolic degradation. Hence, an optimum treatment must be considered for the effective management of ocular viral diseases [3] by increasing the residence time and controlling the drug release to avoid drug loss due to tear drainage and reducing dosing to prevent fluctuations in the ocular drug concentrations and thus decrease systemic side effects [12].

Intravitreal GCV drug administration became the gold standard for drug delivery to the posterior eye tissues and is developed to achieve therapeutic GCV concentrations in the vitreous with minimal systemic toxicities, which leads to considerable improvement and stabilization of HSV and CMV retinitis in treated patients. If a drug is intended to be administered intravitreally, it should possess adequate solubility, long retention, and sufficient permeability to target tissues and cells [2, 11]. Intravitreal therapy is proposed as a safe and well-tolerated alternative route for treating retinitis or retinal infections. However, due to the short vitreal elimination half-life of 7–10h, frequent intravitreal injections are necessary to maintain therapeutic levels of GCV, causing discomfort to the patient. Moreover, these continuous administrations risk emerging cataracts, retinal detachments, hemorrhages, or endophthalmitis [1-3, 5-7].

Besides that, the unique anatomy and physiology of the eye prevent these drug delivery systems from delivering the drug to the site of action. In the ocular drug delivery system, various formulations are available but possess less efficiency in treating the disorders of the anterior and posterior segments of the eye. Despite its high hydrophilicity and minor molecule nature, owing to its low lipophilicity, GCV must struggle to cross lipophilic barriers of the eye, like lipidic and mucin layers of the tear film and tight junctions of the corneal epithelium. The delivery of the drug to the eye is still challenging in the pharmaceutical field due to the presence of static Blood aqueous barrier (BAB), Blood retinal barriers (BRB), and dynamic barriers (Conjunctival and choroidal blood flow, tear turnover, and nasolacrimal drainage). Thus, it is a challenge for the formulator to cross the entire barrier without causing tissue damage/toxicity to the eye [8].

Many strategies have been investigated to improve the corneal permeation of GCV. Many novel formulations have been employed to improve ocular bioavailability. A new ophthalmic delivery system designed which aims at (1) enhancing corneal permeability and ocular bioavailability; (2) prolonging precorneal residence time (more than 2 min); (3) providing constant and sustained release of entrapped drugs; (4) minimizing precorneal drug

loss and side effects; and (5) reducing the recurrence of keratitis [10]. Because of this, various techniques or ways were applied to GCV to improve its lipophilicity or enhance its permeability through barriers to become available at the site of action. These approaches included prodrug formation, liposome, albumin nanoparticle, PLGA microsphere, mucoadhesive nanoparticle, in situ gel, etc. Most of these approaches explore the possibility of enhancement of lipophilicity, thereby increasing the permeability of GCV via ocular cellular barriers [8]. In this review study, various alternative strategies are employed to achieve therapeutic goals for GCV **(**Table 1**)** [10].

Table 1. Ganciclovir formulation for ophthalmic drug delivery system.

No	Drug Delivery Systems		Formulation	Cells	Target site	Mechanism	Ref.
1	Liposomes (LPs)	Transferrin (Tf)-conjugated liposomes loaded GCV (Tf-GCV-LPs)	Intravitreal injection and topical instillation	ARPE-19 and MRC-5	Retina	Intracellular uptake in the ARPE-19 cells indicated that Tf-GCV-LPs were taken up by the cells via TfRs-mediated endocytosis and inhibited the activity of CMV Glycoprotein B in the infected cells.	[6]
2	Microspheres (Ms)	Ganciclovir chitosan micropheres (GCV-CS-Ms)	An ophthalmic topical preparation	-	Retina and cornea	The mucin on the surface of the cornea and conjunctiva carries a negative charge. The positively charged chitosan from GCV-CS-Ms interacts with sialic groups and sulfonic acid substructures of mucin and acts as an adhesive force on the eye surface. Subsequently, the positive charge interacts with the cell membrane and helps the drug permeate the corneal surface.	[3]
		Ganciclovir (GCV)-loaded poly(lactide-co-glycolide) (PLGA) micropheres (Ms)	Intravitreal administration	-	Retina	-	[1-2]
3	Microemulsion (ME)	GCV o/w ME GCV w/o ME GCV o/w ME with chitosan (CSME)	Topical solution (eye drops)	SIRC and ARPE-19	Retina and cornea	The drug loaded in the ME exists mainly in the internal phase. The drug can be distributed among dispersed phase, continuous phase, and surfactant interphases. The lachrymal drainage does not eliminate the adsorption of the nanodroplets on the cornea and acts as a reservoir of the drugs. After installation, the oil drops act as reservoirs that continuously release the drug to the tear film as the drug gets absorbed into the ocular epithelia. Positive charge from chitosan facilitates binding and allows electrostatic interaction	[8] [21-23]

No	Drug Delivery Systems		Formulation	Cells	Target site	Mechanism	Ref.
						with negatively charged groups in the mucus of the cornea.	
4	Polymeric Nanoparticles (NPs)	Ganciclovir Loaded Albumin nanoparticles (GCV-Albumin-NPs)	Intravitreal administration	-	Retina	-	[7, 24]
		Gancivlovir Loaded Chitosan Nanoparticles (GCV-CS-Nps)	-	-	Cornea	CS has a positive surface charge, which can form electrostatic interactions with the negatively charged mucin of mucous on the surface of the cornea and conjunctiva.	[25-27]
		Ganciclovir Loaded PLGA Nanoparticles (GCV-PLGA-NPs)	Topical administration	HCEC cells	Cornea	-	[10]
5	Gold Nanoparticles (AuNPs)	Ganciclovir (GCV) Loaded Glutathione (GSH)-Modified AuNPs	Topical administration	ARPE-19 cells	Retina and Cornea	AuNPs can cross the blood-retinal barrier, reach all retina layers, and bind to cell membranes. Topical AuNPs in guinea pig eyes can penetrate the corneal cell layer without changing the morphology of the endothelium.	[28-31]

2. Materials and Methods

The data used in writing this journal review was collected using the literature study method from primary and secondary literature. Literature searches were conducted using online-based library search instruments such as NCBI-PubMed, Google Scholar, ScienceDirect, and Elsevier from May 2023 to July 2023. The keywords and phrases used during the search were ““Ganciclovir Nanoparticle””, ““Drug Delivery of Ganciclovir””, ““Ganciclovir Nanoparticle””, and ““Ophthalmic Delivery System of Ganciclovir””. The works of literature collected were then arranged according to the framework, the Ganciclovir Formulation for Ophthalmic Drug Delivery System data were compiled in tabular form, and journal review writing was carried out according to the format provided. From the literature study results, the journals used were from 2001-2023, which contained information about the Ganciclovir Formulation for Ophthalmic Drug Delivery System, shown in **(**Table 1**)**.

3. Results and Discussion

Nanoparticles (NP) are providing a new tool to overcome an unmet clinical need, especially in the ophthalmic field, with significant improvements in drug delivery [13]. NPs consist of a solid core with polymer around it, and their size ranges from 100 to 1000nm [14, 15]. There are two types of NPs: nanospheres and nanocapsules (Figure 1). Nanospheres are matrix systems where the drug is adsorbed on the surface or evenly dispersed into the matrix. In contrast, a nanocapsule is defined as a vesicular system where the inside of the core can be different from the outer polymer. In such systems, the drug is usually dissolved in the particle

core but can also be adsorbed on the surface [15-17]. The nanocapsule consists of an oil-filled cavity surrounded by a polymer with a size ranging from 10 to 1000nm [14, 15].

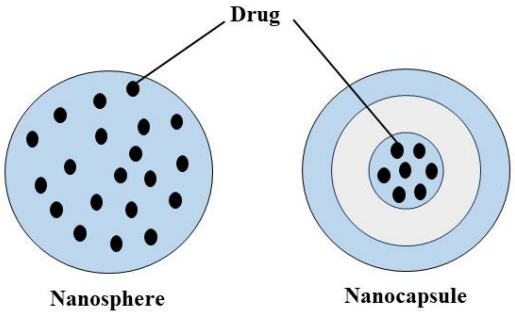

Figure 1. Nanosphere and Nanocapsule [17].

NPs are coated with hydrophilic polymers or functional antibodies, which enable targeted drug delivery [15, 16]. NPs are defined as particulate dispersions generally produced from biodegradable polymers with sizes of 10-1000nm [18]. NP has been used for drug delivery because [19] it has broad advantages as a drug delivery system compared to conventional systems, such as biocompatible, biodegradable, easy to manufacture, non-toxic, non-immunogenic, controlled release, increased drug stability, and bioavailability, as well as site-specific targeting to specific organs or tissues [18, 20]. Properties of NPs such as hydrophobicity, size, charge, mucoadhesion, and surface ligands, as well as the route of administration and suspension media, influence their ability to overcome ocular barriers and distribute them in the eye and must be carefully designed for specific target tissues and eye diseases [20].

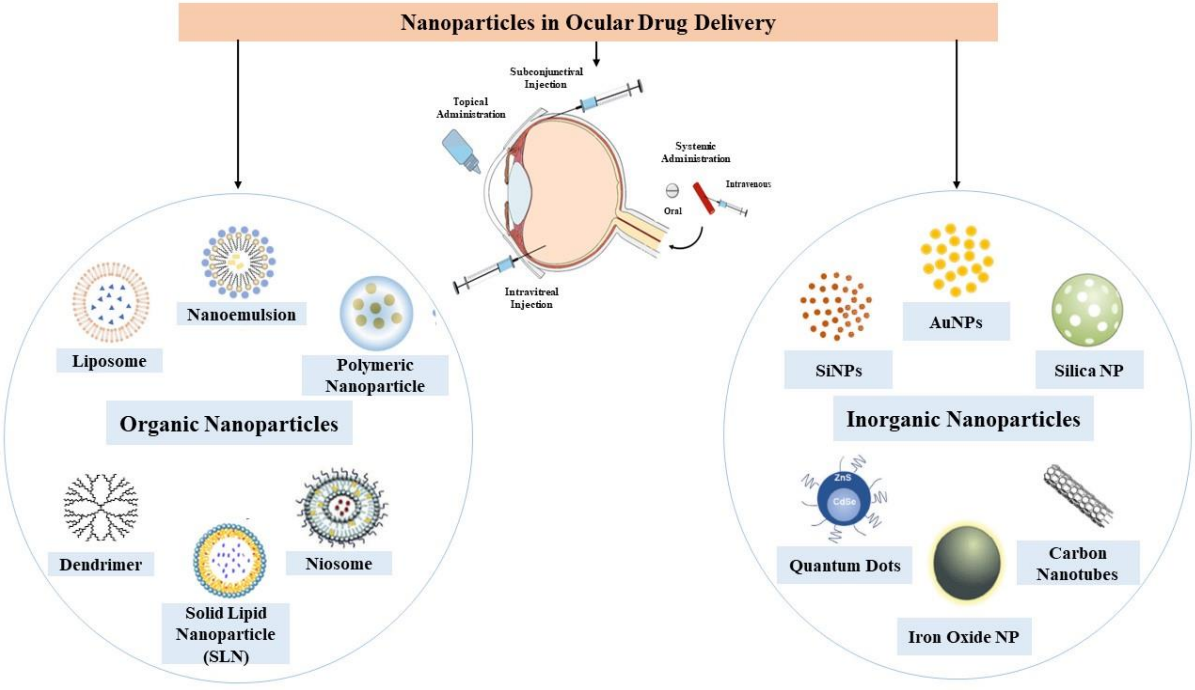

Figure 2. Nanotechnology-based strategies for treating and diagnosing ocular disease [13,19].

NPs for drug delivery were classified into organic and inorganic NPs (Figure 2). The organic NPs group consists of liposomes, niosomes, dendrimers, solid-lipid NPs, polymers, and protein/peptide-based NPs made from proteins, lipids, carbohydrates, or other organic compounds. Organic NPs offer several advantages to facilitate drug or gene delivery to the ocular surface. Meanwhile, inorganic NPs consist of metallic NPs and quantum dots. Metallic NPs have also received much attention during the last decade. They can be categorized into

four distinct groups: metal NPs, metal oxide NPs, doped metal–metal/oxide–metal NPs, metal sulfide, and metal–organic. Metal NPs such as silver (Ag), gold (Au), copper (Cu), magnesium (Mg), titanium (Ti), platinum (Pt), zinc (Zn), and iron (Fe) NPs have been investigated in various research fields and has been successful as an efficient and stable drug delivery with minimal side effects [13, 19].

3.1. Liposomes.

Liposomes (LPs) as lipid nanoparticles are the most widely used in cosmetics, agriculture, and medical imaging and are investigated for targeted drug delivery [32]. LPs are biocompatible, non-toxic, and biodegradable entities that help to enhance efficacy, improve stability, and minimize the toxicity of encapsulated drugs [33]. Conventional liposomes (primary forms) **(**Figure 3**)** are considered first-generation liposomes, composed of a phospholipid bilayer with anionic, cationic, or neutral phospholipids and cholesterol enclosing an aqueous space [34-35]; they are formed by self-assembly of hydrophilic head groups and hydrophobic acyl tails of lipids to spontaneously form closed unilamellar or multilamellar vesicles of a wide range of sizes in aqueous media. With the protective effect of the unique self-assembled structure from external mechanical forces and the similarities between cell membranes and lipid bilayers, liposomes have been widely used in drug delivery (e.g., anticancer, anti-inflammatory, antibiotics, anesthetics), vaccines (e.g., mRNA-based Covid-19 vaccines), and tissue-engineering applications [32].

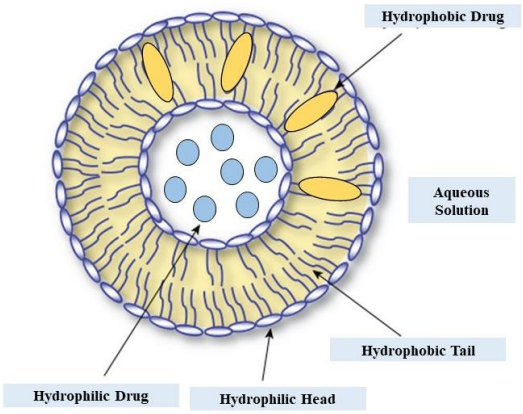

Figure 3. Structure of a liposome and schematic representation of possible drug incorporation [35].

One of the significant disadvantages of these traditional LPs is the short life span during circulation due to uptake by the reticuloendothelial system (RES). With the help of opsonins, phagocytes identify them as foreign bodies and engulf them quickly [36]. The surface of liposomes can conjugate with small molecules, receptors, vitamins, carbohydrates, peptides, proteins, antibodies, aptamers, and enzymes (Figure 4). These advanced LPs exhibit more excellent solubility, higher stability, long-circulating time, and specific drug-targeting properties in the retina. They also facilitate drug absorption through the ocular tissue, giving higher drug bioavailability in the posterior eye segment. They can control and sustain drug release to the targeted ocular tissue, leading to decreased drug administration frequency [6, 33].

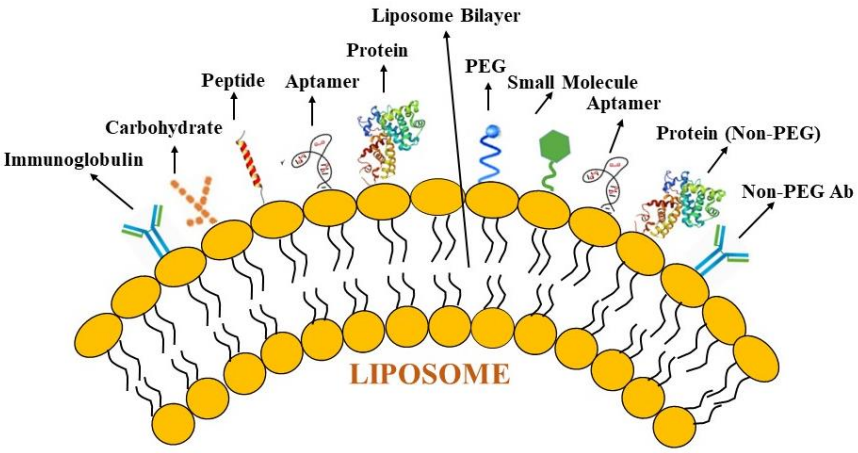

Figure 4. Modification of liposome surface with different ligands (targeted liposomes) [33].

Formulations of transferrin (Tf)-conjugated liposomes loaded GCV (Tf-GCV-LPs) for intravitreal injection and topical instillation having particle sizes lower than 100nm with a negative value of zeta potential [6]. The smaller GCV LPs could penetrate the cornea more easily than the more giant liposomes [37]. The *in vitro* cytotoxicity tests of Tf-GCV-LPs were safe for the ARPE-19 cells with a percentage cell viability of 80-100%. Intracellular uptake of Tf-GCV-LPs in the ARPE-19 cells indicated that Tf-GCV-LPs were taken up by the cells via TfRs-mediated endocytosis and showed inhibitory activity on CMV Glycoprotein B (gB) in the infected cells (MRC-5 cells) [6]. gB, an abundant glycoprotein of human herpesviruses, plays a role in the HMV entry process [38]. Tf-GCV-LPs as a promising drug delivery system for targeted GCV delivery to the retina by retarding recognition and drug removal from blood circulation by phagocytic cells in the treatment of CMV retinitis [6]. A study of the ocular pharmacokinetics of GCV LPs in albino rabbits was also obtained. Ocular bioavailability and *in vitro* transcorneal permeability of GCV LPs were found to be 1.7-fold and 3.9-fold, respectively, higher than that of the GCV solution in the eyes of Albino rabbits. The higher transcorneal permeability is because of interactions between liposomes and the corneal epithelial surface, thereby increasing drug penetration. Liposomal incorporation of GCV increased ocular tissue distribution and drug concentration by 2- 10-fold in the sclera, cornea, iris, lens, and vitreous humor compared with GCV solution. Besides that, liposomes require low concentrations of GCV for effectiveness in CMV retinitis (Figure 5) [37, 39].

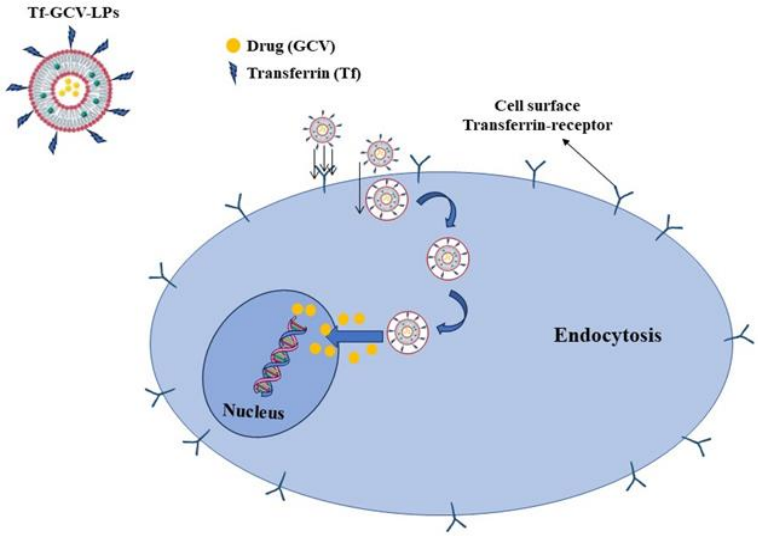

Figure 5. Uptake of liposomes by cells via receptor targeting and cell penetration [39].

3.2. Polymeric microspheres.

Over the past 25 years, much research has been focused on degradable polymer microspheres for drug delivery. The microspheres are the preferred delivery system for ocular drug delivery. Drugs are encapsulated in a mucoadhesive polymer to overcome the poor ocular bioavailability of conventional eye drops. Administration of medication via polymer microspheres is advantageous because microspheres can be ingested or injected, are easy to administer in liquid form, are used for controlled release drug delivery, and can even provide an organ-targeted release [3, 40, 41]. Polymeric microspheres also diffuse rapidly and have better ocular tissue internalization. The entrapped drug in the form of a monolithic type or reservoir type in the microspheres can act as a depot and sustain the release of the drug [3].

Microspheres (Ms) are small spherical particles prepared by polymeric, waxy, or protective materials with diameters ranging from 1 to 1000µm and can be manufactured from various natural and synthetic polymeric materials. They can encapsulate many types of drugs, including small molecules, proteins, and nucleic acids, and are easily administered through a syringe needle [41].

Using natural polymers (such as cellulose, chitin, and chitosan (CS)) can achieve the biocompatibility of a drug or by employing polymers made from naturally occurring monomers such as lactic and glycolic acids. Synthetic polymers derived also show excellent delivery properties, including polylactic acid (PLA), Poly(lactide-co-glycolide (PLGA), and polycaprolactone (PCL) [40].

Chitosan microspheres (CS-Ms) have many advantages over other microsphere formulations with starch, gelatin, or albumin. The inherent biological activity of chitosan [poly (β- (1 → 4)-2-amino-2-deoxy-D-glucose)] signifies its role in ocular therapeutics. Compared to other biodegradable polymers, chitosan is the only one with a cationic and mucoadhesive character. A biodegradable polymer with hydrophilic improves stability and enhances precorneal retention and interaction with eye mucosa, prolonging drug release time [3, 42-44].

The modified water-in-oil emulsification method was a suitable and straightforward technique for encapsulating ganciclovir using chitosan (Figure 6) [45-46]. More than 60% of the deacetylation of chitosan is ideal for ocular delivery, as a decrease in deacetylation leads to the decreased water solubility of the polymer [44].

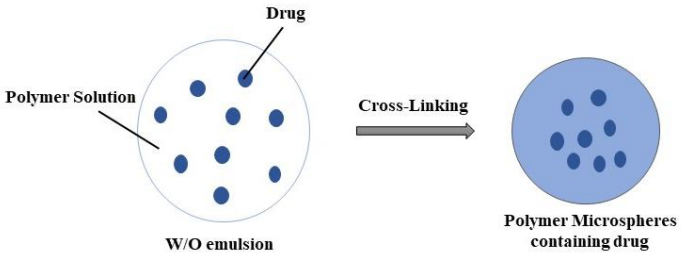

Figure 6. The schematic process of water-soluble polymeric microspheres containing drugs [46].

GCV, as a microsphere formulation, can improve antiviral effectiveness. The *in vitro* data showed ganciclovir release from GCV chitosan microspheres (GCV-CS-Ms) 50% in a few minutes and then slowly release over a few hours (up to 90%), indicating GCM minimizes dosing frequency by sustained ganciclovir release for better efficacy [3]. The drug release process in gelatin microspheres is faster, with almost 70% of the drug released in the first 1h [9]. The microsphere's appropriate physicochemical properties help achieve adequate drug bioavailability and biocompatibility with ocular mucosa. The positive zeta potential can facilitate an adequate adhesion to the cornea surface. It could improve some limitations related

to ocular administration, such as preventing tear washout (due to tear dynamics). A mucus film as a thin fluid layer covers the surface of the cornea and conjunctiva. The mucin carries a negative charge at physiological pH. The positively charged chitosan from GCV-CS-Ms interacts with sialic groups and sulfonic acid substructures of mucin and acts as an adhesive force on the eye surface **(**Figure 7**)**. Subsequently, the positive charge interacts with the cell membrane. It helps permeate the drug through the corneal surface and improves intraocular drug bioavailability [3, 44, 47].

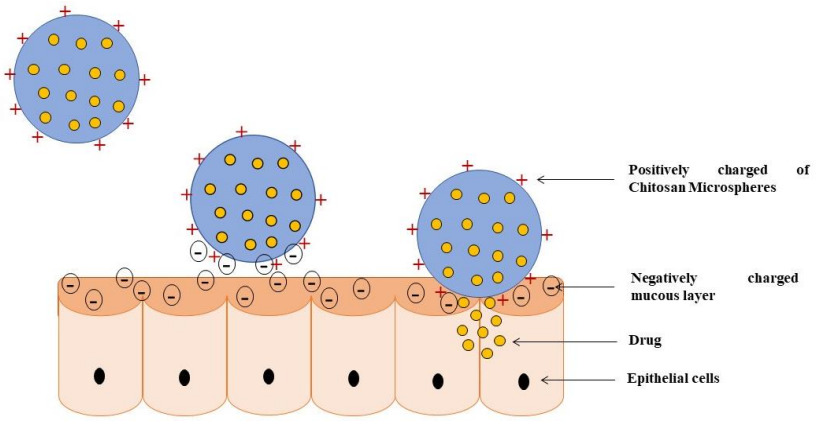

Figure 7. Schematic representation of ganciclovir loaded Chitosan Microspheres (GCM) structure and interaction with the mucus layer. From left to right: CS-NP, upon reaching the mucosal layer, binds to the negatively charged mucus under electrostatic attraction and releases the drug over time [47].

The optimized formula was also stable in long-term and accelerated storage conditions. The ocular irritation test showed that the GCV-CS-Ms sample was well tolerated in the right eye of the Wistar rats. The in vivo ocular pharmacokinetic studies and the histopathology report demonstrated the efficacy and tolerability of the formulation. GCM formulation significantly improved the intraocular bioavailability of ganciclovir in Wistar rats [3].

Besides that, in recent years, PLGA has also been extensively studied as a common biodegradable polymer made from the polymerization of lactic acid (LA) and glycolic acid (GA). Because of its outstanding biocompatibility, biodegradability [48,49], non-toxicity, good plasticity [49], protection of the drug from degradation, the possibility of sustained release, the opportunity to modify surface properties to provide better interaction with biological materials, the possibility of targeting to specific organs or cells [48]. PLGA has received approval from the US Food and Drug Administration (FDA) as well as the European Medicines Quality Agency (EMA) as a superior drug carrier [49].

Recently, microspheres of PLGA loaded with GCV were developed using the oil-in-water (O/W) single emulsion and solvent evaporation methods [1, 2]. Controlled delivery systems can help achieve goals safely, effectively, and predictably, which are of immense therapeutic value. PLGA microspheres in thermo-gelling PLGA-PEG-PLGA gel can deliver GCV to the vitreous over prolonged periods following an intravitreal administration [2].

The release of the GCV shows a triphasic release pattern, i.e., an initial burst, a diffusive phase, and a second burst. The initial burst occurs within the first 2 days of immersion. After the burst, the release is by diffusion for up to 13 weeks, followed by another burst release, which signals the onset of bulk degradation of the PLGA polymer [1].

The degradation of PLGA involves chain scission of ester bond linkages in the polymer backbone by the hydrolytic attack of water molecules and autocatalytic degradation [1, 50, 51]. Studies on the degradation of PLGA samples have shown a heterogeneous degradation mechanism, with the degradation products generated in the device's interior autocatalytically

accelerating the degradation process [1, 49]. It is due to increased carboxylic acid end groups, which are responsible for faster degradation in the center of the device than at the surface. These studies were carried out on pure PLGA polymers without any drugs. The presence of drug molecules often influences the rate of water penetration, thus affecting the rate of hydrolytic degradation. In the case of GCV-loaded microspheres, GCV decreased the rate of PLGA degradation. It was believed that the basic nature of the amino groups in GCV neutralizes the acidic by-products of PLGA degradation [1].

Slower in vivo GCV release from the PLGA microsphere in thermo-gelling PLGA-PEG-PLGA gel formulation results from the vitreous's static nature. Drug distribution and diffusion from the formulation in vivo could be owing to its inherent diffusivity in the vitreous. As deposition of the formulation is deposited near the retina, most released GCV could be eliminated instantaneously, resulting in an underestimation of the amounts of drug released. The dispersion of GCV-loaded PLGA microspheres could allow for an intravitreal administration of an exact GCV dose and may diminish particle migration inside the vitreous [2].

3.3. Microemulsion (ME).

Microemulsion (ME) has been researched extensively in ophthalmology as a potential medication delivery for the anterior and posterior of the eye. They offer the advantage of being a thermodynamically stable system, which, compared to other nanoformulations [8, 22, 52], ease of preparation, spontaneous formation and scale-up, thermodynamic stability, enhanced drug solubilization, and bioavailability. Microemulsions also enhance the therapeutic efficacy of drugs and reduce the volume of the drug delivery vehicle, thus minimizing toxic side effects [53].

ME is a continuous droplet-type system made up of oil and water, which mimic the architecture of cellular membranes. Because of the small particle sizes, microemulsions appear as transparent or translucent solutions and thermodynamically stable fluid systems capable of loading hydrophilic and lipophilic drugs [8, 22, 52, 54]. The particle size of microemulsions ranges from about 10 to 300nm (0,01–0,30μm) [53, 54].

ME can be prepared by mixing water, oil, and surfactant/co-surfactant in different mixing ratios. The amphiphiles (surfactant/co-surfactant mixture) lower the oil–water interfacial tension by interfacial adsorption, thus minimizing the positive-free energy change of dispersion associated with the formation of a surface. The presence of surfactant as a significant component of the microemulsion facilitates drug absorption by elevating the permeability of the cell membrane. Besides, in the case of lipophilic drug administration, the ability of the cell membrane to solubilize lipophilic components tremendously aids its absorption [53]. Microemulsion also exhibits a high solubilization capacity for both lipophilic and hydrophilic drugs; thus, more drugs can be loaded into the microemulsion, which increases the concentration gradient [53, 55].

Depending upon the nature of the dispersed particles, microemulsions are classified into two types: 1) Water-in-oil microemulsion (w/o ME) is a water dispersion or aqueous solution in a water-immiscible liquid. The water is, in this case, the "discontinuous" (inner) phase, and the oil is the "continuous" (outer) phase. 2) Oil-in-water microemulsion (o/w ME) is a dispersion of a water-immiscible liquid (always called oil) in an aqueous phase. The oil is, in this case, the "discontinuous" (inner) phase, and the aqueous phase is the "continuous" (outer) phase **(**Figure 8**)** [53,57]. GCV was formulated as oil in water (o/w) type ME, and the

most convenient topical ocular dosage form is aqueous eye drops. Nevertheless, because of its hydrophilic nature and to modulate drug release from ME, water in oil (w/o) type ME offers a unique opportunity to modulate drug release on contact with tear fluid into liquid crystal form, which enhances corneal retention and slow release of drug from dosage form [56].

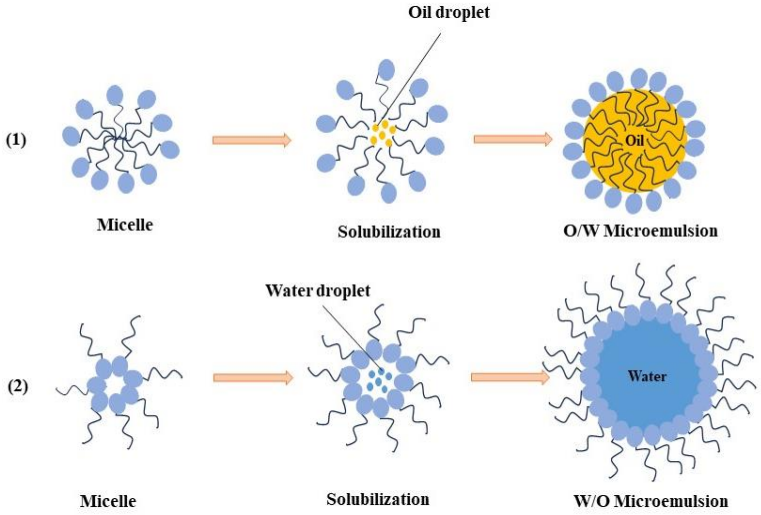

Figure 8. Representation of different types of microemulsion [57].

In recent studies, using mucoadhesive polymer in topical ocular formulations provided better outcomes regarding ocular bioavailability and tolerability [58,59]. Among many explored mucoadhesive polymers, chitosan has been widely accepted as a biocompatible material for dosage forms [60,61]. Thus, its impact was also examined upon incorporation in o/w ME [8].

All microemulsions (GCV o/w ME, GCV w/o ME, and GCV o/w ME with chitosan (CME) were relatively stable, transparent, and homogenous systems. They showed similar drug release patterns (after 24 h) [8]. The w/o ME showed higher initial drug release than that of CME, followed by o/w ME. GCV is a hydrophilic molecule, and this caused easy drug release from GCV Solution into surrounding media owing to the concentration gradient. In o/w ME, the continuous phase was water, allowing it for higher drug release than rest MEs but lower than GCV Solution because of its droplet-type nature. In w/o ME, the continuous phase was oil, which posed restrictions on drug release [62]. Adding mucoadhesive polymer (chitosan) can retain the cornea's surface for a longer duration, thus resulting in slow GCV release from its formulation [8]. This was one of the reasons for maintaining appropriate concentration at the precorneal site for permeation through the cornea, on account of the concentration gradient, which did not cause any side effects due to the burst drug release effect [63, 64].

ME droplets exist in micelle form and various structures, such as droplets of oil or water, ordered structures, or lamellar structures. The drug loaded in the ME exists mainly in the internal phase. However, at the equilibrium state, the drug can be distributed among dispersed phase, continuous phase, and surfactant interphases [21]. The mechanism of the action is based on the adsorption of the nanodroplets on the cornea, which are not eliminated by the lachrymal drainage and act as reservoirs of the drugs [22]. After installation, the oil drops act as reservoirs that continuously release the drug to the tear film as the drug gets absorbed into the ocular epithelia [23].

The highest *ex vivo* goat corneal permeation was shown on a chitosan-coated microemulsion. The *ex vivo* mucoadhesion investigation was carried out to ensure that the formulation would adhere to the goat cornea for a period at the place where it would be

absorbed. A formulation's mucoadhesive quality depends on the availability of functional groups that interact with mucin to promote mucoadhesion [65]. A positive charge from cationic polymers chitosan facilitates binding. It allows electrostatic interaction with negatively charged groups in the mucus of the goat cornea, thus leading to better mucoadhesion than other formulations (GCV o/w ME, GCV w/o ME) [8]. Besides that, microemulsion formulations were found to be non-irritant in *in vitro* cell viability assay on Statens Serum Institut Rabbit Cornea (SIRC) and Human Retinal Pigmental Epithelium (RPE) Cell line (ARPE-19) [8,66], and *ex vivo* corneal irritation study, indicating the potential of using such systems for delivery of drug to eye [8].

3.4. Polymeric nanoparticles (polymeric NPs).

Polymeric NPs are spherical-shaped solid colloidal particles composed of biocompatible and biodegradable polymers ranging from 10nm to 1000nm [67]. Polymeric NP is one of the organic NPs widely used in drug delivery, especially in the eye [19]. Various biodegradable polymers commonly used in the manufacture of Polymeric NP include poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA) copolymers, poly (ε-caprolactone) (PCL), and poly(amino acids) as well as several natural polymers such as alginate, chitosan, gelatin, and albumin [18].

Human serum albumin (HSA) is the most abundant protein in human blood. It is a natural and adequate material for manufacturing nanoparticles for drug delivery because of its high ability to load several non-specific drugs and its tolerability when administered in vivo [68]. Albumin nanoparticles **(**Figure 9**)** are effective for ocular administration of GCV [7, 69].

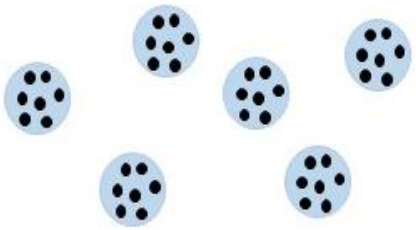

Figure 9. Drug Albumin NP [69]**.**

The size of the albumin nanoparticle formulation is between 200-400nm. GCV loading and albumin nanoparticles offer a higher capacity to carry this antiviral drug. *In vitro*, the release profile of nanoparticles showed a biphasic pattern. The albumin carrier could release GCV sustainably with the initial and rapid release, drug release in 1 hour followed by a sustained release for 5 days and maintained almost constant for 30 days. The presence of trypsin in the release medium increases the concentration of GCV released in acidic or basic media due to the disruption of the covalent bond between GCV and the protein matrix via glutaraldehyde [7]. Further studies were carried out for the *in vitro* release of the activity and cytotoxicity of the formulation containing GCV albumin nanoparticles. Two weeks post-injection in both Wistar rat eyes, large amounts of albumin nanoparticles remained in the vitreous cavity, especially in the thin layer overlying the retina and in areas close to the blood-aqueous barrier and the ciliary body due to the higher porosity of the ocular organs It is the mechanism of elimination of particles through the anterior chamber of the eye. Their prolonged residence in the eye is well tolerated without an inflammatory reaction or change in tissue (i.e.,

cellular infiltration or vascular inflammation). In addition, nanoparticles are not affected by changes in eye cell behavior due to intravitreal injection of nanoparticles [24].

In addition to albumin, chitosan nanoparticles (CS-NPs) can increase complex drug permeability as drug carriers [25]. Chitosan (CS) is the only polysaccharide with a high positive charge density due to the protonation of the amino groups on its backbone. CS is a mucoadhesive polymer that can increase the residence time at the absorption site and has a well-controlled drug-release ability [25, 70].

CS-NP containing GCV was found with a particle size of 121.20nm. The zeta potential was +26.6 mV, confirming that the GCV-loaded CS-NPs were stable. The prepared GCV-loaded CS-NPs have a positive surface charge, which is advantageous for electrostatic interactions with the mucin mucous and provides mucoadhesive characteristics **(**Figure 10**)** [25-27]. Transmission electron microscopy, scanning electron microscopy, and dynamic light scattering techniques reveal spherical particles of uniform size. The *in vitro* release profile found that the drug exhibits a sustained release behavior with a steady increase in cumulative drug release for up to 24 hours [25]. The results show that drug release from CS-NPs follows the Higuchi model, which indicates that drug release is a diffusion process based on Fick's law [71]. Thus, incorporating GCV into CS-NP increased permeability and drug absorption [25].

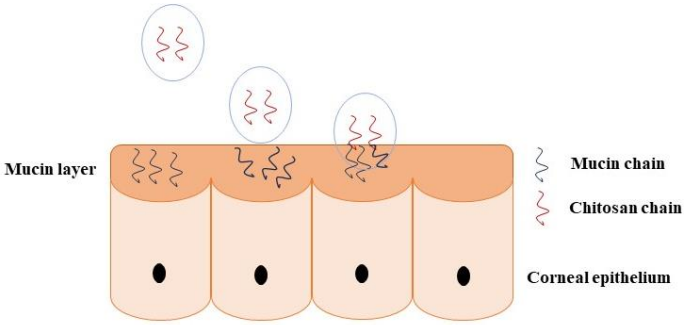

Figure 10. Illustration of chitosan interaction with the mucin layer of corneal epithelium, allowing particle permeation via mucoadhesion [27].

Not only albumin and chitosan but also the development of GCV nanoparticles has been carried out using PLGA polymers [10]. PLGA (Poly(D, L-lactic-co-glycolic acid)) nanoparticles (NP) **(**Figure 11**)** can penetrate the cornea and enhance drug delivery [20]. PLGA NP from the prodrug ganciclovir (GCV) was formulated and dispersed in PLGA thermosensitive gel to treat HSV-1 corneal virus-induced keratitis [10]. PLGA NPs as carriers can effectively overcome ocular barriers due to their specific surface area, surface energy, and surface atoms and have become an effective strategy to overcome ocular barriers' limitations [72-73].

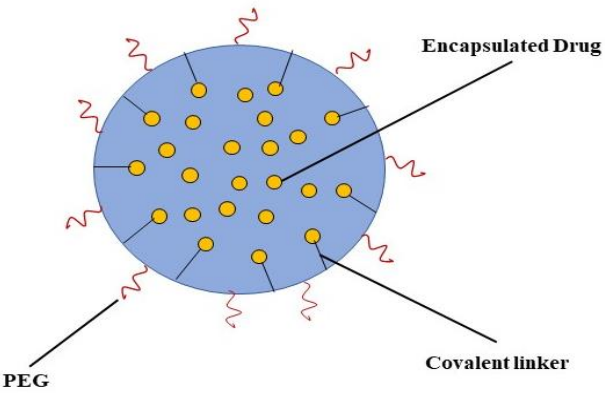

Figure. 11. Schematic representation PLGA-NPs [73]**.**

Drug delivery through PLGA NPs increases the drug amount transported across barriers and can be targeted selectively by changing their particle size [71], optimized particle size results, and zeta potential of GCV-PLGA-NPs <150nm and ≤-15.0mV, respectively. Cytotoxicity studies on HCEC cells showed that the NP PLGA formulation loaded with GCV was non-toxic. The *in vitro* release of GCV from NPs shows a biphasic release pattern with an initial burst phase followed by a sustained phase. The burst effect can be eliminated when GCV-PLGA-NP is suspended in a PLGA-PEG-PLGA thermosensitive gel with close to zero release kinetics. PLGA NPs containing GCV dispersed in a thermosensitive gel may serve as a promising drug delivery system for treating anterior eye disease [10].

The mechanism of PLGA-NP is internalized in cells via fluid-phase pinocytosis and clathrin-mediated endocytosis. PLGA-NP exits the endo-lysosome and enters the cytoplasm **(**Figure 12**)**. Positively charged NPs can detach from lysosomes after internalization and show perinuclear localization. In contrast, negatively charged and neutral NPs prefer to colocalize with lysosomes. Negatively charged PLGA NPs colocalize with lysosomes but can be switched to neutral or positive charges by surface modification, such as PEGylation of the PLGA polymer [74].

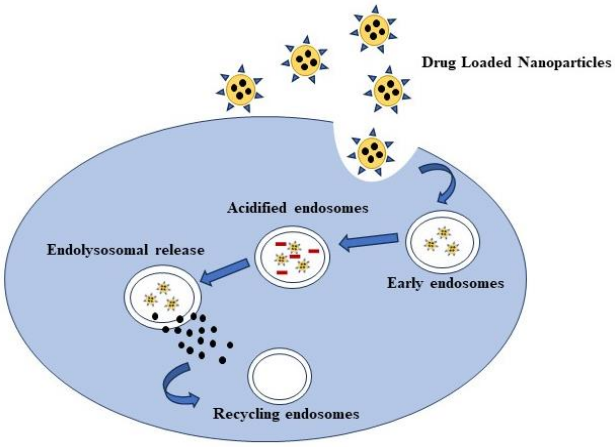

Figure 12. Schematic representation of Polymeric NPs internalization in cells [74].

3.5. Gold nanoparticles (AuNPs).

In the field of ocular drug and gene delivery, inorganic NPs such as gold nanoparticles (AuNPs) [19, 87] have demonstrated potent antioxidant capabilities with high safety profiles after delivery to the eye, high chemical stability [13], water solubility, and broad size and shape controllability. AuNPs have unique optical properties and uniform size distribution in the nanometer (1 to 800 nm) range size and have different morphological shapes, including spheres, rods, prisms, tetrapods, dog bones, cubes, shells, and several hollow structures [75-77, 87]. AuNPs also have a solid binding affinity for organic molecules with thiol and amine groups [78-80]. Drug conjugation with AuNPs can be achieved either by direct drug conjugation with AuNPs or by drug conjugation with surface-modified AuNPs [81-84]. Also, electrostatic or hydrogen bonding of the drug with AuNPs is a better strategy [85]. AuNPs can be administered differently via corneal, intravitreal, subretinal, intravenous injection, topical application, perfusion, or incubation (for *in vitro* studies) [29].

The AuNPs surface can be modified with glutathione. Then GCV was loaded onto glutathione (GSH)-modified AuNPs via a reaction between the carboxyl group (GSH) and the amino groups of GCV **(**Figure 13**)**. The particle size distribution of AuNPs ranged from 26.3nm to 31nm. TEM micrographs of AuNPs show that AuNPs are evenly distributed and have a

spherical shape. GCV-loaded AuNPs were successfully prepared and could enhance GCV bioavailability [28].

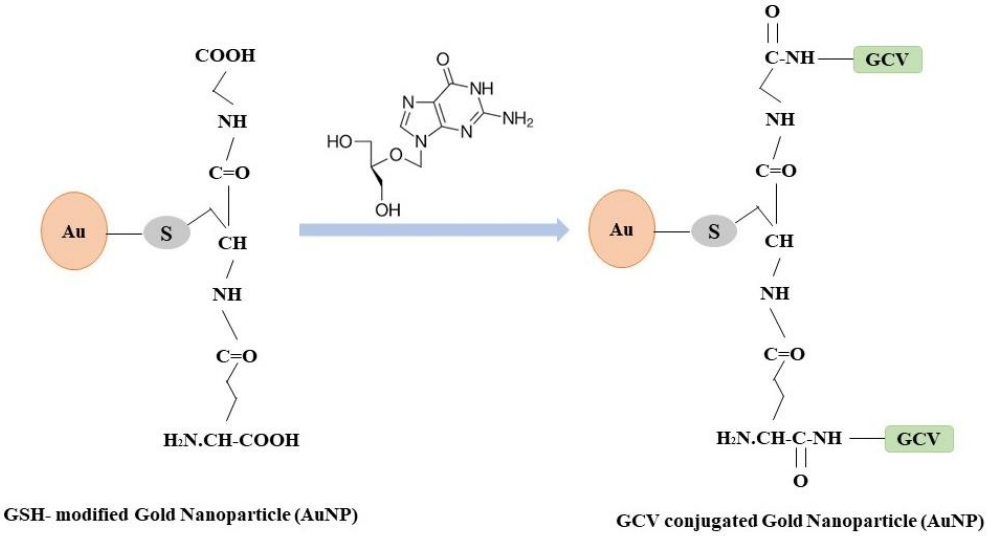

Figure 13. Reaction scheme for GCV loading with GSH-modified AuNPs [28].

The shape and size of AuNPs **(**Figure 14**)** significantly affect their biocompatibility. Internalization efficiency is affected by GNP diameter, and only those smaller than 30nm in diameter are highly internalized (more than 86%) [29]. The size of AuNPs affects their distribution in the posterior segment of the rat eye [86]. Given intravenously, auNPs with a size <30nm can cross the blood-retinal barrier, reach all retina layers, and bind to cell membranes [29]. AuNPs (20-30nm) applied topically in vivo to guinea pig eyes can penetrate the corneal cell layer without changing the morphology of the endothelium. AuNPs were administered twice daily for 2 weeks and were still present in the cornea after more than 30 days [29,30]. AuNPs cytotoxicity studies demonstrated no visible inhibition or toxic effects on ARPE-19 proliferation [3].

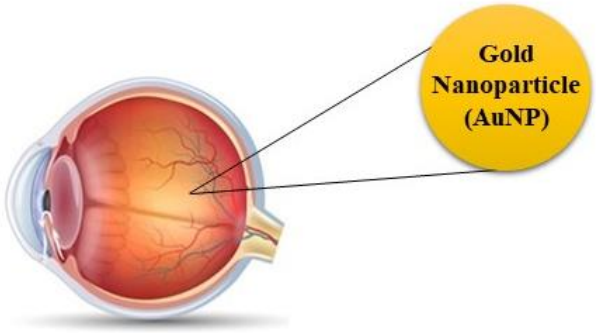

Figure 14. Schematic illustration of the use of AuNPs in the eye [87].

4. Conclusions

GCV is a drug used to treat viral infections of the eye, especially HSV and CMV, including class III BCS, which have poor ocular bioavailability. In addition, it is difficult for the drug to pass through the eye barrier without causing tissue damage/toxicity, which prevents delivery of GCV to the site of action, and conventional formulations have several limitations. Several strategies are carried out by formulating GCV in nanoparticle-based drug delivery systems such as liposome, microemulsion, nanoparticle microsphere, polymeric nanoparticles,

and gold nanoparticles, which can increase the limitations of conventional GCV formulations by increasing GCV bioavailability in the ocular.

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Conflicts of Interest

The authors declare no conflict of interest.

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