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REVIEW ARTICLE

A review on ophthalmic delivery systems containing flavonoids for the treatment of eye diseases

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HIGHLIGHTS

> Flavonoids have very beneficial effects on eye health, and also in the treatment of the eye diseases.
> A decreased antioxidant capacity, oxidative stress and inflammatory mechanisms have a significant role in the development and progression of the ocular diseases.
> Ophthalmic delivery systems can increase the ocular bioavailability of flavonoids.

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ABSTRACT

Flavonoids, polyphenolic compounds, have many biological effects, including antioxidant, free-radical scavenging properties, antiviral, antibacterial, anti-inflammation, anti-allergic, and anti-carcinogenic effects, anti-platelet, anti-thrombotic, and vasodilating actions. A decreased antioxidant capacity, oxidative stress, and inflammatory mechanisms in the ocular tissues are considered to have a significant role in the development and progression of the ocular diseases. Flavonoids have very beneficial effects on eye health, and also the treatment of the eye diseases due to their antioxidant, anti-inflammatory and ocular blood flow enhancing properties. Most flavonoids have low bioavailability associated with low water solubility. It is important to develop effective ocular drug delivery systems containing flavonoids for application directly to the eye. This delivery systems can increase ocular bioavailability and enable flavonoids to reach the internal structures of the eye more effectively. Furthermore, considering the sensitive nature of flavonoids as antioxidant agents, especially nano-sized formulations have in particular become potential carriers for preserving them and improving their bioavailability and therapeutic efficacy. This review will focus the published studies that have investigated the development of delivery systems containing flavonoids for the treatment of eye diseases. In addition, within the scope of this review, flavonoids, common eye diseases, and the materials used in the preparation of the ophthalmic delivery systems containing flavonoids are briefly mentioned.

1. Introduction

Flavonoids are a big family of plant-derived polyphenolic compounds with diphenylpropane skeletons that are widely distributed in vegetables and fruits, thus, regularly consumed in the human diet. The number of different flavonoids identified until now is over 4000 [1]. Flavonoids, polyphenolic compounds, have several subgroups depending on the positions of the substitutes present on the parent molecule, which include flavonols, flavanones, flavones, isoflavones, flavanols or catechins, and chalcones [2,3]. These compounds have many biological effects, including antioxidant and free-radical scavenging properties, anti-platelet, anti-thrombotic, and vasodilating actions, antiviral, antibacterial, anti-inflammation, anti-allergic, and anti-carcinogenic effects [1,3–5]. They are also capable of strengthening capillary walls and reducing fluid retention [6].

In a study, the *in vitro* antioxidant, anti-inflammatory, and antibacterial activities of the flavonoid fraction extracted from the leaves of *Abutilon theophrasti Medic.* (*A. theophrasti*), commonly used for the treatment of inflammation and joint pain in China, was evaluated. Their obtained results showed that the fraction has *in vitro* antibacterial, antioxidant, and anti-inflammatory effects and

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it might be beneficial in the adjuvant treatment of oxidative stress and also, inflammatory and bacterial diseases [7].

In another study, the extracts (ethanol, ethyl acetate and heptane) *of Euphrasia officinalis L.*, which is traditionally used in folk medicine and especially in the treatment of eye disorders, were obtained. Later, the effects of these extracts on human corneal epithelial cells were investigated *in vitro*. After 24 hours of incubation, the extracts (ethanol or ethyl acetate) reduced pro-inflammatory cytokine expression (TNF-α IL-6, and IL-1β) of human corneal cells. The biological effects of these extracts are based on the presence of highly active compounds such as flavonoids, essential oils, iridoids, phenolic acids. It was stated that the application of the extracts of *Euphrasia officinalis L.* as a complementary therapy for eye disorders is promising [8].

In addition, it has been shown a select group of flavonoids (quercetin, luteolin, fisetin, 3,7-dihydroxyflavone, and baicalein, etc.) protect retinal pigment epithelial cells from oxidative stress-induced death with low toxicity and high potency, and also many of these flavonoids induce the expression of phase-2 detoxification proteins that may serve as an additional protection against oxidative stress [9].

The previously published studies reported that flavonoids cause beneficial effects on reduction of the growth of malignant carcinomas, on inhibition of tumor angiogenesis, and on protection from cardiovascular disease, coronary heart disease, menopause syndrome, hypertension, and cerebral thrombosis [1,5,10].

Recently, in *in vitro* and preclinical studies, it has been reported that decreased antioxidant capacity, oxidative stress, and inflammatory mechanisms in the ocular tissues are considered to have a remarkable role in the development and progression of the ocular diseases with etiology including hypoxia, decreased blood supply to ocular tissues, free radical mediated oxidative damage and in certain conditions, increased vascular permeability, angiogenesis, and leakage of vascular contents (diabetic retinopathy, age-related macular degeneration etc.). Therefore, some phytochemicals such as flavonoids may be useful due to their antioxidant, anti-inflammatory and ocular blood flow enhancing properties in the treatment of the ocular diseases [6]. Xiao et al. [11] evaluated the suppression effect of daidzin, an isoflavone found in some plants, including kudzu root and soy plants, on corneal inflammation and oxidative stress in the dry eye rat model developed by removing the lacrimal gland of rats. They found that oxidative stress in the cornea was reduced due to the tyrosyl radical scavenging activity of daidzin. Daidzin improved corneal erosion and also restored tear volume in the dry eye rat model. Xiao et al. [11] emphasized that daidzin might be useful in the treatment of dry eye due to protection of the cornea by the suppression of oxidative stress and inflammation in a dry eye rat model. In another study, which is a population-based prospective cohort study included 2856 adults aged 49 y and over at baseline for prevalence analysis and 2037 adults re-examined (follow-up) 15 y later for incidence analysis, the independent associations between the prevalence and 15-y incidence of age-related macular degeneration (AMD) with dietary intake of flavonoids (the median intake of total flavonoids: 875 mg/d) were assessed. This study reported that a higher intake of flavonoids (especially flavanones and flavonols) was associated with reduced odds of the prevalence of AMD, thus, the intake of flavonoids might have a role in the prevention and progression of AMD [12]. Baicalin is a flavonoid found in *Scutellaria baicalensis Georgi*, an herb that has been used in traditional Chinese medicine for many years. It has various biological activities such as antibacterial, antiviral, anti-inflammatory, and anti-cataract effects that may be beneficial in the treatment of eye diseases [13,14].

On the other hand, Pawlowska et al. [15] emphasized that most of the evidence for the effect of dietary polyphenols on the progression of AMD comes from *in vitro* and animal studies. Therefore, the necessity of conducting prospective interventional studies to confirm these results was stated by the authors.

In addition, Davinelli et al. [16] conducted a systematic review (16 studies included after literature search) and meta-analysis (11 intervention trials involving 724 participants) to evaluate the effect of dietary flavonoids on primary eye disorders. They stated that flavonoids could improve the clinical manifestations related to ocular diseases, but further clinical studies are needed to examine and confirm the effect of flavonoids on different eye disorders.

There are enzymatic [glutathione peroxidase (GSH-Px), super oxide dismutase (SOD) and catalase (CAT)] and non-enzymatic (such as β-carotene, vitamin C and E) antioxidant defences responsible for scavenging free radical [17]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the most important oxidants generated by various metabolic pathways, biological factors, and pathological conditions [18]. The mitochondria is one of the main sources of cellular ROS and an imbalance between antioxidant defenses and ROS causes to oxidative stress [19]. Oxidative stress leads to damage lipids, proteins, DNA, and mitochondria and is associated with some age-related disease, because, with age, mitochondria become gradually more incompetent [20]. In addition, an excess amount of ROS can lead to functional and morphological impairments in retinal pigment epithelium (RPE), endothelial cells, and retinal ganglion cells (RGCs). Oxidative stress can initiate and develop ocular tissue/cell (cornea, endothelial cells, retinal pigment epithelium, and retinal ganglion cells etc.) damages resulting in a decrease in visual acuity, or even vision loss [20,21]. The one of major target of the ROS/RNS attack is the eye, because, it (especially, its anterior segment, mainly the cornea) is exposed to a number of environmental factors such as oxygen, ionizing radiation, UV light, visible light, air pollution, irritant, environmental toxins, cigarette smoke, and pathogenic microbes, which are able to alter the redox status of a cell to oxidizing conditions [18,19,21]. On the other hand, the retina is also highly sensitive to oxidative damage, because, (a) there are an abundance of the polyunsaturated fatty acids, whose double bonds are primary targets for peroxidation reactions, in its membrane bilayers, (b) free radical formation and peroxidation reactions can be initiated by photoexcitation as a result of the periodical exposure of the retina to continuous light, and (c) the retina that is a highly metabolic tissue requires a high rate of blood flow for sufficient oxygen supply [6]. Atmospheric oxygen, environmental chemicals, constant light irradiation, and physical abrasion contribute to oxidative stress which is responsible for disruption of blood–retinal barrier (BRB), microvascular abnormalities, retinal neovascularization, increased vascular permeability, and apoptotic loss of retinal capillary cells [22]. As a result, the oxidative stress has a significant role in the development and progression of ocular diseases such as AMD, cataract, retinopathy of prematurity (ROP), and diabetic retinopathy (DR) [20,21].

When the antioxidant defense systems are depleted/dysfunctional, visual impairment can be observed. Therefore, antioxidants are important agents in the prevention/treatment of oxidative diseases [22].

1.1. Age-related macular degeneration (AMD)

AMD, which is caused by the loss of RPE cells and photoreceptors in large zones of the macula, is a multifactorial disease of the retina [15,23]. It is a leading reason for blindness and the number of people affected by AMD will increase to 288 million by 2040 [24]. AMD especially affects people over 65 and is the main cause for legal blindness and vision loss in the elderly in developed countries. It affects the macula which is a highly specialized area of the central retina responsible for color and fine vision [15]. Oxidative stress, age, gender, race, diabetes, obesity, hypertension, smoking, hyperopia, pyroptosis, iris-color, genetics, and sun-light are risk factors for AMD [24]. AMD is characterized by drusen accumulating between the RPE and Burch's membrane. In this accumulation, carboxyethylpyrrole adducts, which is produced by the oxidative modification of fatty acids in photoreceptor tips, and advanced glycation end-products, a transmembrane receptor that applies pro-inflammatory functions thru nuclear factor-κB (NF-κB) signaling, have been found [23]. Therefore, oxidative stress and oxidative stress-induced inflammation play a major role in the pathogenesis and progression of AMD [15,23,24].

1.2. Retinopathy of prematurity (ROP)

ROP, which is a retinal vasoproliferative disease and frequently found in prematurely born infants, is the significant cause of visual impairment and blindness in these infants. Its incidence rate is about 98% among babies born with very low birth weight (<750 g) and about 68% among infants born with birth weight <1200 g [25].

It is a retinal vasoproliferative disease [26,27]. ROP might be divided into an early retinal ischemic stage and a late retinal neovascular stage [27]. Birth weight, gestational age, oxidative stress, prolonged mechanical ventilation, bronchopulmonary dysplasia, pulmonary complications, deficiency of vitamin E, maternal diabetes mellitus, hypertensive disorders of pregnancy, smoking, gender, blood transfusion, race/ethnicity, anemia, bacterial and fungal sepsis, medical interventions, intraventricular hemorrhage, and genetic factors are risk factors for the development and progression of ROP in premature infants [28]. High supplemental oxygen must be given to premature infants due to their immature cardiopulmonary system [29]. Oxygen has a critical role in the development of ROP, because, it causes tissue injury through the formation of ROS and lipid peroxidation [27,28,30].

1.3. Cataract

Cataract, which is a multifactorial eye disease associated with several risk factors such as oxidative stress, age, ultraviolet (UV) radiation, low antioxidant defence capacity, diabetes mellitus, obesity, female gender, smoking, and steroid consumption, is one of the leading causes of preventable blindness in the world today [31–33]. It refers to lens degradation characterized by clouding, with consequent blurred vision. It is stated that 35.1 million out of 191 million people with visual impairment globally have this debilitating disease. The prevalence of cataracts increases exponentially after the age of 40, and it is 3.9% in the 55-64 age group, while it is 92.6% for 80 years and older [31]. ROS produced by H_2O_2 causes protein degradation and epithelial cell damage. In cataract, there are the aggregation of oxidatively damaged proteins, and thus, oxidation of lens proteins is a main risk factor in cataract formation. Using antioxidant supplements is a protective strategy against oxidative stress. It was shown that ghrelin, which is a growth hormone-releasing peptide and may reduce ROS, may protect human lens epithelial cells against oxidative stress and prevent the progression of cataract [34].

1.4. Diabetic retinopathy (DR)

DR is one of the leading reasons for acquired blindness in working-age adults in the world. In DR, there are structural and functional changes in the retina [35]. The retina's oxygen uptake and glucose oxidation are higher than that of other tissues; which makes it more susceptible to oxidative stress. Atmospheric oxygen, physical abrasion, environmental chemicals, and, continuous light radiation contribute to oxidative stress [22]. Multiple factors such as hyperglycemia, and oxidative stress are included in DR pathophysiology. Hyperglycemia promotes changes in neuronal and vascular structures through ischemic/hyperosmotic damage, it also heads oxidative stress. Neovascularization is the major clinical change in proliferative DR [36]. Oxidative stress plays a significant role in the development and pathogenesis of DR. NADPH oxidase 2 is one of the isomers activated in the retina of person with diabetes. Ras-related C3 botulinum toxin substrate 1 (Rac1) is an obligatory component of the multicomponent cytosolic NADPH oxidase 2. Activation of Rac-1-NADPH oxidase 2-mediated cytosolic ROS production causes to mitochondrial damage, and the development of diabetic retinopathy [37].

1.5. Oral bioavailability of flavonoids

Flavonoids, which has a central ring (C-ring) in their structure and are largely decomposed by bowel flora, have poor bioavailability [38]. The bioavailability of flavonoids may be even lower and vary severely among different flavonoid classes due to their complex structures and larger molecular weights. Their bioavailability depends primarily on the type and position of sugar moiety in their structure and also their dietary source [39]. The flavonoids, which are in the ingested matrix after consumption of foods/beverages containing flavonoids, should pass from the gut lumen to the circulatory system for their absorption

[40]. Flavonoids are substrates for conjugating and hydrolyzing enzymes in liver, small intestine, and colon. Their conjugation first becomes in the small intestine and then in the liver where they are metabolized and the produced sulfate and glucuronides derivatives facilitates their excretion through urine and bile [39]. However, most of the flavonoids, except flavan-3-ols, exist as glycosides, thus, the attached sugar in their structure must be removed before their absorption [40]. The glycosides are generally hydrolyzed to their aglycones to show effects *in vivo* [41]. Flavonoid-O-glycosides that are commonly too large and polar structure to be absorbed are converted into an aglycone through hydrolysis by bacterial enzymes. Then, the aglycones can be absorbed or continue breakdown by a C-ring fission process to give smaller ring fission products that can be readily absorbed [38]. The chemical structure and molecular weight of flavonoids and also their glycosylation and esterification are significant factors for flavonoid absorption [39].

There are several approaches to improve the bioavailability of active compounds; these are changing their absorption site, improving their metabolic stability [such as quercetin, it is rapidly cleared after oral intake due to undergoing extensive metabolism [42], increasing their intestinal absorption, use of different routes for their administration, etc. Drug delivery systems (microparticles, nanoparticles, microemulsion, solid dispersions etc.) are commonly used to achieve these purposes [39].

2. The studies on the development of drug delivery systems containing flavonoids for eye diseases

The human eye is anatomically divided into two parts: the anterior segment (cornea, aqueous humor, iris, and lens) and the posterior segment (the back of the sclera, choroid, retina, and vitreous body). After systemic administration of drug/compound, it can be delivered to the anterior segment of the eye with very low ocular bioavailability due to the presence of the anterior blood-aqueous barrier (BAB), and also a systemically administered drug/compound must cross the posterior blood-retinal barrier (BRB) to reach the posterior segment of the eye. On the other hand, although the topical application of drugs to the eye surface is more convenient and simpler, the drug must pass the corneal barrier consisting of different layers (primarily epithelium, stroma, and endothelium) to reach the inner segments of the eye; the hydrophobic epithelium restricts the penetration of hydrophilic drugs into the cornea, while the more hydrophilic stroma limits the passage of lipophilic drugs. However, topically applied ophthalmic drugs can enter the anterior segment of the eye after absorption through the conjunctiva/sclera (compared to the cornea, they have a larger surface area and high permeability) [13]. Furthermore, other factors limiting the passage of the drug into the inner segments of the eye include the possible elimination of the drugs by lymphatic and conjunctival blood flow, and the size of drug molecules. Besides, ophthalmic bioavailability of drugs is also reduced by metabolic degradation, removal of drug from the surface of the eye due to tear clearance or nasolacrimal drainage. Other alternative routes used for drug administration to the eye (intracameral and subconjunctival, intravitreal routes) have some disadvantages such as discomfort and pain that may limit patient compliance [13]. As mentioned above, several treatment options for eye diseases are available, but multiple factors (the existence of anatomical barriers in the eye, the disadvantage of the alternative ocular routes) could limit their efficacy. The development of ophthalmic drug delivery systems (particularly nano-sized systems) has been useful to overcome these limitations of existing ocular drug application methods. Antioxidant agents such as flavonoids, which are used in ophthalmology due to their capacity to scavenge free radicals, prevent cellular and tissue damage caused by oxidative stress [13]. Previously published studies suggest that the flavonoids have very beneficial effects for eye health, and also the treatment of the eye diseases [1,42–44]. The flavonoids such as quercetin, genistein, and chrysin are rapidly cleared following oral ingestion due to undergoing extensive metabolism, and thus, they have limited systemic effects [42]. Therefore, it is important to develop effective ocular drug delivery systems containing flavonoids for application directly to the eye. This delivery systems can increase ocular bioavailability and enable flavonoids to reach the internal structures of the eye more effectively. Furthermore, considering the sensitive nature of antioxidant agents, nano-sized formulations have in particular become potential carriers for preserving antioxidant agents and improving their bioavailability and therapeutic efficacy [22]. A nano-sized delivery system such as like nanoliposomes, polymeric nanoparticles, or micelles can prevent premature degradation or removal of the antioxidant agents by the eye's protective mechanisms. These nano-carrier systems carry the antioxidant agents towards the internal structures of the eye that are generally difficult to reach using the conventional formulations of antioxidant agents [22,43,45].

This review will focus the published studies that have investigated the development of delivery systems containing flavonoids for eye diseases. In addition, within the scope of this review, the materials used in the preparation of the ophthalmic delivery systems containing flavonoids are briefly mentioned.

The design and preparation/synthesis of effective modern delivery systems is of great importance in fields such as pharmaceutics, food, and cosmetics. Advances in especially nanotechnology and the production of new materials have synergistically fueled advances in the design/preparation of these systems. The preparation of biocompatible, biodegradable, targeted and environmentally friendly delivery systems has been made possible by innovations in material chemistry [46]. Materials such as polymers, lipids, and metals are often used in the preparation of these delivery systems due to their high biocompatibility and biodegradability properties, synthetic polymers (poly (lactic-co-glycolic acid), poly-L-lactic acid, etc.) and natural polymers (alginate, chitosan, etc.) are widely used in the preparation of the delivery systems [47]. Delivery of drug/biologically active compounds/nutraceuticals is a vital issue for healthcare. Advantages such as the modified release of drugs/biologically active compounds, increasing/improving their stability and bioavailability, overcoming the solubility problem of hydrophobic compounds, and targeting of drugs/the compounds have been achieved especially with the development of nano-sized delivery systems [46,48].

Cyclodextrin (CD) complexes, liposomes, polymeric micelles, nanocomplexes, and polymer-/lipid-based nanoparticles as delivery systems are frequently prepared, to overcome the limitations of especially hydrophobic drugs/compounds.

2.1. Polymeric delivery systems

Developments in polymer science are very important for the design and preparation of polymeric delivery systems. Most flavonoids have low bioavailability associated with low water solubility, poor stability, extensive metabolism, limiting the activity and potential health benefits of flavonoids. There are different approaches to increase the solubility of active substances/compounds with poor water solubility; the use of water-soluble complexing agents is one of these approaches. Various bonds/interactions (covalent bonds or non-covalent forces such as electrostatic interactions, hydrophobic interactions, dipole forces, hydrogen bonds or van der Waals forces) are involved in the formation of these complexes. CDs and polyvinylpyrrolidone (PVP) are complexing and solubilizing agents commonly used in pharmaceutical formulations. PVP is a hydrophilic polymer that can form intermolecular cross-links with active substance/compounds and is suitable for the formulation and therapeutic applications of poorly water-soluble active substance/compound. PVP is also used in some ophthalmic suspensions and solutions due to its functions as a viscosity increaser and stabilizer [49].

Wang et al. [49] prepared nanocomplex containing naringenin using polyvinylpyrrolidone (PVP) K-17 PF (Kollidon® 17 PF). Naringenin, a flavanone with anti-inflammatory, anti-cancer, antioxidant, and antiviral effects widely found in some fruits such as citrus, cherries, tomatoes, bergamot, and cocoa [50,51]. Naringenin's therapeutic potential is limited due to its hydrophobic nature, which leads to poor water solubility and very low bioavailability. It rapidly converts to its crystalline form with low absorption from the gastrointestinal tract and has a short half-life. Furthermore, naringenin in an aqueous solution has the potential for oxidative modification and degradation, limiting its therapeutic use. Recently, it has been shown that naringenin may have potential use in the treatment of eye diseases (inhibition of corneal neovascularization, prevention of retinal damage in diabetic retinopathy, and treatment of uveitis, etc.) due to its antioxidant and anti-inflammatory activities [50]. PVP can improve its water solubility by forming complexes with poorly water-soluble naringenin. For this purpose, Wang et al. [49] firstly dissolved naringenin and PVP 17 PF in ethanol for the preparation of nanocomplex. After the evaporation of solvent, thin dry film was hydrated with a buffer solution (pH 6.5) under normal pressure. Later, the non-complexed naringenin was removed by passing the prepared mixture through a 0.22 μm filter and then re-filtered using 0.22 μm filter to obtain a sterile ophthalmic nanocomplex formulation containing naringenin. It was determined that the optimum formulation obtained using PVP 17 PF and naringenin at a ratio of 20:1 (w/w) had a complexation efficiency of 98.51%, had a small mean particle size of 6.73 nm, and showed a homogeneous size distribution (PDI:0.254). It was determined that the *in vitro* antioxidant activity, *in vitro* membrane permeability, and *in vivo* anti-inflammation efficacy of naringenin were significantly improved with the use of naringenin-containing nanocomplex. Besides, they evaluated the ocular biodistribution of the naringenin-containing nanocomplex administered topically to the rabbits'eyes and found that the levels of naringenin in the cornea, aqueous humor, conjunctiva, and iris-ciliary body after application of the nanocomplex to the rabbit's eye were 5.22-, 10.16-, 7.43-, and 34.02-fold higher at 30 min, and 2.31-, 8.33-, 1.54-, and 1.57-fold higher at 60 min, respectively, when compared with the use of free naringenin solution [49].

CDs are relatively large molecules consisting of (α-1,4)-linked α-d-glucopyranose units. They are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic central cavity and form water-soluble inclusion complexes with many lipophilic compounds. α-Cyclodextrin, β-cyclodextrin, and γ-cyclodextrin, which contain six, seven, and eight glucopyranose units, respectively, are commonly found as natural cyclodextrins and differ in cavity size, their molecular weight, and solubility. Its low cost and complexing ability make β-cyclodextrin a very useful pharmaceutical complexing agent. Their toxicity especially at high concentrations limits the use of cyclodextrin complexes and thus the dose level. As the cyclodextrin complexes' formation requires specific molecular properties this approach may not work for some compounds [52]. CDs can be easily modified to change their physicochemical properties [53]. Cyclodextrin derivatives [hydroxypropyl (HP)-β-CD, sulfobutyl ether (SBE)-β-CD, HP-γ-CD, etc.] with improved properties are available but tend to be expensive [52]. They are also versatile oligosaccharides that can be used in combination with other excipients such as polymers to achieve a synergistic effect. CDs have been shown to be compatible with many polymers (synthetic or natural) [53]. By preparing CD complexes of poorly soluble active substances/compounds, their water solubility and dissolution characteristics can be changed/improved. Thus, CDs play an important role in improving the oral bioavailability of active substances/compounds whose absorption is limited by the dissolution rate. Also, in recent years, CD inclusion complexes have been investigated for ocular delivery. CD complexes have been shown to be useful in the treatment of eye diseases such as glaucoma, eye infection, corneal inflammation and AMD. For example, HP-β-CD has been extensively researched for its potential to show lower toxicity, improve the solubilization and stability of drugs/compounds, reduce ocular irritation, and increase ocular drug/compound permeability and bioavailability [53]. A study in which the ocular pharmacokinetics of baicalein was evaluated after topical application. Baicalein, a flavone originally extracted from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*, is a poorly water-soluble compound, thus, HP-β-CD that form inclusion complex with hydrophobic drug molecule was used to improve the solubility of baicalein. After the administration topically of baicalein suspension (1%) or baicalein-HP-β-CD formulation (1%) to rabbits'eye, cornea and aqueous humor were collected to determine baicalein concentrations using HPLC. It was found that compared with baicalein suspension, baicalein- HP-β-CD formulation caused significant solubilization of baicalein in the precorneal area, thereby providing a significant increase in baicalein levels in the rabbit's cornea and aqueous humor [54].

For the effective ocular delivery of hesperidin, changes in its physicochemical properties and *in vitro* ocular tissue permeability were investigated by preparing the complex of hesperidin with various ratios of HP-β-CD by Majumdar and Srirangam [55]. Hesperidin has potential in the treatment of cataracts, diabetic retinopathy and AMD. However, it shows poor water solubility. The poor solubility of hesperidin was dramatically improved with the use of HP-β-CD. In addition, Majumdar and Srirangam [55] performed a corneal permeation study using a side-by-side diffusion apparatus and various ocular tissues (cornea, sclera and sclera with RPE) isolated from the rabbits. The permeability of hesperidin in the presence of HP-β-CD across the sclera was about ten-fold higher than that across the cornea and sclera with RPE. It was observed that the corneal permeability of hesperidin in the presence of HP-β-CD from apical to basolateral direction and vice versa was not statistically different and as a result, no carrier-mediated process (*influx* or *efflux*) was involved in the corneal permeation of hisperidine [55].

In addition, poly(ethylene glycol) (PEG), a synthetic polymer with hydrophilic properties, PEG has been used both alone or in combination with other polymers such as PLGA to prepare ocular delivery systems [56]. PEG nanocomplexes can be prepared to obtain long-acting and sustained-release formulations of active substances/compounds [57]. In a study on the use of catechin, a naturally occurring flavonol, in dry eye disease, the therapeutic effect of the nanocomplex of catechin and PEG in the treatment of dry eye disease in a dry eye mouse model was investigated. Catechin and PEG nanocomplexes containing different ratios of PEG (catechin:PEG=1:1, 1:5, and 1:10 w/w) were prepared. They stated that the ratio of PEG is important for the effectiveness of nanocomplexes and the highest catechin-PEG weight ratio (1:10) is the most convenient system for the treatment of dry eye model. It was found that catechin:PEG nanocomplex (1:10) increased tear production and anti-inflammatory activity, and also stabilized corneal epithelium in the dry eye model. Catechin, a potent antioxidant, its propensity to act as a dose-dependent prooxidant, and its low bioavailability attributed to poor solubility in water can be enhanced by the use of PEG-catechin nanocomplexes [57].

Biopolymers are attracting great interest in many application fields such as food or pharmaceutics. In this context, proteins and polysaccharides are widely used for drug delivery/nutraceuticals [58]. Chitosan nanoparticles have been commonly prepared as delivery systems for active substances, nutraceuticals, gene, and protein delivery. Chitosan, a polyaminosaccharide obtained by N-deacetylation of chitin, is used in various fields (pharmaceutical, biomedical, food, etc.) due to its, biocompatibility, biodegradability, safety profile, bacteriostatic, and mucoadhesive properties. Ionotropic gelation is the mostly used method for the preparation of chitosan NPs. It is a method based on the electrostatic interaction between negatively charged polyanions (tripolyphosphate, hexametaphosphate, dextran sulfate, etc.) and positively charged amino sugar monomeric units of chitosan. While chitosan NPs have many benefits as drug delivery systems, there are many hurdles that need to be resolved to realize the clinical potential of these NPs [59].

Another study was conducted in which naringenin-containing sulfobutylether-β-cyclodextrin/chitosan ophthalmic nanoparticles were prepared for topical application. For this purpose, naringenin was first complexed with sulfobutylether-β-cyclodextrin, which can significantly increase the solubility of poorly soluble compounds. Secondly, chitosan nanoparticles were prepared as a result of the ionic gelation of the obtained complex with chitosan. The average particle size of the prepared nanoparticles was about 446 nm and their zeta potential value was +22.5 mV. It was observed that the nanoparticles were non-irritant for the rabbit eye and increased the residence time of naringenin in the eye compared to the naringenin suspension [60].

Gelatin, obtained by partial hydrolysis of collagen, is a natural biopolymer and has been widely used for the encapsulation of drugs/nutraceuticals/biologically active compounds to treat various disease such as cancer, eye diseases [43,61,62]. Gelatin nanoparticles for topical application are preferred because gelatin is biodegradable and biocompatible and also collagen, which is the source of obtaining gelatin, is the main component of the corneal stroma [62]. Tseng et al. [63] prepared cationic gelatin nanoparticles for ocular delivery and evaluated the nanoparticles *in vitro* and *in vivo*. They showed that the cationic gelatin nanoparticles were non-toxic to human corneal epithelium cells in *in vitro* study, and non-irritating to the eyes of rabbits. The cationic gelatin nanoparticles efficiently adsorbed on the negatively charged cornea of the rabbits and retained in the cornea longer than other ophthalmic solutions [63].

Chuang et al. [43] prepared kaempferol containing-gelatin nanoparticles by a two-step desolvation method using glutaraldehyde (GA) as a cross-linker for the treatment of corneal neovascularization that is one of the main causes of vision loss. Kaempferol is a dietary flavonoid and has antioxidant and antitumor effects. The particle size, zeta potential, and encapsulation efficiency of the prepared nanoparticles in optimum condition (0.4% (v/v) GA for 3 h; 1% (*w/v*) gelatin Type A) were about 85 nm, +25.6 mV, and 95%, respectively. The authors also investigated the effects of kaempferol containing-gelatin nanoparticles on corneal neovascularization model induced in mice. They reported that the mice treated with kaempferol containing-gelatin nanoparticles showed a lower corneal neovascularization ratio that indicates a better therapeutic condition for inhibition of vessel formation and the use of nano-formulation as eye drops may be useful for effectively treating corneal neovascularization [43].

The epigallocatechin gallate, is a major flavonoid in green tea, has antioxidant, anti-inflammatory, and pleiotropic effects [13,64]. It is therapeutic in various inflammatory diseases (dry eye syndrome, atherosclerosis, and arthritis) and inhibits inflammation-related autoimmune disorders [62]. Epigallocatechin gallate is effective on the condition of the eye and the physiology of vision [62]. The symptoms of dry eye syndrome can be summarized as an unstable tear film, visual disruption, and discomfort. There is inflammation of the ocular surface, tear hyperosmolality, and potential damage to the ocular surface. Inflammatory cytokines (such as IL1β, IL6, TNFα, and IFNγ) are known to play a role in the ocular inflammation associated with dry eye syndrome [62]. Nano-sized systems have been developed for epigallocatechin gallate to protect it against undesirable environmental factors (oxygen, humidity, light), avoid degradation, extend its shelf life, and provide a modified release [13]. Huang et al. [62] developed epigallocatechin gallate-containing gelatin nanoparticles with surface decoration by hyaluronic acid for the treatment of dry-eye syndrome. The mean particles size and zeta potential values of the obtained nanoparticles with different concentrations (0-250 µg/mL) of hyaluronic acid ranged from about 142-323 nm and (+)23.2-(-)35.2 mV. The zeta potential values decreased proportionally with the addition of hyaluronic acid (the zeta potential values of the nanoparticles were (+)9.2 mV and (−)35.2 mV at the hyaluronic acid concentrations of 62.5 µg/mL and 250 µg/mL, respectively). The encapsulation efficiency values were more than 97%. The authors selectively used the nanoparticles with added 62.5 µg/mL hyaluronic acid for further experiments to maintain the cationic surface charge of the NPs. The anti-inflammatory effect of the selected nanoparticles on lipopolysaccharide-stimulated human corneal epithelium cells were evaluated *in vitro*. It has been reported that the nanoparticles down-regulate gene expression of IL6, TNFα, and IL8 in inflamed human corneal epithelium cells. Besides, they examined the therapeutic effect of these nanoparticles in a dry-eye syndrome rabbit's model. The ocular surface of the rabbits treated with nanoparticles topically twice daily exhibited normal corneal architecture with no appreciable change in inflammatory cytokine levels in corneal lysate, improving associated clinical signs such as tear secretion. Therefore, it was emphasized by the authors that epigallocatechin gallate-containing gelatin nanoparticles with surface decoration by hyaluronic acid have the potential to be used as eye drops (the suspension of nanoparticles) for the treatment of dry-eye syndrome [62].

Polymeric micelles are delivery systems prepared using amphiphilic block copolymers. They are characterized by a core-shell structure resulting from the self-assembly of in aqueous solutions [65,66]. Polymeric micelles have lower critical micelle concentration and higher kinetic stability compared to micelles formed by low molecular weight surfactants. Easy preparation, nano-size, and good solubilization properties of polymeric micelles are beneficial factors for their administration by different routes. Polymeric micelles are used in the pharmaceutical field to increase the solubility of drugs with poor water solubility, improve the bioavailability of drugs, provide modified drug release and reduce their side effects. Amphiphilic diblock copolymers such as PEG and polystyrene, and triblock copolymers such as poloxamers, and ionic copolymers such as PEG-poly(ε-caprolactone)-g-polyethyleneimine) are widely used for the preparation of polymeric micelles. There is a nanomicellar formulation of cyclosporine (CEQUA™) for ocular administration approved by the FDA in 2018 to increase tear production [66].

Myricetin, a natural flavonol compound, has beneficial bioactivities such as anti-inflammatory, antioxidant, and antimicrobial activities. However, its clinical use is limited due to the characteristics of myricetin such as poor water solubility, poor stability in aqueous/physiological media, and poor bioavailability [67]. Sun et al. [67] developed myricetin-containing polyvinyl caprolactam–polyvinyl acetate–PEG graft copolymer micelles using the thin-film hydration method to increase the efficacy of myricetin in eye disease treatments. The size, zeta potential, and encapsulation efficiency of the selected polymeric micelle formulation (polymer:myricetin weight ratio=18:1) containing myricetin for further studies were 60.72 nm, (-)2.29 mV, and 99.5%, respectively. They found that micelles significantly increased the water solubility and stability of myricetin, which provides flexibility in the design of eye drops for myricetin. Sun et al. [67] reported that myricetin-containing polymeric micelles greatly enhanced the *in vitro* cellular uptake, *in vivo* corneal permeation (in mouse eyes), and *in vivo* anti-inflammatory activity (in rabbit eyes with the inflammation induced by sodium arachidonate solution) of myricetin.

2.2. Lipid-based delivery systems containing flavonoids

Besides, the use of lipid-based delivery systems (liposomes, solid lipid nanoparticles, nanoemulsion, etc.) provides improvements/increases in the solubility, absorption and bioavailability of poorly water-soluble drugs/compounds [68]. Liposomes contain a phospholipid bilayer surrounding an aqueous core. Liposomes, biodegradable and biocompatible, are delivery systems that can be prepared from nano size to micro size using different preparation methods such as thin-film method, reverse phase evaporation method, membrane extrusion, sonication, microfluidic technique [69,70]. Liposomes with different compositions [using different phospholipids (such as egg or soybean phosphatidylcholine, dipalmitoylphosphatidylcholine, phosphatidylserine) and cholesterol content], structure (large unilamellar vesicle, small unilamellar vesicle, multilamellar vesicle*)* and charge (e.g. using phospholipids with different charges) can be prepared. Cholesterol is added to the formulation to increase the stability by improving the rigidity of the membrane. They can contain a wide variety of hydrophilic and hydrophobic diagnostic, active substances or biologically active compounds, protecting the encapsulated agents from environmental conditions and metabolic processes [69,71]. Because of their versatile nature, liposomes offer some advantages for the treatment of both anterior and posterior segment eye disorders. By binding to the corneal surface and increasing residence time, they can improve the permeation of poorly absorbed compounds/active substances and increase the therapeutic effect and reduce higher dose-related toxicity [72].

Other common lipid-based delivery systems are solid lipid nanoparticles and nanostructured lipid carriers [73]. Lipid-based nanoparticles have been developed to overcome some of the limitations of polymeric nanoparticles, such as the high cost and the scarcity of safe polymers with legal approval. These lipid nanoparticles have the potential to improve the performance of pharmaceuticals, neutraceuticals, and other compounds due to their small size with large surface area, and interaction of phases at interfaces [74]. Solid lipid nanoparticles are prepared by dispersing physiological lipids in water or an aqueous surfactant solution. Potential disadvantages were seen with solid lipid nanoparticles, such as the relatively high water content of the dispersions (≥70%), poor drug loading capacity, and active substance/compound expulsion during storage. The structure of the lipid matrix, the solubility of the active substance/compound in the lipid melt, and the polymorphic state/arrangement of the lipid matrix limit the drug loading capacity of the conventional solid lipid nanoparticles. Therefore, higher drug loading requires the use of more complex lipids [74]. Solid lipid nanoparticles are particularly useful in ocular delivery as they can improve the ocular bioavailability of hydrophilic and lipophilic drugs/compounds by increasing their corneal absorption. Another advantage of solid lipid nanoparticles is that they allow autoclave sterilization. Sterilization is necessary step for the ophthalmic formulations [75]. Nanostructured lipid carriers were developed to overcome potential difficulties with solid lipid nanoparticles. Biodegradable and compatible solid lipids (such as steroids, fatty acids, monoglyceride, diglyceride, triglyceride, and waxes) and liquid lipids (such as oleic acid, castor oil, Miglyol® 812, olive oil) and surfactants for their preparation were used [74,76]. The presence of liquid lipids in the formulation leads to structural imperfections of solid lipids and a less regular crystal arrangement, thereby preventing drug/compound leakage, and providing a high drug/compound loading. They are promising delivery systems for oral, ocular, parenteral, pulmonary, topical and transdermal use [76].

Nanoemulsions, kinetically stable system, have nanosized (generally about 200-600 nm) droplet size. Nanoemulsion formulations are prepared using different surfactants, oil phases, and aqueous phases. Their composition and structure might be controlled for the encapsulation and efficient delivery of active substances/compounds. Nanoemulsions have potential application in the pharmaceutical, cosmetic and food fields. They are widely used for the delivery of active substances/compounds and nutraceuticals. By preparing nanoemulsion formulations of poor-water soluble active substances/compounds, the solubility and bioavailability of these compounds are increased [77]. Nanoemulsions are potent delivery systems for ophthalmic use due to their high ability of active substances/compounds penetration into the deeper layers of the ocular structure and the aqueous humor [78].

Quercetin, a common flavonol, has been found to have potent anti-inflammatory, antioxidant, and anti-fibrotic effects in various tissues in *in vitro* and *in vivo* studies. Especially, studies evaluating the efficacy of quercetin for the treatment of dry eye, corneal inflammation, keratoconus, and the neovascularization of the cornea draw attention [42]. It protects eye cells from oxidative damage [79]. After oral administration, its exposure to intense metabolism limits the systemic effects of quercetin [42]. For this reason, some studies have been carried out on the preparation of different dosage forms containing quercetin to be administered by different application routes.

In a study, quercetin-containing lipid-based nanoparticles (solid lipid nanoparticles and nanostructured lipid carriers) and quercetin-containing hot melt cast films were prepared and also evaluated the permeability of quercetin for nanostructured lipid carrier and hot melt cast film formulations across rabbit cornea in a side-by-side diffusion apparatus. The particle sizes, zeta potentials, and the entrapment efficiencies of nanostructured lipid carriers and solid lipid nanoparticles were 46.1 and 65.4 radius in nm, (-)16.2 mV and (-)12.3 mV, and 93.4% and 90.9%, respectively. Transcorneal flux values were found to be 0.026 μg/min/cm^2 for quercetin (control), about 0.036 μg/min/cm^2 for quercetin-containing nanostructured lipid carrier, and about 0.144 μg/min/cm^2 for quercetin-containing hot melt cast films. As the result of this study, quercetin-containing hot melt cast films showed a better transcorneal permeability. It was stated that lipid-based nanoparticles and hot melt cast films might be beneficial for topical application of quercetin to the eye [45].

Wang et al. [80] prepared puerarin and scutellarin-loaded cationic lipid nanoparticles for ophthalmic application. Puerarin, an isoflavonoid compound, is derived from the root of *Pueraria lobata* (Willd.) Ohwi, commonly known as Gegen (Chinese name) in traditional Chinese medicine. It is widely used in the treatment of cancer, cerebrovascular disorders, cardiovascular diseases, diabetes and diabetic complications, Parkinson's disease, and Alzheimer's disease. It also has protective effects against oxidative damage, inflammation, osteonecrosis, hyperlipidemia, fever and disorders caused by alcohol and reduce intraocular pressure [80,81]. Scutellarin, a flavonoid, is derived from *Erigeron breviscapus* (Vant.) Hand.-Mazz, and is used in the traditional Chinese medicine. Breviscapine, a flavonoid extract of *Erigeron breviscapus*, contains ≥90% scutellarin and ≤10% apigenin-7-O-glucronide and is classified as a prescription drug in China. Data from clinics and experimental studies have demonstrated the benefits and efficacy of breviscapine and scutellarin in the treatment of cardiovascular diseases, cerebrovascular diseases, and diabetic complications. It has also been shown to be effective in regulating intraocular pressure. However, the low bioavailability of scutellarin due to its low solubility and its short half-life in biological systems limit the efficacy of scutellarin in some clinical applications [82]. Puerarin and scutellarin are used to treat retinopathy and glaucoma [80]. Wang et al. [80] stated that scutellarin can help protect the optic nerve and improve optic nerve microcirculation, and puerarin can reduce intraocular pressure, and therefore, with the co-administration of these two compounds, it can be a focal point in relief of symptoms and the treatment of ocular diseases. They evaluated the preocular retention time of puerarin and scutellarin-loaded cationic lipid nanoparticles using a noninvasive fluorescence imaging system, besides they performed corneal permeation study using excised rabbit corneas [80]. Wang et al. [80] prepared the puerarin and scutellarin-loaded cationic lipid nanoparticles using 1-oleoyl-rac-glycerol, lecithin, cholesterol, Tween 80, pluronic F127, Gelucire® 44/14, and octadecyl quaternized carboxymethyl chitosan. The mean particle size of puerarin and scutellarin-loaded cationic lipid nanoparticles was found to be 181 nm with a polidispesity index of about 0.22 and their zeta potential was 23.8 mV. The particle size and distribution are important parameters for uptake of nanoparticles through the cornea. The positive zeta potential value of the nanoparticles is due to the use of octadecyl quaternized carboxymethyl chitosan. Moreover, the use of F127 as a stabilizer has a beneficial effect on increasing the stability of the nanoparticle dispersion. In corneal permeation study, it was found that compared with puerarin solution and scutellarin solution, puerarin and scutellarin-loaded cationic lipid nanoparticles exhibited a

2.01-fold and 1.23-fold increase in the apparent corneal permeability coefficient, respectively. Therefore, the nanoparticles were more taken up by the rabbit cornea. Due to the high viscosity of puerarin and skutellarin-loaded cationic lipid nanoparticles, an increased retention time on the cornea was observed for nanoparticles. It was found that compared with puerarin solution and scutellarin solution, puerarin and scutellarin-loaded cationic lipid nanoparticles exhibited a 2.33-fold and 2.32-fold increase in the AUC values, respectively [80].

However, for hydrophilic compounds such as epigallocatechin gallate, the lipophilic nature of the corneal epithelium is a hindrance. To overcome this hindrance, positively charged lipid nanoparticles containing epigallocatechin gallate were prepared in the literature [62,83]. Fangueiro et al. [83] prepared cationic lipid nanoparticles containing epigallocatechin gallate using softisan® 100 , glyserol, Lipoid® S75, ascorbic acid, poloxamer 188, and cetyltrimethylammonium bromide/dimethyldioctadecylammonium bromide, by multiple emulsion (w/o/w) technique. The particle size, and zeta potential values of epigallocatechin gallate-loaded cationic lipid nanoparticles containing cetyltrimethylammonium bromide or dimethyldioctadecylammonium bromide were found to be about 149 and 144 nm, and 20.8 and 25.7 mV, respectively. The polidispersity index values of epigallocatechin gallate-loaded cationic lipid nanoparticles were less than 0.25 [83]. They performed e*x vivo* permeation study using isolated rabbit sclera and cornea as a membrane mounted on Franz-type diffusion cell and found that epigallocatechin gallate showed higher permeation than retention in the sclera and cornea when cationic lipid nanoparticles containing cetyltrimethylammonium bromide were used. However, when using cationic lipid nanoparticles containing dimethyl dioctadecyl ammonium bromide, retention in the sclera and cornea was higher than penetration. The results showed that cationic lipid nanoparticles could improve the residence time and bioavailability of epigallocatechin gallate [83].

As a result of the electrostatic interaction between the negatively charged ocular mucosa and the positively charged nanoparticles, the retention time of the compounds such as epigallocatechin gallate on the eye surface and their ocular absorption can be improved [62].

Nanoemulsion and solid lipid nanoparticle formulations for ocular quercetin delivery were prepared by Liu et al [79] to take advantage of the antioxidant capacity of quercetin by providing its physical and chemical stability. The particle size, polydispersity index, zeta potential, and entrapment efficiency % values for quercetin-loaded solid lipid nanoparticles were determined as 143 nm, 0.27, (-)16.57 mV, and 66.56%. On the other hand, the droplet size, polydispersity index, zeta potential, and entrapment efficiency % values for quercetin-containing nanoemulsion formulation were found to be 138.3 nm, 0.25, (-)23.97 mV, and 74.26%. In addition, quercetin-loaded solid lipid nanoparticles showed the lowest cytotoxicity on retinal ganglion and cornea cells. Compared with free quercetin, quercetin-loaded solid lipid nanoparticles effectively protected cornea and retinal ganglion cells from oxidative damage induced by H_2O_2. Besides, scleral and corneal delivery of these formulations was confirmed using *ex vivo* porcine eyes. Quercetin-loaded solid lipid nanoparticles had a higher transcorneal flux for quercetin (158.5 µg/cm^2/day) compared to the quercetin-containing nanoemulsion formulation (130.7 µg/cm^2/day). Solid lipid nanoparticles have been shown to be more suitable for transocular delivery [79].

Surgery for the treatment of cataract, one of the leading causes of blindness globally, is accompanied by some complications, especially intraocular infections. Oxidative stress is one of the important causes of cataract development. Huang et al. [84] stated that nanoformulations may be beneficial for delaying or preventing cataracts, and therefore, they prepared chitosan-coated liposomal formulations containing a combination of lanosterol (It is a tetracyclic triterpenoid compound effective in preventing/reversing protein aggregation in cataracts) and hesperetin by thin film evaporation-active extrusion method. Hesperetin is a bioactive flavanone that is found in some vegetables and fruits (young citrus fruit, tomatoes, apples etc.), being thus easily accessible and isolated at low cost. Active hesperetin can be obtained from hesperidin hydrolysis by the intestinal bacteria. Similar to other flavonoids, it has many biological activities (anti-inflammatory, antioxidant, anti-diabetic, anticancer, estrogenic, neuroprotective, vasoprotective and cardioprotective effects, lipid lowering abilities) beneficial to human health. However, it has poor water solubility due to its hydrophobic nature and does not sufficient stability in the gastrointestinal tract; therefore it has low oral bioavailability [85].

The mean particles size of a combination of lanosterol and hesperetin-loaded chitosan-coated liposomes prepared by Huang et al. [84] was 224 nm with a polydispersity index of 0.25. They found that the coated-liposomes were cytocompatible and showed slow and sustained release of lanosterol and hesperetin from chitosan-coated liposomes. The retention time of the chitosan-coated liposomes in the cornea was more than a week. The authors also investigated the effect of lanosterol and hesperetin-loaded chitosan-coated liposomes in preventing/delaying selenite-induced cataract in rats and reported that the coated liposomes increased the retention time for lanosterol and hesperetin in cornea and allowed the upregulation of antioxidant status and ultimately led to a delay in cataract progression [84].

In another study, for the ophthalmic delivery of baicalin, different vesicular systems such as liposome, penetration enhancer vesicles or transfersomes were prepared by thin film hydration technique using soybean phosphatidylcholine (SPC) with/without cholosterol and/or sorbitol or SPC and labrasol (penetration enhancer) with/without cholosterol and/or sorbitol or SPC and bile salts (such as sodium taurocholate) with/without cholosterol and/or sorbitol. The mean vesicle size and zeta potential values of baicalin-containing vesicular systems were in the range of 667.9- 2712 nm and (-)14.4- (-)31.5 mV, respectively. The EE% values obtained for baicalin-containing vesicular systems were ranged from 25.96 to 99%. They carried out Draize test and found that all baicalin vesicular systems and baicalin control solution had good ocular tolerance and were non-irritant. Besides, the ocular pharmacokinetics parameters for the vesicular systems were studied in rabbits. For this purpose, sterile vesicular formulations or baicalin control solution were dropped into the conjunctival sac, then aqueous humor samples were collected at certain time intervals, and the concentration of baicalin in the samples was analyzed by

HPLC. The author found that the C_{max} values were found to be 4.073 µg/mL for liposomes, 2.313 µg/mL for penetration enhancer vesicles, 1.503 µg/mL for transfersomes, and 2.228 µg/mL for the baicalin control solution. The $AUC_{0-\infty}$ values of penetration enhancer vesicles, transfersomes, or liposome were 5.4, 4.6 and 4.4-fold higher, respectively, compared to the $AUC_{0-\infty}$ value of the baicalin control solution [86].

Liu et al. [14] prepared the solid lipid nanoparticles containing baicalin for ocular administration by emulsification/ultrasonication method using soya phospholipids SL-100, and triglyceride as oil phase, and Poloxamer 188 as surfactant. They found that the solid lipid nanoparticles were spherical and the encapsulation efficiency, particle size, and zeta potential values of these nanoparticles were approximately 62%, 91 nm, and (-)33.5 mV, respectively. Baicalin-loaded solid lipid nanoparticles enhanced the corneal permeability of baicalin. Furthermore, they carried out pharmacokinetic studies in rabbits and found that the bioavailability of baicalin can be significantly increased with the use of solid lipid nanoparticles [14].

2.3. Metallic nanoparticles

Gold is a multifunctional material used in medical applications due to its antioxidative, bacteriostatic and anticorrosive photoacoustic and photothermal properties. Due to the presence of thiol and amine groups, it can be functionalized by allowing conjugation of various compounds such as antibodies or active substances and produced at nanoscale [87]. Spherical gold nanoparticles have large surface-to-volume ratio, excellent biocompatibility, optoelectronic properties, and low toxicity properties regarding their shape and size. The loading of active substances/compounds onto gold nanoparticles can be accomplished via covalent conjugation or non-covalent interactions. Non-covalent interactions (affinity, electrostatic and hydrophobic interactions) are widely used in the fields of delivery and sensing due to reversible nature and their ease of release; covalent conjugation of compounds to gold nanoparticles is utilized in imaging. It has been shown that cell membrane penetration can be regulated by surface ligand arrangement on these nanoparticles. Therapeutics can be delivered to cells via passive or active targeting mechanisms [88].

Li et al. [89] developed poly(catechin) capped-gold nanoparticles containing amfenac to treat dry eye disease. Catechin as a flavonol has an antioxidant effect, while amfenac is a nonsteroidal anti-inflammatory drug. They aimed to use these nanoparticles to simultaneously prevent (to achieve a synergistic effect) the inflammation induced by cyclooxygenase enzymes, and also oxidative stress induced by reactive oxygen species, which are the causes of dry eye disease. As a result of this study, they showed that poly(catechin) capped-gold nanoparticles containing amfenac have both antioxidant and anti-inflammatory effects. In addition, the researchers conducted *in vivo* studies to demonstrate the biocompatibility and the dual effects of the nanoparticles. They showed that the nanoparticles had good tolerability and provided a rapid recovery in the rabbit dry eye model [89].

3. Conclusion

Flavonoids appear to be promising compounds in the prevention/treatment of various ocular diseases. However, most flavonoids are poorly soluble in water and have low bioavailability and stability, limiting their use. *In vitro* and preclinical studies have shown that nano-sized delivery systems improve the water solubility, bioavailability, stability, and consequent *in vivo* activity of flavonoids. However, more studies and especially well-constructed clinical studies are required to examine the effects of flavonoid-containing delivery systems (especially nano-sized systems) on ocular diseases.

Compliance with Ethical Standards

There is no conflict of interest to disclose.

Conflict of Interest

The author(s) declares no known competing financial interests or personal relationships.

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RESEARCH ARTICLE

The use of unmanned aerial vehicles in the detection of forest fires with a gas detection technique

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HIGHLIGHTS

> It is aimed at bringing a new and more precise perspective based on visuals to forest fire early detection systems.
> The ability to detect CO and CO_2 gas, which is the earliest sign of fire diagnosis, has been added to the unmanned aerial vehicle.
> Metal oxide CO_2 detector was produced by screen printing technique in order to detect the CO_2 gas.

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ABSTRACT

Unmanned Aerial Vehicles (UAV) are used in many applications such as forest fire, atmosphere research, ocean observations, geological surveys, weather forecasting, and especially in military applications. While designing Unmanned Aerial Vehicles, which are generally used for reconnaissance, surveillance and operational purposes, in addition to performance and efficiency increasing studies, today researches are also conducted on which type of aircraft is most suitable for what type of mission.

In this study, it is aimed at bringing a new and more precise perspective based on visuals to forest fire early detection systems. In this period, since the number of systems used for utilizing unmanned aerial vehicles technology is increasing day by day, it would be appropriate to use unmanned aerial vehicles with perception capability to minimize the destruction of forests, which are the lungs of the world, except for the natural flow, and to manage the workforce and time resources in the best way.

In this study, metal oxide CO_2 detector was produced by screen printing technique to detect the CO_2 gas from the sensors to observe and made the necessary controls in case of a fire in the areas that can be reached or not reached by the UAV.

1. Introduction

UAV is a kind of aircraft with no pilot and no passengers, carrying only the equipment suitable for the purpose (video camera, camera, global navigation satellite system, laser scanning device, etc.) to perform its task remotely and/or automatically [1].

Unmanned aerial vehicles are increasing in importance and are mentioned in more areas. The success of the tasks they are assigned and expected to perform depends on their best performance. In this context, the place of unmanned aerial vehicles in aircraft design is increasing [2].

UAV is used in many applications such as forest fire, atmosphere research, ocean observations, geological surveys, weather forecasting, especially in military applications [3].

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Today, while designing the UAV, which is generally used for reconnaissance, surveillance and operational purposes, studies are carried out to increase the performance and efficiency. Also, some researches which type of aircraft is most suitable for what kind of mission [4].

The history of UAV started with the construction of air balloons and their use for various purposes, and with the developments in aircraft technology in the following years, it took the form of unmanned aircraft and today's modern unmanned aerial aircraft were produced. Initially, its use for military purposes came to the fore, then it is used for various purposes in meteorological studies and now in many civilian areas including agriculture. UAVs are classified according to altitude and flight range or according to their wing structures and are used in a wide variety of fields, both military and civilian [5].

While forests are of vital importance for the protection of soil and water and for the survival of all living things, forest fires are seen as the biggest threat to these resources. Air temperature, relative humidity, precipitation, wind direction and speed, etc., are effective in the formation of forest fires. Since it is important, risky areas can be determined by following these data [6].

In this study, it is aimed to monitor the fires from the air with UAV and to detect and extinguish fires at an early stage. The ability to detect carbon monoxide (CO) and carbon dioxide (CO_2) gas, which is the earliest sign of fire diagnosis, has been added to unmanned aerial vehicle. The most basic problem in common algorithms used in fire detection is the high rate of false notification and oversight. Confirming the result extracted from the image taken and defining an extra verification step will increase the reliability of the system and guarantee the accuracy of the result. Thanks to the mobile vision of the unmanned aerial vehicle, the viewpoint will be controlled by the control point, where the data can be taken from the place, clearly and continuously.

Metal oxide CO_2 detector was produced by screen printing technique in order to detect the CO_2 gas from the sensors required in order to observe and make the necessary controls in case of a fire in the areas that can be reached or not reached by the UAV used in this study.

The necessity of choosing economical and effective production methods was taken into consideration as the purpose of choosing the screen-printing technique as the sensor construction technique. Screen printing technique allows synthesizing and converting the smallest possible size films into gas detectors for gas detector applications. In addition to being one of the most economical methods for the production of gas sensor components, the screen-printing technique has several advantages such as the production of semiconductor oxide structures with excellent homogeneity and the addition of high purity additives.

2. Materials and Methods

2.1. Screen-Printing Technique

A thick film gas detector consists of three main components. These are the heater, the electrode and the sensitive layer.

While applying this technique, at each stage (heater, electrode and sensitive layer construction), the selected material is screen-printed on the substrate and the heat treatment of the created structure is performed.

In the fabrication of the CO_2 thick film gas detector made within the scope of this study, the screen-printing method was used and the planar structure of the thick film to be produced is given in Figure 1.

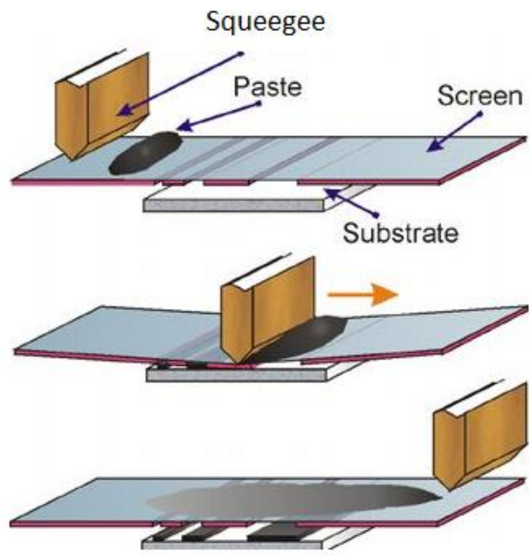

Figure 1. The basic screen-printing process

The order of application in the screen-printing process is as follows [7];

• Screen printing of the heater on the substrate and heat treatment of this layer

• Screen printing of the insulation layer and heat treatment of this layer

• Screen printing of electrodes on the substrate and heat treatment of this layer

• Screen printing of the active layer on the substrate and heat treatment of this layer

2.2. Mask

A crucial part of screen-printing equipment, the mask identifies the pattern of the printed film and also measures the amount of paste that is planned to be passed on to the underside.

The selection of a suitable mesh number for a mask is a very important criterion. The mesh itself is usually based on a flat mesh pattern as shown in Figure 2 [7].

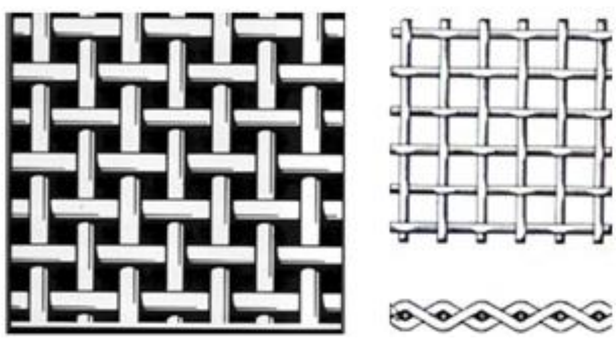

Figure 2. Flat pattern mesh for a typical thick film screen.

The selection of a suitable mesh number for a mask is a very important criterion. The type of paste used and the model to be applied are factors that may affect this choice. A typical mesh number for general purpose work is around 200 threads per inch.

It is possible to obtain the screen mesh aligned in several orientations, the most common three mesh angles are 22.5°, 45°, and 90°.

Many screen manufacturers apply the emulsion by hand coating, a skilled process which can accurately produce a standard range of thicknesses (typically between 5 to 25 µm). The specifications of screens such as mesh count, mesh angle, tension, emulsion thickness, and size of frame normally comes with a label attached to the rear of the screen.

Figure 3 shows the mask used in the preparation of the active layer in this study.

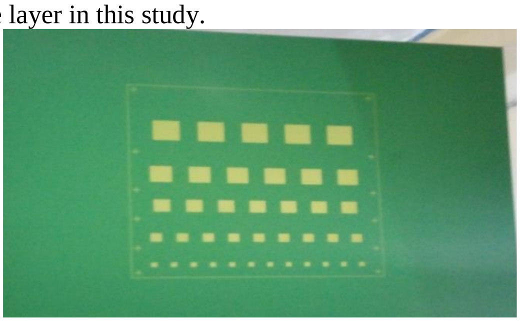

Figure 3. The mask used in the active layer

2.3. Screen Printing Process

The screen-printing process, which gave its name to the method in this technique, consists of masks designed according to the type of gas to be detected and metal oxide to be used. A small amount of organic compounds called paste is placed on the mask and pulled with the help of a rubber arm.

As can be seen in Figure 4. The prepared paste is applied to the upper part of the pattern to be used on the mask placed in the screen-printing machine, and the shape of the pattern is drawn on the substrate by applying the drawing process.

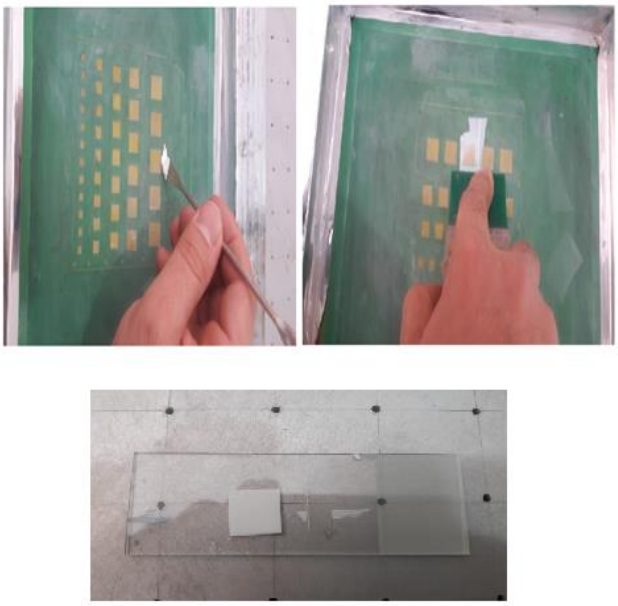

Figure 4. Pulling process by adding paste on the mask

2.4. Heat Treatment

The active layer, heater or electrodes screen-printed on the substrate are subjected to heat treatment. The temperature and waiting times in the process steps to be applied in the heat treatment vary according to the characteristics of the material.

The heat treatment steps are as follows;

- Rest
- Drying
- Annealing
- Cooling

2.5. Preparation of Binder and Paste

Both of the bonding preparation techniques described in the screen-printing method were used for comparison. The rates encountered in the literature study [7–10] to determine the proportions of chemicals to be used in the compounding were tested one by one, and the rates decided to be used in this study are as given in Table 1.

Table 1. Chemicals and ratios used in binder preparation

Binder made with linseed oil		Binder made with ethyl cellulose	
	wt (%)		wt (%)
linseed oil	85	Ethyl cellulose	10
m–xylene	12,5	α–terpineol	90
α–terpineol	2,5		

For the preparation of the binder, first, linseed oil and m–xylene were mixed and they subjected to magnetic stirring at 40 °C on hot plate. The stirring process was continued for about 4 hours.

After that α–terpineol was added then the temperature of was decreased to 30°C. The stirring was continued for about 2 hours to obtain a sticky liquid form binder.

Thick film gas sensor pastes usually comprise a semiconducting metal oxide powder, inorganic additives and organic binders.

In this research, tin oxide (SnO_2) was used as the base sensitive metal oxide powders.

Sensitive powder (SnO_2) was mixed with 40 wt% and binder was mixed with 60 wt% and they subjected to magnetic stirring at 40 °C on hot plate for 24 hours.

2.6. Film Preparations

The screen-printing process, which has given its name to the method in this technique, consists of masks designed according to the type of gas to be detected and the metal oxide to be used.

The base to be used during the screen-printing process is placed on the screen-printing machine used in the serigraphy technique.

A number of factors need to be considered when choosing the mesh angle. 45° mesh size was selected in this study. A 45° mesh provides the maximum flexibility for the stretched fabric but many produce a serrated edge on conductor lines which are aligned in parallel or at 90° with the direction of the squeegee

The reason for using the screen-printing machine is to prevent the base from sticking to the mask while screen-printing, since it has a vacuum feature.

The SnO_2 thick films were prepared by screen print technique using Al_2O_3 substrate. Silver electrodes were printed on an Al_2O_3 substrate. After printing the electrodes, it was kept at room temperature for 10 minutes. After these electrodes were kept at 125 °C for 10 minutes for drying. Finally, electrodes were kept at 350 °C for 20 minutes for annealing.

Sensitive layer was printed with sensitive paste on electrodes. After printing the sensitive layer, films were left at room temperature for 20 min to insure the paste is leveled off and settled and then the films are subjected to a drying and annealing process.

The sensitive layer was dried in three steps: first it was subjected to 50°C for 5 minutes and then temperature was increased to 100 °C for another 5 minutes, and finally it was dried at peak temperature of 125 °C for last five minutes. Finally, film was kept at 450 °C for 30 minutes for annealing.

2.7. UAV Aerial Platform

In this study, the investigation of the effectiveness of the use of UAV in the fire detection study was carried out using a modified 4-engine helicopter model as a platform. The UAV system used in the fire detection study consists of an air platform and a ground control station.

A rotary wing 4-engine (quadrotor) mini helicopter was used as an aerial platform. The model used has a suitable place for mounting electrical - electronic (avionic) systems and camera systems such as camera, video transmitter and standard radio transmitter. Figure 5 shows the DJI Phantom 2 Vision Plus UAV used as an aerial platform.

Figure 5. DJI Phantom 2 Vision Plus

3. Results and Discussion

3.1. XRD Analysis of Samples Produced with SnO_2

The structures of the SnO_2-based samples produced by the screen-printing method were examined by the XRD technique and are shown in the figures given below, respectively. (Figure 6).

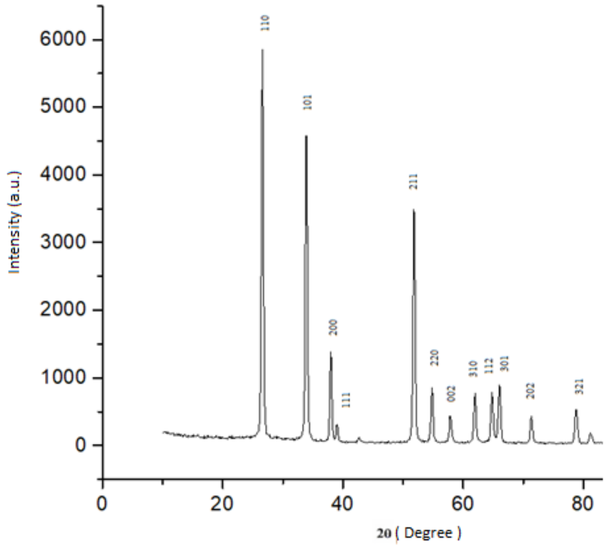

Figure 6. XRD graph of the SnO_2 sample

In the graphic shown in Figure 6. It has been observed that SnO_2 reflects in (110), (101), (211), (220), (002), (310), (112), (301), (202) and (321) planes. When these planes are compared with other studies [11–15] and with the JCPDS 71-0652 card number XRD database phases, it was observed that they are reflective peaks of pure SnO_2.

3.2. Gas Detection Measurements of SnO_2 Based Gas Sensor

The main purpose of the gas detection measurement system is to measure the change in sample resistance as a result of the interaction of the sample and the target gas.

The values used during the gas detection measurement of the SnO_2 sample measured at 300 °C temperature are given in Table 2.

Table 2. Values used during the measurement of the SnO_2 sample

Temperature	300 °C
Time to measure	400 s
External gas sweeping time	400 s
Gas measured	CO_2
External gas	Air
Period (Per Ppm)	1
PPM list	10000
Voltage source level	1 V

In order to clean the environment from various gases, nitrogen gas was sent to the environment for 40 seconds during the measurement process of the SnO_2 sample. Then, measurements were taken by giving the carbon dioxide gas to be measured together with nitrogen gas at the rate of 10000 ppm in the 40th second and shown in Figure 7.

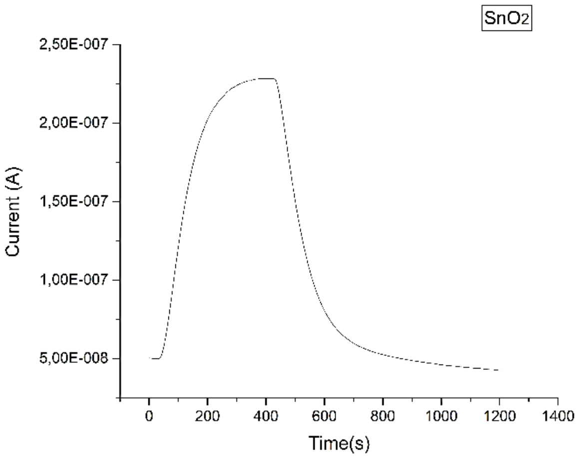

Figure 7. Gas sensor measurement chart of SnO_2 sample under 10000 ppm CO_2

The measurements of the SnO_2 sample used in this study were made at 10000 ppm CO_2 concentrations and measurement temperatures, and similar and better results were obtained with the data in this range. When the response times of the measurements were examined, it was observed that it was between 50 and 65 seconds after the fire started.

4. Conclusion

In the fabrication of the CO_2 thick film gas detector made within the scope of this study, the heater, electrode and sensitive layer, which are the three main components of a TiO_2 and SnO_2 doped thick film gas detector, were produced by using linseed oil, xylene, alpha terpineol and ethyl cellulose starting materials.

Screen printing method was used in film preparation, films were coated on glass and alumina (Al_2O_3) substrate and annealed at various temperatures. Structural analyzes were obtained by X-Ray Diffraction (XRD), the morphological properties of the films were analyzed by Scanning Electron Microscope (SEM) and their electrical properties were examined in detail.

The results obtained by interpreting the observations during the studies and the data obtained after the studies are as follows;

From the XRD results, SnO_2 phases in anatase structure were determined and it was observed that the peak intensity of these phases increased depending on the appropriate heat treatment process.

As a result of the study, low power consumption gas sensors using SnO_2 metal oxides as sensitive active materials were designed, produced and characterized. As a result of the tests, it has been shown that the developed sensors are sensitive to the materials specified in the literature. In accordance with the increase in gas concentration, an increase in sensor response was also observed.

At the end of the study, the gas detection feature of the produced SnO_2 sample was examined under the specified gases, and it was concluded that it can be used simultaneously with fire detectors to increase the detection reliability and reduce the alarm time with today's fire detectors.

Compliance with Ethical Standards

There is no conflict of interest to disclose.

Conflict of Interest

The author(s) declares no known competing financial interests or personal relationships.

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REVIEW ARTICLE

Nanotechnology in cosmetics: Opportunities and challenges

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HIGHLIGHTS

> The use of nanotechnological applications in the cosmetic industry was examined.
> The use of different nano-sized materials in cosmetics was examined.
> The safety requirements of cosmetic products were determined.

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ABSTRACT

The word "nano" is derived from the Greek word "nanos" meaning "dwarf" and corresponds to a size of 1 x 10^{-9} meters. The technology dealing with these tiny substances is also called "nanotechnology". The famous American physicist Richard Feynman (1918–1988) proposed for the first time that there is a nano-sized world. Feynman addressed the mystery of nanoscales for the first time in his speech titled "There is more room below" at a conference in 1959. The scientist reported that many new discoveries would emerge if he could work in the atomic and molecular dimensions he imagined. In this speech, which is accepted as the beginning of nanotechnology, it was emphasized that nanoscale measurement and production methods should be developed first in order to be engaged in nano-scale studies. Nanotechnology is the creation of functional structures at nanometer scales by combining atoms and molecules. Nano is a scale, nanotechnology means technologies developed at that scale. Therefore, dealing with nanoscience and nanotechnology requires a multidisciplinary field. Its target is not a specific subject; it creates a wide coverage area that requires collaboration and includes many researches. With atoms and their arrangement; it means making innovations in materials, production techniques and developing new products with superior features suitable for needs. Nanotechnology is widely used in cosmetics, especially in moisturizers, sunscreen and anti-aging products: Apart from these, there are different uses such as make-up products, perfumery, oral and hair care products. The number of products containing nanoparticles is increasing day by day in the cosmetics industry.

1. Introduction

Nanotechnology can be explained as a field in which popular and widely used raw materials, devices and systems with a size range of 1-100 nm are designed, structurally examined and their applications are investigated. Nanotechnology is known as the technology of the future and it was around the same time that the cosmetic industry met it. It has been seen that it is used in the sector, especially in areas such as protection against harmful sun rays, long-lasting cosmetic products, pigment production and skin penetration Therefore, sectoral development in which nanotechnology is used in cosmetics is gaining more and more importance day by day [1]. The reason why nano-sized products are preferred in cosmetics is the color they gain with their size, durability, transparency and antimicrobial properties [2]. With these attractive properties, nanomaterials are becoming preferred for the cosmetic industry.

2. Nano Diversity in Cosmetics

2.1. Mineral-Based Cosmetic Ingredients with Nano Dimensions

Cosmetics containing sunscreen are mineral content. Its effectiveness varies depending on the size of the nanoparticles in the product used. Titanium and zinc

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containing nanoparticles can be added to sunscreens with sizes around 20 nm. It is known that these products provide different advantages, one of these advantages is to obtain sufficient UV protection effect in less use [3,4].

2.2. Other Nano-Sized Materials Used in Cosmetics

Many well-known cosmetic companies claim that their products contain different nano-sized materials such as nanoparticles, liposomes, fullerenes, nanotubes and quantum dots [3,4].

Types of Nanomaterials Used in Cosmetics Are:

2.2.1. Liposomes

Structurally, liposomes are natural or synthetic phospholipids that are purely GRAS (generally considered safe) products of the aqueous volume. These phospholipids form bilayer vesicles with a lipid bilayer. By their arrangement, the lipid bilayer of liposomes is similar to the cell membrane, which supports the release of its contents. For this reason, they can easily pass through the cell membrane [5,6]. Thus, they are preferred in formulations in the field of cosmetics due to their rapid absorption. Of the liposomes, transferosomes, niosomes, and etosomes are examples of the most rapidly absorbed [6–9].

2.2.2. Nanoemulsions

It is obtained by dispersing a liquid in another liquid as nanoscale droplets [10]. Stability may vary according to synthesis methods. In addition, GRAS products are used in its synthesis. Depending on the size of the obtained nanoemulsion, its properties such as stability, content carrying capacity, absorption and shelf life may change [9,11].

2.2.3. Nanocapsules

On the other hand, nanocapsules are nano-sized structures consisting of a polymeric capsule surrounding an aqueous or oil-based core. Experiments on pigskin have shown that the use of nanocapsules reduces UV filtered octyl methoxycinnamate penetration compared to non-nanosized ones [12].

2.2.4. Solid Lipid Nanoparticles (SLN)

They are nanocomponents that are solid at body temperature, containing stabilizing agents and lipid droplets in their structure. They are known to be used to protect encapsulated structures. In addition, it has been understood that the active ingredients in formulations prepared using SLN can more easily pass into the stratum corneum [13]. Studies with SLM have shown that it increases hydration on the skin compared to placebo [1].

In another study, it was found that when used with sunscreens, they increase the UV filtration properties. In a study conducted using this method, it was observed that the UV filtration property of 3,4,5-trimethoxybenzoylkit increased when included in SLNs [14].

2.2.5. Nanocrystals

Their structures are clustered formations of between 100-10,000 atoms. The sizes of these clusters usually have a scale of 10-400 nm. Due to their size, their chemical and physical properties may differ. When used, they cause controlled, safe and effective absorption through the skin [15].

2.2.6. Nanosilver and Nanogold

One of the structures commonly used in the cosmetics industry is nanosilver. Cosmetic manufacturers widely benefit from the antimicrobial property of this structure. Examples of personal care and cosmetics produced for this purpose are products such as antimicrobial armpit dedorants with 24-hour effect. It is understood that it can be used in the development of mouthwash and oral care products as a different application area. Another structure, nanogold, is seen to be used in cosmetic products such as hair dyes and masks. It is also known to be added to mouthwashes and toothpastes as a personal care product [16].

2.2.7. Dendrimers

Dendrimers are about 20 nm in size, branched structures with a symmetrical backbone and contain functional groups at their ends. The usage areas vary according to these functional groups. The structures of dedrimers can be monomolecular, monodisperse and micellar. They gain functionality according to the structure of their functional groups [17,18].

2.2.8. Cubosomes

Cubosomes are cubic-shaped structures with a biphasic structure. They usually contain sulfates and phospholipids and crystal structures in their structures. When evaluated in terms of size, they are classified as nano or submicron [19]. Cubosomes are formed as a result of the reaction of the material selected as a sulfactant and the crystal structure in the presence of microstructures in the aqueous medium. Their surface areas are quite large due to their geometry. They have a low density and also very dilute solutions can be prepared easily. Both polar and non-polar groups on them provide advantages and they also have high heat stability [1,12,20]. They are widely preferred in the commercialization of cosmetic products due to the stated reasons and also due to the low cost.

2.2.9. Hydrogels

Structurally, they are three-dimensional polymers that swell in water or biological fluids linked by chemical or physical cross-links. They are widely used in rejuvenating care products due to their net-like systems and hydrophilic nature [20].

2.2.10. Bucky Balls

Fullerelen (C60) is the most remarkable and widely used nanomaterial. Structurally, its diameter is around 1 nm. It is used in expensive anti-aging creams due to its strong radical scavenging effect [1].

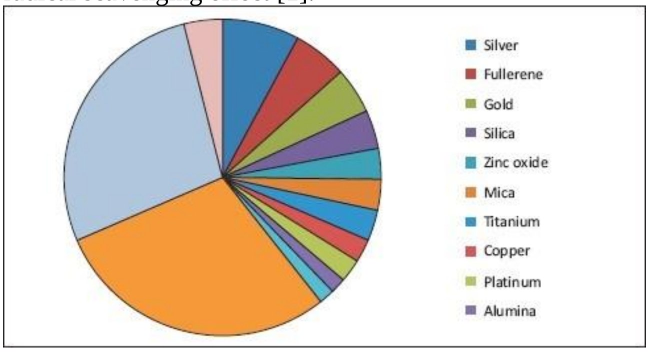

Figure 1. Basic nanomaterials used in cosmetics

3. Characterization Methods for the Safety Assessment of Nanoparticles in Cosmetics

The views of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) relate to risk analyses (25) to assess potential adverse health and environmental impacts of nanotechnology products, as well as research into nanomaterials [21]. The particular properties of nanomaterials will require new testing methodologies to identify potential injury treatments they may cause.

The following parameters can be listed in the safety evaluation of nanomaterials:

3.1. *Physical and Chemical Properties of Nanomaterials*

Physical properties of nanomaterials such as shape, size, agglomeration status, surface area, size, morphology should be determined [22].

3.2. *Mathematical Modeling*

These predictive models range from simple, experimental algorithms to complex mathematical equations that sometimes require knowledge and estimation of parameters that are not available experimentally. However, since none of these models include data compounds, they can't be applied with confidence as they cannot predict what might happen when such substances come into contact with the skin [23].

3.3. *Microscopic Techniques*

More useful information can be obtained from *in vitro* studies by microscopic examination of the skin after treatment. Although absolute quantification is not possible, visualization of the tissue to which an active has been applied can provide valuable information [23,24]. The analysis methods for evaluation of samples are given in Table 1 below.

Table 1. Methods used for imaging techniques [23].

Techniques used	Advantages
Laser scanning confocal microscopy (LSCM)	• 3D views of the skin samples obtain with high resolution electron microscopy images. • Demonstration of efficacy in formulation containing nanomaterial in cosmetic product. • Visualize the affinity of particulate vectors for follicular opening
High-resolution transmission electron microscopy	• Visualize individual particles in ultra-thin sections of tissue • Can use X-ray analysis to identify the chemical composition of the visualized vector
Particle induced X-ray emission (PIXE)	• Large fields of view • Ease sample preparation • Facile elimination of artefacts
Radio labeling with the positron emitter ^{48}V	• Ultra-sensitive • Easy to use • Provides different fields of view • Shows individual positron tracks • Useful for localizing particles to specific structures in/on the skin

3.4. *In Vitro Methods*

Although there are various methods and technologies for studying the molecular mechanisms involved in the biological activity of compounds, only approved methods are allowed for cosmetic products. These approved methods should be used for testing the safety assessment of cosmetic ingredients [23,24]. The proven *in vitro* tests used in the field are given in the Figure 2 [23].

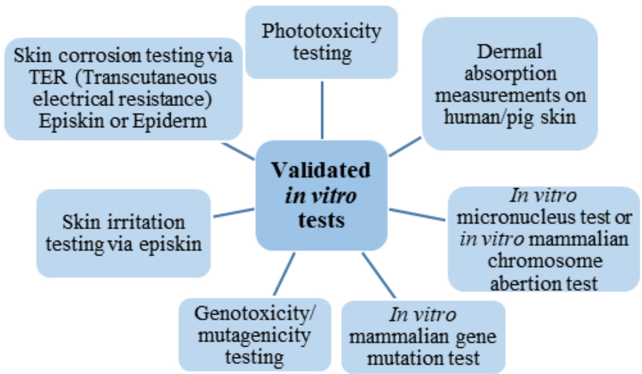

Figure 2. Some *in vitro* methods used [23]

Relevant toxicological endpoints considered important for nanomaterials are given in Figure 3 below [24].

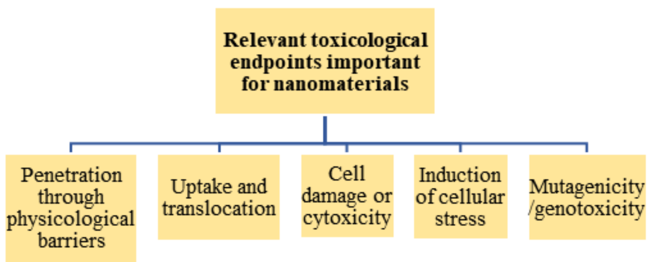

Figure 3. Relevant toxicological endpoints important for nanomaterials

4. Safety Requirements for Cosmetic Products

Cosmetic manufacturers should not lose sight of the fact that there may be some uncertainty using nanotechnology, both from a consumer response and a regulatory perspective. Leading scientific organizations such as the UK's most prestigious scientific institution, the Royal Society, and the US Food and Drug Administration, agree that nanocosmetics can have health risks and therefore require extensive research before product commercialization [25].

Although the use of nanomaterials in cosmetic products has increased day by day, no different regulation has been made regarding the safety assessment. It is involved in regulating the safety of ingredients in products in the cosmetic industry within the framework of the National Industrial Chemicals Notification and Evaluation Plan (NICNAS) in Australia. However, there is no distinction between nano or bulk sizes. The EU's Scientific Committee included animal experiments in its safety assessment of nanomaterials in cosmetic products for Consumer Products (SCCP) and evaluated whether previous findings should be corrected [26].

The European Parliament approved the re-amendment of the EU Cosmetics Directive in EU legislation. Within the framework of the new regulation requested by the European Parliament, a safety assessment procedure was required for all products containing nanomaterials. In addition, it was ruled that cosmetic products containing nanomaterials should be banned if there was a risk to human health (31). Some parts of the law are listed below:

Some *following terms:*

• Decision defines a nano-material as “an insoluble or biologically persistent and intentionally produced material with one or more external dimensions or an internal structure on the scale of 1 to 100 nm”.

• Responsible person, restrictions listed in Annexes, animal testing, safety, product information dossier, notification, safety assessment, sampling and analysis, GMP, CMR, traces of nanomaterials, and labelling, public information, claims, SUE on substances.

Before the cosmetic material is presented to the customer, it is required to submit the following information to the Commission:

• Presence of nanomaterials

• Chemical name of materials (IUPAC) and ingredients

• Projected exposure conditions

• If the Commission has negative opinions about the safety of nanomaterials, it asks them to submit an perspective on the safety of them and the exposures for the relevant cosmetic products.

• The form of the nanomaterial will be clearly stated in the ingredient substance of product. The names of these components will be written as the "nano" word.

• Special attention will be paid to its toxicological effects.

• Dimensions of particles including nanomaterials

• Impurities of the ingredients;

• Interaction of materials [27,28]

5. Conclusion

The use of nano-sized materials in the cosmetic industry is increasing day by day. Due to the superior properties of nanomaterials, it has become the focus of attention of the cosmetics industry and has taken its place in the production of cosmetics. In addition, the introduction of nanomaterials into cosmetics has caused great concerns in terms of reliability. For the purpose of ensuring the safety and effectiveness of all products, the European Union has made serious changes to the Cosmetics Directive since 2012. With this regulation, only Nanocosmetic products whose safety has been demonstrated were allowed to enter the market, and inaction was ensured for suspicious ones.

Compliance with Ethical Standards

There is no conflict of interest to disclose.

Conflict of Interest

The author(s) declares no known competing financial interests or personal relationships.

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REVIEW ARTICLE

Environmental opportunities of aquatic insects in nanotechnology

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HIGHLIGHTS

> Aquatic Insects in Nanotechnology.

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ABSTRACT

Nanotechnology plays an important role opportunity in several industries, biotechnology, medicine, and environments by creating new opportunities. These roles may be developed for recyclability, to prevent environmental pollution and performance of countless products. For example, in terms of environmental protection, nanotechnology has every possible method. The most influential members of the environment are insects in terms of species numbers-varieties. For this reason, in terms of the sustainability of these areas, it is important to analyze insects in more detail, especially in freshwater. If these details are obtained, it can only be feasible with nano-scale research that current and evolving technology. The purpose of this review is to investigate to take lead in the formation of ideas that will enable the discovery of new features about correlations between aquatic insects and nanotechnology. It is expected that this review will enable us to explore the latest ecological data and will lead us to new methodological approaches on nano-scale for aquatic insects.

1. Introduction

Over the long history of human evolution, various insects (hexapods) have adapted to the unique habitats and conditions created by humans in and around the household. This group of organisms has also successfully adapted to their natural habitats. The adaptation was accomplished through the ability of hexapods associated to utilize food resources and harborages with humans [1]. In all ecosystems, biotic concussion is expected gradually to increase worldwide due to human activities [2]. These adaptations have taken place in an ecosystem balance. However, human activities negatively affect all terrestrial and aquatic ecosystems [3].

Freshwater is an essential resource for life [4] in aquatic ecosystems. These areas support approximately 10% of all species in the World [5]. Especially macroinvertebrates constitute an important part of the biotic parts in freshwaters. Hexapods cover the majority of macroinvertebrate communities [6]. Hexapods, without its means to collapse food production [7], have some exemplary living beings in analyzing the structure and function of the freshwater ecosystem due to their high abundance, huge biomass, and rapid colonization of freshwater habitats [8]. In addition, they are responsible for much of the transfer of organic matter [9] in freshwaters.

Some insects are commonly used as indicators of ecological conditions to describe recent habitat transformation [10]. Therefore, freshwater ecosystems have been studied to better understand their current and future importance [11] through new methodological and technological studies. The first of these new technologies is nanotechnology. Nanotechnology includes running manipulation of materials at atomic/molecular level in the range of 1-100 nm. Objects in this level display

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incomparable and distinct physical, chemical, or biological characters over their mass form. The 21st era may be concerned as a new centenary of nanotechnology [12].

In this study, information is given about widely use of aquatic insects in nanotechnology. Our results are believed as an important gain in ecological and nano-scale dimensions. In terms of all these, evaluating using, purposing, and opportunities of aquatic hexapods in nanotechnology are the subject of this review. The review examines aquatic insects in wetlands, which have not yet been totally discovered in nanotechnology. It is expected that important data will form basis for some scientific studies. It is believed that anatomical structure, physiological properties and adaptive abilities of these insects will shed light on some unexplored new discoveries in nanobiotechnology.

2. Main body and discussion

Our current era can be easily regarded as a new era of nanotechnology in research and development with its potential applications ranging from electronics to material science. This field enables to development of novel nanoscale-based manufacturing processes (Figure 1), nanostructured materials, and nanoelectronics. Nanomaterials have definite physical, chemical, or biological properties over their massive form (Figure 2). Nano-scale opportunities open a fresh corridor in the area of innovative product development and are considered to the uplift the economy and development of the country significantly in near future [13,14].

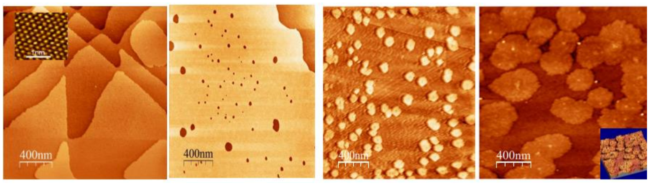

Figure 1. Images on nano-scale under SEM [15].

Enzyme-imitation catalytic nanoparticles, more commonly known as NanoZymes [16], so using enzyme mimicking on aquatic hexapods is possible to preventing invasive organisms and pollution in terms of bio-indicator. Nanotechnology intervenes in these circumstances by all means. In visual research, a new hybrid apposition/superposition lens system borrows much of its design inspiration from the compound lens structure found in hexapods such as the dragonfly [17]. Nanoscale fibrillary building on hexapod pods and geckos are the principal components, which endow them with such extraordinary adhesive wall walking properties. Fibrillary structures take on a hairy appearance that varies in size and surface density between species. The weight of animals has ordered the evolutionary path of adhesive design, supplying smaller hexapods with large adhesive structures, and geckos with fine nanometer-scale hairs [18].

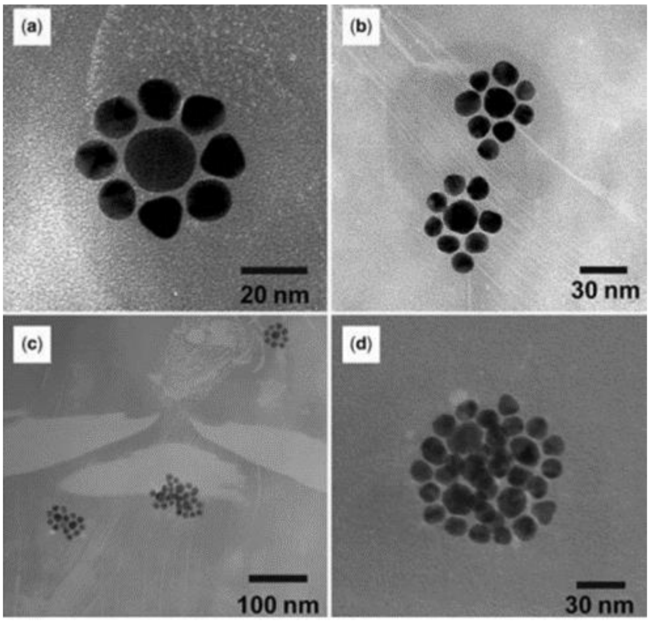

Figure 2. Various nano sizes [19].

Examples of particles levels, nanoparticles have been used for a very long time. For example, carbon black is the most well-known example of a nanoparticulate material that has been produced in quantity for decades. In old Indian medical practice, the therapeutic effects of gold and silver were known and put to use [20]. Nanoparticles have captivated big attention for biomedical applications including disease diagnosis and treatment [21]. These particles own prominent physical, biological and chemical properties associated with their atomic strength [22]. Goswami et al. experiment was purposed at pedigree and testing of lipophilic entomotoxic silica nanoparticle (SNP) in tropical climates and value addition for urban and intensive agriculture and poultry industries [20]. In summary, the experiment is not a hexapod infestation, which is found in the SNP treated stored rice even after 2 months, so SNP can also be used as an excellent seed-protecting agent. Hexapod control and nanoparticles should be accelerated toward the introduction of faster and eco-friendly pesticides in next near days [23]. Nano-scale (biological) control applications in environments will change public awareness and increase its implements in the future.

Ecosystems differ in their dynamics [24] and animal influence on the ecosystem dynamics remains widespread, especially hexapod herbivory has a substantial impact on some environment dynamics [25]. Aquatic macroinvertebrates act important roles in many ecological processes in their living area [26]. For example, aquatic hexapods (Figure 3) can build up inland waters [27]. Coleoptera order comprises some 250,000-known species, many of which are able to exploit human-made, are now important pests. Pest species are known from a wide range of commodities, including dried fish, skins, woolen articles, museum specimens, and cereal grains [28]. It is very important to conduct nano-scale research on this family, which is important especially in aquatic areas with its prevalence and known annoyances.

Figure 3. An aquatic hexapod [29].

Coleoptera, which is expressed as "hexapods" or "hard-winged", is the hexapod group represented by the most species on earth. Percentages 40 of existing hexapods are in this order [30]. Some of these living creatures undergo holometabolic metamorphosis and their life phases are egg-larva-pupa and adult [31,32]. Studies on the biology of Dytiscidae family hexapods have been researched. These hexapods provide necessary oxygen by creating air spaces as hunting [33–37]. This is important for nanoscale studies. Helophoridae, which has a wide distribution area, are represented with 200 species (approx.) in the world [38–53]. Hexapods of the Hydrophilidae family; these members have nutritional value for fish and waterfowl, found in lakes, small puddles, and shallow parts of fast-flowing water [45,54–57]. Elmidae family has 1497 species in 147 genera worldwide. They have also been reported that there are five fossil records belonging to two genera [58]. They prefer to live on rapid-flow Rivers and are sensitive to the various pollutants [59,60]. Adults and larvae feed on diatoms, rotten algae detritus, and plant residues [61]. Therefore, it is known that they were clean-water [60,62]. Doubtlessly, it is nanotechnologically inevitable that this expansion will bring many scientific and technological opportunities. Heteroceridae family live in the muddy or sandy parts of the aquatic habitats where galleries they have opened in soil [63–66]. If these galleries are examined at the nanoscale, it is likely that there will be unexpected discoveries.

In aquatic ecosystems, some mycelium contains a hydrophilic segment. These micelles have low resolution, low-level features. Therefore, Micelles pharmaceutical products. It is inexpensive to manufacture and can be used widely. More allows small sizes to roam alongside [19]. This is a risk to the environment. The mechanisms of the aquatic hexapods feeding on these micelles to dissolve the toxic substances in the micelles can be explored on a non-scale. Otherwise, there are several communities were associated with these aquatic areas. Firstly, extremophiles organisms are described that are accommodated to grow selectively at or near the extreme ranges of environmental variables [67]. Nanoscale characters in the life of extremophiles are the subject of research. Else, another aquatic area, microbial communities may be given a second example from other communities. Microbial communities play pivotal roles in biogeochemical processes [68]. Precursory research included modeling enthusiastic behavior of using prokaryotic channel proteins and to date, the first ion channels purified have been sodium and potassium channel [69,70] from *Escherichia coli*.

Once more, heavy metal deposition is a pervasive environmental problem because heavy metals are non-biodegradable and have the potential to accumulate in macro-organisms. Most of these metals are drastically toxic even at low concentrations depending on the solubility of heavy metal compounds in the aquatic areas [71]. Some heavy metals such as Cadmium, Copper, Lead, and Zinc are essential for the growth and survival of the organisms [72]. Many studies have been carried out due to the problems caused by heavy metals, the environment, and human health. There are bacteria that bind heavy metals in the gut microbiota of some aquatic hexapods [73], in particular, it has great value in the nano-scale prevention of environmental pollution. As it is thought that heavy metals are effortlessly aggregated in edible parts of leafy vegetables, as compared to grain or fruit crops [74], as *Exiguobacterium*. *Exiguobacterium* bacteria are below growth, Gram-positive, facultative anaerobes. Matching of expansion of *Exiguobacterium* strains isolated from cold and hot environments indicated that all could grow within a temperature range of 20–37 °C. Nevertheless, the least possible temperature permissive of growth appeared to vary noticeably [75]. Three *Exiguobacterium* sp. defined, it has attracted the attention of researchers as an important resource for developing environmentally friendly biological alternatives, because of its ability to survive in changing environmental conditions and to tolerate heavy metal stress including arsenic. Nano-scale manipulation is understood how important of agricultural and environmental.

If we talk about pesticide use by utilizing the technology of nano-scale, accession for leading of hexapod pest has become the need of our current. As to be envisioned in the implementation of nanotechnology in agriculture, it can be suggested that the use of nanomaterials will result in the development of efficient and potential approaches toward the management of hexapod pests [76].

Divergently, since 1950 annually plastic demand has risen at defensible rates [77], with growing trouble of waste disposal and high cost of pure substrates in polyhydroxyalkanoates (PHA) production. It has caused to future need of upgrading waste streams from different industries into the role of feedstock for the production of PHA [78]. In previous studies, bacterial species were discovered; these bacteria effectively degrade plastic from hexapod guts [79]. Also, *Exiguobacterium* spp. has the potential to degrade synthetic products such as plastics. [77,80].

Magnetic research has indicated that hexapods have ferromagnetic resonance, which is temperature-dependent. Magnetic material is present in the head, thorax, and abdomen of some hexapods. Magnetic nanoparticles in the social hexapods act as geomagnetic sensors [81]. It is aware of the behavior of a great variety of higher animals is influenced by changes in the local magnetic field within their environment. If we give more interesting examples, it has been displayed that honeybees (*Apis mellifera* Linnaeus) use geomagnetic field information for orientation, homing, and foraging [82].

Intercalary, in forestry industries, nanotechnology is in its fresh stage. For this reason, it has countless studies opportunities for innovations like the development of intelligent paper/wood-based products along with in-built sensors, manufacturing pulp, paper, and wood/fiber-based products, building functional lignocellulosic surfaces, nano dimensional building blocks of higher strength and lighter weight [83]. The cardboard sector has been evolved with use of microfibers and clay fillers that substantially improves their performances. These features make lignocellulose an outstanding material for forest-based research. Nanotechnology has the potential to produce valuable wood-based materials such as engineered wood and fiber-based materials that can effectively replace non-renewable materials used in the manufacturing of plastic, metallic or ceramic products. Thereby, fulfilling social demands and improving forest health as well [12].

Nonetheless, chitosan is not toxic, a bio-harmonious polymer that has found a number of applications in drug delivery [84], use of chitosan in nanotechnology is prevalent [85]. Chitosan is an amino polysaccharide and exoskeleton of some animals as hexapods. This organic object is not toxic. The biocompatible polymer has found a number of applications in drug delivery [86]. Chitosan can bind to DNA and take part in gene transfer (Figure 4).

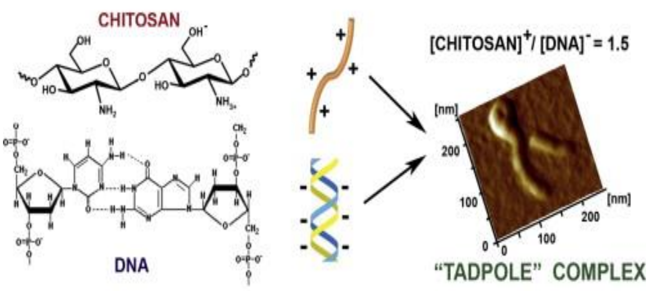

Figure 4. Chitosan–DNA complexes [87].

Additionally, chitin-like substances are obtained from aquatic hexapods [88].

Finally, these data in industry and technology, which have been published, constitute the main idea of our review.

3. Conclusions

Hexapods, the most populated group in the living world, has been attracted attention with their various characteristics by researchers. When we regard many more unexplored nanoscale characters of aquatic hexapods in their life forms, it is believed that many features of nanotechnological significance are still unsolved. Many undetermined hidden nanoscale properties of these creatures are likely to be used in agriculture, industry, medicine, defense, medicine, and even astronomy (on the extremophile aspect). In the next few years, we expect that it will be realized with more research to be done by nanotechnology.

Compliance with Ethical Standards

There is no conflict of interest to disclose.

Conflict of Interest

The author(s) declares no known competing financial interests or personal relationships.

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