A Review of Anti-Aging Nanoformulations: Recent Developments in Excipients for Nanocosmeceuticals and Regulatory Guidelines

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ABSTRACT: Skin aging is the progressive biological process generally characterized by the appearance of wrinkles, age spots, sagging of skin, and dryness. Since skin is an essential part of physical appearance, this has led to increased concerns about skincare. Anti-aging products help in improving the quality and health of the skin by nourishing it. However, due to large particle size they are less efficacious. Nanotechnological approaches for topical anti-aging products have a significant effect on the product performance. Lipidic, polymeric, and metallic nanoparticles have shown potential advantages like enhanced stability and efficacy due to their smaller size. The excipients used in these nanoformulations play an important role in improving the efficacy and shelf-life of the product. The optimal selection of excipients plays a major role in the nanoformulation approach for their enhanced efficacy and stability. For the past three decades the ingredients of natural origin for cosmetic formulations have been widely recognized for being safe and less toxic. The objective of this article is to review the nanoformulations used in anti-aging along with the potential excipients used, currently marketed formulations, and patents filed for cosmetic use. Recent updates related to regulatory aspects of the nanocosmetics have also been highlighted.

KEY WORDS: anti-aging, excipients, nanoformulation, regulatory guidelines, anti-oxidant, lipidic nanocarrier

I. INTRODUCTION

Cosmeceuticals are cosmetics that contain biologically active ingredients with therapeutic benefits. In today’s world, where physical appearance has become increasingly important, people are concerned about skin nourishment in addition to living a healthy lifestyle. The cosmeceutical skin products are designed to improve the aesthetic appearance of the skin and to alter the health of the skin. This helps to maintain an individual’s youthful appearance, which also has an impact on one’s self-esteem. This segment of cosmeceuticals has seen a great demand along with the other cosmetic products in all strata of society. The global cosmetic market has expanded significantly. In 2018, it was worth USD 507.75 billion and it is expected to reach USD 758.45 billion by 2025, with growth of 5.9% with the highest market share in skincare. Specifically, the global
anti-aging market was worth $58.5 billion in 2020 and is expected to be worth $88.30 billion by 2026, with a CAGR of 7.10% from 2021 to 2026. According to Goldstein, Market Intelligence, India’s cosmetics market was valued at nearly USD 11.6 billion in 2017 and is expected to grow at a 5.91% annual rate between 2017 and 2030.

Skincare products include anti-aging creams, skin brightening creams, sunscreens, and body lotions that help in improving the quality and health of the skin by nourishing it. These products act by removing dirt, sebum, microorganisms, exfoliated corneocytes (cleansing); reducing unpleasant skin symptoms (soothing); restoring of damaged skin (restoring); and reinforcing the undamaged, damaged, and vulnerable skin cells (skin reinforcing). Skin anti-aging products generally include anti-wrinkle products (creams, dermal fillers, and botulinum toxin A injections), anti-pigmentation products (chemical peels and creams), and sunscreen products. Neutrogena Rapid Wrinkle Repair® Night Face Moisturizer with Retinol, Hyaluronic Acid, Roc Retinol Correxion Deep Wrinkle Anti-Aging Retinol Night Cream, NeoStrata Triple Firming Neck Cream, and Kumkumadi miraculous beauty fluid ayurvedic night serum are a few of the marketed products used in anti-aging.

Skin aging is the progressive biological process generally characterized by the appearance of wrinkles, age spots, sagging of skin, and dryness. The aging process of the skin is generally caused by intrinsic and extrinsic factors. Intrinsic factors include a slowdown of metabolic process, disease state, mitochondrial DNA damage, hormonal activity, etc. Extrinsic factors include ultraviolet (UV) radiation, smoking, pollutants, lifestyle, etc. This leads to dryness, uneven skin texture, and also increase in visibility of pores, increase in sebum flow. UVB radiation is the primary reason for skin aging that increases oxidative stress. Cellular damage mechanisms are triggered by oxidative stress, resulting in cell senescence, which can lead to photoaging. There is a reduction in skin integrity due to decreased blood supply to the skin because of flattening of the dermal–epidermal junction. Likewise, increased production of matrix metalloproteinase (MMP) due to UV radiation and also decreased collagen I and III caused by reduced estrogen triggers a reduction in skin integrity. Anti-aging products include actives of natural and synthetic origin. Synthetic compounds consist of titanium dioxide, zinc oxide, oxybenzone, avobenzene, cinoxate, dioxybenzone, which are used as UV filters and protect skin from damage. However, they exhibit some toxicity due to their interaction with skin components. In recent decades, there has been increasing awareness of the role of natural origin ingredients for skincare as they are believed to have minimal or no toxicity. This shift in demand for natural origin ingredients for the skin cosmeceuticals has opened up huge market opportunities for all major leading multinational giants like Garnier, L’Oréal, Unilever, Proctor and Gamble, and Johnson & Johnson. The phytochemicals that have proven scientific data for their significant effect on skin health and the treatment of aging skin include resveratrol, quercitin, curcumin, and many more. These phytochemicals provide various properties like anti-oxidant, skin rejuvenation, purifying skin surface, anti-wrinkle, protection of skin from sunburn, and skin nourishment.

Cosmetics are one of the oldest market sectors in which the concept of nanotechnology was first applied. In recent times it has been observed that the application of
nanotechnology is escalating in the area of cosmetics. This can be attributed to the fact that there is a need to overcome certain drawbacks of conventional products like particle size and stability of actives that may lead to harmful reactions. Also, the larger particles are less efficient due to poor penetration through the skin pores and hence high doses of actives are required. It has been noted that sales of cosmetic products containing nanomaterials were about $2.6 billion in 2015; this is expected to grow to $55.3 billion by 2022. Nanotechnology helps to reduce the particle size in the range of 1–100 nm. This reduced size plays a crucial role as the nanoparticles can travel through the intercellular spaces. Small skin appendages on the surface of the skin provide direct delivery of these particles due to their depth. It has been observed that lipidic nanoformulations penetrate into the deeper layers of skin using the intracellular route. A schematic representation of the various routes for the drug absorption from the surface of the skin has been illustrated in Fig. 1. Nanoformulation also helps to improve stability, provide control release of actives, and improve skin hydration and protection of skin barrier function through film formation on the skin. Along with nanotechnology, the excipient used also plays a significant role in achieving the above desired characteristics. The concentration and class of excipients also affect the stability of the actives. It is important to understand the physicochemical properties of excipients along with the skin anatomy for designing a successful nanoformulation strategy.

Skin has a total area of about 2 m² and is the biggest organ in the human body, with a lipophilic nature and numerous pores. The epidermis and dermis are the two layers that act as barriers for topically applied products. The epidermal layer is composed of stratified squamous keratinized epithelial cells (keratinocytes and non-keratinocytes), whereas the dermal layer is subdivided into four sublayers (stratum basale, stratum spinosum, stratum granulosum, and stratum corneum). The dermal layer protects the skin from UV light and has several immunological functions (melanocytes, Merkel cells, and Langerhans cells).

FIG. 1: Schematic representation of major pathway for absorption of drug from the surface of the skin

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In this review, details of the different excipients used in anti-aging nanoformulation such as lipids and polymers are covered. This paper also highlights the use of protein, peptides, and vitamins in anti-aging products. Authors have attempted to discuss the use of anti-oxidants as preservatives, importance of emollients, and significance of surface modification of nanocarriers for their applications in anti-aging products. Various nanoformulations like nanoemulsions, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), liposomes, niosomes, ethosomes, phytosomes, polymeric nanoparticles, nanospheres, nanofibers, and gold and silver nanoparticles have been discussed. Current marketed formulations and patents that have been granted have also been discussed. The particle size of nanomaterials has the potential to alter the distribution pattern and the bioavailability of the therapeutic products. The nanosized carrier systems tend to distribute more uniformly as compared to the macrosized carriers with same chemical composition. In addition, the uptake, absorption, and biodistribution of the material may be altered, leading to potential systemic exposure, which can further cause toxicity. Hence, in the later part of the review, regulatory guidelines for manufacturing of nanocosmeceuticals have been discussed. Various countries have different guidelines to regulate nanocosmeceuticals. These guidelines need to be more stringent and streamlined in the future. This would help manufacturers and consumers to make careful decisions so as to address the safety and toxicity issues related to the nanoformulations.

II. EXCIPIENTS USED IN NANOFORMULATION FOR ANTI-AGING

Excipients have a tremendous impact on the stability and morphology of the lipidic nanoparticles, hence it becomes very important for the formulator to have thorough knowledge about the excipient’s behavior and its chemistry. Surfactants, lipids, and polymers are excipients that have been used widely in nanoformulations. Lipid and polymers enhance the stability of the active compounds by encapsulating or forming matrix system. They also help to provide sustained release of active compounds. Surfactants enhance the stability of the nanoformulation by their property of interfacial surface tension, which also impacts the particle size of the nanocarrier. Various excipients used in the nanoformulations for anti-aging products are discussed below.

A. Lipid

Lipids are a major component of lipidic nanoformulation used in anti-aging. Various lipids including fatty acid, monoglycerides, diglycerides, triglycerides, and waxes have been utilized. An important advantage is their physiological similar nature, thus reducing toxicity concerns. They also offer advantages like sustained release, enhanced stability, and reduced transepidermal water loss. The decisive factor for choosing a particular lipid in a formulation generally depends on the solubility and compatibility of the active compounds. The actives can lodge themselves in the structural defects of the lipid due to lipid polymorphism, thus influencing the lipid nanoparticle system. The commonly used lipid-containing nanoformulations include nanoemulsions, solid lipid nanoparticles,
nanostructured lipid carriers, and vesicular systems like liposomes, niosomes, and phytosomes. Lipids are further classified into solid lipids and liquid lipids.

1. Liquid Lipid

Liquid lipids used in nanoformulations are of both synthetic and natural origin and they are liquid at room and body temperature. Oils obtained from natural origin are non-toxic and also have inherent properties for skincare. Liquid lipids derived from plants are divided into two categories: fixed oils (vegetable) and essential oils (volatile).\textsuperscript{25} These oils mainly include jojoba oil, sunflower oil, olive oil, argan oil, etc. Some of the natural oils along with their cosmeceutical applications have been highlighted in Table 1. They have the property to penetrate the skin and interact with the cell membrane, causing certain modifications. These modifications can boost skin properties by forming a physical barrier, acting as an anti-oxidant agent, and providing fatty acid to different skin layers. The modifications also help in activation of peroxisome proliferator-activated receptor-\(\alpha\) signaling or the reduction of cutaneous inflammation.\textsuperscript{26} Besides, they also provide a synergistic effect when used as an excipient. The oils can cause mild skin irritation, whereas their oxidation products can cause dermal sensitization in humans. The potentially damaging effects may also differ depending on the dose, its form (water or fat soluble), and the site of application (hair follicles, sebaceous gland). All of these factors will influence absorption rates as well as the accumulation of bioactive compounds in various skin layers and systemic circulation.\textsuperscript{26}

### TABLE 1: List of various liquid lipids (oils) obtained from natural sources and their applications\textsuperscript{26}

<table>
<thead>
<tr>
<th>Oils</th>
<th>Plant</th>
<th>Application</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil</td>
<td>Cocos nucifera</td>
<td>Skin moisturizer, anti-oxidant, anti-bacterial</td>
<td>27,28</td>
</tr>
<tr>
<td>Palm oil</td>
<td>Elaeis guineensis</td>
<td>Anti-oxidant, anti-aging</td>
<td>29</td>
</tr>
<tr>
<td>Argan oil</td>
<td>Argania Spinosa Skeels</td>
<td>Emollient, anti-oxidant</td>
<td>30</td>
</tr>
<tr>
<td>Olive oil</td>
<td>Olea europaea</td>
<td>Skin conditioner, anti-oxidant</td>
<td>14,31</td>
</tr>
<tr>
<td>Jojoba oil</td>
<td>Simmondsia chinesis</td>
<td>Moisturizer, fine wrinkle, acne</td>
<td>32</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>Sesamum indicum</td>
<td>Anti-oxidant, demulcent, and emollient</td>
<td>33</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>Helianthus annuus</td>
<td>Skin hydration, anti-oxidant</td>
<td>34</td>
</tr>
<tr>
<td>Grape seed oil</td>
<td>Vitis vinifera</td>
<td>Anti-bacterial, anti-oxidant, anti-aging</td>
<td>35</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>Glycine max</td>
<td>Anti-human tyrosinase activity, anti-oxidant</td>
<td>36</td>
</tr>
<tr>
<td>Pomegranate seed</td>
<td>Punica granatum</td>
<td>Anti-oxidant, anti-aging</td>
<td>35</td>
</tr>
<tr>
<td>Tea tree oil</td>
<td>Myrtaceae alternifolia</td>
<td>Anti-oxidant, anti-acne, antiseptic</td>
<td>37</td>
</tr>
</tbody>
</table>
Sesamol is a natural phenolic compound obtained from *Sesamum indicum* seed oil, which is widely been studied as liquid lipid phase in preparation of NLCs. A comparative study was performed by Puglia et al., in which sesamol was incorporated into two different types of NLCs comprising liquid lipids: Miglyol 812 and sesamol oil. The particle size analysis and drug release studies did not show any significant difference with the use of either oil. However, the drug encapsulation efficiency and anti-oxidant activity of NLCs using sesamol oil were comparatively higher than miglyol 812. The higher drug encapsulation efficiency was attributed to the higher affinity between sesamol oil and sesamol over the combination of miglyol 812 with sesamol. The higher anti-oxidant activity is because of the composition of sesamol oil, which contains anti-oxidant compounds like lignans and tocopherols exhibiting synergistic activity.\(^{38}\) Tou et al. formulated Coenzyme Q10 (CoQ10) nanoemulsion cream containing evening primrose, linseed, and olive oil. These oils contain several fatty acids like linoleic acid oleic acid (OA, omega-9), (LA, omega -6), and \(\alpha\)-linoleic acid (ALA, omega-3). It was reported that nanoemulsion comprising of linseed oil showed high permeation of CoQ10 because of the presence of omega-3, 6, and 9 fatty acids.\(^{39}\)

Palm olein is the liquid fraction that is obtained from palm oil. It consists of a high amount of oleic acid. Since palm olein also contains natural anti-oxidants such as \(\alpha\)-tocopherol and \(\alpha\)-tocotrienol, Ramli et al. prepared nanoemulsion loaded vitamin E as an anti-aging active and palm olein as oil phase. It was observed that the anti-oxidant activity of palm olein with vitamin E can enhance the anti-oxidant activity of the nanoemulsion system.\(^{40}\) Che Sulaiman et al. used palm kernel oil ester synthesized from palm oil for cosmetic application and guava seed oil for the formulation of *Clinacanthus nutans* leaves extract nanoemulsion.\(^{41}\) Similarly, refined palm olein was used for incorporation of \(\beta\)-D-glucan into a nanoemulsion that facilitated to sustain higher stability of \(\beta\)-D-glucan at lower concentration along with Kolliphor® RH40, Labrafil® M1944-CS.\(^{42}\) Chaiyana et al. incorporated *Ocimum sanctum* extract in NLCs in which tea seed oil was used as liquid lipid. NLCs prepared from tea seed oil were a suitable formulation for dermal delivery of rosmarinic acid in the *Ocimum sanctum* with anti-aging properties.\(^{43}\) Chu et al. formulated pumpkin kenaf seed oil-based NLCs containing UVA filters like (Uvinul A Plus Granular and UVB filter: Uvinul1 T150). This formulation can be used as a photoprotective agent in cosmetics.\(^{44}\) Red palm O/W nanoemulsion was prepared using Tween 80 and Span 80. The study concluded that red palm nanoemulsion can be used as a natural source of vitamin E for skin care and cosmeceutical products.\(^{39}\)

Pinto et al. formulated placebo NLCs and \(\alpha\)-tocopherol (TOC) NLCs using four vegetable oils including sunflower oil (SF), coconut oil (CO), sweet almond oil (SA), and olive oil (OV). These lipid nanoparticles and placebo NLCs demonstrated good anti-oxidant activity, with scavenging activity values exceeding 56.7%, which was enhanced by TOC encapsulation (scavenging activity values above 64.3%). The formulations containing SF and SA had the highest anti-oxidant values, 70.2% ± 3.2 and 68.8% ± 0.4, respectively, which were slightly improved by the encapsulation of TOC (71.2% ± 4.1 and 70.5 % ± 4.0).\(^{45}\) Grapeseed oil was used as an active to formulate nanoemulsion. It contains vitamin E and oligomeric proanthocyanidins (OPC), which provide
anti-oxidant properties. It was observed that vitamin E in grapeseed oil prevented skin aging by its anti-oxidant property.46

Some studies also suggest that the use of functional liquid lipids in nanoformulation provides synergism in anti-aging. Tichota et al. used argan oil as a liquid lipid to formulate nanostructured lipid carrier, which was further incorporated into hydrogen. The in vivo skin evaluation studies demonstrated that the formulation showed increase in hydration via synergistic effect of NLC occlusion and argan oil hydration.47 Nanostructured lipid carrier containing bocaiuva almond oil showed synergistic activity against sun protection.48 Sesame oil also shows synergistic anti-aging effects when used with vitamin E.49 Chu et al. formulated nanostructured lipid carrier using carnauba wax, beeswax, pumpkin seed oil, and UV filter (Uvinul A Plus B). It was observed that addition of PSO showed a synergistic effect with Uvinul A Plus B, which helped to boost the formulation’s photoprotective properties while also exhibiting good anti-oxidant activities.50

Synthetic oils used in nanoformulation for anti-aging consist of ethyl oleate (Crodamol EO), Capmul MCM (medium chain mono- and diglycerides), isopropyl myristate, Caprylic/capric triglyceride (Miglyol 812), Glyceryl triacetate (Captez 500). These oils have been used to incorporate various actives including vitamins, herbal extracts, and Coenzyme Q10. Capmul MCM is a blend of medium-chain (mostly caprylic) fatty acid of mono and diglycerides. It is prepared by esterification of caprylic/capric acid with mono and diglycerol. It is an excellent solvent for a wide range of organic compounds and an effective emulsifier in water–oil (w/o) emulsion systems. Capmul MCM was used to formulate nanoemulsion to incorporate vitamin C or E along with curcumin.51 It was also used to incorporate orobal in NLC, an isoflavone having anti-aging properties.52 Caprylic/capric triglyceride (Miglyol 812), a medium-chain triglyceride has been employed to incorporate various actives used in anti-aging. Synthetic oil caprylic/capric triglyceride can also be used as an emollient.53 Miglyol 812 has been used in the formulation of nanoemulsions and nanolipid carriers of different actives like Jabotica extract ellagic acid, 20 (S) – Protopanaxadiol and it can be also used in various anti-aging nanoformulations.54,55 Synthetic oils used in marketed formulations like Intensive Care Cocoa Radiant Spray and Cleure Day Cream include isopropyl myristate, caprylic capric triglyceride. These oils have the potential to be used further in nanoformulations.56,57 Natural oils are more suitable compared with synthetic oils due to their low toxicity; however, oxidation of natural oils must be prevented by using a suitable anti-oxidant.

2. Solid Lipid

Solid lipids containing fatty acid, monoglycerides, diglycerides, triglycerides, and waxes make up the major proportion of SLNs and NLCs.58 In solid lipids the actives accumulate in the structural defects of the lipid due to different polymorphic crystal lattice forming a lipid matrix thereby giving a sustained release effect. It has been observed that metastable lipids tend to transform into more stable form, which leads to drug expulsion.
Therefore, the rate at which there is transition from metastable to stable crystalline lattice is an important parameter in selection of solid lipids while formulating solid lipid nanoparticles. Various solid lipids used in anti-aging formulations include glyceryl monostearate, stearic acid, shea butter, cetyl palmitate, glyceryl behenate, glyceryl palmitostearate, and stearic acid. Various solid lipids used in anti-aging formulations include glyceryl monostearate, stearic acid, shea butter, cetyl palmitate, glyceryl behenate, carnauba wax, glyceryl palmitostearate. Glyceryl behenate, glyceryl monostearate, glyceryl palmitostearate, and stearic acid are generally recognized as safe (GRAS) and so can be widely used in nanoformulation.

Glyceryl diheneamate (docosanoic acid, diester with glycerin) is a mixture of glycerol esters and behenic acid. The melting range of Compritol 888 ATO is 69–74°C. High loading of actives is possible due to its crystal lattice. Compritol 888 ATO has been used for development of various nanoformulation to incorporate curcuminoids; sesamol; and idebenone with entrapment efficiency of 86.67%, 91.2%, and 82.58% with particle size of 149.9 ± 15.1, 169.2 ± 18.6, 605 ± 4.01 and zeta potential −7.2 ± 0.9, −38.0 ± 2.62, −13.91 ± 2.70 respectively. Cetyl palmitate is a plant-derived wax that is prepared through the esterification of palmitic acid and cetyl alcohol. Cetyl palmitate has melting point of 54°C. It has emollient properties and can be used for loading of various lipophilic actives. Cetyl palmitate has been used in incorporating Ocimum sanctum extract and 20(S) - Protopanaxadiol (PPD) oil in nanostructured lipid carrier. Entrapment efficiency was found to be 87.4% and 78.2%, while particle size 261.0 ± 5.3 and 148.7 ± 1.5 zeta potential were −45.4 ± 2.4 and −11.5 ± 1.6 for Ocimum sanctum extract and PPD respectively. Precirol ATO 5 is composed of mono-, di-, and triacylglycerols of palmitic and stearic fatty acids with a melting range of 50 to 60°C. Precirol ATO 5 has used to incorporate curcuminoids, Ridolfia segetum Moris essential oil with entrapment efficiency 96.89% and 100% while particle size was found to be 111.7 ± 7.9 and 143 ± 5 and zeta potential −4.1 ± 0.6 and −16.3 ± 0.6 respectively.

In formulation of curcuminoid loaded NLCs using either Compritol 888 ATO or Precirol ATO 5 as a solid lipid, it was observed that relatively high amount of curcuminoids was incorporated in Precirol ATO 5 as compared to Compritol 888 ATO. In human dermal fibroblasts (HDF), a comparative analysis was conducted to assess the potential cytotoxic effects of various nanostructured lipid carrier (NLC) compositions. In this study, different blank NLCs were prepared using Glyceryl palmitostearate, Compritol 888 ATO, Glyceryl monostearate, Tristerin, and Softisan 100. The Guava Via-Count Assay was used to assess the biocompatibility/cytotoxicity of the NLCs in UVA exposed and non-exposed HDF. NLC formulated with Compritol 888 ATO was the one with a cytotoxicity neutrality that was not exacerbated by UVA exposure, despite the mild pro-oxidant effect. The moisturizing and occlusion effects of SLNs and NLCs can be determined using magnetized water and deionized water. A comparative study was conducted on SLNs and NLCs formulated using various lipids such as Tripalmitin, Compritol 888 ATO, and Precirol ATO 5 along with emulsifiers such as Poloxamer and Tween 80 for determining moisturizing effect of lipids. Skin hydration measurement using corneometer revealed that SLN containing 5% compritol 888 ATO showed maximum moisturizing effect in vitro. However, 5% SLN Precirol ATO 5 had the most...
moisturizing effect in vivo. Raman mapping of lipids was carried out in which it was observed that Precirol ATO 5 had an aging effect (storage condition).

B. Polymer

Polymers are widely used excipients in cosmetic formulations. These macromolecules are made up of several repeating units (monomers) that are normally arranged in a chain. In cosmetic formulations, polymers are used as rheological modifiers, film formers, skin-feel beneficial agents, conditioners, emulsifiers, foam stabilizers, stimuli-responsive reagents, fixations, anti-microbials. Along with these properties they have the ability to encapsulate active compounds and provide desired effects. Hence, there is an increase in their use in nanocosmeceuticals. Some of the widely used polymers are discussed in Table 2.

Chitosan is a linear heteropolysaccharide composed of poly (1,4)-linked 2-amino-β-D-glucose (GlcN) formed by partial chitin deacetylation. In cosmetics, chitosan is used as an excipient and also as a biological active agent due to properties like low toxicity, biodegradability, and biocompatibility. It has been observed that degree of acetylation (DD) and molecular weight (MW) play a significant role in biological effects. There is an increase in anti-oxidant, anti-microbial, mucoadhesive, and skin penetration property as DD increases. As the MW increases, there is an increase in mucoadhesive properties, and as the MW decreases there is an increase in anti-oxidant and anti-microbial properties.

Chitosan with a high molecular weight (MW) has a film-forming property that reduces cutaneous water loss and improves skin smoothness and elasticity. Quaternized carboxymethyl chitosan/organic montmorillonite (QCOM) was prepared and skin hydration studies were conducted. QCOM nanocomposite has been found to have significant moisture absorption and retention capacity and a good UV protective efficiency. Acrylic nanocapsules penetrated the deeper layers of skin due to the cationic surface charge of chitosan. Quaternized cyclodextrin-grafted chitosan nanoparticle associated with hyaluronic acid were found to be a promising skin penetration vehicle. Chitosan oligosaccharide showed preventive effect in in vivo studies of UV

| TABLE 2: Properties and applications of various polymers used in cosmeceuticals |
|-------------------------|-----------------------------------------------------------------------------------|---|
| Polymer                  | Properties/application                                                              |Refs.|
| Chitosan                | Biodegradable, moisturizing elastic film, anti-microbial                           |91  |
| Starch                  | Emulsifying agent, film former                                                     |73  |
| Hyaluronic acid         | Moisturizer, anti-aging                                                             |86  |
| Poloxamer               | Thermoreversible hydrogel, non-ionic surfactant                                    |92  |
| Polyvinyl alcohol       | Film former ability, biodegradable and enhance mechanical properties              |93  |
| Polyvinyl pyrrolidone   | Film former, viscosity enhancement, stabilizer                                    |94  |
| Polyethylene oxide      | Biodegradable, enhance mechanical properties                                        |95  |
damaged hairless mouse dorsal skin. Similarly, chitosan along with hydroxyapatite showed protection from UV radiation. Chitosan film in the presence or absence of hyaluronic acid neutralized with citrate buffer showed skin exfoliation due to its bioadhesive property. Marked formulation containing chitosan includes ChitoCare Body Lotion and ChitoCare Beauty Face Cream.

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan that consists of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked via glycoside bond in the alternate arrangement of β-(1→4) and β-(1→3) bonds. Hyaluronic acid has the property to hold 1,000 times its weight of water due to its structure. In cosmeceuticals, HA is used as a skin hydrator, wrinkle treatment, anti-aging agent, and collagen stimulator, and in skin augmentation. HA also holds and maintains moisture, turgor, and elasticity in the skin. HA is co-administered with active compounds and excipients to provide synergistic activity. Hyaluronic acid along with epigallocatechin-3-gallate (EGCG) was incorporated into nano-transfersomes to improve its anti-oxidant and anti-aging property in UV damaged skin. According to in vitro skin permeation and deposition study, co-entrapment of HA in the formulation increased both skin permeation and EGCG deposition. Actives in combination with hyaluronic acid and chitosan showed promising application in anti-aging treatment. Jegasothy et al. experimented to study the topical efficacy of nano-hyaluronic acid on humans. Nano-hyaluronic acid showed substantial benefits in reduction of wrinkles (up to 40%), increasing hydration of skin (up to 96%), and improving skin elasticity and firmness (up to 55%) at the end of the eight weeks. Therefore it was concluded that as the molecular weight of HA decreases, there is an increase in skin penetration and skin hydration. Polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polylactic acid (PLA), and polyethylene oxide (PEO) are some of the polymers that can be used in the nanoformulation of excipients. Marketed formulations containing hyaluronic acid include Hydro Mega Hyaluronic Cream and Isntree Hyaluronic Acid Toner.

C. Surfactant

Surfactants are amphiphilic compounds with both polar and non-polar heads, used for stabilizing the nanocosmeceuticals. Surfactants are also known as surface-active agents (SAAs); at low concentrations, they are adsorbed at the interface of water and oil and reduce the surface tension. As the concentration of the surfactants increases, they reach a threshold (critical micelle concentration) at which they start forming micelles. As a result, a stable system is formed. SAAs are classified as anionic, cationic, and non-ionic based on their head group. An effective surfactant decreases the interfacial tension between lipid and aqueous phases and therefore creates emulsion droplets smaller in size, which leads to the production of smaller-sized nanoparticles when cooled. In addition, the particles formed can be stabilized by forming a sterie barrier on the particle surface that creates an impediment to the coagulation of the particles. The partition coefficient or distribution is defined as the ratio of surfactant concentration in the oil phase to that in the water phase at equilibrium. Partition coefficient decreases as the surfactant’s oil...
solubility decreases or water solubility increases or oil solubility decreases more than the respective water solubility.\textsuperscript{99} The partition coefficient of a surfactant plays a critical role as the surfactant adsorbed on the interface depends on the partition coefficient. It can be affected by additives like alcohol.\textsuperscript{100} Surfactants can also be used as penetration enhancers in topical delivery of cosmeceuticals in order to increase skin penetration.\textsuperscript{101} Surfactants can also cause toxicity depending on the charge and solubility of the molecule. The polar heads present on the surface of the ionic surfactant react with keratinocyte and lead to toxicity. The toxicity may increase as the solubility of the ionic surfactants increases.\textsuperscript{102} Surfactants enhance skin permeation through solubilization or extraction of the lipid barrier in the stratum corneum, increase the fluidity of the membrane, and cause changes to the stratum corneum’s tight junction properties.\textsuperscript{103,104}

In lipid-based nanoformulations, it has been observed that polyoxyethylene sorbitan fatty acid esters (polysorbates), sorbitan, and poloxamer are the most widely used surfactants. Poloxamer and Polysorbate 80 are GRAS by United States Food and Drug Administration (FDA).\textsuperscript{105,106} Polysorbates are non-ionic surfactants used in the emulsification of o/w emulsions, which are produced by addition, via polymerizing ethylene oxide to sorbitan fatty acid esters.\textsuperscript{107} Sorbitan esters are non-ionic surfactants used for the preparation of water in oil emulsion, and are prepared by reacting sorbitol and stearic acid.\textsuperscript{108} They are also used in combination with polysorbates with varying concentrations to produce oil in water emulsion or creams of varying consistencies. Poloxamers are nonionic triblock copolymers made up of a central polyoxypropylene hydrophobic chain flanked with two polyoxyethylene hydrophilic chains. They are used as solubilizing, emulsifying, and wetting agents.\textsuperscript{109,110}

It has been observed that combination of surfactants showed smaller particle size and high stability of the nanosystems as compared to nanosystems formulated using a single surfactant. Coenzyme Q10 was loaded in nanoemulsion and stabilized by a surfactant mix of Tween 80 and Span 80, since there is a direct relation between o/w interfacial tensions and as the interfacial tensions decrease, the droplet size decreases. Tween 80, being hydrophilic, and Span 80, being a lipophilic surfactant, were used in combination to reduce the interfacial tension, forming a stable system.\textsuperscript{39} Similarly, Coenzyme Q10 was loaded in nanoemulsion with surfactant mix Tween 80 and transcutol HP. Transcutol HP, which was used as a cosurfactant, enhanced the permeation.\textsuperscript{103} Likewise, in the formulation of catechin nanoemulsion, surfactant (Span 80) and cosurfactant (transcutol CG) were added to form a stable nanoemulsion. Kim et al. observed that when the concentration of surfactant and liquid lipid was increased, the solubility of actives was increased thereby giving better entrapment efficiency. The surfactant used in this formulation was Tween 20 along with Poloxamer 188 for steric stabilization.\textsuperscript{55}

The hydrophilic–lipophilic balance (HLB) of a surfactant molecule is the balance of the size and strength of its hydrophilic and lipophilic moieties. The HLB scale has a scale of 0 to 20. Surfactants with HLB values ranging from 3.5 to 6.0 are better suited for use in w/o emulsions. Surfactants with HLB values ranging from 8 to 18 are commonly used in o/w emulsions.\textsuperscript{111} Chong et al. used polysorbate 80 and sorbitan 80 during the formulation of tocotrienol-rich palm oil-based nanoemulsion. Polysorbate 80 and
Sorbitan 80 have HLB values of 15.0 and 4.3 respectively. Literature suggests that an HLB value within the range of 10–12 is suitable for the preparation of nanoemulsion. A mixture of Tween 80 and Span 80 provided a synergistic activity at HLB value 11. This can be attributed due to the difference in the size of head groups; small molecule surfactant can be packed well with large surfactant at the interface between the oil and water phase. Also by reinforcing the interfacial film, the mixed surfactant enhances the stability of nanoemulsion.\cite{112,113} It was also found by Rebolleda et al. that the droplet size of mixed surfactant (T80 and S80, HLB 11) was small as compared to single surfactant (T80 HLB 15 and T20 HLB 20).\cite{29} A similar study was carried out by Abbas et al., in which solid lipid nanoparticles were formulated using two different grades of poloxamer (188 & 407). It was observed that when P188 and P407 were used in combination there was a decrease in polydispersity index (PDI) value and better drug entrapment as compared to uni-surfactant. This was due to the non-ionic nature of poloxamer 188 and poloxamer 407, which provided steric stabilization of nanoparticles via molecular polarization and surfactant adsorption on the lipid/water interface, as well as the formation of electric double layer forces. Also, the drug entrapment was improved due to higher HLB values, which provide better aqueous solubilization of the drug.\cite{114}

NLCs loaded with curcuminoids were formulated using either Polysorbate 80 or Poloxamer 407. Ex vivo permeation studies carried on pig skin revealed that polysorbate 80 exhibited greater penetration of curcuminoids than poloxamer 407. The increase in penetration of curcuminoids is because of the chemical nature of polysorbate 80. Polysorbate 80 (polyoxyethylene monooleate (20) sorbitan) has a chain of 18 carbons with a cis-unsaturation in position 9–10 that can intercalate between the lipid chains of the stratum corneum, causing a “fluidization” of the lipid matrix, and increase in lipid disorder.\cite{64} Various surfactants like Tween 80, Span 60, Span 80, and Poloxamer 188 were used in formulation of α-tocopherol loaded NLCs using vegetable oils (sunflower oil, olive oil, sweet almond oil, coconut oil). Span 60 showed a significant increase in particle size when compared to other surfactants. Similarly, for all vegetable oil NLCs, the PDI values obtained with this hydrophobic surfactant were significantly higher, suggesting a more heterogeneous size distribution. These findings could be attributed primarily to differences in the chemical structure of the surfactants used, as well as their compatibility and conformational rearrangement with the lipid matrix.\cite{45}

**D. Peptide, Protein, and Vitamins for Anti-Aging**

Skincare products’ peptides are chemical compounds made up of short amino acid chains.\cite{115} Peptides have become prominent in cosmetics due to their bioactive properties, which include the ability to interact with skin cells through multiple pathways, high potency at low dosage, and moderate penetration into the upper skin layers. Due to their hydrophilic property, they may be required to undergo certain chemical modifications, such as alkyl esterification, to strengthen penetration.\cite{116} Peptides used in topical cosmeceutical are classified as enzyme inhibitor peptides (Soybean peptide), carrier peptides (Tripeptide-1), neurotransmitter inhibitor peptides (Acetyl hexapeptide-3), and signal
peptides (Palmitoyl Tetrapeptide-7). However, the efficacy of bioactive peptides when added directly to anti-aging products is limited. High molecular weight and polarity of bioactive peptides causes hindrance for their penetration in the stratum corneum. Furthermore, peptides are prone to degeneration and inactivation when stored. Hence, peptides are incorporated in nanocarrier to overcome these drawbacks. Acetyl hexapeptide-3, palmitoyl tripeptide-5, and carnosine were loaded in nanoliposomes, and Heptapeptide was loaded into solid lipid nanoparticles to improve their efficacy. These formulations are promising for peptide delivery.

In skincare products, proteins are widely used because of their anti-aging properties. The first protein used in the cosmetic sector was milk protein. With their film-forming properties, proteins increase water in the stratum corneum. The exposure of many hydrogen binding sites available for water linkage may be responsible for this property. This reduces wrinkles caused by dehydration and improves the thickness and quality of the skin barrier. Gelatin and collagen are the most widely used proteins in anti-aging formulations. They are used as actives or excipients while preparing nanoformulations. Collagen is the human body’s most abundant structural protein, and supports several tissues, including tendons, skin, and teeth. It has anti-scarring, anti-aging, anti-acne, anti-wrinkle, and wound healing characteristics. Effective topical delivery is hampered by its hydrophilic nature and high molecular weight. To overcome this, a topical nano-surfactant dispersion (NSD) was developed to deliver collagen. NSD has been prepared using cholesterol and sorbitan monostearate (Span 60). The NSD treatment demonstrated a 2.5-fold increase in skin hydration test compared to collagen gel treatment in the stratum corneum (SC) thickness of the skin. It has also been used with chitosan to prepare anti-aging gel using aloe vera. Hence, collagen can be safely used in the preparation of various nanoformulations. Gelatin is a biopolymer obtained from discarded or unused materials (skin, tendons, cartilages, bones, and connective tissues) of bovine, porcine, ovine, poultry, and marine industrial farms. It is a natural, elastic, biocompatible polymer that is hydrophilic, biocompatible, and biodegradable. It has anti-aging properties such as reduction of stretch marks or wrinkles, and rejuvenation and refilling of the skin. Gelatin was used in the preparation of a nanofibers-based mask for loading gold nanoparticles. Since electrospinning of gelatin is difficult. Therefore, it is required to combine gelatin with polymers that have a high molecular weight such as polycaprolactone (PCL), polyvinyl alcohol (PVA), or polyethylene oxide (PEO).

Vitamins are organic compounds with low molecular weight that are required for life activity in trace amounts for essential metabolic reactions. Vitamin deficiency results in a variety of disease symptoms, including skin damage. Vitamins used in the formulation of skincare mainly include retinyl acetate, retinyl palmitate, retinol, retinal and retinoic acid (vitamin A); L-ascorbic acid (vitamin C); tocopherols α, β, γ (vitamin E); and lipoic acid (vitaminoids N). These vitamins help in the treatment of skin care by topical application. They act by inhibiting the UV-induced, MMP-mediated collagen breakdown and protect against UV-induced decrease in procollagen expression. Therapeutic efficiency can be increased by using a combination of various vitamins, with an increase in stability, and improved skin penetration. Vitamins are incorporated
into various lipidic and polymeric nanoformulation systems for improving the therapeutic outcomes. Vitamin C, vitamin E along with curcumin, CoQ10, and retinaldehyde were incorporated into nanoemulsion and nanostructured lipid carrier to improve problems associated with skincare.\textsuperscript{51,129}

E. Natural Anti-Oxidant

1. Natural Anti-Oxidant Used as Anti-Aging Agents

Ultraviolet radiation, pollution, and cigarette smoke cause oxidative damage to proteins, lipids, carbohydrates, and DNA by generating free radical species. They also stimulate the formation of matrix metalloproteinases (MMP1, MMP3, MMP8, and MMP9), and activating enzymes like elastase, collagenase, xanthine oxidase, and tyrosinase, that results in degradation of elastin and collagen, damaging dermal connective tissue, and leads to formation of premature skin aging.\textsuperscript{130,131} These radical damages can be controlled by endogenous anti-oxidant compounds like glutathione peroxidase, glutathione dismutase, catalase, and superoxide dismutase, or exogenous, primarily administered through diet and topical products.\textsuperscript{131} Exogenous anti-oxidants from natural origin are derived predominantly from plant sources, include carotenoids and polyphenols, and have been proved to be efficient for the treatment of anti-aging.\textsuperscript{132} Phytochemicals like resveratrol,\textsuperscript{133} curcumin,\textsuperscript{134} quercetin,\textsuperscript{135} genistein,\textsuperscript{136} are potential anti-oxidants that can be used in the treatment of anti-aging. Ocimum sanctum extract,\textsuperscript{43} Cordyceps militaris extracts,\textsuperscript{137} jaboticaba extract (Plinia peruviana),\textsuperscript{54} green tea and roselle extracts\textsuperscript{138} are some of the plants extract that have has anti-oxidant properties. These phytochemicals and plant extracts are incorporated into suitable nanoformulation systems to increase their stability and skin penetration in anti-aging treatments.\textsuperscript{132}

2. Natural Anti-Oxidant Used as Preservatives

Cosmetics can oxidize their components themselves and thus get degraded. Preservatives are therefore necessary to limit the oxidation reactions of these products.\textsuperscript{139} Preservatives are compounds that are widely applied to everyday cosmetic formulations with the primary purpose of preventing the product from degradation due to microbial contamination and oxidation.\textsuperscript{140} Anti-oxidant compounds are also known for their preservative property.\textsuperscript{141} They are generally used to prevent or delay rancidity or oxidation spoilage of the product. The anti-oxidants BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) are among the most commonly used.\textsuperscript{140,142} Synthetic anti-oxidants as preservatives can cause harmful side effects and lead to toxicity. Hence there is a need for use of natural anti-oxidants as preservatives. There are some essential oils that have proved to be promising as preservatives. Essential oils from Aframomum danielli, Aframomum citratum, Monodora myristica, and Piper capense have been shown to be useful in the preservation of agricultural products. An evaluation of their preservative
efficacy was conducted in the formulation of cosmetics. All essential oils demonstrated effective preservation on the oxidation of the cream. The essential oil of *Monodora myristica*, which contains α-phellandrene, was found to be the most effective in preventing cosmetic cream from accelerated oxidative degradation. Donut seed oil with a concentration of 0.2% had shown strong anti-oxidant activity, which was almost equal to that of BHT. Other preservatives from natural sources that can be used as preservatives include ascorbic acid and tocopherols. There is a need for further research to use antioxidants as a preservative derived from natural sources in anti-aging nanoformulations.

**F. Emollients**

Emollients are the compounds that provide exogenous lipids to the skin that helps in improving barrier properties, reducing evaporative water loss, and protect the stratum corneum. Despite the fact that the words “moisturizer” and “emollient” reflect separate processes, they are often used interchangeably. The difference between a moisturizer and an emollient is that a moisturizer adds moisture to the tissue while an emollient softens and makes it flexible. Both moisturizers and emollients have the potential to soften and moisturize the skin. Saturated triglycerides function as an emollient to moisturize the skin by flattening the dry curled edges of corneocytes and filling the gaps between them. Some of the widely used excipients that have emollient properties are paraffin; cetyl/stearyl alcohol, and stearalkonium chloride; medium chain triglycerides; glycol and glycerol esters, and ethoxylated derivatives and alkyl carboxylic esters; rutin and linoleic acid; starch; glycerol monostearate; glycerin; *Amaranthus cruentus* and pumpkin seed oil containing ω-fatty acids; hydrogenated lecithin and d-α-tocopheryl polyethylene glycol 1000 succinate; *Coffea arabica* oil; microalgae extracts; aloe vera gel; red raspberry seed oil; and vitamin A and vitamin E. Retinol Correxion Deep Wrinkle Filler and Retinol Correxion Deep Wrinkle Daily moisturizer with sunscreen broad spectrum SPF 30 are two of the marketed formulation using stearyl alcohol, glyceryl stearate, tocopherol acetate, and retinol.

**G. Excipients Used in Surface Modification of Nanoformulation**

The surface of the lipid carrier consists of a charge that depends on various factors like composition of the lipid carrier, strength, and valency of the ions, pH, and hydrogen ion concentration in the medium. The zeta potential is a measurement of the net charge on a particle’s surface. Its measurement is used to assess the dispersion and aggregation processes in the application that affect particle stability. The surface charge present on nanocarriers can be altered by using compounds like surfactant and polymer-based coatings. The most common coatings are polysaccharides such as chitosan, starch, dextran, derivatives of dextran, and other synthetic polymers such as polyethylene oxide. These ingredients can modify the stability, biodistribution, particle uptake, and pharmacokinetics of actives.
Quaternized chitosan (QCS) is a chitosan (CS) derivative obtained by quaternary modification of N-substituted CS with N-(3-chloro-2-hydroxy-propyl) trimethyl-1-ammonium chloride (GTMAC). Since CS carries a positive charge, it may interfere with the anion of cell surface glycoproteins that allow the tight junction to expand reversibly and improve actives percutaneous penetration. Liang et al. formulated Coenzyme Q10 loaded NLCs by using Cetyl palmitate, Caprylic/Capric triglyceride, Caprylyl/Capryl Glycoside (APG), Tween 80, and QCS. It was observed that the zeta potential of NLCs was increased from $-13.46 \pm 0.9$ (1.5% APG solution) to $26.29 \pm 5.63$ by the addition of 0.1% QCS solution. CoQ10 loaded with QCS-APG-NLC showed higher stability and significantly protected the degradation of CoQ10. Confocal laser scanning microscopy images showed CoQ10 loaded NLC penetrated the deeper layer of the skin. Hence, skin penetration and storage stability of CoQ10 NLCs were increased significantly.

Retinyl palmitate was incorporated into SLN and the surface of the SLNs was modified using negatively charged dicetyl phosphate (DCP). DCP is recognized as a safe excipient in cosmetic delivery that has applications in facial moisturization, moisturizing sunscreens, hair color-bleaching agents, and hand creams. DCP imparts negative charge on the surface of the SLNs and affects the delivery efficiencies of the carrier. It was observed that 2.5% DCP provided the optimal charge required for the physical stability of the formulation. DCPmod-SLN reached a greater depth and increased the skin distribution of SLN by 4.8-fold compared to neutral SLNs. The surface of the niosome containing Centella asiatica extract and liposome containing coenzyme Q10 and alpha-lipoic acid was coated using hyaluronic acid and chitosan respectively to increase the skin penetration and stability of the formulation.

III. NANOTECHNOLOGY BASED CARRIERS FOR COSMECEUTICALS USED IN ANTI-AGING

Nanotechnology based formulation is an approach to formulate a delivery system with a particle size below 100 nm. The application of nanotechnology is to formulate different nanocosmeceuticals for anti-aging that include solid lipid nanoparticles, nanostructured lipid carriers, niosomes, phytosomes, liposomes, nanofibers, nanocapsules, nanoemulsions, gold, and silver nanoparticles. These formulations tend to disintegrate into their molecular components upon application to the skin. Some of the most significant applications of nanotechnology in cosmetics and dermatology include moisturizers, anti-aging and anti-wrinkle treatments, anti-oxidants, and anti-microbials.

A. Lipid Based Nanoparticle

Lipid-based nanoformulations are nanosystems consisting of various fatty acids and their derivatives, cholesterol, etc. Being lipophilic in nature, they provide various advantages due to their physicochemical properties resembling skin components. This helps to increase the chemical stability and high drug loading capacity of actives. Some of
the lipid nanoparticles used in cosmetics highlighted here include nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers, liposomes, phytosomes, niosomes, and ethosomes in lipid based nanoparticles.60

1. Nanoemulsion

Nanoemulsions are translucent or transparent emulsions. The smaller droplet size provides an advantage for the Brownian motion of nanoemulsion particles to overcome the physical destabilization. Nanoemulsions have an intrinsic bioactive property to decrease transepidermal water loss and reinforce barrier function (Table 3).170–172 Nanoemulsion based body lotions provide better skin hydration and more freshness than conventional body lotions. Nanoemulsions are stable at temperature changes or dilution as compared to the rest of the emulsion systems. They do not contain thickening agents, have a transparent appearance, require low emulsifier concentration, and also, they have high aesthetic value.170,173

Coenzyme Q10 (CoQ10) is a lipid soluble anti-oxidant found in mitochondria. It acts as a free radical scavenger, used in cosmetics for the treatment of wrinkles. Its application is limited due to its instability when exposed to free radicals and light. In order to overcome this problem, CoQ10 was incorporated into nanoemulsion. Kaci et al. formulated nanoemulsion using xanthan gum (XG) and carboxymethylcellulose (CMC). Through electrophoretic mobility and polydispersity index (PDI) it was found that the addition of XG and CMC increased the negative surface charge, leading to increased stabilization. XG enhanced cell growth by improving glucose metabolism. However, CMC, as a cellulose derivative, was not metabolized. Therefore, CMC was not suitable for cosmetic formulation.174 Biosurfactant has a significant role in stabilizing and modifying the surface properties of the colloidal system. Lewinska et al. formulated a nanoemulsion in the form of a self-emulsifying drug delivery system (SEDDS) with a natural biosurfactant (surfactin obtained from Bacillus subtilis). The optimized batch of SEDDS comprised of surfactin, transcutol, and oil phase (Capmul MCM and active compounds like vitamin C or E, curcumin) in the ratio 5:3:2. It was been observed that surfactin with 20% and 50% in 100-fold dilution leads to the drastic reduction of particle size.51

Natural herbs are non-toxic and potent but due to lack of stability and penetration, their uses are limited. A study by Septiyanti et al. suggested that different concentrations of extract would require different compositions and types of surfactants. However, further studies are required to improve the stability at a higher temperature.175 Centella asiatica extract containing triterpenoids has low skin absorption. To overcome this problem, water-in-oil nanoemulsion was formulated using the phase titration method. Along with capryol 90, the major component of the oil phase, wheat germ oil, walnut oil, and grape seed oil were added to enhance cosmetic application. It was observed that surfactant (Span 80) and cosurfactant (labrasol) in the ratio 1:1 provided maximum area of nanoemulsion in the pseudo ternary phase diagram. Capryol 90 (oil phase) and labrasol (stabilizer) provided high permeation.176

Particle size is an important factor that needs to be taken into consideration while formulating nanoemulsions. An experiment was carried out by Samson et al. in which an
The artificial neural network was applied in the study design to optimize the composition of the virgin coconut oil (containing active copper peptide) nanoemulsion by using particle size as the response parameter. A three-layer genetic algorithm neural network was used to model and process experimental data in order to determine the effect of variables on particle size. The variables that were considered include virgin coconut oil (10–20% w/w), xanthan gum (0.5–1.0% w/w), Tween 80: Pluronic F68 (10–15% w/w), and water.

**TABLE 3:** List of various anti-aging actives formulated as nanoemulsions for topical use

<table>
<thead>
<tr>
<th>Active compound</th>
<th>Excipient</th>
<th>Results/conclusion</th>
<th>Refs.</th>
</tr>
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<tbody>
<tr>
<td><em>Cordyceps militaris</em></td>
<td>Sugar squalene, Tween 85</td>
<td>Nanoemulsion enhanced the stability and skin delivery</td>
<td>137</td>
</tr>
<tr>
<td>Extracts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clinacanthus nutans</em></td>
<td>Palm kernel oil ester, xanthan gum, Span 80, Tween 80, guava seed oil (GSO)</td>
<td>In vivo studies showed significant increase in collagen content</td>
<td>41</td>
</tr>
<tr>
<td>leaves extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>Ethyl oleate, Span 80, transcutol CG</td>
<td>The relative bioavailability of catechin was increased by 894.73%</td>
<td>177</td>
</tr>
<tr>
<td>Vitamin C or E and curcumin</td>
<td>Surfactin, Transcutol and Capmul MCM</td>
<td>Stability studies showed that the emulsion was stable for 195 days</td>
<td>51</td>
</tr>
<tr>
<td>Grapeseed oil</td>
<td>Grapeseed oil, Tween 80, PEG 400</td>
<td>The use of nanoemulsion improved the moisture level, evenness, pore size, and the number of spots and wrinkles</td>
<td>46</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Isopropyl myristate (IPM), Tween 80, Transcutol HP</td>
<td>IPM has the ability to penetrate through lipid skin layers by disrupting the arrangement of the stratum corneum due to presence of aliphatic ester</td>
<td>103</td>
</tr>
<tr>
<td>β-D-glucan</td>
<td>Refined palm olein, Kolliphor®RH40, Labrafil®M1944-CS</td>
<td>At lower concentration, β-D-glucan-loaded nanoemulsions showed high stability</td>
<td>42</td>
</tr>
<tr>
<td>Red palm oil</td>
<td>Red palm oil, Tween 80, Span 80, glycerol</td>
<td>Homogenization pressure effects the droplet size significantly whereas concentration of surfactant or glycerol (co-solvent) showed insignificant difference</td>
<td>29</td>
</tr>
<tr>
<td><em>Achyrocline satureoides</em></td>
<td>Egg lecithin, medium chain triglyceride, vitamin E, Tween 80</td>
<td>Flavonoids were found to be retained in the skin at approximately 2 μg/cm²</td>
<td>178</td>
</tr>
<tr>
<td>extract</td>
<td>Miglyol 812 N, Tween 80</td>
<td>Jaboticaba nanoemulsions demonstrated significant phenolic, flavonoid and ellagic acid concentrations, with encapsulation efficiency values above 90%</td>
<td>54</td>
</tr>
</tbody>
</table>
(64.008–79.291%). The most significant factor controlling particle size was the concentration of xanthan gum followed by Tween 80: Pluronic F68, virgin coconut oil, and water. The predicted and actual particle size was 124.16 nm and 122.70 nm, respectively.\(^{27}\) Various anti-aging compounds formulated as nanoemulsions are discussed in Table 3.

2. Solid Lipid Nanoparticle

Solid lipid nanoparticles are derivatives of lipid nanoparticles in which liquid lipid (oil) is replaced by solid lipid from o/w emulsions. SLNs are prepared by various methods like solvent evaporation/emulsification, supercritical fluid extraction of emulsions, high pressure homogenization, spray drying, and ultrasonication or high extract of emulsions.\(^{179,180}\) They can incorporate both hydrophilic and hydrophobic drugs. SLNs provide many advantages like physical stability, controlled release, chemical versatility, low cost, ease of preparation, high scale production, good release profile, reliable and biodegradable nature of lipids, less requirement of organic solvents, and no or little toxicity of lipid carrier system.\(^{181–183}\) SLNs enhance skin hydration by being occlusive, with the ability to block UV radiation and form a skin film barrier.\(^{184}\)

Luteolin, a flavonoid found in different fruits and vegetables, has anti-oxidant and anti-aging properties. To enhance the stability and efficacy of luteolin it was incorporated in SLN. Jeong et al. used different types of cationic lipids to increase the transdermal delivery of luteolin. Solid lipid consisted of different types of cationic lipids (single-, double-, triple-tailed cationic lipid) like trimethyloctadecyl ammonium bromide, dimethyl dioctadecyl ammonium bromide, and methyl trioctadecyl ammonium bromide. It was observed that as the lipid tail length increases from single to triple, there was a decrease in the particle size of the SLNs. Cellular uptake studies revealed that the presence of a positive charge increased cellular uptake of SLNs containing triple-tailed cationic lipid, resulting in high binding affinity towards the cell surface. As a result, it can be used as an efficient transdermal delivery system for luteolin.\(^{185}\)

N-6-furfuryl adenine (N6FA) a natural plant growth hormone promotes cell division and also acts as an anti-oxidant. It has anti-aging properties and it also helps in reducing wrinkles. In order to improve its efficacy, it was incorporated in SLN formulated by the hot microemulsion technique. \(Ex\ vivo\) skin permeation studies revealed that N6FA loaded in SLN permeated 3 times more than the conventional cream. \(In\ vivo\) studies indicated that there were no wrinkles on UV irradiated mice after the treatment with N6FA loaded SLN.\(^{186}\) SLN loaded with CoQ10 was formulated using 13% lipid (stearic acid/cetyl palmitate) and 8% surfactant (Tego Care 450/Tween 80) by high pressure homogenization method was dispersed in cream (o/w) containing CoQ10. This formulation was compared with the conventional cream containing CoQ10. The \(in\ vitro\) drug release profile revealed that CoQ10-loaded SLN had an extended release time compared with simple cream. \(In\ vivo\) studies suggested there was increase in skin humidity by 120% and elasticity by 125% after 2 months of SLN cream use.\(^{187}\)

Yeo et al. formulated silicone-based crosslinking elastomer for the treatment of skin wrinkles as an elastic artificial skin. They developed a biocompatible lipid-based nanocarrier for improving adenine (AD) skin permeation and a formulation containing the
lipid-based carrier incorporated in elastic artificial skin. It was observed that SLNs and elastic artificial skin had sustained drug release for 48 hours. The amount of AD released from SLNs and elastic artificial skin was approximately 10 times and 5 times greater than that released from AD solution, respectively. Caffeic acid (CA) a potent anti-oxidant was incorporated into SLNs using tristearin and poloxamer 188. In vitro diffusion study was carried out using nylon membrane and was compared with CA–water. CA–SLN showed diffusion 7 times slower in comparison to CA–water. Aloe vera is a xerophytic plant with high anti-oxidant levels. In order to improve its photoprotective potential, aloe vera was incorporated into SLN. The ex vivo drug permeation profile was carried on excised rat skin. The drug permeation was found to be 80.10% over a period of 8 h. Similarly, resveratrol loaded SLNs were prepared using stearic acid, soy lecithin, and poloxamer 407. Ex vivo skin permeation studies were carried out using pig ear skin. Around 45% of the resveratrol was permeated through the skin after 24 hrs. Various active compounds formulated as solid lipid nanoparticles for anti-aging have been discussed in Table 4.

3. Nanostructured Lipid Carrier

Nanostructured lipid carriers (NLCs) are second-generation solid lipid nanoparticles. In nanostructured lipid carriers, the solid lipid is replaced by liquid lipid, and is formulated using a mixture of solid lipid and liquid lipid. This increases drug loading potential and

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Aloe vera</td>
<td>Glycerl Monostearate, Tween 80</td>
<td>The release profile demonstrated improved topical withholding of aloe vera over a long period of time</td>
<td>190</td>
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<tr>
<td>Transresveratrol</td>
<td>Soy phosphatidylcholine SPC, stearic acid, poloxamer</td>
<td>Transresveratrol loaded NLC has a potential use in therapies against skin disorders like aging and hyperpigmentation</td>
<td>191</td>
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<tr>
<td>Curcumin</td>
<td>Cholesterol, Tween 80</td>
<td>Curcumin SLNs can be used in topical formulations with a sustained release to improve the bioavailability of anti-aging products</td>
<td>192</td>
</tr>
<tr>
<td>Heptapeptide</td>
<td>Shea butter, hydrated lecithin glycerol</td>
<td>SLN loaded with heptapeptide showed skin protective functions and showed the ability for scale up</td>
<td>121</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Oleth 20, glyceryloleate, cetylpalmitate</td>
<td>The amount of solid lipid plays a key role in occlusive property of solid lipid</td>
<td>193</td>
</tr>
</tbody>
</table>
produces firmer active inclusion within the particle matrix during shelf life.\(^\text{194,195}\) It provides numerous advantages like enhancing the chemical stability of active compounds, formation of film on surface of the skin, prevention of transepidermal loss, increased skin hydration, enhancing skin bioavailability, controlled release, and increase in physical stability.\(^\text{196-200}\)

Ijaz et al. attempted to incorporate \(\alpha\)-tocopherol, a potent anti-oxidant, into NLC containing lauric acid, oleic acid, and Tween 80. It was noted that as the solid lipid concentration was increased, while maintaining the total lipid composition, there was increase in the particle size that could be due to increase in the viscosity of the system. Similarly, as the concentration of the \(\alpha\)-tocopherol was increased, there was increase in the particle size since \(\alpha\)-tocopherol is highly viscous. The release profile showed 30\% of the active was released in the first 5 hrs and the remaining 70\% release was attained after 24 hrs, while nanoemulsion showed 10\% release in the first 5 hrs and in total 54\% was released within 24 hrs. Statistical analysis showed that NLCs had significant high release of the active at each point as compared to the nanoemulsion.\(^\text{201}\)

Retinyl retinoate (RR) NLCs were formulated by high pressure homogenization using two different solid lipids and compared. RR was formulated using Precirol 5 ATO and Compritol 888 ATO. Canola oil was used as a liquid lipid since RR showed maximum solubility and it consists of different compounds like omega 3 fatty acids, linoleic acid, and oleic acid. It was observed that RR has more entrapment efficiency and better drug release from Precirol 5 ATO as compared to Compritol 888 ATO. Because RR is a heat sensitive compound, Precirol 5 ATO was the suitable solid lipid as it has low melting point compared to Compritol 888 ATO, as low heating conditions were required during the formulation.\(^\text{202}\)

CoQ10 in oxidized and reduced form was loaded into NLCs to investigate biocompatibility and cytotoxicity of NLCs alone or loaded with CoQ10 in oxidized and reduced form on human dermal fibroblasts. It was observed that NLCs alone and loaded with oxidized CoQ10 would display some cytotoxic effects under the normal and oxidative conditions. The reduced form of CoQ10 helped to maintain the cellular functionality. These observations suggest that there should be continuous assessment of nanoparticles as they can disturb the cell equilibrium leading to toxicity. Lipids and surfactants used in formulation may lead to reduction in cell viability. Hence the choice of excipients is one of the important factors in formulating a safe and stable formulation.\(^\text{203}\) Idebenone, a synthetic analog of CoQ10, was incorporated into NLCs by solvent precipitation method. The use of ethanol as a solvent decreases the dielectric constant of the whole formulation and increases the solubility of idebenone in solid lipid (Compritol 888 ATO). The \textit{in vitro} studies showed that there was initial release of the drug in the first 4 hrs. This may be due to an increase in concentration of liquid lipid that causes a decrease in viscosity of the lipid particle matrix, consequently leading to faster initial diffusion. Therefore, idebenone NLC had a sun protection factor of 23 compared to 14.88 in the reference product.\(^\text{55}\)

Liquid lipid can be replaced by natural functional oil, which can provide synergistic activity. A study was carried out by Tichota et al. using argan oil, which has anti-aging properties including anti-oxidant and hydration effects. Argan oil was incorporated in NLCs as a liquid lipid to promote skin hydration. NLCs loaded with argan oil were
incorporated in hydrogels to increase the long term stability of nanoparticles and consistency of the final formulation. It was observed that the viscosity of hydrogel was decreased after loading NLCs. However it showed synergistic effect (argan oil hydration plus occlusion effect of NLCs) on human volunteers. An attempt was made to improve photostability, water solubility and shelf life of alpha-lipoic acid (ALA) by incorporating into NLCs. ALA-NLC was formulated using hot high pressure homogenization to overcome low solubility in solid lipids. The contact angle measurement showed that the contact angle was decreased from $75.3^\circ \pm 7.8^\circ$ (ALA) to $28.5^\circ \pm 5.2^\circ$ (ALA-NLC). The photochemical stability investigation, the ALA exposed to natural daylight reduced to 0.7% whereas the ALA-NLC was reduced to 88.5%. Various anti-aging compounds formulated as nanostructured lipid carriers are discussed in Table 5.

4. Liposome

A liposome is a self-assembled spherical bilayer structure that consists of cholesterol and phospholipid, in which the aqueous volume is completely enclosed. The amphiphilic nature of the phospholipid helps in the formation of the lipid bilayer in which the lipophilic tails face each other in the presence of water and the hydrophilic head faces towards the aqueous phase. Liposomes resemble cell membrane and follow the same degradation pathway, making them safe and efficient carriers for both drug therapy and cosmetics. They can also act as a penetration enhancer. Liposome without any actives have the ability to hydrate the skin, simply by contributing lipids to the stratum corneum. Liposomes have the property to form a film on the skin surface, which helps in regulating the rate of occlusion. There is a certain limitation of liposome as a drug carrier in physical stability like aggregation, fusion, leaching of actives, and chemical stability like hydrolysis and oxidation.

Gyamera et al. attempted to incorporate green tea and roselle extracts that contains polyphenols in the treatment of anti-aging. Liposomes were prepared using soy lecithin, Tween 80, and cholesterol to increase the stability of the actives by protecting their functional groups. Evaluation studies demonstrated that liposomes prepared only with cholesterol provided a large aqueous pore and thinner wall-shell with the most stable formulation. Similarly, vitamin D3 was loaded in liposomes prepared from egg phosphatidylcholine and cholesterol. The authors found that liposomes can significantly increase the stability of vitamin D3 with skin retention of 1.65 times that of the vitamin D3 solution. In a photoaging model, it showed that vitamin D3 liposomes can repair the surface morphology of skin and promote the formation of collagen fibers. As a result, vitamin D3-loaded liposomes can be used as a skincare product.

5. Niosome

Niosomes are self-assembled spherical vesicles that consist of non-ionic surfactants along with charge-inducing agents and cholesterol. The major components of niosomes are non-ionic surface-active agents like sorbitan fatty acid (Span), alkyl ethers
<table>
<thead>
<tr>
<th>Active compound</th>
<th>Excipient</th>
<th>Results/conclusion</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All trans retinoic acid</td>
<td>Palmitic acid, oleic acid, Tween 80 and lecithin, titanium dioxide</td>
<td>Addition of titanium dioxide enhanced the photostability</td>
<td>205</td>
</tr>
<tr>
<td>CoQ10 and retinaldehyde</td>
<td>Poloxamer® F68, Isopropyl myristate and Compritol 888 ATO</td>
<td>Drug penetrated into the deeper layers of skin without any irritation and provided sustained release</td>
<td>129</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Pluro® stearique, Apifil®, and soft paraffin, liquid paraffin and Capryol™ 90, Tween 80 and Labrafil®</td>
<td><em>Ex vivo</em> studies showed enhanced permeability in Wistar rat skin</td>
<td>206</td>
</tr>
<tr>
<td>Apigenin</td>
<td>Pseudo ceramide, MCT, tego care 450, Tween 80</td>
<td>The occlusion effect of NLCs was much higher than nanoemulsion suggesting that NLCs would prevent water evaporation by forming a lipid film</td>
<td>207</td>
</tr>
<tr>
<td><em>Ocimum sanctum</em> extract</td>
<td>Cetyl palmitate, tea seed oil, plantacare 2000</td>
<td>NLC was suitable formulation for dermal delivery of rosmarinic acid in <em>Ocimum sanctum</em> with anti-aging properties</td>
<td>43</td>
</tr>
<tr>
<td>UVA filter (Uvinul A Plus Granular and UVB filter: Uvinul1 T150)</td>
<td>Pumpkin kenaf seed oils, beeswax, carnauba wax, soy lecithin, poloxamer 188 and Tween 20</td>
<td>Kenaf seed oil loaded NLCs can be used in as photoprotection in cosmetics</td>
<td>44</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>Cetyl palmitate, oleic acid, Tween 80, glycerine</td>
<td>Stability showed that NLC provided better stability than microemulsion</td>
<td>208</td>
</tr>
<tr>
<td>20(S)-Protopanaxadiol (PPD)</td>
<td>Cetyl palmitate, miglyol 812 N and Tween 20, poloxamer 188</td>
<td>Skin deposition of PPD was enhanced by 4.8 times in NLC as compared to lipid solution</td>
<td>55</td>
</tr>
<tr>
<td>CoQ10</td>
<td>Cetyl palmitate, caprylic, Tween 80, Span 80</td>
<td>The most optimal lipid composition was 9:1, which increased the penetration and effectiveness of CoQ10 as an anti-oxidant in anti-aging</td>
<td>209</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>Tristearin, Miglyol, Labrasol, Poloxamer 188</td>
<td>NLC containing miglyol showed less toxicity compared to Labrasol</td>
<td>210</td>
</tr>
<tr>
<td>Active compound</td>
<td>Excipient</td>
<td>Results/conclusion</td>
<td>Refs.</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-------</td>
</tr>
<tr>
<td>Retinoids</td>
<td>Myristic acid, sunflower oil, Span 80, tretinoin (TRT) and adapalene (ADP)</td>
<td>The number of carbons on the fatty acid chain of the solid lipid and surfactant concentration significantly influence the particle size and surface charge of lipid nanoparticles</td>
<td>211</td>
</tr>
<tr>
<td>L-ascorbic acid or Gold Tri.E 30</td>
<td>Stearic acid, oleic acid, Tween 60</td>
<td>NLCs prolonged the shelf life and enhanced the delivery of the actives</td>
<td>212</td>
</tr>
<tr>
<td>Curcuminoids</td>
<td>Precirol ATO 5, Compritol 888 ATO, labrasol, poloxamer 407 and Tween 80</td>
<td>The type of surfactant used affected the in vitro release rate and permeation capability of curcuminoids</td>
<td>64</td>
</tr>
<tr>
<td>Tretinoin</td>
<td>Stearic acid, oleic acid, Tween 80 and Span 80</td>
<td>Cholesterol enhanced the stability and entrapment efficiency</td>
<td>213</td>
</tr>
</tbody>
</table>
including Brij 30 (polyoxyethylene (4) lauryl ether), polyoxyethylene sorbitan fatty acid esters (Tween), and sucrose esters.\textsuperscript{220} Niosomes have numerous advantages like increased skin permeation, enhanced stability of the actives, sustained release of the drug, improved surface adhesion, increased dermal penetration and bioavailability.\textsuperscript{221,222} Rice [\textit{Orizya sati} L.(Gramineae)] bran, which contains \( \gamma \)-oryzanol (O), phytic acid (P), and ferulic acid (F), was entrapped in niosomes by supercritical carbon dioxide fluid (scCO\textsubscript{2}). The prepared niosomes were incorporated in gel, cream, and cream containing rice bran orizya. This formulation showed an increase in skin hydration and enhancement of skin thickness, lightening, elasticity, and roughness, on 30 human volunteers for 28 days of application.\textsuperscript{223} Collagen, a high molecular weight, hydrophilic protein was incorporated into nanosurfactant dispersion using cholesterol and Span 60 (sorbitan monostearate) by ethanol injection method followed by probe sonication. \textit{In vivo} skin hydration demonstrated 2.5-fold and 3-fold increase in stratum corneum (SC) thickness compared to collagen gel–treated and untreated skin respectively.\textsuperscript{222} Gallic acid was incorporated in neutral and cationic niosome. Neutral niosome was formulated from Brij 52/cholesterol in ratio 7:3 while the cationic niosome was formulated using Brij 52/cholesterol/CTAB in ratio 7:3:0.65. The release profile studies showed that gallic acid incorporated in neutral and cationic CTAB niosomes released gradually over 24 hours. This study showed cationic CATB niosome to be more effective in anti-aging (anti-wrinkle) compared to free gallic acid and gallic acid loaded in neutral niosome.\textsuperscript{224}

\textbf{6. Ethosome}

Ethosomes are vesicular systems that mainly consist of ethanol and phospholipids. They are flexible in nature due to the presence of ethanol and phospholipids. They are non-invasive carriers, and have higher transdermal efflux and better skin penetration.\textsuperscript{225,226} In ethosomes ethanol disrupts the lipid bilayer of the skin, allowing the vesicle to penetrate the stratum corneum.\textsuperscript{227,228} Furthermore, high concentration of ethanol allows the lipid membrane to pack less densely while maintaining the stability of liposomes, which aids in delivery of actives in stratum corneum. It provides various advantages like enhanced permeation and incorporation of macromolecules, and is non-invasive and can be widely used in cosmeceutical applications.\textsuperscript{229}

To overcome the permeation rate, rutin, a potent anti-oxidant, was incorporated into ethosomes. In tape stripping assay, rutin incorporated in ethosomes was found in high amount in the deeper layers compared to rutin in a free state. Even though the \textit{in vitro} assay demonstrated anti-oxidant action, in \textit{ex vivo} studies there was no anti-oxidant activity from the rutin-loaded ethosomes.\textsuperscript{230} Arora et al. applied a quality by design approach to formulate resveratrol loaded ethosomes. The concentration of phospholipids and ethanol was selected as critical material attributes. Vesicle size, entrapment efficiency, permeation flux, and drug deposition in the dermal layer were selected as critical quality attributes. It was found that ethososomal gel significantly enhances the skin permeation and skin deposition of resveratrol compared to conventional cream.\textsuperscript{231} Caffeic acid (CA) ethosomes were prepared using soybean lecithin (90% phosphatidylcholine) (PC). \textit{In vitro}
studies demonstrated CA release from ethosomes was 18-fold slower than SLN. The $t_{lag}$ of ethosomes-CA was longer as compared to CA-solution. These findings suggest that PC vesicles take longer to associate with lipids in the stratum corneum than CA solution and that ethanol merely acts as a penetration enhancer, opening pores inside the stratum corneum.\textsuperscript{189}

7. Phytosome

Phytosomes are lipid-based vesicular systems (similar to liposomes) in which herbal extracts or phytoconstituents are incorporated. Herbal active compounds are hydrophilic in nature hence they have poor penetration. To increase skin permeation and stability of the phytoconstituents, they are incorporated into the vesicular system. Phytosomes provide various advantages like increase in stability, enhancement of bioavailability, etc.\textsuperscript{232,233} Aloe vera extract was incorporated into phytosomes composed of lecithin. \textit{Ex vivo} permeation studies on rat skin suggested better permeation and flux profile of phytosomal gel compared to conventional gel. Phytosomal gel can be used as a potential carrier for the delivery of herbal extracts.\textsuperscript{234} Surini et al. formulated grape seed extract (GSE) phytosome serum to increase the GSE in the skin. GSE phytosome serum was formulated using a thin-film hydration technique. It was observed that GSE was not entrapped in the vesicle but bound on the polar surface of phosphatidylcholine. It was concluded that phytosome could enhance the penetration of total phenolic compounds in the grape seed extract in a serum dosage form.\textsuperscript{233} Various anti-aging compounds formulated as vesicular systems for are discussed in Table 6.

B. Polymeric Nanosystem

Polymeric nanoformulation is formulated by using various synthetic, semi-synthetic, and natural polymers. These polymers help to promote a variety of functions in anti-aging products that include emulsifiers, thickeners, rheology modifiers, foam stabilizers and destabilizers, film formers, and conditioners.\textsuperscript{234,239} These polymers are used in formulating different types of nanosystems like nanoparticles, nanospheres, and nanofibers.

C. Nanoparticle

Polymeric nanoparticles (matrix system) generally prepared from biodegradable polymers have wide applications in anti-aging. These are nanosized solid colloidal particles prepared from polymers. They are formulated using spray drying, solvent evaporation, emulsion-solvent diffusion, nanoprecipitation, salting out, and supercritical fluid technology.\textsuperscript{240} Polymeric nanoparticles have several advantages, including protecting actives from degradation from adverse environments, improvement in physicochemical properties of actives, and controlled release, all of which result in increased effectiveness and bioavailability.\textsuperscript{241,242} Olive oil extracts were incorporated in poly (lactic acid) (PLA) by emulsification–solvent evaporation technique. PLA is an aliphatic polyester,
biodegradable, biocompatible, lipophilic polymer. *In vitro* studies showed that olive oil extracts diffused at a constant rate for 7 days from nanoparticles after the initial burst.\textsuperscript{242} Similarly, phytoconstituents like curcumin and quercetin have been incorporated in poly (lactic-co-glycolic) acid (PLGA) and PLGA-TPGS (D-α-tocopherol polyethylene glycol 1000 succinate) respectively for treating UVB induced skin damage.\textsuperscript{243,244}

### D. Nanosphere

Nanospheres have a core–shell structure and are spherical particles. The actives are encapsulated, dissolved, attached, or entrapped in nanospheres, and they also protect actives from degradation by enzymes and chemicals.\textsuperscript{245} This system is promising because it has the ability to convert poorly absorbed, labile biologically active substances and poorly soluble active substances into efficacious deliverable drugs. Nanospheres’ cores can be filled with a variety of actives.\textsuperscript{246} Nanospheres are also used in skin care products.

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**TABLE 6:** List of various anti-aging actives formulated as vesicular systems for topical use

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Active compound</th>
<th>Excipient</th>
<th>Results/conclusion</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposome</td>
<td>Rosmarinic acid</td>
<td>Dipamitoyl phosphatidylcholine, cholesterol</td>
<td>Rosmarinic acid loaded liposomes showed antioxidant, anti-collagenase, and anti-elastase effect</td>
<td>235</td>
</tr>
<tr>
<td>Liposome</td>
<td>Retinyl palmitate</td>
<td>L-α-Phosphatidylcholine</td>
<td>Liposomes provided higher skin retention</td>
<td>236</td>
</tr>
<tr>
<td>Niosome</td>
<td>Gallic acid</td>
<td>Cholesterol, Tween 61</td>
<td>There was significant improvement in skin elastic and roughness property</td>
<td>237</td>
</tr>
<tr>
<td>Niosome</td>
<td>Curcumin</td>
<td>Cholesterol, Span 60</td>
<td><em>In vitro</em> and <em>in vivo</em> studies showed increase in moisture retaining capacity of the skin, anti-oxidant activity and improvement of overall pharmacokinetics providing anti-wrinkle effect</td>
<td>238</td>
</tr>
<tr>
<td>Ethosome</td>
<td>Naringin</td>
<td>Soy phosphatidylcholine, ethanol</td>
<td>Ethosomes loaded with naringin showed enhanced skin permeation</td>
<td>227</td>
</tr>
<tr>
<td>Phytosome</td>
<td>Aloe vera extract, jojoba oil, vitamin E, grape seed extract</td>
<td>Phosphatidylcholine</td>
<td>Formulation containing aloe vera extract and coconut water showed high anti-oxidant activity compared to marketed formulation</td>
<td>232</td>
</tr>
</tbody>
</table>
to deliver active ingredients deeper into the skin and more precisely and efficiently deliver their beneficial effects to the affected area of the skin. Anti-aging protection is significantly enhanced by these microscopic fragments.247

Chitosan, being a non-toxic, biodegradable, biocompatible, and low-cost biomaterial, was used to incorporate α-tocopherol to form nanospheres. It was prepared using the emulsion formation method. Anti-oxidant activity was determined using the superoxide dismutase (SOD) activity method. The anti-oxidant activity of the placebo formulation of chitosan nanospheres was 9.07 SOD units/g, whereas the anti-oxidant activity of the α-tocopherol-loaded chitosan nanospheres was 40.92 SOD unit/g. These results show that chitosan has inherent anti-oxidant properties but it cannot be used solely in cosmetics. There was a sustained release of α-tocopherol for 24 hrs due to cross-linking and swelling of chitosan with a cumulative release of 64%.248 Vitamin K1, which has anti-oxidant properties, was encapsulated in poly(epsilon-caprolactone) (PCL) (MW = 65,000). Ex vivo skin penetration using female porcine skin demonstrated that a greater amount of actives was in the dermis layer of the skin for up to 8 hrs.249

1. Nanofiber

Nanofibers are generally fibers with a diameter of less than 100 nm, developed with the aid of electrospinning.250 Electrospinning is a low-cost, simple, versatile technique for formulating nanofibers with diameters ranging from micrometers to nanometers by using various polymer solutions like cellulose acetate,251–253 chitosan,254 hyaluronic acid,255 and synthetic polymers, namely, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and polyethylene oxide (PEO).126 As the diameter of the polymeric nanofiber shrinks to nanometers, a larger specific surface area is obtained. This property of nanofibers has much application in the development of facial masks, perfumes, deodorants, and anti-perspirants. The ease of incorporating actives into electrospun fibers makes them convincing for cosmetic applications.250

Pomegranate seed oil and sea buckthorn oil were incorporated into polylactide (PLA-based fibers) and polyvinyl pyrrolidone (PVP-based fibers) nanofibers by using electrospinning technique. The anti-oxidant of the formulated nanofibers was determined using 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH). It was observed that encapsulated oils showed higher anti-oxidant activity compared to the pure active compound. This was because the finer dispersion of active compounds was achieved by forming nanofibers. The oils were immiscible with PLA, but this had no significant effect on anti-oxidant properties. PLA fibers preserved more anti-oxidant properties of oils than PVP, which could be attributed to the hydrophobicity/hydrophilicity of basic polymers.256 Manatunga et al. for the transdermal delivery of nanogold formulated a nanofibrous cosmetic face mask. Initially, gold (Au) nanoparticle was synthesized using green tea (Gt) and orange peel extract (Op). Later, these nanoparticles were incorporated into polyethylene oxide and gelatin (PEO-Gel) fiber mat by electrospinning technique. Along with PEO-Gel, ascorbic acid (AA) and collagen (COL) was added to improve the

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efficacy. The results of scanning electron microscopy showed that adding ascorbic acid and collagen enhanced the incorporation of gold nanoparticles. *In vitro* release studies on Strat membrane revealed that the PEO-Gel-AA-COL-orange peel Au (OpAu) system has a higher content of Au release than the green tea Au (GtAu) NP system, with a maximum release time of 10–30 minutes.\(^{126}\)

### 2. Gold and Silver Nanoparticle

Gold and silver nanoparticles have a particle size ranging from 5 nm to 400 nm. They also show various shapes such as nanospheres, nanocubes, nanopentagons, and nano-triangles.\(^{257}\) Gold and silver nanoparticles have gained interest due to various properties like anti-inflammatory, anti-aging, and anti-oxidant.\(^{258,259}\) Due to their strong anti-bacterial and anti-fungal properties, gold and silver nanoparticles have been studied as promising materials in the cosmeceutical industry. These nanoparticles can be used in a wide range of cosmeceutical products, including creams, lotions, face packs, deodorants, and anti-aging creams.\(^{221}\) A recent trend suggests that there is an increase in the use of green sources for the synthesis of gold and silver nanoparticles. Gold and silver precursors are reacted with plant extract to obtain a stable nanoparticle system. Some of these are discussed below.

To reduce the toxicity of nanoparticles, green synthesis approaches are used for the formulation of gold and silver nanoparticles. In this experiment precursor salts used for the synthesis of AuNPS and AgNPs were gold (III) chloride trihydrate (HAuCl3.3H2O) and silver nitrate (AgNO3) respectively. Ginseng berry extract (GBE) containing polysaccharides and phenolic compounds was used to stabilize and functionalize nanoparticles. Evaluation studies showed that GBAgNPs have high anti-oxidant, anti-tyrosinase, and anti-bacterial activity as compared to GBAuNPs. However, GBAgNPs were found to be more toxic on B16 and HDF cells as compared to GBAuNPs. Therefore, GBAuNPs can be used as a potential agent for anti-bacterial, anti-oxidant, and anti-tyrosinase activities.\(^{260}\) Similarly, Ben et al. synthesized gold nanoparticles using plant extracts (green synthesis). *Hubertia ambavilla* extract contained polyphenolic compounds that reduced metal salts into nanoparticles. It was observed that gold nanoparticles were non-toxic to fibroblasts and dermal cells and efficiently scavenge the dermal cells. Regulatory tests ensured that gold nanoparticles are non-irritant, non-genotoxic, non-photo toxic, non-toxic, and non-sensitizing according to OECD guidelines. Hence, it can be used as an ingredient in anti-aging cosmetics.\(^{261}\) Likewise, results were obtained in the synthesis of gold nanoparticles using Panax ginseng leaves.\(^{262}\)

### IV. MARKETED NANOFORMULATIONS FOR ANTI-AGING

In the last few decades, various anti-aging products developed with the application of nanotechnology have been marketed. Various anti-aging marketed nanoformulations are illustrated in Table 7.
<table>
<thead>
<tr>
<th>Nanoformulation</th>
<th>Product name</th>
<th>Manufacturer/marketed by</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoemulsion</td>
<td>Bepanthol Protect Facial Cream Ultra</td>
<td>Bayer Healthcare</td>
<td>Moisturizer and anti-aging</td>
</tr>
<tr>
<td>Nanoemulsion</td>
<td>Precision-Solution Destressante Solution Nano Emulsion Peaux Sensitivity</td>
<td>Chanel</td>
<td>Moisturizer</td>
</tr>
<tr>
<td>Nanoemulsion</td>
<td>Coni Hyaluronic Acid and Nanoemulsion Intensive Hydration Toner</td>
<td>Coni Beauty</td>
<td>Skin hydration</td>
</tr>
<tr>
<td>Nanoemulsion</td>
<td>Phyto-Endorphin Hand Cream</td>
<td>Rhonda Allison</td>
<td>Softens and smooths the skin</td>
</tr>
<tr>
<td>Nanoemulsion</td>
<td>Nanovital Vitanics Crystal Moisture Cream</td>
<td>Vitacos Cosmetics</td>
<td>Skin moisturizer and lightening effect</td>
</tr>
<tr>
<td>Solid lipid nanoparticle</td>
<td>Allure Body Cream</td>
<td>Chanel</td>
<td>Moisturizer</td>
</tr>
<tr>
<td>Solid lipid nanoparticle</td>
<td>Soosion Facial Lifting Cream SLN Technology</td>
<td>Soosion</td>
<td>Anti-wrinkle</td>
</tr>
<tr>
<td>Solid lipid nanoparticle</td>
<td>Phyto NLC Active Cell Repair</td>
<td>Sireh Emas</td>
<td>Hyperpigmentation, moisturizing and skin firming</td>
</tr>
<tr>
<td>Nanostructured lipid carrier</td>
<td>Cutanova-Cream Nanorepair CoQ10</td>
<td>Dr. Rimpler</td>
<td>Smoothing of lines, anti-aging</td>
</tr>
<tr>
<td>Nanostructured lipid carrier</td>
<td>Intensive Serum Nanorepair CoQ10</td>
<td>Dr. Rimpler</td>
<td>Anti-wrinkle, aging</td>
</tr>
<tr>
<td>Nanostructured lipid carrier</td>
<td>Iope Supervital Extra Moist Softener</td>
<td>Amore Pacific</td>
<td>Moisturizes dry and rough skin</td>
</tr>
<tr>
<td>Nanostructured lipid carrier</td>
<td>Olivernol Anti Falten Pflegekontrat</td>
<td>Dr. Theiss/ Medipharma Cosmetics</td>
<td>Anti-wrinkle and skin tightening</td>
</tr>
<tr>
<td>Nanostructured lipid carrier</td>
<td>Surmer Crème Légere Nano-Protection</td>
<td>Isabelle Lancray</td>
<td>Intensely hydrating</td>
</tr>
</tbody>
</table>
### TABLE 7: (continued)

<table>
<thead>
<tr>
<th>Liposome</th>
<th>Russell Organics Liposome Concentrate</th>
<th>Russell Organics</th>
<th>Hydrating, makes skin firmer, softer, and smoother</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposome</td>
<td>Dermosome</td>
<td>Microfluidics</td>
<td>Moisturizer</td>
</tr>
<tr>
<td>Liposome</td>
<td>Capture Totale</td>
<td>Dior</td>
<td>Anti-wrinkle and removes dark spots</td>
</tr>
<tr>
<td>Liposome</td>
<td>Natural Progesterone Liposomal Skin Cream</td>
<td>NOW Solutions</td>
<td>Maintenance of healthy feminine balance</td>
</tr>
<tr>
<td>Liposome</td>
<td>Kerstin Florian Rehydrating Liposome Day Crème</td>
<td>Kerstin Florian</td>
<td>Moisturizer</td>
</tr>
<tr>
<td>Liposome</td>
<td>Advance Night Repair Protective Recovery Complex</td>
<td>Estee Lauder</td>
<td>Skin repair</td>
</tr>
<tr>
<td>Niosome</td>
<td>Mayu Niosome Base Cream</td>
<td>Laon Cosmetics</td>
<td>Whitening and moisturizing</td>
</tr>
<tr>
<td>Niosome</td>
<td>Eusu Niosome Makam Pom Whitening Facial Cream</td>
<td>Eusu</td>
<td>Skin whitening</td>
</tr>
<tr>
<td>Niosome</td>
<td>Anti-Age Response Cream</td>
<td>Simply Man Match</td>
<td>Anti-wrinkle</td>
</tr>
<tr>
<td>Niosome</td>
<td>Niosome + Perfected Age Treatment</td>
<td>Lancome</td>
<td>Anti-wrinkle</td>
</tr>
<tr>
<td>Nanosphere</td>
<td>Nanosphere Plus</td>
<td>Dermaswiss</td>
<td>Anti-aging and anti-wrinkle</td>
</tr>
<tr>
<td>Gold nanoparticle</td>
<td>Nano Gold Energizing Face Cream</td>
<td>Chantecaille</td>
<td>Anti-wrinkle, anti-oxidant, anti-aging</td>
</tr>
<tr>
<td>Gold nanoparticle</td>
<td>Nano Gold Firming Treatment</td>
<td>Chantecaille</td>
<td>Anti-wrinkle, anti-oxidant</td>
</tr>
</tbody>
</table>
V. PATENTS

There has been an increase in the number of patents filed in the field of nanocosmeceuticals. Some of the recent patents filed are listed in Table 8.

VI. REGULATORY ASPECTS

The application of nanomaterials and nanotechnology in different fields of medicine and cosmeceuticals has increased over the past 10 years. The advent of the nanotechnological field has been shown to be highly beneficial in meeting various human healthcare needs. In spite of this, there appears to be little regulatory guidance regarding the safe use of nanomedicine. There are various challenges in the development of regulatory guidelines for nanocosmetics used in anti-aging. These challenges have been illustrated in Fig. 2. Establishing regulatory guidelines ensures the safety and ethical use of nanotechnological aspects in medicine and cosmeceuticals. Nanocosmetics specifically require strong regulatory guidelines since they can show harmful effects due to skin sensitization. The safety and quality of nanocosmetics are regulated by different bodies in various regions. Cosmetic products do not require pre-market approval from the US Food and Drug Administration (FDA) under FDA regulations. However, certain laws put forward by the FDA regulate these products. If any of the ingredients in these cosmetics cause changes in body function, they are classified as a “drug” under the Food, Drug, and Cosmetic Act (FD&C). The industry producing these products is responsible for ensuring their safety.

In July 2007, the FDA issued an assessment of scientific and regulatory considerations prepared by the Nanotechnology Task Force on the safety and efficiency of FDA-regulated nanomaterial products. The Task Force also recommended the issuance of guidelines to describe the safety concerns that manufacturers should take into account to ensure nanomaterial cosmetics are safe and not adulterated. Hence, “Guidance for Industry Safety of Nanomaterials in Cosmetic Product” was issued in 2014.

Nanomaterials may have different physical, chemical, or biological properties than large-scale materials with the same chemical composition. Changes in electrical, optical, or magnetic activity, as well as increased structural integrity and changes in chemical or biological activity can result from such differences. Analytical methods are not validated for nanomaterials. This raises safety concerns for cosmetic products. Hence, FDA has recommended non-binding safety assessment guidelines for cosmetic products using nanomaterials. These include:

- physicochemical characteristics
- agglomeration and size distribution of nanomaterials under the conditions of toxicity testing and as expected in the final product
- impurities
- potential routes of exposure to the nanomaterials
- potential for aggregation and agglomeration of nanoparticles in the final product
- dosimetry for in vitro and in vivo toxicology studies
### TABLE 8: Patents for nanoformulations on anti-aging

<table>
<thead>
<tr>
<th>Patent No.</th>
<th>Title of patent</th>
<th>Description</th>
<th>Year</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN104434551A</td>
<td>Preparation method of curcumin flexible liposome cream</td>
<td>Curcumin flexible liposome cream was prepared by the following three steps: preparation of curcumin flexible liposome, preparation of freeze dried powder from the curcumin flexible liposome, and preparation of a curcumin flexible liposome cream</td>
<td>2015</td>
<td>266</td>
</tr>
<tr>
<td>KR20160014315A</td>
<td>Cosmetic composition having anti-wrinkle effect containing nanoemulsion of walnuts and chestnuts fermented extract</td>
<td>Nanoemulsion was formulated by preparing aqueous phase (hydrogenated lecithin, potassium cetyl phosphate, emulsifying aid and dispersing aid of glycols) and oil phase (cholesterol, emulsifier, macadamia oil and shea butter)</td>
<td>2016</td>
<td>267</td>
</tr>
<tr>
<td>US20170157005A1</td>
<td>Novel anti-wrinkle and anti-aging nano formulation and method of preparation using novel nano co-delivery system</td>
<td>A multilayer nanocarrier was formulated by using natural plant extracts and biological active agents, vitamins, and others</td>
<td>2017</td>
<td>268</td>
</tr>
<tr>
<td>CN105001433B</td>
<td>A kind of preparation method and applications of chitosan nano suspension</td>
<td>Chitosan nanoparticle suspension was formulated by three steps: preparation of chitosan acetic acid aqueous solution; preparation of chitosan nano-coarse suspension using sodium tripolyphosphate; filtration and homogenization of chitosan nano-coarse</td>
<td>2018</td>
<td>269</td>
</tr>
<tr>
<td>CN104606125A</td>
<td>Plant nano-emulsion anti-freckle cosmetic cream</td>
<td>In this work, natural plants are nano-pulverized to produce nano-emulsion with particle sizes ranging from 30–100 nm, and also nano-emulsion prepared with cosmetics</td>
<td>2015</td>
<td>270</td>
</tr>
<tr>
<td>US20150238403A1</td>
<td>Low viscous cosmetic composition using a natural emulsifying agent</td>
<td>In this present invention, a cosmetic nanoemulsion was formulated by using a naturally derived saccharide-based surfactant in replacement of chemical surfactant to provide safety to the skin</td>
<td>2015</td>
<td>271</td>
</tr>
<tr>
<td>Patent No.</td>
<td>Title of patent</td>
<td>Description</td>
<td>Year</td>
<td>Refs.</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>US9700042B2</td>
<td>Nanoformulation of musk-derived bioactive ingredients for nanocosmetic applications</td>
<td>Hyaluronic acid and fatty acid cross-linked with ultralow molecular weight chitosan were used to formulate nanoformulation encapsulating isolated compounds from musk and their combinations for cosmetic use as anti-aging, anti-microbial, and fragrance</td>
<td>2017</td>
<td>272</td>
</tr>
</tbody>
</table>
- *in vitro* and *in vivo* toxicological data on nanomaterial ingredients and their impurities, dermal penetration, potential inhalation, irritation (skin and eye) and sensitization studies, mutagenicity/genotoxicity studies.277

The Indian Government has invested heavily in the Nanoscience and Nanotechnology Initiative (NSTI) in order to create a highly efficient setup in national laboratories, academic institutions, and R&D centers.278 The application of nanotechnology in pharmaceuticals and cosmetics has great potential as it can improve efficacy and reduce the toxicity of drugs. Efforts have also been made to develop guidelines in nanopharmaceuticals. In 2019, the Government of India released guidelines for the evaluation of nanopharmaceuticals. However, cosmetics containing nanotechnology applications are exempted from these guidelines.279

European Commission Regulation (EC) No. 1223/2009 on cosmetic products aimed to ensure a high level of human health safety. Cosmetic goods must be safe under reasonable or normal foreseeable conditions of use, according to EU regulations. It mandates the premarket notification of cosmetics containing nanomaterials, including their toxicology data. Besides that, all ingredients in a cosmetic product that are nanomaterials must be identified in the ingredient list by adding the prefix “nano” to the ingredient name. The labeling is not intended to indicate any danger or risk, but to help the consumers to make informed decisions when purchasing products.282
representation for the safety assessment of a cosmetic product containing nanomaterials is depicted in Fig. 3.

The International Cooperation on Cosmetics Regulation (ICCR) is a voluntary international organization composed of cosmetics regulatory authorities from the United States, the Republic of Korea, Japan, the European Union, Chinese Taipei, Canada, and Brazil. This group of regulators meets annually for discussion of issues on the safety and regulation of cosmetics, as well as for constructive dialogue with the relevant industry associations in the field of cosmetics.\textsuperscript{283}

The purpose of the ICCR Working Group report is to inform those seeking to assess the safety of cosmetic nano-scale ingredients. In 2013, ICCR provided information regarding the Safety Approaches to Nanomaterials in Cosmetics. It was suggested that the ADME parameters of nanomaterials be studied in order to investigate the fate and behavior of nanoparticles in the body and to identify the likely target organs. The methods used to investigate the toxicology of conventional materials are also applicable to nanomaterials with certain modifications. \textit{In vitro} and \textit{in silico} modeling are also required to generate high-quality data on the physicochemical properties, biokinetic behavior, and toxicological effects of nanomaterials.\textsuperscript{284}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Schematic outline for the safety assessment of nanomaterials in cosmetics based on Scientific Committee on Consumer Safety (SCCS) (modified)\textsuperscript{280,281}}
\end{figure}
VII. SAFETY, EFFICACY, AND TOXICITY STUDIES

Nanoformulations formulated from synthetic and natural sources have various functions in anti-aging. Particle size in the nano range provides them unique properties compared to bulk. However, they may also possess certain risks. The US FDA has recommended guidelines for the characterization of nanoformulation used in cosmetics. In addition, *in vitro* and *ex vivo* and pre-clinical studies must be carried out to determine the potential toxicity.

Particle size has a great impact on the toxicity profile of the nanoformulation, hence it becomes crucial to monitor the particle size of the nanoformulation. It has been reported that, as the particle size decreases below 100 nm, solubility and adhesion increase. Also, particles above 100 nm enter the cell by macrophages hence very limited particles can enter the cell, while particles below 100 nm enter through endocytosis, which leads to an increase in biodistribution. Biodegradability of the material is also an important parameter for toxicity studies. Non-biodegradable nanomaterials remain in the cell for a longer duration after internalization by cells. Based on these properties, a nanotoxicological classification system was developed that places the nanoformulation into four groups (Table 9). Toxicity can also be caused by various other factors like dose, surface area, concentration, particle chemistry, and crystalline structure of actives and excipients used in formulation along with the surface coating.

There are various methods that are used for the evaluation of the safety and efficacy of the formulation. *In vitro* and *ex vivo* studies are economical and less time-consuming to determine the potential toxicity and efficacy data. These include various studies to determine the safety of the nanoformulation. Cell culture studies carried out through MTT assay provide biocompatibility and safety of materials and nanoformulations. Irritation studies are carried out by hen’s egg test on the chorioallantoic membrane (HET-CAM) and Dermal Irritector® Assay System. In order to determine the *in vitro* anti-oxidant efficacy (1,1-diphenyl-2-picrylhydrazyl) and DPPH radical-scavenging activity, ferric reducing anti-oxidant power (FRAP) assay, 2,2-Azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, and determination of lipid peroxidation inhibition by ferric thiocyanate (FTC) assay are used. Skin permeation and skin distribution are tested using excised skin of animals including pig ear, rats, and goats. *In vivo* studies are carried out in suitable animal models. Skin irritation is done by applying topical formulation on the skin and irritation scores are given based

| TABLE 9: Nanotoxicological classification system |
|-----------------|-----------------|-----------------|
| Class | Particle size (in nm) | Nature of material | Risk |
| I | > 100 | Biodegradable | No or low risk |
| II | > 100 | Non-biodegradable | Medium low risk |
| III | < 100 | Biodegradable | Medium risk |
| IV | < 100 | Non-biodegradable | High risk |

Nanotoxicological classification system based on particle size and nature of material.

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on the observation. A similar study is carried out to determine anti-wrinkle efficacy of
the formulation. Skin hydration studies are measured using Tewameter and corneometer.
The anti-oxidant activity is evaluated by estimating the level of catalase (CAT),
reduced glutathione (GSH), and superoxide dismutase (SOD) reactive substances af-
ter sacrificing the animals. Methods used to evaluate anti-aging in humans involve
high-quality image analysis, expert evaluation, horny protein changes, transepidermal
water loss, and clinical data of dryness and erythema. In the future, it will be neces-
sary to improve current evaluation approaches and create a new paradigm in the field
of anti-aging cosmetics.

VIII. CONCLUSION AND FUTURE PROSPECTS

Cosmetics involved in anti-aging consist of four types of actives: moisturizing, free rad-
ical removing, cell repairing, and UV absorption types. To increase the efficacy of these
actives, nanotechnology is used for their formulation along with selecting a reasonable
excipient. Anti-oxidants are widely used actives in anti-aging due to their radical scav-
enging activity and they can be also used as preservatives. Synthetic anti-oxidants may
show toxicity hence the use of natural anti-oxidants is increasing. Oils play a significant
role in formulating nanoemulsion and nanostructured lipid carriers. It has been found
that oils from natural origin have inherent properties of anti-aging. Therefore, more
studies should be carried out on these oils as they have less or no toxicity and can pro-
vide synergistic activity when used as an excipient. Polymers have numerous properties
as they are used to modify the surface properties of colloids to enhance the stability of
formulation as well as having inherent anti-aging properties. Peptides, proteins, and vi-
tamins also have a great potential in formulating anti-aging products as they can be used
as actives as well as excipients.

Nanoformulations have proven to be promising in anti-aging due to their supe-
rior penetration into the skin and reliable delivery of actives, along with their bio-
compatibility, stability, and anti-oxidant properties. This has led to the integration
of nanostructures into various beauty care products including anti-aging products.
As the application of nanoformulation is increasing in anti-aging products there is
a need for further investigation due to safety concerns. It is essential to characterize
them in terms of their physicochemical properties, as their nanosize can cause sys-
temic effects and accumulation in living tissues. There are guidelines provided by
the FDA and EU for the application of nanotechnology in cosmetics. However, addi-
tional guidelines on product labeling, physicochemical evaluation, and post-market-
ing monitoring may help to raise the standards for the manufacturing of anti-aging
products. There is a need for international collaboration of regulatory agencies in
sharing information on cosmetic ingredients, and both academics and industry ex-
erts can provide knowledge through interdisciplinary work for the development
of guidelines on analysis, quality control, and safety studies. Consumers should
be more vigilant and should report adverse reactions to products to the concerned
authorities.
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