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The Role of Nanoparticles in Sunscreen: UV Protection and Particle Size

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Abstract

Sunscreen formulas are essential for protecting the skin from damaging UV rays, which reduces the risk of sunburn, skin cancer, and premature aging. However, conventional sunscreens frequently lack further advantages including anti-aging and antioxidant capabilities. Strong polyphenolic component epigallocatechin-3-gallate (EGCG), present in green tea extracts, has anti-inflammatory, antioxidant, and photoprotective properties. Similar to this, poly amino acids and hyaluronic acid (HA) have hydrating, anti-aging, and skin-repairing qualities. In this work, we investigated the co-delivery of EGCG and HA/poly amino acids in sunscreen formulations using nano-carriers, namely transferosomes and polymeric nanoparticles. To increase the effectiveness of these nano-carriers, a number of formulation factors, such as the drug-polymer ratio, surfactant concentration, homogenization speed, and sonication time, were improved.

When compared to normal EGCG solutions, the optimized nano-formulations showed superior properties such reduced particle size, higher encapsulation efficiency, and improved skin permeability. Studies on cytotoxicity showed that the nano-formulations were not harmful to skin cells and that they increased cell viability when compared to EGCG in its purest form. Because the nano-formulations contained EGCG and HA/poly amino acids, they offered considerable protection against skin damage caused by UV radiation. The spherical shape of the nano-carriers was shown by transmission electron microscopy, and the lack of interactions between EGCG and excipients was validated by differential scanning calorimetry and Fourier-transform infrared spectroscopy.

Keywords: Sunscreen, Nano-carriers, EGCG, Hyaluronic Acid, Photoprotection.

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Introduction

Electromagnetic radiations such as UV radiation, visible light and heat are obtained from sun light. UV radiations are broadly classified into three bands namely UVA (ranging from 315- 400) nm, UVB (ranging from 280-315 nm) and UVC (ranging from 100-280 nm) [1] Among them, UVB radiation (18 mJ/cm2) is beneficial to our daily life. UVA radiations are long waves with lower energy and high penetration power. They generate free radicals, cause DNA damage indirectly and damage the collagen fiber in extracellular matrix of the biological system. UVA is the main etiological factor in malignant melanoma. UVB radiations are medium waves with lower penetration than that of UVA and they produce erythema or sun burn. Depending on the intensity and length of the exposure UVB also cause skin cancer (malignant melanoma) through indirect DNA damage [2]. One positive effect of moderate doses of UVB is the production of vitamin D and vitamin K. UVC radiations are short waves with high energy and low penetration power. They are completely absorbed by ozone layer, however excessive exposure of UVC cause skin cancer like UVA and UVB.

Harmful biological effects of UVR can be divided into two types depending on their range and duration of exposure to UVR. Acute reactions are sunburn and tanning. Prompt photoaging, skin pigmentation irregularities (premalignant actinic keratosis), immunosuppression, and skin cancer are considered chronic consequences. The American Cancer Society has expressed that skin cancer is significant medical condition among different sorts of malignancies.

Natural approaches to photo carcinogenesis (skin cancer), photo-aging, photosensitivity and immune suppression would resolve the problems associated with the currently used sunscreen products. Antioxidants are substances which can scavenge free radicals produced in the body. Many natural antioxidants are photoprotectors and anti-aging agents. [3]. Based on this, various antioxidants have been investigated for their potential in sunscreens for photoprotection, to prevent or treat photo-aging and for antioxidant activity. Antioxidants can be supplied to the skin through the diet or by oral supplementation. Because of the problems associated with oral delivery like poor solubility, low absorption and low bioavailability, the amount of antioxidant to be delivered skin is low. [4] So, it is desirable to apply them directly on skin. Regular application of sunscreens containing antioxidants helps the skin also fight against exogenous oxidative stress occurring in daily life besides having advantages of photoprotection and properties. Polyphenols, for example, (-)- epigallocatechin-3-gallate (EGCG), (-)- epicatechin (EC), (-)- epicatechin-3-gallate (ECG), (-)- epigallocatechin (EGC), naringin, sylimarin, rutin, hesperedin, morin, curcumin, , quercetin and resveratrol have different kinds of pharmacological properties including antioxidative, calming, hostile to mutagenic, against cancer-causing, against angiogenic, apoptotic, hostile to weight, hypocholesterolemic, hostile to arteriosclerotic, against diabetic, hostile to bacterial, against viral and against maturing impacts. These mixtures have additionally shown promising outcomes in assurance from UV radiation.

Epigallocatechin-3-gallate (EGCG) is a characteristic polyphenolic compound from green tea, has been depicted as hostile to oxidative, calming, chemopreventive and photoprotector. Numerous studies have demonstrated that tea catechins are effective free radical scavengers. Orally administered EGCG is metabolized in the gastrointestinal tract and liver. Poor solubility and gatro-intestinal permeability results in low bioavailability on oral administration. EGCG has short half-life due to strong systemic clearance [5]. Thus, topical application of this polyphenol could provide an alternative way to resolve these problems.

Literature Review

Antoniou et al. (2008) underscored how urgent sunscreens are for forestalling skin harm brought about by UV radiation. While choosing sunscreens, they focused on that purchasers ought to be aware of significant attributes including water opposition, expansive range protection, sun protection factor (SPF), and appropriate application strategies [6]. This study underlines how significant sunscreen is as the principal protection against UV radiation.

Bouwstra et al. (2001) investigated new viewpoints on the construction of the skin obstruction. They focused on how the layer corneum, the epidermis' peripheral layer, controls the skin's porousness. The meaning of proteins, lipids, and tight intersections in safeguarding the honesty of the skin hindrance was canvassed in the review [7]. To make skincare items that work, particularly sunscreens, understanding both the underlying and utilitarian parts of the skin barrier is basic.

Brandner (2009) inspected how the mammalian epidermis utilizes tight intersections and tight intersection proteins. A fundamental capability of tight intersections is to control sub-atomic vehicle over the epidermal hindrance [8]. The skin's boundary capability can be endangered by disturbance of tight associations, making the skin more

helpless against natural damage, especially UV radiation. This study underlines that it is so urgent to safeguard tight intersection respectability to support strong and sound skin.

Burnett and Wang (2011) gave a basic examination of the discussions that are at present happening around sunscreens. The productivity of sunscreens in lessening UV-prompted skin harm, the security of sunscreen parts, and new stresses over nanoparticle innovation were totally researched in this review [9]. This examination underlines the need for more examination to fill in information holes and assurance the viability and wellbeing of sunscreen organizations.

Dransfield (2000) underlined the capability of inorganic sunscreens in forestalling UV beam harm. Zinc oxide and titanium dioxide are instances of inorganic sunscreens that capability by scattering and reflecting UV radiation. When contrasted with substance sunscreens, these sunscreens are much of the time picked due to their lesser possibility causing skin bothering and expansive range protection [10]. To amplify the viability and security of inorganic sunscreens in skincare definitions, understanding their qualities and methods of action is urgent.

Drug Profiles

Epigallocatechin-3-Gallate (EGCG)

EGCG, a polyphenol, is a significant substance of green tea. It displays pharmacological properties like cancer prevention agent, photograph defensive, against maturing, mitigating, resistant modulatory, anticancer, neuroprotective, cardioprotective, antidiabetic, lipid bringing down impact, antiviral, antibacterial and allergy medicine impacts. The molecular mechanisms involved in skin protection properties of EGCG under UVB induced skin edema are modulations in the MAP kinase and NF-kB signaling pathways after topical administration. EGCG also decreases GSH levels in dose as well as time dependent manner [11]. It is also effective on non-malignant and malignant tumors without affecting host epidermis of skin. The physicochemical and pharmacokinetic properties of EGCG are listed.

Hyaluronic Acid (HA)

Hyaluronic acid (HA) or hyaluronan is a normally happening straight polysaccharide that is made out of subunits of two sugars (glucosamine and glucuronic acid). HA is non-sulfated glycosaminoglycan, exceptionally hydrated polyanionic macromolecule and present in human and creature connective tissues, extracellular grid of ligament tissues; particularly in the skin, it has defensive, structure-settling and shock-engrossing capabilities. It additionally goes about as humectant in beauty care products. Because of properties like viscoelasticity, biocompatibility and non-immunogenicity, HA is more favorable in other clinical circumstances too. HA likewise has been utilized in the treatment of joint inflammation as supplementation of joint liquid, in eye a medical procedure as a careful guide, in maturing where it goes about as a filler to eliminate wrinkles and furthermore utilized in the therapy of wound mending. [12] Its moisturizing property, viscoelastic property and excellent biocompatibility have made HA as suitable candidate in topical preparations and supplements in cosmetic products, viz., moisturizers, creams, shampoos, conditioners and bath oils. HA present in the cosmetic products is expected to provide anti-wrinkle effect on skin by its moisturizing effect. It also facilitates restoration of elasticity of skin. In addition to these benefits, HA also promotes hair growth and restores hair color. HA-based sunscreens may be capable of providing anti-aging effect besides sun protection due to its free radical scavenging properties. Since HA is hydrophilic in nature, it can be easily incorporated into sunscreen creams or other cosmetic products in the concentrations of 0.01 to 0.05 %.

Preformulation Studies

Preformulation is fundamental step in the formulation development process, which is helpful in formulation of quality pharmaceutical products. It is also helpful in the selection of excipients to be used in a particular formulation. In this process, physical, chemical and mechanical properties of new drug are characterized alone as well as in combination with excipients to investigate the physical or chemical interaction between drug molecule and excipients. This stage of formulation gives the idea to develop stable, safe and effective dosage. Analytical Method Validation Parameters and Results shown in Tab 1 and Plasma Stability Assessment of Analytical Method: Benchtop, Autosampler, Freeze-Thaw, and Long-Term Stability shown in Tab 2.

UV-Visible Spectroscopy

a) UV spectroscopy of EGCG in Milli-Q water (Fig. 1): The maximum wavelength (λmax) for EGCG was observed to be 280 nm.

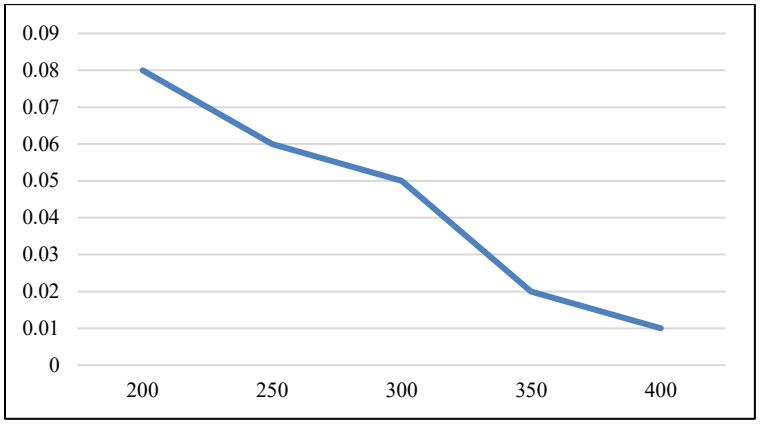


Fig. 1: UV Spectrum of EGCG in Milli-Q Water

b) UV spectroscopy of HA in Milli-Q water (Fig. 2): The maximum wavelength (λmax) for HA was observed to be 212 nm.

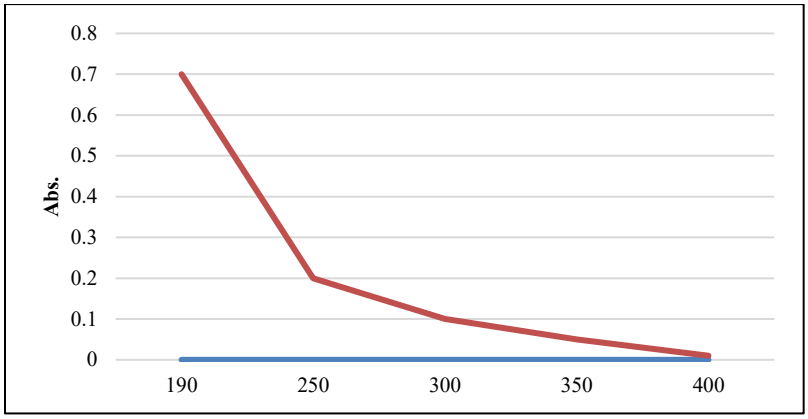


Fig. 2: UV Spectrum of Hyaluronic Acid in Milli-Q Water

Table 1: Analytical Method Validation Parameters and Results

Validation Parameters	Results	Acceptance criteria
Calibration range	100-5000 ng/mL	-
Linearity	r2: 0.9985±0.003	r2 ≥ 0.98
System suitability Tailing Factor Theoretical plates/meter (N)	%CV Area ratio < 1.36 %CV RT (analyte) < 0.25 %CV RT (ISTD) < 0.21	%CV ≤ 2.0 for area ratio RT of analyte or any other component under examination
Sensitivity	%CV: 1.42 Mean % nominal conc.: 96.45%	%CV ≤ 2.0 0 Mean % nominal conc. Should be 80-120%
Intra batch accuracy %CV	LLOQ 95.78 LQC 96.23 MQC 98.56 HQC 99.35	% Nominal concentration should be within 85-115% except at LLOQ where it should be within 80-120%
Inter batch accuracy %CV	LLOQ 94.45 LQC 96.78 MQC 99.14 HQC 100.05	% Nominal concentration should be within 85-115% except at LLOQ where it should be within 80-120%
Intra batch precision %CV	LLOQ 0.9 LQC 1.07 MQC 0.8 HQC 0.6	%CV should be ≤ 15% except at except LLOQ where it should be within 80-120%
Inter batch precision %CV	LLOQ 1.78 LQC 0.98 MQC 1.32 HQC 2.14	%CV should be ≤15% except at except LLOQ where it should be within 80-120%
Stock solution stability	Mean % change after 7 days is 0.05%	Mean % change must be within ± 10%

Table 2: Plasma Stability Assessment of Analytical Method: Benchtop, Autosampler, Freeze-Thaw, and Long-Term Stability

Plasma Stability				
Parameters	**Level**	**% Nominal**	**% Change**	**Acceptance criteria**
Bench top (8 h) stability	LQC	99.45	-0.82	% Nominal concentration should be within 85- 115% and % change should be within ± 15%
	HQC	99.31	-0.45	
Autosampler stability	LQC	98.25	-1.78	
	HQC	99.39	-0.87	
Freeze thaw stability	LQC	99.17	-1.67	
	HQC	99.53	-1.35	
Long term stability 15 days -80 ° C	LQC	92.76	-6.89	
	HQC	95.28	-5.78	
Mean % recovery	Mean % recovery: 92.68±2.56 (analyte) and 96.78± 0.78 (ISTD) % CV: 1.67 (analyte) and 0.35 (ISTD)			% Recovery should be consistent and reproducible

Drug-Excipient Compatibility Studies

Drug-excipients similarity study is the significant phase of pre-definition studies. It is by and large explored to choose appropriate excipients for the advancement of powerful measurements structures with thought of physicochemical properties and great strength. Nanoparticulate drug conveyance frameworks like liposomes, transfersomes and nanoparticles were created to diminish poisonousness and corruption. In this way drug-excipients similarity studies is the significant activity with standard conventions in pre-plan lab. Differential scanning calorimetry (DSC) is one of the significant strategies, which includes utilization of warming and cooling sign to test and a norm. The change of intensity stream to the example and reference was seen against time and temperature. The similarity studies were closed in view of change in energy related with different warm occasions, for example, dissolving point, glass progress temperature and crystallization and so on. DSC is a fast strategy to assess physicochemical cooperations among drug and excipients. Notwithstanding DSC, FTIR Spectroscopy is additionally utilized as one more procedure to decide drug-excipients similarity studies. In which, standard FTIR spectra of medication and with excipients were thought about and change in practical gatherings were thought of. In the event that there is no variety in utilitarian gatherings, it was supposed to be viable.

Fourier Transform Infrared Spectroscopy (FTIR)

A broadening peak at 3439.19 cm -1 corresponding to the H-built Goodness bundle, a C-H stretching out at 2899.11 cm-1, a medium reaching out at 1633.76 cm-1 for the amide II assembly, a peak at 1413.87 cm-1 for the carboxylate social event, and a medium expanding peak at 619.17 and 1041.6 cm-1 for the C-O-C get-together were all visible in the raw HA spectrum. In addition, the methylene pack-related specialized band at 2920 cm-1 was recorded.

Specific peaks at 2360 cm-1 (C-H alkane stretch), 2890 cm-1 (aliphatic C-H stretch), 1649 cm-1 (amide C stretch), 2850 cm-1 (alkenyl C-H stretch), 3423 cm-1 (amide N-H stretch), 1464.22 cm-1 (C=C stretch), and 1273.06 cm-1 (C-O-C stretch) were observed in the simple peptide dendrimer scope.

The FTIR range of the actual mix for transfersomes of EGCG and HA/peptide denrimer showed the characteristic pinnacles of EGCG. Fig. 3(a) and Fig. 3(b) shows, the FTIR spectra of the actual mix for nanoparticles of EGCG and HA/peptde dendrimer showed the characteristic tops for EGCG. These perceptions showed the shortfall of any compound connections between the EGCG and HA/PD or other excipients utilized in the planning of transfersomes and nanoparticles.

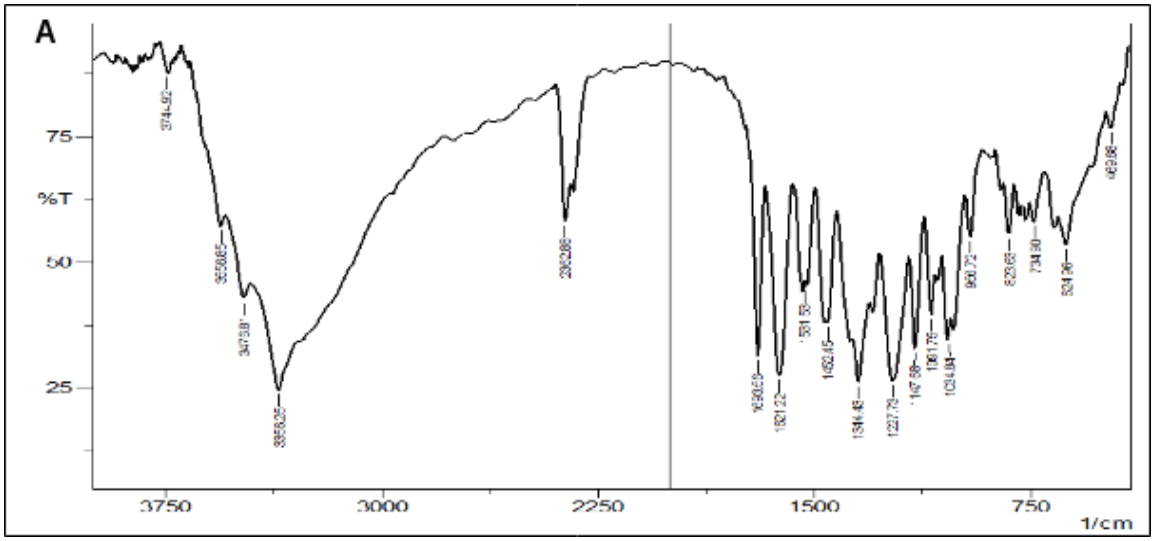


Fig. 3: (a) FTIR Spectrum of EGCG

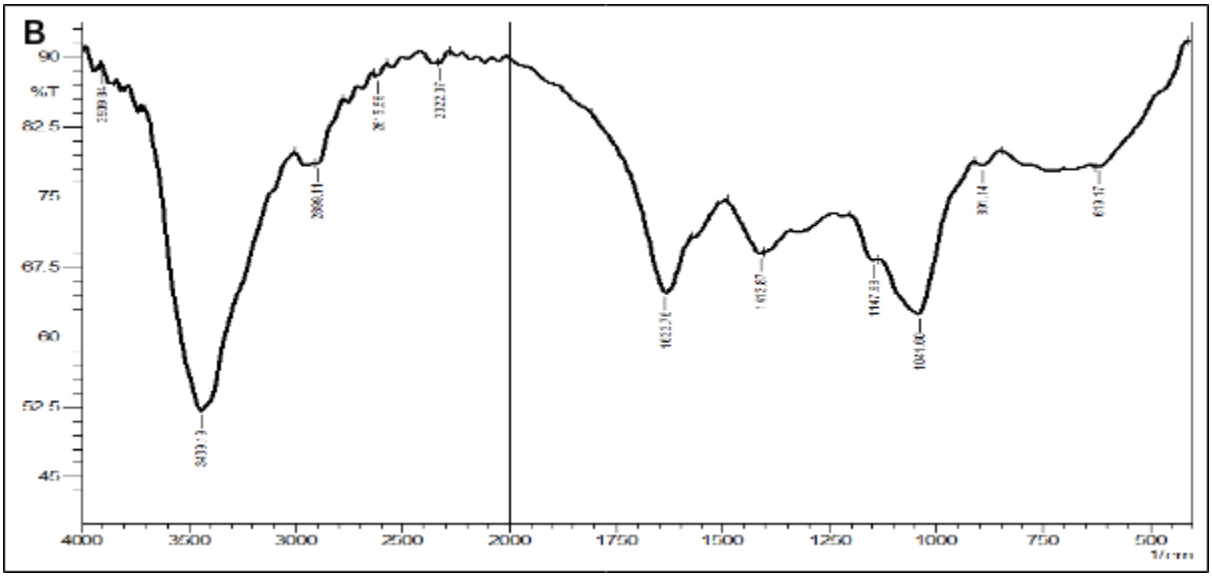


Fig. 3: (b) FTIR Spectrum of HA

Preparation And Optimization of Transfersomes

Preparation and Optimization of Transfersomes Containing EGCG and HA

Transfersomes containing EGCG and HA were prepared by changed small film hydration strategy and overhauled by Plan of Examinations using Box-Behnken exploratory arrangement. Free figures picked this study were number of phospholipids (X1), proportion of surfactant (X2), proportion of EGCG (X3) and proportion of hyaluronic corrosive (X4); however subordinate variables were vesicle size (Y1), polydispersity list (Y2), zeta potential (Y3), % catch viability (EE) of EGCG (Y4) and % EE of hyaluronic corrosive (Y5). Subordinate variables are huge key limits to propel the transfersomes.

a) Effect of Drug Concentration

As part of the continuing study, the amount of EGCG and HA coordinated into the transfersomes had an effect on %EE. In the hydration medium, HA was hydrolyzed, and EGCG was isolated from the lipid and surfactant mixture. On one hand, we observed a %EE value of 76.53±2.68% (ETF20) and 42.21±2.34% (ETF26) for EGCG [13].

Vesicle zeta potential and average atom size were both affected by the concentration of EGCG. Separately, ETF13 had a mean particle size of 234.6±8.71 nm and ETF24 had a mean particle size of 91.5±6.12 nm.

The stacking of EGCG has no significant impact on the zeta potential. These findings align with further data that has been shared. Likewise, when the amount of HA in the stacking proportion was increased from 0.5 mg (ETF5) to 1.75 mg (ETF20), the zeta capacity of the vesicles was slightly reduced, going from -52.0±3.87 mV to -44.8 ±4.24 mV. However, when the amount of HA in the stacking proportion was further increased to 3 mg (ETF17), the zeta ability of the vesicles was expanded to -48.9±4.26 mV. There was no general effect on the zeta potential properties from joining HA at different locations. Short, terrifying vesicle-to-vesicle interaction and subsequent avoidance of combination is characterized by very negative or positive zeta potential. Accordingly, the zeta capacity of the whole large number of nuances was determined to be around -30 mV in the current study, which is considered a good reason for the vesicles' actual stability.

b) Effect of SPC/ Sodium Cholate Ratio

The ETF20 group, which was made with 85 mg of SPC and 15 mg of SC, exhibited very abnormal percentages of EE for EGCG and HA, respectively, at 76.53±2.68% and 48.57±4.35%. Therefore, ETF20 was deemed a redesigned bunch since this group also had a small atomic size (101.2±4.82 nm). Using a transfersomal definition, Patel et al. detail an almost equivalent impact of phospholipids and edge activator on curcumin entrapment viability. In that study, there were clear objectives for the amount of surfactant and SPC, and the percentage EE of curcumin increased as SPC obsession increased and dropped as surfactant center grew. We stopped short of specifying the amount of surfactant since doing so might disturb the vesicles and lead to a reduction in %EE.

Phospholipon90G (SPC) is a negatively charged lipid that is often complex in the context of transfersomes for transdermal transport. The current study employed SPC at doses ranging from 75 mg to 95 mg each dose.

Preparation and Optimization of Polymeric Nanoparticles

Preparation and Optimization of Polymeric Nanoparticles Containing EGCG and HA

PLGA nanoparticles containing EGCG and HA were prepared by modified double emulsion solvent evaporation technique with varying formulation parameters such as drug- polymer concentration, volume of PVA, homogenization speed and sonication parameters.

Effect of Drug-polymer Ratio: Molecule size and %EE were impacted by expanding medication to polymer proportion in the planning of polymeric nanoparticles. EGCG and PLGA polymer were broken up in natural dissolvable, emulsified with PVA arrangement containing HA. At the point when the medication to PLGA polymer focus was expanded from 1:10 (ENP1) to 1:40 (ENP3), the %EE of EGCG and HA were likewise expanded significantly because of expanded viscosity could increment drug embodiment. The expanded centralization of polymer additionally builds the size the emulsion beads during diffusing into the fluid phase and shaped nanoparticles. The biggest and littlest molecule size of nanoparticles was viewed as 945.5±4.87 (ENP3) and 134.3±4.56 (ENP6) separately. Higher %EE values for EGCG were found for ENP3 (41.48±7.69%) and ENP8 (43.68±4.24%). The base %EE an incentive for EGCG was viewed as 6.87±3.96% for ENP7. Likewise, the %EE of HA was viewed as most noteworthy and least with ENP3 (29.56±2.17%) and ENP7 (2.68±1.77%), individually. The medication polymer proportion of 1:10 (ENP18) was chosen as enhanced proportion for PLGA nanoparticles containing EGCG and HA by considering more modest molecule size and higher %EE of both EGCG and HA. The cluster ENP5 was chosen as improved clump for PLGA nanoparticles containing EGCG alone. The impact of various factors on molecule size examination and %EE of EGCG and HA is shown.

Effect of Surfactant: The concentration of PVA play very important role in preparation of polymeric nanoparticles. Higher concentration of PVA reduces the particle size resulting decreasing the %EE of drugs especially hydrophilic drugs. In present study, the volume of PVA (1% w/v) was increased from 10 mL to 100 mL (ENP4 to ENP7), the particle size of nanoparticles was found to be decreased from 10 mL to 50 mL (ENP4 to ENP6). But at PVA volume at 100 mL (ENP7), particle size was increased considerably. Then again, the %EE of both EGCG and HA were diminished with expanded volume of PVA in the arrangement of nanoparticles. It could be because of arrangement of more modest emulsion drops by decreasing the interfacial pressure between natural/watery phase and loss of medication in the fluid phase.

Homogenization Speed/ Sonication Time

Sufficient homogenization speed is required to reduce the particle size by increasing shear stress at constant energy during the formation of emulsion droplets. In the current review, the size of the nanoparticles was streamlined by changing homogenization speed from 10000 rpm to 17500 rpm (ENP8, ENP9, ENP10 and ENP11). The molecule size was diminished with expansion in the homogenization speed. Likewise, %EE of both EGCG and HA was

diminished with speed up. In the current review, 17500 rpm was viewed as the ideal homogenization speed in the arrangement of polymeric nanoparticles.

The influence of the sonication amplitude and time on physicochemical properties of nanoparticles was also investigated comparing the results of different batches for nanoparticles prepared with varying amplitude (40 W, 60 W and 80 W for ENP12, ENP13 and ENP16, respectively) by keeping sonication time (40 min) and pulse (9) as constant. In the present study, there was no significant change in particle size and %EE for both EGCG and HA when amplitude was increased from 40W to 60W, but a drastic reduction in the particle size without affecting the %EE was observed when amplitude was increased to 80 W. The particle size of nanoparticles was also influenced by sonication time without affecting %EE of both EGCG and HA. The particle size was decreased with increasing the sonication time by 10 min to 60 min (ENP14, ENP15, ENP16 and ENP11) with constant amplitude (80W) and pulse (9). The results indicated that batch ENP11 showed lowest particle (145.3±2.29 nm) and higher %EE for EGCG (33.68±3.27) and HA (15.76±3.15). Therefore, ENP11 was considered as an optimized batch among the different batches of nanoparticles. Similarly, nanoparticles containing EGCG alone and EGCG with PD (ENP18 and ENP20) were optimized with the same process parameters as that of ENP11 and results are shown.

Characterization of Polymeric Nanoparticles

a) Measurement of Size, Polydispersity Index (PDI) and Zeta Potential

The PLGA nanoparticles went from 133.3±2.99 nm to 945.5±4.87 nm in normal size, from 0.112±0.03 to 0.423±0.09 in polydispersity file and from - 12.9±3.83 mV to - 43.6±5.87 mV for zeta likely qualities. The molecule size, PDI and zeta capability of nanoparticles containing EGCG alone (ENP18) was viewed as 133.3±2.99 nm, 0.17±0.05 and - 33.7±2.38 mV individually. The substance known as PLGA nanoparticles with EGCG and HA (ENP11) within, measuring 145.3±2.29 nm in size, PDI of 0.268±0.04, and zeta ability of - 24.5±2.68 mV, was considered to be in a state of overhaul. An improved bunch for the nanoparticles containing EGCG and PD was PLGA nanoparticles (ENP20) with an atom size of 174.1±3.58 nm, a PDI of 0.112±0.03, and a zeta ability of - 43.4±2.88. The physicochemical features of the PLGA nanoparticles were optimized by adjusting the drug-polymer extent, surfactant volume, homogenization strain, and sonication time, as mentioned in the previous segment.

b) Transmission Electron Microscopy

The overhauled nanoparticles were moreover undeniably clear without blend or collection as shown in Fig. 4. The size of still up in the air by TEM was somewhat lesser than that by DLS technique.

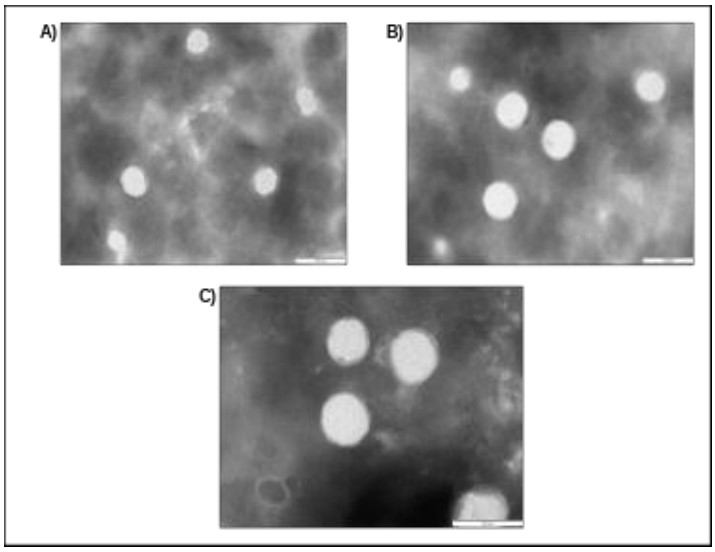


Fig. 4: TEM Images of Optimized PLGA Nanoparticles: A) ENP18, B) ENP11 and C) ENP20

In Vitro Cell Culture Study

Cytotoxicity Study by MTT Assay

This is accompanied with the outcomes of the in vitro cytotoxicity analysis. At various concentrations of EGCG (ranging from 0.625 to 40 µg/mL), the cytotoxicity of plain EGCG, transfersomes (EGCG-TF, ETF20, and ETF32),

and polymeric nanoparticles (ENP11, ENP18, and ENP20) was examined on HaCaT cells. As EGCG's fixation grew, its cell plausibility decreased. At 0.625 µg/mL, the cell viability was about 100%, at 10 µg/mL, the concentration of EGCG was optimal for >80% of the phones, and at 40 µg/mL, the phone sensitivity was 55.65%±2.80. There was a significant decrease in the cytotoxic impact on HaCaT cells when comparing plain EGCG to EGCG-TF, ETF20, and ETF32, all of which contained EGCG with or without HA or PD. Separately, at a concentration of 10 µg/mL of EGCG, 93.48±2.36% of HaCaT cells were compatible with ETF20, 91.98%±4.10 with ETF32, and 84.91%±4.77 with EGCG-TF transfersomes. In the same way, when treated with ETF20, ETF32, and EGCG-TF transfersomes separately, 76.15±2.74%, 70.53±2.56%, and 66.71±1.63% of HaCaT cells were suitable, using 40 µg/mL of EGCG for each. The inclusion of hyaluronic acid and phospholipid structure in ETF20 may explain its lower cytotoxic impact, while the presence of peptide dendrimer and phospholipid organization in ETF32 may explain its higher cytotoxic effect.

The cytotoxic impact on HaCaT cells was typically smaller in polymeric nanoparticles containing EGCG+HA (ENP11), EGCG alone (ENP18), and EGCG+PD (ENP20) compared to plain EGCG. With ENP11, ENP18, and ENP20 nanoparticles, respectively, 87.49±3.65%, 85.93±3.87%, and 90.34±2.66% of HaCaT cells were viable at an EGCG concentration of 10 µg/mL. In addition, when treated with ENP11, ENP18, and ENP20 nanoparticles individually, 69.89%±3.11, 62.19±3.79%, and 65.29±2.67% of HaCaT cells were viable with 40 µg/mL of EGCG. It is possible that the peptide dendrimer or polymeric structure accounts for ENP20's lower cytotoxic impact, whereas the presence of a hyaluronic acidic and polymeric network in ENP11 nanoparticles contributes to this effect.

These outcomes demonstrated non-harmful nature of streamlined transfersomes and polymeric nanoparticles on HaCaT cells contrasted with plain EGCG, which are as per past reports where epitomized bioactive atom showed expanded cell practicality than in its plain structure in Fig. 5.

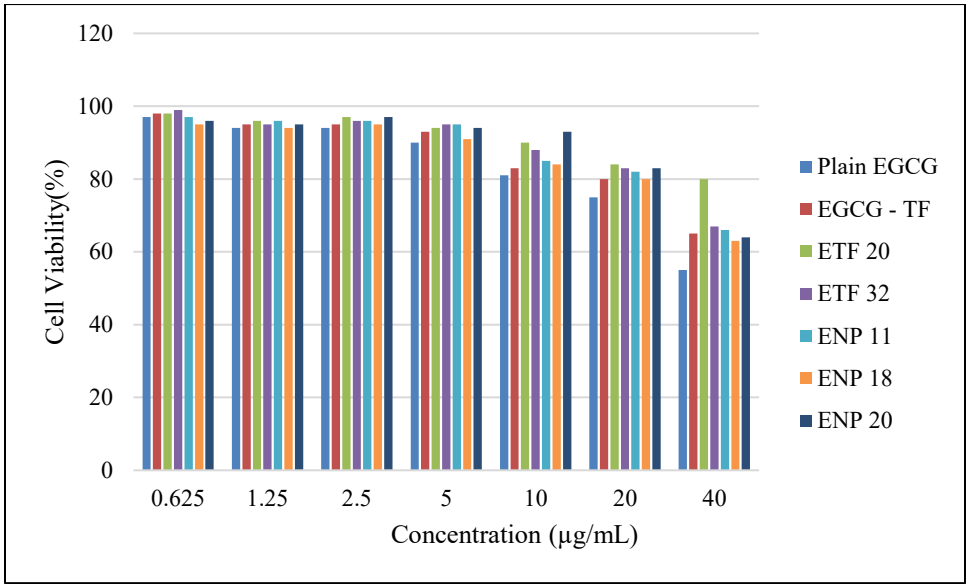


Fig. 5: Cytotoxicity Study of Plain EGCG and Different Nano Formulations

Lipid Peroxidation Assay

As a result of photo carcinogenesis and photo developing, lipid peroxidation is an indicator of oxidative stress and a major robotic route for UV radiation-induced skin cell damage. Specialists in cancer prevention oversee lipid peroxidation, which includes substances like polyphenols, glutathione, β-carotene, polyunsaturated unsaturated fats, and more. Sunlight causes lipid peroxidation to rise, which in turn causes DNA damage in skin cells, explosion of cell layers, loss of transparency, and inactivation of film proteins. Afterwards, we identified photo oxidative damage by measuring lipid peroxides. The results reveal that in HaCaT cells, smoothed out transfersomes (ETF20 and ETF32) provided better protection from UV radiation compared to plain EGCG and EGCG-TF transfersomes. Beyond that, compared to simple EGCG and ENP18 nanoparticles, polymeric nanoparticles (ENP11 and ENP20) significantly protected HaCaT cells from ultraviolet radiation. Transfersomes and PLGA nanoparticles containing a combination of EGCG and HA provided better protection against UV radiation-induced skin damage. Generally speaking, peptide dendrimers that were near to EGCG provided better protection against skin photodamage caused

by UV radiation. According to an earlier study, quercetin deformable liposomes improved intracellular MDA level in HaCaT cells more effectively than plain quercetin, which is an ongoing consequence of lipid peroxidation.

Fig. 6 shows MDA Levels in the Cells After Different Treatments (Nmol, Normalized to Total Cell Protein).

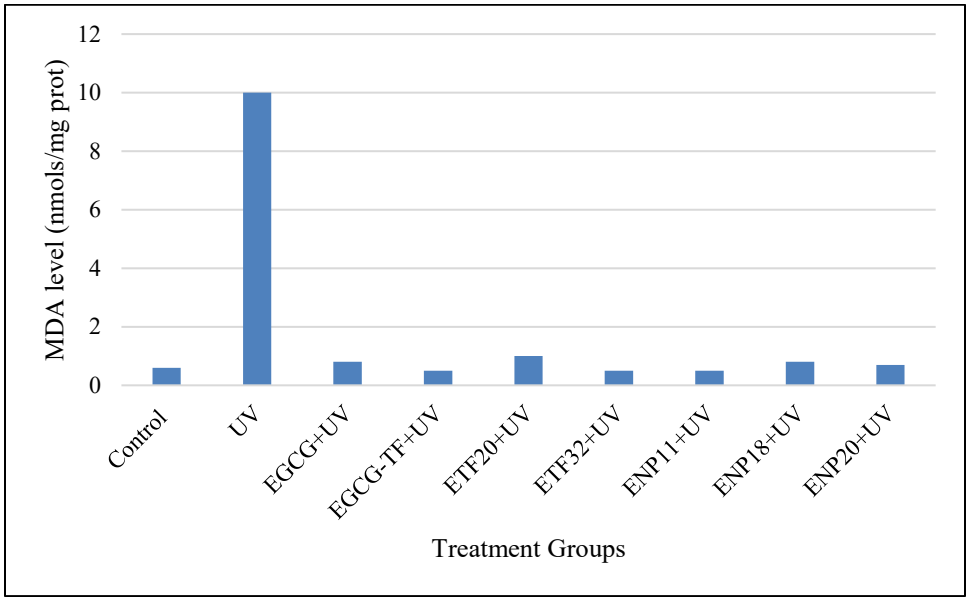


Fig. 6: MDA Levels in the Cells After Different Treatments (Nmol, Normalized to Total Cell Protein)

All the Values are Presented as Mean ± SD (n = 3);

*p<0.05 compared to UV irradiated group, #p> 0.05 compared to plain EGCG treatment group.

In Vitro Skin Permeation and Deposition Study

The skin invasion characteristics of simple EGCG, its transfersomes and its polymeric nanoparticles are illustrated. EGCG game plan showed a Q12 (full proportion of EGCG entered close to the finish of 12 h) value of 56.08±4.42 µg/cm2; of course, EGCG-TF (EGCG alone) transfersomes showed a Q12 worth of 159.90±6.90 µg/cm2, demonstrating ≈2.8 times upgrade in EGCG infiltration.

The permeation of EGCG from the transfersomal suspension (ETF32) was increased due to the presence of peptide dendrimer (PD). The amino acids present in the peptide dendrimer are not only responsible for enhancement in skin permeation of co-encapsulated EGCG but also responsible for anti- aging effect.

Correspondingly, Q12 values for polymeric nanoparticles suspension containing EGCG and HA (ENP11), polymeric nanoparticles suspension containing EGCG alone (ENP18) and the polymeric nanoparticles suspension containing EGCG and PD (ENP20) were found to be 124.28±9.65 µg/cm2, 95.78±7.99 µg/cm2 and 109.72±6.67 µg/cm2 respectively. These Q12 values are ≈ 2.2 times, ≈1.7 times and ≈ 1.9 times higher than Q12 values of plain EGCG (from solution) respectively. The particle size of EGCG nanoparticles as well as high lipophilicity lead to greater extent of skin penetration by intracellular or trans appendageal route. The enhancement in skin permeation of EGCG from ENP11 was more than that from ENP18 and ENP20. Q12 values for polymeric nanoparticles were considerably decreased when compared to transfersomes. The slow and prolonged release of EGCG from the polymeric nanoparticles could be reason for decreased permeation of EGCG from polymeric nanoparticles than that of transfer some carriers.

Fig. 7 indicate the Good skin deposition of EGCG (20.76±2.33 µg/cm2) was also found with polymeric nanoparticles containing EGCG alone and with polymeric nanoparticles containing EGCG+PD (25.38±2.76 µg/cm2). Polymeric nanoparticles showed significantly (p< 0.05) increased skin deposition of EGCG compared to plain EGCG solution. The increased skin deposition of polymeric nanoparticles suggests accumulation of nanoparticles within the skin reservoir instead of penetration into deep layers of skin. It could be beneficial for photoprotective and anti-aging effects.

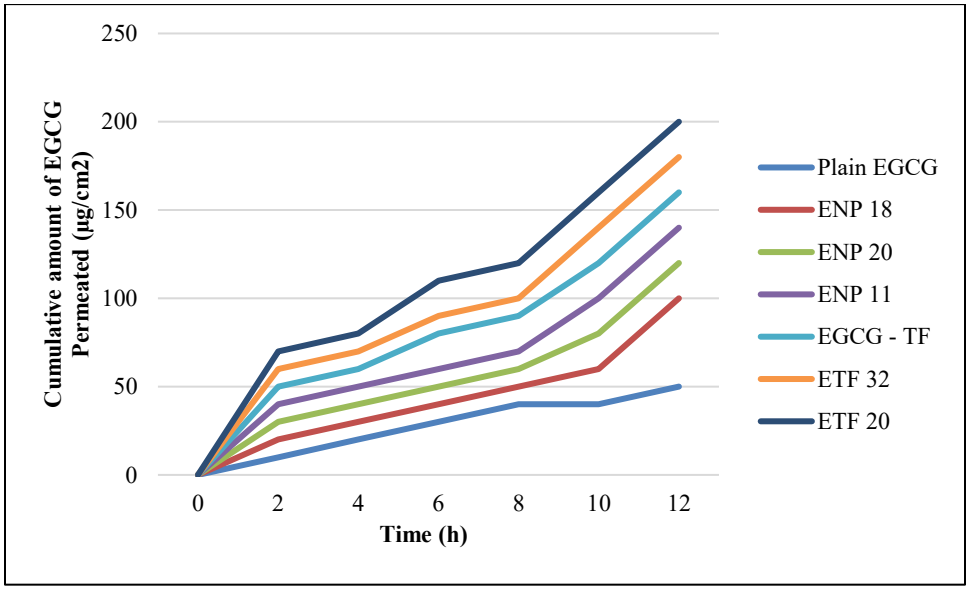


Fig. 7: Skin Penetration Profiles of Polymeric Nanoparticles, Transfer Somes, and Ordinary EGCG Solution in Vitro

Conclusion

Sunscreen products are topical formulations, widely used to prevent sunburn and to reduce premature photoaging, skin cancer risk and longer exposure to sunlight. The majority of sunscreen agents used in currently available sunscreen creams are inorganic and organic sunscreens. There are many reports explaining the side effects of presently available sunscreen products. Moreover, currently used sunscreen products rarely provide additional desirable effects like antioxidant and anti-aging properties along with UV radiation protection [14].

Epigallocatechin-3-gallate (EGCG) is a normally happening polyphenolic compound. It is most widely available in green tea extracts. EGCG has been described as anti-oxidative, anti-inflammatory, anti-aging, chemopreventive and photoprotector. Hyaluronic acid is a natural compound found within the body in connective tissues and fluids. As skin ages, it loses its elastic qualities and becomes wrinkled, and hyaluronic acid has been shown to be effective in reducing UV-light-induced wrinkles. Hyaluronic acid also has wound healing and skin repair properties. It is a good moisturizer and is effective on skin ulcers.

Amino acids like histidine, arginine, lysine, proline, glycine, leucine, and so on are the particles that make up proteins like collagen and elastin which are vital for sound and lovely skin. Amino acids go about as regular free extreme scavengers which kill free revolutionaries and assist with forestalling the harm brought about by them. Amino acids are likewise described as one more class of against maturing specialists.

For better efficacy and multiple advantages with single sunscreen cream, we planned to co-deliver EGCG and hyaluronic acid/ poly amino acids to skin by making use of nano- carriers such as transfersomes and polymeric nanoparticles. We used amino acids in the form of peptide dendrimers (PD), which contain more than one amino acid and will cleave into individual amino acids once they enter the skin. There are no reports on available on the use of EGCG and HA/ peptide dendrimer in nanoparticulate sunscreen products. Thus, the current work was embraced to create nanoparticulate sunscreen creams containing transfersomes/polymeric nanoparticles of EGCG and HA/PD alongside for the most part utilized sunscreen specialists (titanium dioxide and zinc oxide) to have antioxidant and against maturing impacts other than their photoprotective property.

Nano-transfersomes containing EGCG and HA (ETF20), nano-transfersomes containing EGCG and peptide dendrimer (ETF32), polymeric nanoparticles containing EGCG and HA (ENP11) and polymeric nanoparticles containing EGCG and HA (ENP20) were improved in view of size, zeta potential, poly dispersibility index and medication content [15]. There was no connection among EGCG and excipients of nanoparticulate plan as confirmed by FTIR and DSC studies. DSC and XRD studies revealed the amorphizaton of bioactive molecules in nano-formulations [16]. The nano-carriers exhibited spherical shape as assessed by transmission electron microscopy.

Future Scope

The investigation encompassed preclinical sub-acute and chronic toxicity assessments of the optimized sunscreen creams, evaluating their safety profiles over extended exposure periods. Additionally, long-term stability studies were conducted to scrutinize the formulations' robustness and efficacy under various storage conditions. Subsequently, clinical studies were undertaken to evaluate the performance and tolerability of the optimized sunscreen creams in human subjects, assessing their efficacy in providing protection against harmful UV radiation and any potential adverse reactions on the skin.

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