RESEARCH PAPER

Development and optimization of transdermal patches loaded with Ellagic acid nanosuspension

Mr. Vivek H. Rathod[1]*, Dr. Bhushankumar S. Sathe[2], Dr. Rajesh Khathuriya[3]

[1] Research Scholar, Pacific Academy of Higher Education and Research, Udaipur, Rajasthan, 313001

[2] Institute of Pharmaceutical Education and Research (D.Ph), Borgaon (Meghe), Wardha, Maharashtra, 442001

[3] Professor, Pacific Academy of Higher Education and Research, Pacific University, Udaipur, Rajasthan 313001

**Author for correspondence:*
Mr. Vivek H. Rathod
Research Scholar, Pacific Academy of Higher Education and Research, Udaipur, Rajasthan, 313001, Email: vivekhrathod@gmail.com

ABSTRACT
The present investigation aims to develop and optimize transdermal patches loaded with nanosuspensions (NSPs) of Ellagic acid (EGA). NSPs are intended to enhance the solubility of hydrophobic actives. Preformulation investigation of EGA were conducted to assure its identity and required formulation aspects, such as λmax determination, FT-IR spectroscopic analysis, standard calibration curve and saturation solubility. These investigations assured purity, identity and compatibility of EGA. NSPs were prepared by precipitation method, by generating batches using Box-Behnken design. Independent parameters included poloxomer and sodium lauryl sulfate concentrations, and speed of homogenization, and the dependent variables included particle size, zeta potential and drug content. The formulated NSP batches of EGA were subjected to particle size and zeta potential, drug content, and drug loading efficiency determination, SEM and in vitro dissolution studies were also performed. Results suggested that NSPs from EGA batch S2 (600 mg poloxomer, 550 mg SLS and 7000 rpm) were optimum and were further use. EGA NSP (Batch S2) showed highest cumulative percent drug dissolved (99.36%). Transdermal patches using optimized batch of EGA NSPs were prepared using PVP, ethyl cellulose combination, dibutyl phthalate as plasticizer and oleic acid as penetration enhancer. These patch batches were subjected to various evaluation tests including, physical appearance, folding endurance, film thickness, drug content, tensile strength, SEM, in vitro drug release testing and ex vivo drug diffusion study. It was revealed that all transdermal patches were able to sustain the release of drug from formulation. EGA formulations P6 showed 99.23 ± 0.43 % drug release in 12 hrs. The results of in-vitro and ex-vivo studies of different batches revealed that sustained release of drug is due to varying concentrations of permeation enhancer i.e., oleic acid. Formulations containing 5% of oleic acid showed better sustained release. The optimized transdermal patches of EGA and RTN were found to be stable under accelerated stability conditions conducted for 3 months.
Keywords: Nanosuspensions, Ellagic acid, Box-Behnken design, transdermal patches, penetration enhancer

How to cite this article: Rathod VH, Sathe BS, Khathuriya R. Development and optimization of transdermal patches loaded with Ellagic acid nanosuspension. Int J Drug Deliv Technol. 2026;16(54s): 28-37. DOI: 10.25258/ijddt.16.54s.5

INTRODUCTION
Ellagic acid is an occurrence of polyphenolic compound that is natural and is prevalent in fruits, vegetables, and medications plants. It is related to the category of hydrolysable tannins and occurs in the hydrolysis of the ellagitannins. Pomegranate, strawberries, raspberries, blackberries, walnuts, pecans, and grapes to name a few, are rich sources of diet, with pomegranate being among the richest sources of diet1. Ellagic acid is a naturally occurring compound with considerable popularity in

nutrition, pharmacology, and pharmaceutical sciences because of its health-promoting properties. Ellagic acid is a compound with four hydroxyl groups and two lactone rings in which it is chemically a dilactone of hexahydroxydiphenic acid2. This organic group offers intensive antioxidant capability that allows the compound to donate hydrogen atoms and eliminates reactive oxygen species (ROS). Consequently, ellagic acid serves to defend against oxidative stress and cell damage in the biological systems3.

Ellagic acid has a great deal of pharmacological activities. Its antioxidant properties have been predominant in the prevention of diseases that are linked to oxidative stress including cancer, cardiovascular diseases, diabetes and neuro degenerative diseases. It inhibits lipid peroxidation, cell membrane protection and DNA damage4. Besides antioxidant properties, ellagic acid exhibits a great anticancer property. It suppresses the growth of numerous cancer lines of cells such as the breast, prostate, colon and lung cancer. It also demonstrates positive effects in metabolic disorders as it enhances insulin sensitivity, glucose metabolism regulation, and cardiovascular protection. Ellagic acid, though having therapeutic potential, has a low bioavailability that inhibits its clinical use5. The bioavailability is the proportion of a drug that gets into the systemic circulation in its original form. To ensure successful treatment, a medication needs to be soluble in biological fluids, be able to pass through biological membranes, and have to be stable in the body6.

Ellagic acid is not very soluble in water hence its solubility in gastrointestinal fluids is greatly low. This limitation leads to a low availability of drugs because dissolution is a requirement to absorption. Also, the absorption of ellagic acid is limited by its characteristics, as its permeability through the biological membranes is low, owing to its physicochemical properties7. The other critical constraint is prolonged metabolism. Once orally taken, intestinal microflora quickly convert ellagic acid into urolithins, decreasing the level of unmodified drug in the bloodstream. These are combined to cause low oral bioavailability and low therapeutic efficacy.

Nanosuspension technology has been identified as one of the strategies that help to overcome the challenges of solubility and bioavailability of poorly water-soluble drugs such as ellagic acid. A nanosuspension is a colloidal suspension of vehicles of a drug in the nanometer range (10 -1000 nm) that is stabilized by surfactants or polymers. Decrease in particle size also increases the surface area of the drug particles and this results in an increase in the rate of dissolution as per the principles of surface area enhancement8. This enhances solubility and absorption of the drugs. Also, nanosuspensions raise the level of saturation solubility because surface energy is enhanced at the nanoscale. Nanosuspensions are also beneficial in terms of enhancing bioavailability through better adhesion to biological membranes and residence time at sites of absorption. They can also offer depot and continuous drug delivery and this can help to enhance better treatment9.

Transdermal drug delivery systems (TDDS) represent another way of transporting drugs over the skin to the systemic circulation10. This method has a number of benefits to oral administration, especially when working with drugs that have low oral bioavailability, such as ellagic acid. The first one is the absence of first-pass metabolism which makes drug availability much better. Transdermal patches are also known to deliver controlled and sustained release of drugs, which maintain a constant plasma level of drug and low frequency of dosing11. Also, the transdermal systems enhance compliance in a patient because they are non-invasive, painless, and user-friendly12. Their safety can be boosted by the fact that the patch can be removed in case of side effects. These are the benefits that make TDDS a promising method in the delivery of ellagic acid. Overall, the research aimed to prepare and maximize an ellagic acid nanosuspension and integrate it in a transdermal patch based system to improve drug delivery and therapeutic effects.

MATERIALS AND METHOD

Materials

EGA (purity 98 and above) was acquired from Otto Chemie Pvt. Ltd. (Mumbai, India). Poloxamer 188 (analytical grade), a stabilizing agent in the nanosuspension formulation was acquired at BASF (Germany). Sodium lauryl sulfate (SLS) was purchased as a surfactant to enhance the dispersion and stability of the drug and it is produced from Loba Chemie Pvt. Ltd. (Mumbai, India). Polyvinyl pyrrolidone (PVP) that was used as the film forming polymer on the preparation of transdermal patches was provided by Colorcon Asia Pvt. Ltd. (Goa, India). Ethyl cellulose (rate controlling polymer 10 cps) was obtained at Himedia Laboratories Pvt. Ltd. (Mumbai, India). The plasticizer was Dibutyl phthalate that was sourced from Merck Life Science Pvt. Ltd. (India). Methanol (analytical grade solvent) was acquired from Merck. We made phosphate buffer solution (pH 7.4) by the dissolution of potassium dihydrogen phosphate and sodium hydroxide of analytical grade in the presence of phosphate buffer solution.

Methods

Preparation of Nanosuspension of Ellagic acid:

Ellagic acid nanosuspensions were prepared through the antisolvent precipitation method (Nanoprecipitation method). Briefly, Ellagic acid (100 mg) was dissolved completely in chloroform to prepare the organic phase and the solution was then passed through a 0.45-μm filter to remove the possible impurities. Meanwhile, the antisolvent phase was prepared by dispersing stabilizer Polaxomer-188 and surfactant SLS in distilled water. At a fixed temperature, 1 ml of organic solution was quickly injected by syringe into 30 ml of anti-solvent using a high-speed homogenizer at 8000 rpm for 1 hour. Immediately, drug particles precipitated from the anti-solvent. Prepared Nanosuspension was then stirred under high-speed homogenizer at room temperature for 1 h to evaporate organic solvent13. The batches were prepared according to the formulation design in Table 1.

Table no.1: Composition of EGA NSPs (S1 -S15) according to Box-Behnken Design (BBD)

Formulation code	A: polaxomer-188 (mg)	B: sodium lauryl sulphate(mg)	C: speed (rpm)
S1	600	550	9000
S2	600	550	7000
S3	650	600	7000
S4	550	600	9000
S5	600	650	7000
S6	550	600	7000
S7	550	650	8000
S8	650	550	8000
S9	650	650	8000
S10	550	550	8000
S11	600	600	8000
S12	650	600	9000
S13	600	600	8000
S14	600	650	9000
S15	600	600	8000

Evaluation of EGA NSPs

Particle size, zeta potential and total drug content determination

The prepared EGA NSPs were evaluated for particle size, zeta potential, total drug content and percentage drug loading. Average particle size of nanosuspensions were measured using dynamic light scattering in a Malvern zeta sizer (Nano ZS; Malvern Instruments, UK). For analysis, 0.1 mL of respective formulation was diluted ten times with triple distilled water. For measurement of zeta potential, Zetasizer (HORIBA, SZ100, Japan) was used. Nanosuspension sample (1ml) was taken and dispersed in double distilled water. To prevent the agglomeration, the dispersed solution was placed for 5 minutes in ultrasonicator bath. Then the sample was taken in the glass cuvette and zeta potential was measured by using zetasizer. For total drug content determination, an aliquot (0.5 ml) was evaporated to dryness. The residue was then dissolved in methanol and filtered with 0.45 μm filter paper. The samples were analyzed using UV spectrophotometer (Shimadzu-1700, Japan) at λmax of 276.6 nm. Total drug content (TDC) and % TDC were calculated using following equation.

TDC=(Total volume of nanosuspension)/(Volume of aliquot)×Amount of drug in aliquot

Drug loading efficiency:

A fixed amount of Ellagic acid nanosuspension (10 ml) was taken with a pipette (10 ml, Borosil), and transferred into a centrifuge tube and centrifuged at 14000 rpm for 10 min at 20°C. The absorbance of the drug in the supernatant was determined spectroscopically using UV-VIS Spectrophotometer (Shimadzu) at 276.6 nm.

Drug excipient compatibility studies:

EGA and excipients were initially triturated and uniformly dispersed with potassium bromide. KBr coupled EGA-excipient compacts were prepared by compression method. These compacts were subjected to scanning between 4000-400cm-1 on a FTIR spectrophotometer instrument (Model No. 84005 Schimadzu Asia Pacific Pvt. Ltd, Singapore).

In-vitro drug dissolution studies:

USP type II paddle type dissolution apparatus was employed to perform in vitro drug dissolution testing (Electro lab Dissolution Tester USP TDT-10L) at 100 rpm, and 37±0.5°C. Accurately weighed NSPs (approximating 10 mg of EGA) were placed in dialysis bag and sealed. The bag was placed in 500 ml buffer pH 7.4, and the dissolution test apparatus was run. Periodically, 5 ml aliquots were withdrawn and equal amount of buffer was poured. Then, the collected aliquots were screened using 0.1 μm filters, followed by dilution. The drug content was quantified using UV spectrophotometer at λmax 276.60 nm.

Scanning Electron Microscopy:

To examine the particle nature in NSPs in desiccated form, samples were analyzed by using a Scanning Electron Microscope (Hitachi S-3700N). The surface morphology (roundness, smoothness, and formation of aggregates) was also determined.

Preparation of EGA nanosuspension loaded transdermal patch:

PVA backing membrane, was molded by adding pouring 12 ml 5 %w/v (PVA in water). on a surface of petri plate and dehydrated at 42°C for one day. EGA (Batch S2) NSPs (equivalent to 50 mg EGA) loaded

transdermal patches were casted via solvent vaporization method using a film former. The required amount of Polyvinyl pyrrolidone and ethyl cellulose were dissolved in chloroform (Table no. 2). EGA NSPs were added to the above polymeric blend and stirred for 4h. To this resulting solution mixture ratios of dibutyl phthalate (plasticizer) and oleic acid (penetration enhancer) was added. This mixture was added over preformed PVA backing film and dehydrated at 42 °C for overnight. To regulate the solvent vaporization, an upturned funnel was kept on the plate. The prepared patches were stored at controlled humidity.

Table no. 2. Compositions of formulations (P1-P6) of nanosuspension Loaded transdermal patch of EGA

Formulation code	Ethyl cellulose (mg)	Polyvinyl pyrrolidone(mg)	Dibutyl phthalate (%w/w)	Oleic acid (%w/w)
P1	377	127	20	10
P2	377	127	30	15
P3	377	127	40	10
P4	377	127	20	15
P5	377	127	30	10
P6	377	127	40	15

Evaluation of EGA NSPs embedded transdermal films:

The casted NSP loaded transdermal films were visually inspected for surface texture, color, clarity, and pliability. Film thickness was determined by employing screw gauge, at three random areas and a mean value was recorded. Folding Endurance was quantified by iteratively creasing the patch in same dimension until broken. The count resulting in patch breakage gives this value. Folding iteratively signifies the patch ability to endure breakage. Drug content uniformity was estimated by following method. Transdermal patches were solubilized in 4 mL dichloromethane, further adjusting it to 10 ml using buffer 7.4, thereafter dichloromethane was vaporized. These mixtures were clarified using a 0.45 µm filter medium and after dilution they were subjected to UV-Vis spectrophotometric estimation, against blank. To quantify tensile strength, the film was stretched using a pulley system. Three circular patches were cut of radius 2 cm^2. Force was gradually increased by adding weights to the pan until the patch fractures. The tensile strength can be given by.

$$\text{Tensile strength}(S) = \frac{(Applied\ force)}{(Cross\ section\ area)}$$

In-vitro drug release study of EGA from NSP loaded transdermal patches

The drug release studies of EGA NSPs loaded transdermal patch were performed using the USP dissolution test apparatus V (paddle over disc method) at a rotation of 50 rpm. Studies were carried out in 500.0ml of pH 7.4 phosphate buffer and temperature maintained at 37 ± 0.5°C for a period of 12 hrs. The film was adhered to an inverted petri dish using epoxy adhesive, permitting drug diffusion solely from the top surface, and was positioned at the base of a vessel containing 500 ml of buffer pH 7.4. periodically, 5ml aliquots were taken at 5 min, 15 min, 30 min, 1hr with regular interval of 1hr for 12hrs, replenishing with fresh medium. The samples were analyzed spectrophotometrically at respective wavelengths for EGA and calculations were performed using equation from standard curve.

Ex-vivo drug diffusion study of EGA from NSP loaded transdermal patches:

Ex-vivo drug diffusion investigation was done using Franz diffusion cell having receptor compartment of 20 ml and diffusion area 3.14 cm^2. Excised goat abdominal cadaver was used, which was placed between donor-acceptor section of diffusion cell, followed by experimental patch along with 7.4 buffer. The entire setup was placed on magnetic stirrer to run at 500 rpm, at 32 ± 0.5°C. The aliquots were removed at specific intervals (5min, 15min, 30min, 1hr, 2hr upto 12hr) and quantified for amount of drug diffused, using spectrophotometer. The aggregate quantities of drug diffused from films was scaled against time.

RESULTS AND DISCUSSION

Preformualtion studies

EGA was investigated to confirm its identity by determining its melting point and solubility. The results of all these tests were in compliance with specification provided by the manufacturer. Based on the initial identification test, it was determined that the drug meets the official standards. The polymers Polaxomer-188, Polyvinyl pyrrolidone (PVP), Ethyl cellulose all excipients provided by supplier confirmed by their identification test official in IP and BP. All the excipients showed result in compliance with standard specification. From the scanning of EGA in pH 7.4 buffer and in methyl alcohol the λmax was found to be 276.60 nm and 277.6 nm respectively.

Formulation and optimization of EGA nanosuspension using Box-Behnken Design (BBD)

BBD was applied to detect the impact of three input factors on output variables.

Following the constraint exhibited (Table No.12 and 13), the Design Expert software generated 15 runs, that were formulated and quantified for the output responses, i.e. the independent variables were amount of polymer Polaxomer-188(X1), amount of surfactant SLS(X2) and Speed(X3). While Particle Size(Y1), Zeta potential(Y2) and Total drug content (Y3) were used as dependent variables (responses).

The responses indicated, that with increasing poloxomer concentration, zeta potential increases, whereas particle size and drug content reduces with increasing surfactant concentration, speed lowered the particle size and zeta potential, and enhanced drug content (Figure no. 1).

Equation generated for correlating particle size with input variables is

(Particle size=220.40-37.35 A-22.29 B-57.64 C-23.60 AB+38.08 AC-85.00 BC+0.4250.10 A^2-1.75B^(2)+103.30C^2)

Where, A - Poloxamer 188, B - Sodium lauryl sulphate, C - Speed

F-value of 6.95 reveals significant value, with only 1.75% probability of noise that could occur with this value.

Equation generated for correlating zeta potential with input variables is

Zeta potential=-13.70-1.20 A-2.17B+ 1.18 C-1.53 AB-2.07 AC-0.7750 BC-2.51A^2-1.76B^2-0.6625C^2)

Where, A - Poloxamer 188, B - Sodium lauryl sulphate, C - Speed

F-value of 1.44 reveals significant value, with only 35.84% probability of noise that could occur with this value.

Equation generated for correlating total drug content with input variables is

(Total drug content=89.29-1.82 A+0.9325 B-3.28 C-8.82AB-13.06 AC-28 BC-5.12 A^2-9.79 B^2-7.71C^2)

Where, A - Poloxamer 188, B - Sodium lauryl sulphate, C - Speed

F-value of 2.65 reveals significant value, with only 20.40% probability of noise that could occur with this value.

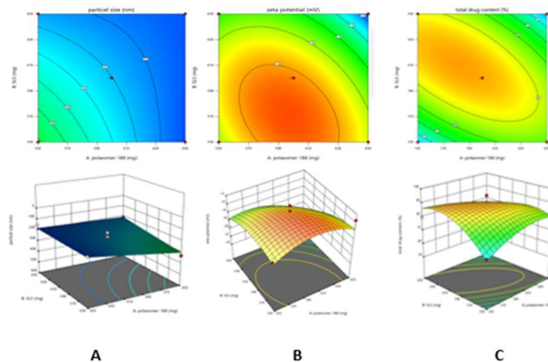


Figure 1. Contour and 3D plot showing A. Effect of the amount of Poloxamer 188 and SLS concentration on particle size of EGA nanosuspension, B. Effect of the amount of Poloxamer 188 and SLS concentration on zeta potential of EGA nanosuspension, C. Effect of the amount of Poloxamer 188 and SLS concentration on total drug content of EGA nanosuspension

Inference drawn from the findings of the optimization conducted by BBD 15

The results suggests that the concentration of surfactants and the rate of processing is very important with regard to decreasing the size of the particles, probably because the surfactants are more effective in stabilizing and size reduction is efficient during the homogenization process. Smaller size is also a benefit because it increases the rate of dissolution and bioavailability of poorly soluble drugs, such as ellagic acid.

Conversely, the zeta potential model was not found significant (F-value = 1.44) and this means that the variables chosen did not have a significant impact on surface charge. Nonetheless, the negative zeta potential values obtained indicate moderate stability of the nanosuspension system, which is probably attributed to the repulsive force of the electrostatic force caused by the surfactant system. Even though this was not statistically significant, it is important to keep zeta potential sufficient to avoid aggregation.

The total drug content model likewise was not significant (F-value = 2.65), except the quadratic term B 2 which showed significant nonlinear response of the sodium lauryl sulphate concentration on drug entrapment. The surplus of the surfactant can cause solubilization of the drug not in nanoparticles, thus lowering the encapsulation efficiency.

Evaluation of NSPs 16

Particle size, zeta potential and total drug content and drug loading efficiency were determined (Table no. 3). The results indicated that particle size of all the NSPs was between 174 to 497 nm. The size of particles

depends on the concentration of stabilizers and homogenization speed. Low stabilizer results in formation of clusters and high stabilizer quantum avoids clustering 17.

To examine the surface characteristics of the nanosuspension, an analysis of the zeta potential was conducted. The zeta potential value is crucial for the stability of NPs. More zeta potential signifies a stronger charge on the particle, which leads to particle repulsion and minimizes the likelihood of particles clumping together. Therefore, a high zeta potential value signifies the physical stability of NSP. Zeta potential value of all the NSP batches was in the range of -12.2 to -23.5 mV18. Total drug content was between 59.35 to 96.23 %, which indicates negligible drug loss during the formulation cycle. The drug loading efficiency was in the range of 33.45 ± 0.35 to 62.23 ± 0.51%, which indicates that the drug was uniformly distributed throughout the EGA nanosuspension formulations. Comparison of these evaluation parameters indicated that batch S2 of NSP showed optimum results19.

Table no.3: Observed responses for EGA nanosuspension batches generated using Box-Behnken design

Formulation code	Particle size (nm)	Zeta potential (Mv)	Total drug content (%)	% drug loading efficiency
S1	417.5	-13.2	62.25	47.91± 0.49
S2	154.6	-23.5	96.23	62.23 ± 0.51
S3	380.2	-16.2	84.56	46.63 ± 1.23
S4	191.9	-13.4	75.42	39.86± 0.25
S5	396.4	-20.6	86.29	35.09± 0.56
S6	497.1	-16.5	67.53	33.45 ± 0.35
S7	215.4	-19.5	79.14	62.06 ± 0.27
S8	174.9	-13.4	87.25	39.89 ± 0.19
S9	247.6	-17.4	63.25	58.45 ± 0.45
S10	331.4	-15.5	67.87	23.98 ± 0.03
S11	267.7	-13.5	87.53	24.78 ± 0.14
S12	227.3	-21.4	59.35	43.67 ± 0.31
S13	178.1	-12.2	95.35	60.45 ± 0.27
S14	226.5	-13.3	67.38	35.56 ± 0.32
S15	215.4	-15.4	84.98	50.84± 0.28

Drug-excipient compatibility study

The drug-excipient compatibility study conducted by FT-IR spectroscopy indicated, no significant changes in the prominent peaks exhibited by EGA alone, when combined with all the excipients. The findings are exhibited in Figure no 2.

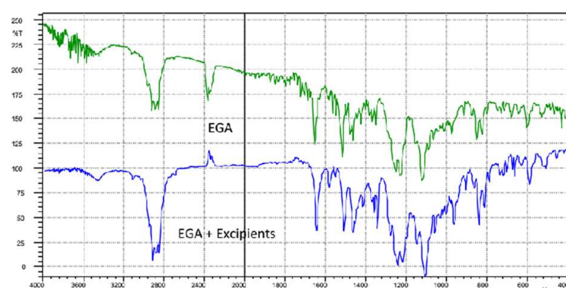
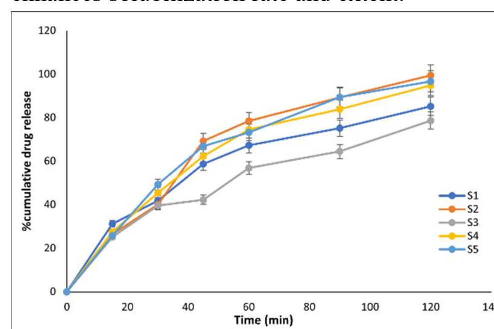


Figure no. 2. FT-IR spectrum of EGA alone and in combination with polymers

In-vitro drug dissolution studies of EGA NSP:

The key advantage of NSPs lies in its ability to enhance the rate of dissolution, which is attributed not only to an increase in surface area but also to a rise in equilibrium solubility. In-vitro dissolution data of EGA NSPs were carried out for 120 min and graphically represented as % drug release v/s time profile. The drug release of optimized batch (S2) was found to be 99.36±0.32% with 120 min. These findings (Figure no. 3) are in congruence with the fact that reduction in particle dimensions enhances solubilization rate and extent.



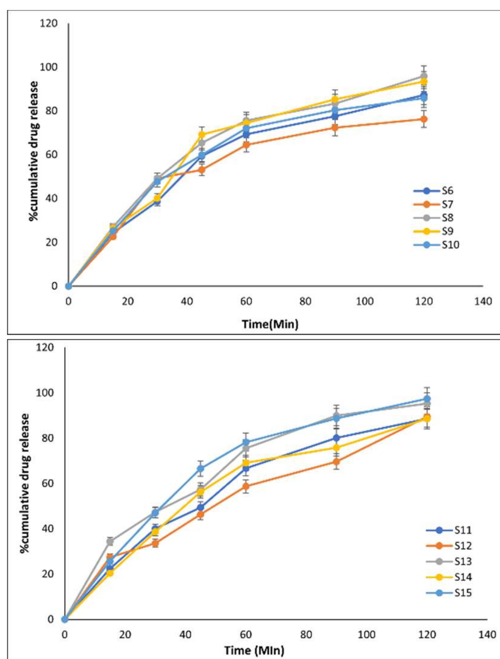


Figure no. 3. In vitro dissolution profile of various bathes of EGA NSPs

Scanning electron microscopy:

SEM was performed to investigate the surface characteristics of particulates. The EGA NSPs revealed a smooth appearance. The surface structure of optimized nanosuspension batches appeared fairly smooth as showed in Figure 4.

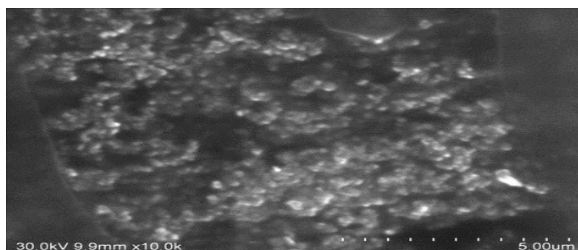


Figure no. 4. Scanning electron microscopy of optimized EGA NSP Batch S2

Preparation of EGA nanosuspension loaded transdermal patch:

EGA NSPs loaded transdermal patch was prepared using solvent evaporation method using PVP and Ethyl cellulose as polymer, Dibutyl phthalate as plasticizer and oleic acid as permeation enhancer in different concentrations. Equivalent concentration of EGA NSPs (50 mg) optimized batch was incorporated in transdermal patch polymeric matrix. Composition of EGA nanosuspension transdermal patch are given in Table no. 2.

Evaluation of EGA NSPs loaded transdermal patches

Physical evaluation of all the transdermal patches was done and were found to be flexible, smooth, opaque and non-sticky (Table no. 4)20. All the prepared NSPs loaded transdermal patch were evaluated for their physical parameters (Flexibility, smoothness, transparency, stickiness), and they were found to be flexible, uniform, smooth, opaque, and non-sticky. EGA nanosuspension loaded transdermal patch gave acceptable folding endurance values. The folding endurance value was observed 120±4.32 which indicate the good mechanical properties of prepared transdermal patches. Use of plasticizer Dibutyl phthalate enhance the flexibility of patch and provide the integrity to prepared formulation. The thickness of prepared batches EGA transdermal patches was in ranged between 0.35 ± 0.19 mm. Very low value of standard deviation in all batches indicated that the prepared patches were uniform in thickness21.
The average value of drug content in prepared formulations was in the range of 93 to 96 % respectively. This result revealed that the uniform dispersion of drug in film and solvent evaporation method used to prepare transdermal patch was capable of producing transdermal patch with uniform drug amount. Tensile strength of transdermal patch formulations P1, P2, and P3 were found to be 0.248 ± 0.063, 0.223 ± 0.085, and 0.385 ± 0.054 respectively; and tensile strength of transdermal patch formulations P4, P5 and P6 were found to be 0.248 ± 0.075, 0.356 ± 0.086 and 0.324 ± 0.096. This result reveals that tensile strength of transdermal patch formulation was satisfactory (Table no. 5)22.
SEM study is qualitative mode to study morphology of EGA NSPs loaded transdermal patch, the visual observations provide an indication of nanosuspension dispersion. At higher magnification aggregates of nanosuspension can be seen. SEM images show uniform distribution of nanosuspension in transdermal patch (Figure no. 5)23.

Table no. 4. Physical evaluation of EGA NSPs loaded transdermal patches

Table no. 5. Physical evaluation of Ellagic acid nanosuspension loaded transdermal patch

Formulation code	Flexibility	Smoothness	Transparency	Stickiness
P1	Flexible	Smooth	Opaque	Non sticky
P2	Flexible	Smooth	Opaque	Non sticky
P3	Flexible	Smooth	Opaque	Non sticky
P4	Flexible	Smooth	Opaque	Non sticky
P5	Flexible	Smooth	Opaque	Non sticky
P6	Flexible	Smooth	Opaque	Non sticky

Formulation code	Thickness (mm)	Folding Endurance	Tensile Strength (Kg/cm^2)	Drug content uniformity (%)
P1	0.29±0.01	86±0.98	0.248±0.063	93.32±1.2
P2	0.33±0.03	99±2.67	0.223±0.085	97.46±0.8
P3	0.28±0.02	116±4.32	0.385±0.054	95.24±0.5
P4	0.34±0.04	79±3.97	0.248±0.075	94.02±0.6
P5	0.35±0.02	117±2.45	0.356±0.086	97.30±1.1
P6	0.28±0.05	107±1.96	0.324±0.096	97.69±1.3

*** Each value represents the mean ± standard deviation (n=3)**

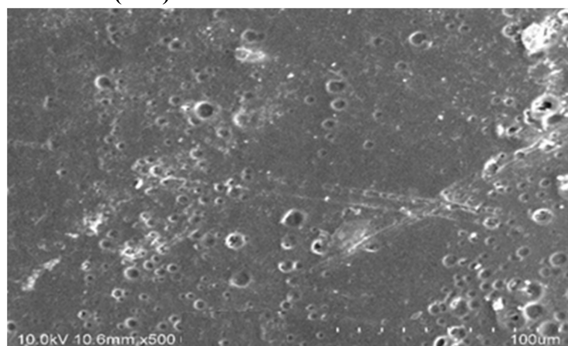


Figure no. 5. Scanning electron microscopy of optimized P6 EGA nanosuspension loaded transdermal patches

In vitro drug diffusion of EGA NSPs loaded transdermal patches24

EGA nanosuspension loaded transdermal patch were prepared with different concentrations of permeation enhancer oleic acid. It was revealed that all transdermal patches were able to sustain the release of drug from formulation. EGA formulations P1, P2 and P3 showed 78.45 ± 0.23, 84.23 ± 0.43 and 95.65 ± 0.65 percent of drug release and P4, P5, and P6 showed 75.62 ± 0.46, 98.43 ± 0.15, 99.23 ± 0.43 percent of drug release (Figure no. 6).

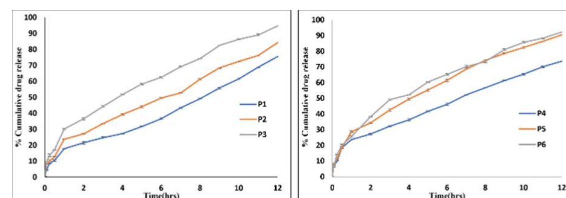


Figure no. 6. In vitro drug diffusion of EGA from NSPs loaded transdermal patches

Ex vivo drug permeation of EGA NSPs loaded transdermal patches[25]

Formulation EGA P6 being the best with highest drug permeated through the membrane at the end of 12 hr. Penetration enhancers typically boost the rate at which a drug permeates through the stratum corneum (Figure 7).

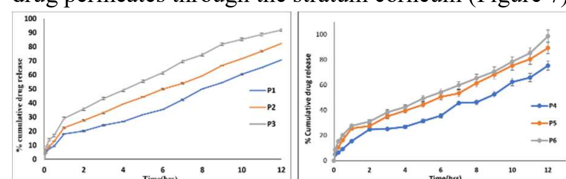


Figure no. 7. Ex vivo drug permeation of EGA NSPs loaded transdermal patches

CONCLUSION

The research was able to create and optimize nanosuspension of ellagic acid and place it in transdermal films having desirable physicochemical characteristics. The optimization showed that the variables included in formulation had a big influence on particle size and little effect on both zeta potential and drug content. The films prepared were uniform in thickness, were flexible and had satisfactory mechanical strength. Sustained drug release and increased skin permeation, especially in formulation P3 was confirmed in in-vitro and ex-vivo studies. In general, the nanosuspension-based transdermal system was effective in enhancing solubility and bioavailability of ellagic acid, which suggests that it has potential as an effective transdermal drug delivery system.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENTS

None

REFERENCES

1. Singh Hallan S, Sguizzato M, Pavoni G, Baldisserotto A, Drechsler M, Mariani P, Esposito E, Cortesi R. Ellagic acid containing nanostructured lipid carriers for topical application. Molecules. 2020;25(6):1449. doi:10.3390/molecules25061449

2. Castellacci R, Maria C. B.. An insight on ellagic acid formulations for transdermal delivery. Pharmaceutics. 2025. doi:10.3390/pharmaceutics17010045
3. Sobhy L, Mostafa M, Fathalla Z, Hussein AK. Ellagic acid-loaded chitosan nanoparticles as an approach for mitigating oxidative stress and liver damage in Poloxamer-407-induced hyperlipidemia in mice: Development and optimization through 3 level full factorial design. Int J Pharm. 2025;677:125659. doi: 10.1016/j.ijpharm.2025.125659.
4. Patel P, Thanki A, Kapoor D U, Prajapati B G. QbD decorated ellagic acid loaded polymeric nanoparticles: Factors influencing desolvation method and preliminary evaluations. Nano-Structures & Nano-Objects, 40, 101378. doi: 10.1016/j.nanoso.2024.101378
5. Ekanayake G, Wijayawardana S, Jayanetti M, Thambiliyagodage C, Liyanaarachchi H, Mendis A. In vitro release kinetics of bioactive compounds (gallic acid, ellagic acid, and eugenol) from chitosan polymer and the bioactivity of herb-loaded chitosan-CuO nanocomposites. Sci Rep. 2025; 13;15(1):35743. doi: 10.1038/s41598-025-19914-7.
6. Bhattacharjee Ananya, Kulkarni Venkatrao H, Habbu Prasanna V, Chakraborty Manodeep, Shabaraya A. Ramakrishna. Protective effect of Ellagic acid against Lead Induced Hepatotoxicity. Research J. Pharm. and Tech 2020; 13(9):4244-4248. doi: 10.5958/0974-360X.2020.00749.0
7. M. Syam Vardhan, K.V.V.S. Krishna, K. Gouri Sankar, S. Rama Krishna, T. Tamizh Mani. Simultaneous Estimation of Ellagic acid and Quercetin in Marketed Ayurvedic Formulations by HPTLC. Res. J. Pharmacognosy and Phytochem. 2017; 9(4): 223-227. doi: 10.5958/0975-4385.2017.00041.3
8. Sarika V. Khandbahale. A Review- Nanosuspension Technology in Drug Delivery System. Asian J. Pharm. Res. 2019; 9(2): 130-138. doi: 10.5958/2231-5691.2019.00021.2
9. Rajashri R. Kulkarni, Dipti G. Phadtare, Ravindra B. Saudagar. A Novel Approach Towards Nanosuspension. Asian J. Pharm. Res. 5(4): 2015;186-194. doi: 10.5958/2231-5691.2015.00029.5
10. Beedha. Saraswathi, Dr. T. Satyanarayana, K. Mounika, G. Swathi , K. Sravika, M. Mohan Krishna. Formulation and Characterization of Tramadol HCl Transdermal Patch. Asian J. Pharm. Tech. 2018; 8 (1):23-28. doi: 10.5958/2231-5713.2018.00004.1
11. Nisha Kumari, Alok Kumar Upadhyay, Shweta Singh, Sachin Kumar. Recent Innovations and the Potential Applications of Transdermal Patches in Drug Delivery Research. Asian Journal of Pharmacy and Technology. 2025; 15(3):305-1. doi: 10.52711/2231-5713.2025.00046
12. G. Vasavi, P. Naga Haritha, B. Chandrashekar. Formulation Development and In vitro Evaluation of Transdermal Patches of Tramadol HCl. Asian J. Res. Pharm. Sci. 2018; 8(3):123-129. doi: 10.5958/2231-5659.2018.00021.8
13. Pawar Pravin, Yadav Adhikrao, Gharge Varsha. Different Techniques for Preparation of Nanosuspension with Reference to its Characterisation and various Applications - A Review. Asian J. Res. Pharm. Sci. 2018; 8(4): 210-216. doi: 10.5958/2231-5659.2018.00035.8
14. Vandana Patil, Krishna Chopde, Amruta Jadhav, Nilay Shinde. Advancements in Nanosuspensions: Bridging Nanotechnology with Medicine to Transform Drug Delivery by Nanonization for Bioavailability Boosting. Asian Journal of Research in Pharmaceutical Sciences. 2025; 15(2):207-4. doi: 10.52711/2231-5659.2025.00032
15. Kassem M, ElMeshad A, Fares A. Enhanced Solubility and Dissolution Rate of Lacidipine Nanosuspension: Formulation Via Antisolvent Nanoprecipitation Technique and Optimization Using Box–Behnken Design. The American Association of Pharmaceutical Scientists. 2016;18(4):983-996. DOI: 10.1208/s12249-016-0604-1
16. Arpita Tupe, S.D. Mankar. Nanosuspension: A Novel Approach to Improve the Solubility, Bioavailability and Pharmacokinetics of Poorly Soluble Drugs. Asian Journal of Pharmacy and Technology. 2023; 13(3):194-0. doi: 10.52711/2231-5713.2023.00035
17. Papdiwal A, Sagar K, Pande V. Formulation and Characterization of Nateglinide Nanosuspension by Precipitation Method. International Journal of Pharmaceutical Sciences and Nanotechnology. 2014;7(4):2685-2691. DOI: 10.37285/ijpsn.2014.7.4.11
18. Sonkambale KG, Katedeshmukh RG, Kumbhar AB, Mane SV, Pawar PA, Kadam JN, Malusare AV. Formulation and evaluation of nanosuspension for enhancing the solubility of poorly soluble Antihyperlipidemic Drugs. European Journal of Molecular & Clinical Medicine.;8(3): 2021.DOI: 10.1155/2014/243575
19. Perween N, Alshehri S, Easwari TS, Verma V, Faiyazuddin M, Alanazi A, Shakeel F. Investigating the feasibility of mefenamic acid nanosuspension for pediatric delivery: Preparation, characterization, and role of excipients. Processes. 2021(4):574-579. DOI:10.3390/pr9040574
20. Maruti MM, Shirsat MK. Formulation and characterization of multilayered controlled release

topical patch for cure of cardiac disease. NVEO-natural volatiles & essential oils Journal| NVEO. 2021 Dec 20:13737-64.

21. Sachin B. Jadhav, Ankita R. Koshti, M. M. Bari, S. D. Barhate. Formulation optimization and Evaluation of Transdermal patch of losartan potassium containing DMSO as permeation enhancer. Asian J. Pharm. Tech. 2019; 9(3):220-227. doi: 10.5958/2231-5713.2019.00037.0

22. Latif MS, Azad AK, Nawaz A, Rashid SA, Rahman M, Al Omar SY, Bungau SG, Aleya L, Abdel-Daim MM. Ethyl Cellulose and Hydroxypropyl Methyl Cellulose Blended Methotrexate-Loaded Transdermal Patches: In Vitro and Ex Vivo. Polymers. 2021 Jan;13(20):3455.DOI: 10.3390/polym13203455

23. Trivedi DG, Patel HJ, Bhandari AK, Shah DA. Preparation and evaluation of transdermal patch of Desloratadine. International Journal of Biomedicine and Research. 2011.DOI:10.22270/ijbr. v2i5.266

24. Sabati AM, Ali MA, Ali BA. Formulation and in-vitro evaluation of baclofen transdermal patches. Asian Journal Pharmacy. 2017 Jan 1;86: 1-28.DOI:10.22377/ajp. v11i01.1105

25. Mo L, Lu G, Ou X, Ouyang D. Formulation and development of novel control release transdermal patches of carvedilol to improve bioavailability for the treatment of heart failure. Saudi journal of biological sciences. 2022 Jan 1;29(1): 266-272. DOI:10.1016/Fj.sjbs.2021.08.088