Original Article

Citric Acid Topical Lecithin Pluronic Organogel used as Anti-skin ageing: Development and Characterization

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ABSTRACT

Background: Skin aging is one of the main issues related with skin as each part of body ages with the time, So, to avoid this problem a novel formulation was designed in which citric acid as exfoliating agent will entrapped in thermodynamically stable, lecithin-based pluronic organogels. **Objectives:** The basic purpose of the study is to formulate a non-irritating and biocompatible citric acid loaded lecithin pluronic organogel for the treatment of skin aging. **Materials and Methods :** All the eight formulations (F1-F8) of lecithin pluronic organogel of citric acid was prepared by fluid filled fiber mechanism with different composition of pluronic f127. The FTIR study was revealed that no interaction observed between the drug and excipients. Among all the formulation F4 showed best results. All the formulation were evaluated through various parameters like Homogeneity test, Washability, pH determination, Viscosity, Spreadability, Drug content. *In-vitro* drug diffusion, Determination of sun protection factor (SPF) by ultraviolet spectrophotometer, rabbit skin irritation test and free radical scavenging assay. **Results:** The citric acid PLO formulation F4 exhibited drug content in maximum of 78.60% + 1.30. F4 formulation was the optimize formulation as there is half of the drug is released in 8 hrs. *In-vitro* drug release was conducted for all formulation shown in 7.4 phosphate buffer and release of citric acid in F4 found to best i.e. 79.57% and spreadability, pH, drug content, viscosity of F4 was found to be 7.85gm^2 cm/sec, 7.08 ± 0.176, 78.60% and 20011cp respectively. The SPF value of the F4 was found 13.312 which were under acceptable ranges. Primary irritation index (PII) of all the eight formulations (F1-F8) were showed slight to no skin irritation. F1- F6 formulation showed high *in-vitro* DPPH radical scavenging activity as compare to BHT and α-tocopherol. By studying all evaluation parameter F4 formulation was found to be best optimized. **Conclusion:** Study concluded that for topical delivery of citric acid PLO was the good delivery system and showed a good anti-ageing property as well.

Key words: Citric Acid, Pluronic Lecithin Organogel, Skin Aging, Topical Delivery, Primary Irritation Index.

INTRODUCTION

The skin is the body's largest organ, forming the outermost enveloping layers and shielding the body from the outer environment. Its capacity is must for endurance, as it is an unexpected organ that connects both physiological and pathological pathways.[1] The epidermis is the skin's most superficial layer, measuring around 100 µm in thickness. It has a distinct keratinized squamous epithelium and its primary work is to shield the body from the climate and reduce fluid loss.[2] The dermis is the basic skin's foundation, sustaining its shallow and deep layers. Its connective tissue layer, to which the epidermis is connected, is around 1-2mm thick. The primary role is to help the vascular system feed nutrients to the epidermis and regulate temperature. The sub-dermis is a deep fat layer beneath the skin that stores fat and heat.[3]

As each bodily part ages with the passage of time, skin is the exterior organ where the

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signs and side effects of ageing are readily apparent. In any case, cosmetics, like medication methods, helped to delay skin ageing. There are two types of ageing.

Intrinsic aging: It occurs as a result of long-term physiological alterations at various but unalterable hereditarily determined rates.

Extrinsic aging: Gravity, repetitive facial expressions, smoking, resting positions, and exposure to sunshine are all factors that contribute to it. Sun exposure is reported to interact with the actual ageing process and cause previous skin ageing.

Skin ageing may be impeded by applying topical formulations to the skin, however the skin acts as a barrier to anti-aging specialists. As a result, a crucial test for skin detailing is, without causing irreversible changes in barrier functions in skin, to increase the sufficient drug penetration. Drug is transported over the epidermal barrier in two ways: transcellular and paracellular. Hydrophilic drugs follow paracellular transport, whereas lipophilic drugs are followed by transcellular route. In the market, there are several creams, balms, and moisturizers for skin ageing treatment. However, research is focused on developing additives that are natural, non-hypersensitive, biocompatible, non-immunogenic, and viable for patients, as well as include ease of assembly, planned quality control and long-term stability.[4]

Thermodynamically organogels are stable, visco-flexible bi-phasic frameworks with a gelator (material suitable for gel shaping) and a non-polar phase, at times, water molecules can be found in the network shaped by the gelator. The hydrogel, on the other hand, has a lower degree of hydration.[5] In the last few years, their biocompatibility and non-irritating properties have become increasingly important in the release of drugs.[6]

Organogels' main solvent system is non-aqueous fluids. This is a great delivery method for lipophilic drugs and aqueous liquids through the skin, and it is also beneficial for hydrophilic drugs included in several pharmacopoeias, as well as skin hydration. Organogel achieved a local and systemic effect by percutaneous ingestion due to the presence of penetration enhancers, and their occlusive effect and lipophilic nature were increased.[7]

The drug release mechanism varies based on the organogel technology used. However, drug release occurs by basic diffusion when a dominant part of the organogel framework is present. The presence of gelator molecules, with their three-dimensional network, limits this diffusion. The degree of crosslinking determines the drug release rate. A higher gelator concentration (more crosslinking) means a slower rate of drug release.[8-10]

Pluronic Lecithin Organogel (PLO): PLO is a pluronic lecithin-based organogel made up of isopropyl myristate / isopropyl palmitate, Pluronic F127 (Poloxamer 407), and water, which is a odourless, yellow-colored, and transparent gel that is quickly absorbed by the skin. It is also thermostable, biocompatible and viscoelastic. Pluronic F127 is a polyoxypropylene / polyoxyethylene copolymer that create a thermoreversible gel at 15-30% concentrations in w/v. Water plays the main role of a design shaper, settling the gel development cycle as it dissolves pluronic F127 and other hydrophilic drugs. Sorbic acid act as a preservative, may be present in both phases of the PLO. As with lecithin organogel, the PLO is made-up of entangled tubular reverse-micelle structures that form temporal three-dimensional constructs.[11]

Citric acid has the capacity to peel the skin. They basically eliminate the "glue" from the upper layer of the skin that clutches the old, dead skin cells. By viably eliminating that top layer of skin the citrus acid serve to help the new skin develops. The chelation properties of citric acid certainty that calcium ions are known to assume a critical part in cutaneous cellular adhesions. Citrus acid decrease the calcium ions fixation in the epidermis and eliminate calcium ions from the cell adhesion by chelation. When calcium ions are decrease than exfoliation take place. Citric acid also controls collagen production and matrix degradation via keratinocyte release cytokines, not simply directly accelerating fibroblasts through collagen synthesis.

MATERIALS AND METHODS

Materials

Citric acid, Soya lecithin, Isopropyl myristate and Polyethylene glycol is procured from central drug house ltd, New Delhi.

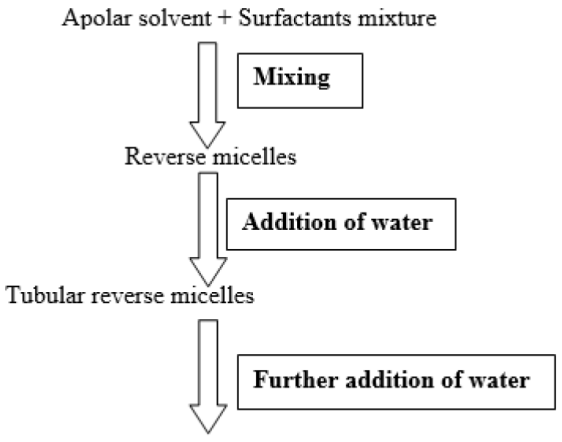

Figure 1: Flow chart of Fluid-Filled Fiber Mechanism.

Table 1: Formulation Table for Citric Acid PLO.								
Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Citric acid(w/v)	25%	25%	25%	25%	25%	25%	nil	25%
PluronicF127(w/v)	33.3%	26.7%	20%	13.3%	6.6%	3.3%	13.3%	nil
SoyaLecithin(w/v)	20%	20%	20%	20%	20%	20%	20%	20%
Isopropyl myristate	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
Propylene glycol	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
Distilled water	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml

Methodology

Fluid-Filled Fiber Mechanism[12]

Preparation of Citric Acid Pluronic Lecithin Organogel

For preparation of lecithin pluronic organogel of citric acid two phase (aqueous phase and oily phase) were prepared, non- aqueous phase prepared as purified lecithin was added with isopropyl myristate at RT and kept overnight for complete dissolution. Aqueous phase prepared using pluronic F127 and propylene glycol was added with water and kept overnight for complete dissolution of pluronic and finally citric acid was added and stirred at 250rpm. The oil phase was slowly added in aqueous phase while being stirrer on a magnetic stirrer at 250rpm (Figure 1). The various formulation of pluronic lecithin organogel developed with different composition (Table 1).

Calibration curve of citric acid

Determination of λ_{max} of Citric acid

Citric acid's UV absorption spectrum revealed absorption maxima (max) of 230nm. In the concentration range of 5-30 mcg/ml, the graph plotted absorbance V/s concentration and was found to be linear. As a result, it seems that the drug follows Lambert's law in this range. Figure 2 depicts the UV spectrum of citric acid, whereas Figure 3 depicts the calibration curve of citric acid in phosphate buffer at 7.4 pH.

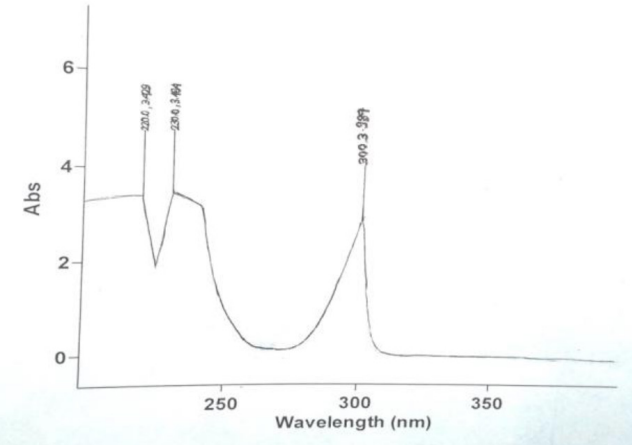

Figure 2: UV Spectrum of Citric acid.

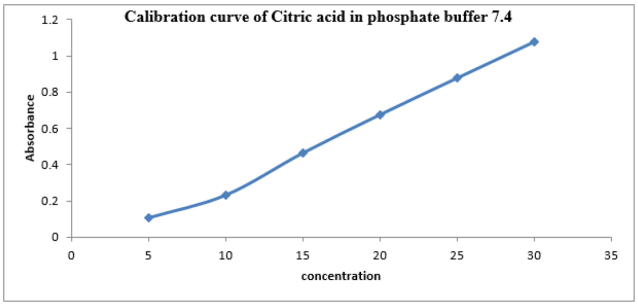

Figure 3: Calibration curve of Citric acid in pH7.4 phosphate buffer.

Evaluation Parameters of Citric Acid PLO

Physiological Evaluation

Oraganoleptic Characteristics

Each formulation of citric acid PLO was tested for color, odor, phase separation and feel upon application (grittiness, stiffness, greasiness and tackiness).

- **Homogeneity Test**

All developed citric acid PLO were tested for homogeneity as a small quantity of gels pressed between index finger and thumb in order to notice the consistency of organogel that any coarse particles being detached or attached on the finger.

- **Washability**

A small amount of PLO (100 mg) was rubbed on the back of the hand's skin, then the organogel was washed with water to see if it was washable.

Fourier Transform Infra-Red Spectroscopy (FTIR) Studies

FTIR spectrophotometers were used to measure the FTIR of pure drug and the blend of drug and added excipients (Perkin Elimer). The goal of the FTIR was to see how the drug interacted with the added excipients

in the formulation. The test groundwork for FTIR is completed by thoroughly mixing powdered drugs and excipients with dry Kbr. The mixer was set to diffused reflectance sampler, and the spectrum was calculated by filtering in the 4000-400 cm wavelength range.

pH determination

pH of the formulations were dictated by utilizing adjusted digital pH meter at 25°C. A solution of 1gm of PLO in 30ml of distilled water was arranged and exposed to pH measurement.

Viscosity

The viscosity of the various citric acid PLO formulations were determined using brookfield Rheometer at RT. The PLO formulations were rotated at 12 to 2 rpm with the spindle. At each RPM, the corresponding reading of viscosity and torque was noted. Experiments were carried out in triplicate.

Extrudability

The gel formulation was put in a collapsible tube and pressed strongly from the crimped end to determine its extrudability. After applying pressure, the mixture extruded to release while the cap was removed. It was calculated that, the ribbon of the formulation (weight in grams) required to extrude a 0.5 cm in 10 sec. In triplicate, the average value of the test was done and calculated. The extrudability was then determined using the formula below.

Extrudability = Weight applied to extrude gel from tube (in gm) / Area (in cm^2).

Spreadability

Spreadability was dictated by the apparatus in which one slide is fixed and another slide is versatile. By this strategy, spreadability was estimated based on 'Slip' and 'Drag' attributes of organogel. A lower glass slide was fixed with the assistance of stand, and about 1gm of organogel was set at the focal point of this slide and afterward the organogel was sandwiched between this slide and another glass slide having the measurement same as the fixed slide.1kg weight was set on the highest point of the two slides for 5 min to oust air and to give a uniform film of the organogel between the slides. The top plate was then exposed to pull of 10 g and the time (in seconds) needed by the top slide to cover a distance of 7.5 cm be noted. A more limited span shows better Spreadability. Spreadability was then determined utilizing the accompanying formula.[13]

$$S = M \times L / T$$

Where, **M =** formulation weight in the pan
S = Spreadability
L = Length covered by the glass slide
T = Time taken to completely separate the slides from one another.

Drug content

Drug content study help to decide the measure of the drug present in the specific amount in the formulation. 100 mg of organogel from every formulation were gauged and it was dissolved in 100 ml volumetric flask with 50 ml of phosphate buffer of pH 7.4 and shaken for 2hrs to get complete solubility of Citric acid and make the volume up to 100 ml of phosphate buffer 7.4. The subsequent solution is separated through Whatman filter paper and appropriate dilution was finished. The citrus acid content was analyzed by UV spectrophotometer.

In-vitro diffusion studies

For these experiments, a Franz diffusion cell was used. Citric acid PLO (500 mg) was uniformly added to the surface of the dialysis membrane. The membrane was clamped between the diffusion cell's receptor and donor compartments. To solubilize the citric acid, freshly prepared 7.4 phosphate buffer solution was poured into the receptor chamber. The diffusion medium in the receptor chamber was agitated at 50 rpm with a magnetic stirrer, and the temperature was kept at 37.5°C. At each calculated time interval, 5ml aliquot was collected. After appropriate dilutions, aliquots were analyzed for Citric acid content using UV visible spectrophotometer. To determine the total amount of citric acid penetration at each time period, cumulative adjustments were applied.

Release kinetic studies

To determine the mechanism of drug release from Citric acid topical Organogel, data occurred through *in-vitro* drug release treated with several kinetic models, including zero order, first order, Higuchi, and Korsemeyer-Peppas. A high regression coefficient value was used as a criteria for selecting the best model.

Determination of sun protection factor (SPF) by UV Spectrophotometer

SPF was determined accurately weighted 1gm of all samples and transferred to a 100 mL volumetric flask diluted with water till volume make up and shaken for 1hr, followed by ultra-sonication for 5 min and then filtered with whatman filter paper. A 5mL of above solution was transferred to 50 mL volumetric flask and diluted to volume make up with water. Then again 5 mL of this last solution was shifted to a 25 mL volumetric

flask and the volume make up with water. The absorption spectra of samples were obtained in the range of 200 to 400 nm using 1 cm quartz cell, and water as a blank, followed by the application of Mansur equation.[14]

$$\text{SPF spectrophotometric} = CF \times \sum_{200}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where: EE – spectrum of Erythemal effect
I – Spectrum of Solar intensity
Abs - Absorbance of sunscreen product
CF – Correction factor (= 10)

EE × I values are constants. They've been Figured out and are listed in Table 2.[15]

In-vivo and *in-vitro* anti-aging evaluation

Closed patch test on rabbit skin irritation

3 large albino male rabbits (1.5–2.5 kg) were housed in a confined rodent facility with natural conditions adjusted to 60–90% RH and 25±2°C, and a 12 hr dark /12 hr light cycle after an acclimatization period of 7 days to confirm their acceptability for the examination. Ad labium access to a commercial rabbit diet was provided to rabbits, and each cabinet was supplied with drinking water. The backs of the rabbits were shaved with an electric trimmer to remove all fur 24 hr before the test. The shaved spots were divided into ten 2.5 x 2.5 cm sites each. On each location, 0.5 g of each sample and a 5% sodium lauryl sulphate arrangement (positive control) were placed. As a negative control, the untreated site was used. The treated areas were wrapped in non-occlusive gauze and coated with gauze. The test samples with gauze were removed after 24 hr, and the treated areas were cleaned several times with sterile water before being air dried. After one hour, the areas were visually examined and skin edema and erythema were measured using a Mexameter. At 24,48, and 72 hr, erythema and edema were scored.[16]

The following equation was used to calculate the Primary Irritation Index (PII):

PII = [(∑ erythema grade at 24/48/72 h + ∑ edema grade at 24/48/72 hr)/ 3 × number of animals].

The degree of irritation was classified as insignificant (PII = 0–0.4), minor (PII = 0.5–1.9), moderate (PII = 2–4.9), or severe (PII = 5–8) based on PII values.

The ethical committee of the School of Pharmaceutical Science, SGRR University, Dehradun, Uttarakhand, examined and approved this study protocol.

DPPH free-radical scavenging assay

PLO's DPPH free-radical scavenging activity was determined using an optimized formulation (F4) of organogel (0.5 ml each) fully mixed with a 1-ml methanol solution containing 0.1 mM DPPH. A UV-Visual spectrophometer was used to measure the absorbance of the reaction mixture at 515 nm against a blank after it had been let be stand for 30 min at 27± 2°C (pH 7.4 phosphate buffer). Positive controls included BHT and a-tocopherol. The experiments were carried out in triplicate.[17]

The activity of scavenging free radicals was determined using the equation:

$$\text{Scavenging (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test sample.

RESULTS

Anticipated topical formulation of citric acid has been resolved by formulating lecithin pluronic organogel. Eight formulations of citric acid were formulated by fluid filled fiber mechanism using different drug surfactant ratio.

Evaluation of citric acid lecithin organogel

Physiological Evaluation

The prepared citric acid PLO formulae were inspected visually the result shows that PLO was yellowish in color with no phase separation, washable when washed with water, upon application in hand feel smooth, odorless, showed good homogeneity in all formulation with absence of lumps.

Fourier Transform Infra-Red Spectroscopy (FTIR) Studies

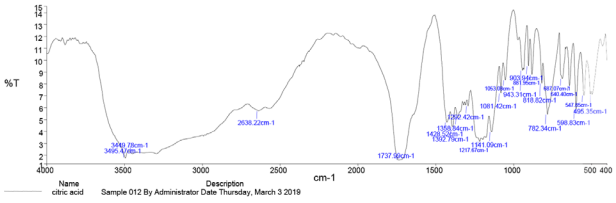

Figure 4: FTIR of Citric acid.

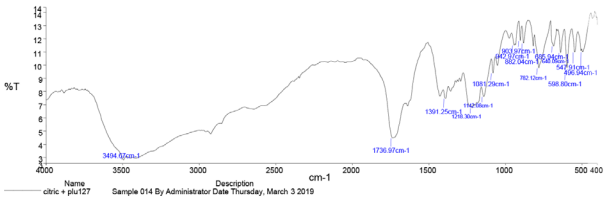

Figure 5: FTIR of citric acid and Pluronic F127.

The drug-excipients interaction reveal no significant changes in absorption bands (peaks), and all of the drug's characteristic peaks are present in the combined drug and excipients spectra, showing that the drug and the excipients used in the formulation are compatible (Figure 4,5,6).

pH determination

All developed citric acid PLO formulae had pH values in the range of 6.68-7.08, which is deemed appropriate to avoid irritation when topically applied to the skin, and there was no significant change in pH values over time in any of the citric acid PLO formulations. (Table 3).

Viscosity, Spreadability, Extrudability and Drug Content

Viscosity is an important physical property of citric acid PLO formulae as affects the rate of drug release. It was observed that on decreasing the concentration of pluronic F127 in formulation, viscosity decrease from 41572.58 to 689.11(Table 3). F8 have not the pluronic as gelator so it showed minimal viscosity. The spreadability indicates that the Citric acid topical organogel is easily spreadable by small amount of shear. The time at which organogel spreadable ranged from 6.25 to 12.8 gm^{2}cm/sec and maximum spreadability was found to be 12.8 gm cm/sec of F6 formulation (Table 3). The degree to which a organogel is extruded out of the ointment tube is referred to as extrudability. The extrudability of the formulation is influenced by the viscosity and consistency of the semisolid preparation. The extrudability and viscosity have an inverse relationship, meaning that the lower the viscosity, the higher the extrudability. Extrudability range of all the eight formulations was found to be 7.70±0.09 to 16.05±0.07 (Table 3). After various formulation of Citric acid POL formulae, drug content of the formulated gel was estimated by Agilent spectrophotometer at 230nm in phosphate buffer of pH 7.4. The content was found to be in range of 41.29 to 78.60% for lecithin pluronic organogel and the maximum drug content was found to be 78.60% of F4 (Table 3).

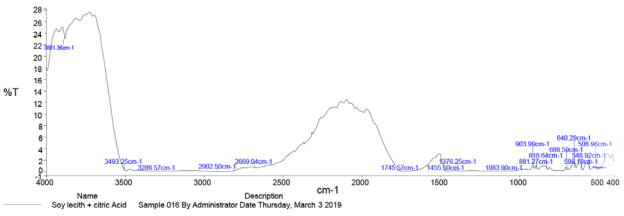

Figure 6: FTIR of Citric Acid and Soy lecithin.

In vitro Diffusion study

Figure 7 shows that drug release from the organogel formulation has an inverse relationship with pluronic (gelator) concentration, i.e., when pluronic concentration (Poloxamar) increases, drug release from the organogel formulation decreases. Pluronic micelles are effective as drug transporters because their assemblies can operate as passive drug containers. After a 12-hr interval, it was discovered that F6 formulation, which had the least amount of pluronic (copolymer) concentration, had the highest drug release percentage, whereas F1 formulation, which contained the most pluronic (copolymer) concentration, had the lowest. F7 formulation used as placebo formulation and F8 formulation not having gelator (copolymer) so result revealed that within 2 hr, >85% drug was released.

In-vitro model fitting release profile

The drug release data was fitted into multiple kinetic models by calculating the release constant and regression coefficients (R). The F4 formulation had the best fit with the Korsmeyer peppas model, with a regression coefficient of 0.9981. This indicates that

Table 3: Evaluation Parameters of Citric Acid PLO.

Formulation code	pH value	viscosity(cp) (Average)	Spreadability (gm cm/sec)	Extrudability (gm/cm^2)	Drug content (%)	SPF Value
F1	7.08±0.175	41572.58	6.25±0.01	7.70±0.09	41.29	9.237
F2	6.68±0.483	35948.50	7.9±0.10	9.10±0.61	67.97	11.312
F3	6.72±0.654	29816.60	6.57±0.05	11.87±0.12	69.17	6.153
F4	7.08±0.176	27235.53	7.85±0.06	13.03±0.42	78.60	13.312
F5	6.71±0.499	25485.58	9.6±0.11	14.61±0.10	76.02	3.672
F6	6.76±0.548	25306.73	12.8±0.15	16.05±0.07	62.10	10.013
F7	6.88±0.231	28542.60	7.10±0.12	13.87±0.08	nil	4.237
F8	7.41±0.342	689.11	7.32±0.65	13.64±0.17	75.09	5.543

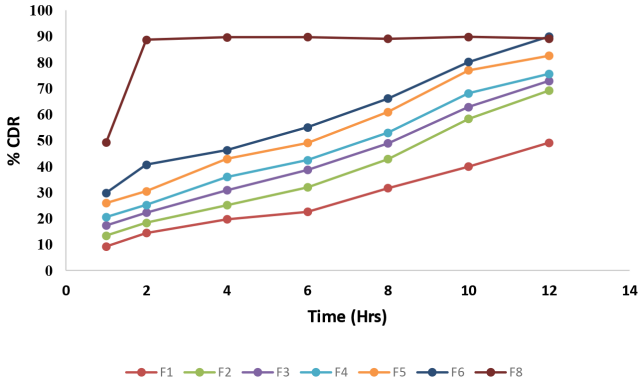

Figure 7: Comparative Release Profile of Citric Acid PLO.

the drug concentration released was influenced by the matrix drug load. The drug release mechanism involved is super case 2, which reflects drug release by diffusion and the influence of polymer relaxation allows drug movement from PLO matrixes. The value of the diffusion exponent (n) found was 1.249, which is greater than 1, indicating that the drug release mechanism involved is super case 2, which reflects drug release by diffusion and the influence of polymer relaxation allows drug movement from PLO matrixes.

Sun protection factor (SPF) by ultraviolet spectrophotometer

Top of Form

SPF, or Sun Protection Factors, is a measure of how well a sunscreen will protect skin from UV rays, the type of radiation that causes sunburn, damages skin and the results ranges from 3.672 to 13.312 and maximum SPF was found to be 13.312 of F4.

In-vivo and *in-vitro* anti-aging evaluation

Closed patch test on rabbit skin irritation

Except for the F1 and F2 (PII = 0.41– 0.76, slight irritation) and the +ve control (5% SLS, PII = 0.73–1.34, somewhat irritant), the calculated PIIs of all citric acid loaded PLO formulations in skin irritation evaluation through the closed patch test were in the range of 0.00–0.39 within 72 hr. (Table 4). Except for the F1 and F2 formulations, none of the developed PLO formulations caused irritation (Figure 8). The irritation was caused by pluronic F127 (Poloxamer 407), which according to the material safety data sheet can cause skin irritation with redness or moderate inflammation. This may be also due to its higher concentrations in the F1 & F2 formulations, which were about 2-2.5 times more than F4 that is the best formulation among all. Other than that 5% SLS also showed the irritation as a positive control.

Table 4: Category of irritation, based on Primary irritation index (PII) of various PLO formulations.

Sl. No	Formulation	Primary Irritation Index (PII)			Category
		24 hr	48hr	72hr	
1	F1 (33.3% pluronic)	0.52	0.65	0.76	Slight Irritation
2	F2(26.7% pluronic)	0.41	0.52	0.64	Slight Irritation
3	F3(20% pluronic)	0.29	0.37	0.39	Negligible
4	F4(13.3% pluronic)	0.20	0.25	0.31	Negligible
5	F5(6.6% pluronic)	0.12	0.16	0.21	Negligible
6	F6(3.3% pluronic)	0.06	0.11	0.14	Negligible
7	F7 (13.3% pluronic)	0.21	0.26	0.32	Negligible
8	F8(0 % pluronic)	0.00	0.00	0.00	Negligible
9	5% SLS, Positive control	1.34	1.05	0.73	Slight Irritation
10	Untreated area (Negative Control)	0.00	0.00	0.00	Negligible

Grading scale for skin irritation effect following OECD Test Guideline 404.
The Primary Irritation Index (PII) was calculated using the following equation:
PII = [(∑ erythema grade at 24/48/72 h + ∑ edema grade at 24/48/72 h)/ 3 × number of animals].

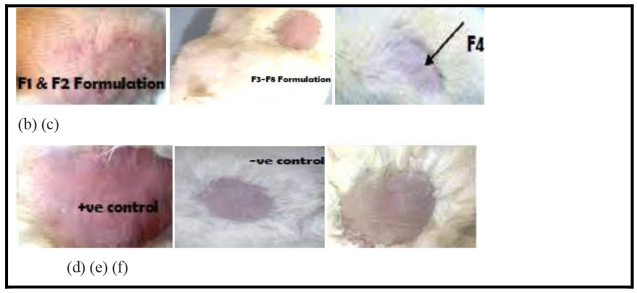

Figure 8: Category of Irritation (a) F1 & F2 formulation shows slight irritation (b)&(c) F3-F8 formulations shows no irritation (d) 5% SLS (+ve control) shows slight irritation (e) & (f) -ve control shows no irritation.

Skin irritant effect grading scale according to OECD Test Guideline 404

The following equation was used to calculate the Primary Irritation Index (PII):

PII = [∑ edema grade at 24/48/72 h + (∑ erythema grade at 24/48/72 h)/ 3 × n. number of animals].

DPPH free-radical scavenging assay

Table 5 shows the SC_{50} values of citric acid pluronic lecithin organogel formulations (F1-F6) in the DPPH radical scavenging assay; F7 is a placebo formulation, and F8 is a formulation without pluronic F127 and the standard antioxidants (-tocopherol and BHT). SC_{50} value

Table 5: DPPH radical scavenging activity of Citric Acid PLO.

S.No	Sample	SC_{50} (µg/mL)
1	Citric acid loaded in PLO (F4)	7.12
2	Blank Pluronic Lecithin Organogel (F7)	nil
3	α-tocopherol	5.43
4	BHT	3.90

of optimized formulation F4 is 7.12 µg/mL, which was somewhat higher than BHT and α -tocopherol (3.90 µg/mL and 5.43 respectively) of about 1.5 to 2 times.

Bottom of Form

DISCUSSION

Citric acid is an alpha hydroxyl and has ability to exfoliate the skin. By its chelation property, reduced the calcium ions concentration in the epidermis and remove calcium ion from the cell adhesions by chelation. When calcium is decreased cellular adhesion are exfoliation take place. It's also worth noting that citric acid not only directly stimulates collagen synthesis by fibroblasts, but it also controls matrix breakdown and collagen synthesis via keratinocyte-released cytokines. The objective of the present work was developed and characterized of citric acid organogel for anti-skin aging topical delivery. Polymers like pluronic F127 and lecithin were incorporated to form organogel.

FTIR study was done and no interaction was seen between citric acid and excipients which showed compatibility with each other. Calibration curve was found that, the estimation of citric acid by spectrophotometrically method at 230 nm in phosphate buffer 7.4 had good reproducibility, at the concentration between 8-24 µg/ml which is the beers range of citric acid.

The pH was calculated by pH meter and all the pH range of the formulation were within range i.e 6.68 to 7.08 which is the required range for the topical drug delivery system.

The viscosity of organogel was determined by using a Brookfield rheometer at different RPM. i.e., (12, 10, 8, 6, 4, and 2) and the rate of shearing is directly proportional to shearing stress and as the shear rate increase the viscosity flow increase which shows that the formulation is non- Newtonian in nature and having Pseudo plastic flow and maximum viscosity was found to be 41572.58 cp of F1 formulation.

The spreadability were ranged from 6.25 to12.8 gm^2cm/sec and maximum spreadability was found to be 12.8gm cm/sec of F6 formulation. The viscosity and extrudability have an inverse relationship, meaning that the lower the viscosity, the higher the extrudability. Range of extrudability of all the eight preparations were found to be 7.70±0.09 to 16.05±0.07.

The drug content was found to be in range of 41.29 to 78.60% for lecithin organogel and the maximum drug content was found to be 78.60% of F4.

The *in-vitro* drug release of the organogel was carried in 7.4 phosphate buffer from 0 to 12hrs by Franz diffusion cell. The release rate of the formulation range from 40.1 to 79.57% and results shows that with decrease in pluronic f127 concentration the release of citric acid increase may be due to decrease in complexity of the organogel and further decrease in the concentration of pluronic f127 decrease in the citric acid release this may be because pluronic f127 also behaves as retarding agent. The best citric acid release shown in F4 i.e 79.57% which follows the Higuchi's and super case reflects the diffusion of drug with polymer relaxation allows movement of citric acid from matrixes of organogel. F4 formulation was the optimize formulation as there is half of the drug is released in 8 hrs. Its R^2 values for zero, first, Higuchi's was 0.975, 0.879, 0.9981 respectively and Korsmeyer peppas n value was 1.249. The SPF value of the different formulation was found to 3.672 to 13.312 and SPF value of F4 was found to be 13.312.

The Primary Irritation Index of all citric acid loaded PLO formulations in rabbit skin irritation was calculated and found that all created PLO formulations gave no irritation, with the exception of the F1, F2 formulation and 5% SLS utilized as a positive control. The irritation was caused by pluronic F127 (Poloxamer 407), which according to the material safety data sheet can cause skin irritation with redness or moderate inflammation. This may be also due to its higher concentrations in the F1 and F2 formulations, which were about 2-2.5 times more than F4 that is the best formulation among all.

The SC_{50} value of optimized formulation F4 in terms of free radical scavenging activity is 7.12 g/mL, which is 1.5 to 2 times greater than the usual antioxidants -tocopherol and BHT (5,43 and 3.90 g/mL, respectively).

CONCLUSION

The topical PLO formulation of citric acid achieved the transdermal absorption by the presence of a copolymer (gelator). Prepared citric acid PLO by Fluid Fiber technique containing soya lecithin, Pluronic F127, IPM, PEG were observed to be non-irritant, no phase separation and cost-effective drug delivery system. The FTIR study was revealed that there was no interaction between the drug and excipients. The citric acid was found to be soluble in 7.4 phosphate buffer. The pH revealed

that formulations were within the range. A rheology study was concluded that the formulation follows Non-newtonian in nature and pseudo plastic flow. *In-vitro* drug release was conducted for all formulation shown in 7.4 phosphate buffer and release of citric acid in F4 found to best i.e.79.57% and spreadability, pH, drug content, viscosity of F4 was found to be 7.85gm^2 cm/sec, 7.08±0.176, 78.60% and 27235.53cp respectively. The SPF value of the F4 was found 13.312 which were under acceptable ranges. By studying all evaluation parameter F4 formulation was found to be best optimized. As the non-aqueous phase of organogel help in penetration of drug by pluronic f127 and lecithin and avoid the microbial growth. Whereas the aqueous phase of organogel help in dissolving citric acid. For anti-ageing property of PLO, *in-vivo* skin irritation test revealed that no irritation observed by optimized formulation (F4) and *in-vitro* free radical scavenging study was shown SC_{50} value of optimized formulation F4 was 1.5 to 2 times more than standard antioxidant i.e. α -tocopherol and BHT. Therefore concluded that for anti-ageing, topical delivery of citric acid pluronic lecithin organogel is best formulation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SPF: Sun Protection Factor, **DPPH:** Diphenylpicryl Hydrazine, **PLO:** Pluranic Lecithin Organogel, **BHT:** Butylated Hydroxytoluene, **PII:** Primary Irritation Index

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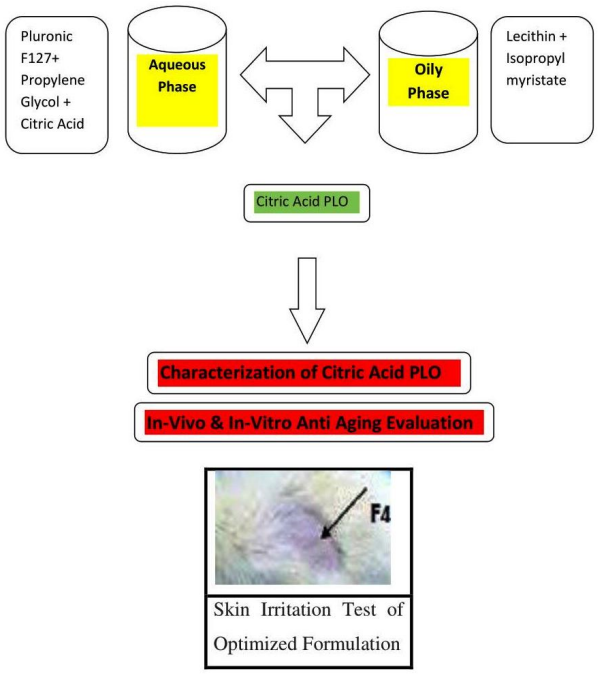
PICTORIAL ABSTRACT



SUMMARY

The purpose of this research is to develop a citric acid-loaded pluronic lecithin organogel for the treatment of skin ageing. The fluid filled fibre mechanism was used to develop eight formulations of pluronic lecithin organogel of citric acid. F4 formulation was found to be the best optimised by evaluating all evaluation parameters. According to the study, PLO was the optimal delivery system for topical citric acid delivery and also has anti-aging properties

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