Formulation and Evaluation of a Topical Emulgel Using Solanum lycopersicum Lycopene Extract for Enhanced Stability

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Abstract
The study aimed to develop a novel, cost-effective extraction technique for lycopene from Lycopersicum esculentum L. fruit, formulate a stable emulgel containing lycopene as the active ingredient, and design an analytical method to quantify lycopene concentration in the emulgel. The emulgel was evaluated for stability under various storage conditions (8°C, 25°C, 40°C, 40°C + 75% RH, and 50°C) over six months. Statistical analysis using two-way ANOVA, Post-Hoc, and paired t-tests at a 5% significance level showed the extraction method yielded 154.83 mg/kg of tomato fruit, with recoveries ranging from 145 to 156 mg/kg. Stability parameters' p-values were <0.05, except at 50°C from day 60 onwards. The analytical method exhibited a linear lycopene range of 1–10 mg/mL, with a detection limit of 0.11 mg/mL and quantification limit of 0.34 mg/mL. Inter-day and intra-day recoveries ranged from 94 to 105%, and relative standard deviation was 5.36%. The extraction technique proved cost-effective and reliable, while the analytical method was simple, robust, specific, and sensitive for lycopene measurement in emulgel. The formulated emulgel remained stable even at elevated temperature (40°C) and high humidity (75% RH).

Introduction

Solanum lycopersicum (tomato) fruit is used as a valuable active ingredient in different topical preparations e.g. emulsions. Here, it serves as an anti-aging and pigment-lightening constituent due to its free radical scavenging property Lyco pene is one of the important constituents of tomato fruit which acts as a potent antioxidant. It belongs to the family of cyclic carotenoids .Tomato fruit contains 0.03–0.14 mg/g of lycopene content.Carotenoids are biologically significant for their roles in preventing certain cancers, cardiovascular diseases, hyperlipidemia, and more.These benefits are independent of their provitamin-A activity and primarily arise from their antioxidant properties, including singlet oxygen quenching and free radical deactivation. Specifically, lycopene plays a crucial role in preventing oxidative damage linked to cardiovascular diseases and treating topical ailments[1].

Various methods exist for extracting lycopene from tomato fruit, but many are time-consuming, require specialized expertise, or involve costly equipment. Additionally, some methods yield low product amounts. Therefore, there is a need to develop an extraction procedure that combines efficiency, simplicity, and cost-effectiveness for optimal lycopene recovery.Various dosage forms are used to deliver lycopene, but topical drug delivery systems offer several advantages.[2] These include selective delivery of the drug to a specific site, avoidance of gastrointestinal incompatibility, and prevention of metabolic degradation associated with oral administration Additionally, topical delivery enhances bioavailability by bypassing liver metabolism and provides consistent drug release over an extended period.Topical products vary widely in formulation and consistency, ranging from liquids to powders, with semisolid preparations being the most popular. Among semisolid forms, transparent gels have gained significant importance in both cosmetic and pharmaceutical applications. Gels are a relatively novel dosage form that entrap a high amount of aqueous or hydroalcoholic liquid within a network of colloidal solid particles. Gel formulations provide faster drug release compared to ointments and creams.[1][2]

However, a key challenge with gels is delivering hydrophobic drugs effectively. To address this, emulgels are developed by combining emulsions with gels, creating a formulation known as an emulgel.

Emulgels for dermatological use possess several favorable properties, including being thixotropic, greaseless, easily spreadable and removable, emollient, non-staining, water-soluble, having a longer shelf life, bio-friendly, visually appealing, and exhibiting better stability.

Currently, scientists face challenges in developing new drug delivery systems because many drugs, either synthesized or identified through high-throughput screening, have poor water solubility.The biopharmaceutical classification system categorizes drugs into four classes based on in vitro solubility and in vivo permeability data. Among these, Class II drugs exhibit poor solubility but high permeability. For Class II drugs, poor aqueous solubility is the primary limitation affecting their overall absorption rate and extent. After solubility, permeability is the next critical factor influencing bioavailability.For topical delivery of poorly water-soluble drugs, emulgels are the preferred choice. Emulsified gels have proven to be stable and effective vehicles for delivering Class II drugs (Jagdale and Pawar, 2017). Fortunately, lycopene is well absorbed topically—such as in creams or lotions—due to its lipophilic nature and small molecular size. Dietary intake alone is insufficient to maximize lycopene's skin benefits because orally consumed lycopene distributes throughout the body, with only a small fraction reaching the skin.[3]

Lycopene is a potent physical quencher of singlet oxygen; however, its stability is relatively low.Researchers are thus focusing on finding the best carrier system for topical lycopene delivery to slow its degradation.

2. Materials

Materials:

Solanum lycopersicumL,acetone, n-hexane, sodium nitrate and sulphuric acidethyl acetate, liquid Paraffin, propylene glycol, triethanolamine and methanol, lycopene standard, carbopol 940, span 20 and tween 20, methyl paraben, purified distilled water.

The method was developed based on the characteristics of lycopene, with the key feature being its solubility in different solvents. Specifically, it dissolves in chloroform, n-hexane, benzene, carbon disulfide, acetone, and petroleum ether, while it does not dissolve in water, ethanol, and methanol, as lycopene is a non-polar substance. The main principle applied in this experiment was the solvent extraction technique.[4]

2.2ExtractionofLycopene:

The extraction method was designed based on the solubility properties of lycopene, which is soluble in solvents such as chloroform, n-hexane, benzene, carbon disulfide, acetone, and petroleum ether, but insoluble in water, ethanol, and methanol, reflecting its non-polar nature. This experiment utilized a modified solvent extraction technique.In brief, 100 g of seedless tomato fruit was sliced and extracted three times with 100 ml portions of acetone, a water-soluble solvent. The residue was collected, dried after filtration, and subsequently extracted three times with 100 ml portions of a water-immiscible solvent mixture of ethyl acetate and n-hexane (3:17 by volume). Each extraction was stirred for 5–10 minutes and then filtered. The combined filtrate was concentrated to a final volume of 20 ml using a rotary evaporator at 40 ± 1°C and 60 rpm One milliliter of the extract was diluted to 100 ml with n-hexane (Dilution 1), and then 2 ml of Dilution 1 was further diluted to 100 ml with n-hexane. The extract was analyzed using a UV–Visible spectrophotometer. Lycopene was precipitated by adding methanol as an anti-solvent at low temperature to reduce solubility, forming a crystalline product which was then filtered and dried.

The extraction procedure was repeated to assess inter-day and intra-day variations with three replicates: intra-day samples were taken three times at equal intervals on the same day, and inter-day samples were taken across three different days.[5]

When a 5% sodium nitrate solution in water and a 1 N sulfuric acid solution in water were added one after the other, the color of the sample solution in acetone changed (Meeting, 2006).

Characterization of lycopene:

The lycopene solution in n-hexane was made and scanned using a UV-VIS spectrophotometer to determine the kmax in the 300–600 nm wavelength range. According to reports, the kmax for this solution is 472 nm. Lycopene's FTIR spectrum was obtained using an FTIR Spectrophotometer.

Wave number ranges of 4000 cm^1 to 650 cm^1 were used to scan lycopene. To identify the corresponding functional groups present, the spectra's major peaks were analyzed.

The extracted lycopene's FTIR spectrum was verified by comparing it to the imported lycopene spectra provided by Lopez-Cervantes et al. (2014) and the prominent peaks.The production of lycopene was then determined using direct mass analysis and Beer Lambert Law,185 ~ 103 L mol~1 cm~1, computed using E (1%, 1 cm) = 3450 (Luengo et al., 2014), b = path length = 1 cm, and c = sample solution concentration in mol/L.

Lycopene crystals were filtered, dried, and their mass was calculated for mass analysis.

Formulation of Emulgel:

Following Steps were involved:

Preparation of the Emulsion

A stable oil-in-water (O/W) emulsion was prepared following previously reported methods with slight modifications

For the test formulation, the oil phase consisted of liquid paraffin (7.5 g), Span 20 (1 g), and lycopene (0.03 g), which were accurately weighed into a beaker. The aqueous phase contained Tween 20 (0.5 g), propylene glycol (5 g), methyl paraben (1.5 g), and distilled water (84.47 g), which were placed in a separate beaker.

Both phases were heated separately to 70–80°C. The oil phase was then added dropwise to the aqueous phase while stirring continuously using a mechanical stirrer at 2000 rpm for 15 min. Subsequently, the stirring speed was reduced to 1000 rpm for 5 min to achieve homogenization, followed by further reduction to 500 rpm for an additional 5 min. The resulting emulsion was allowed to cool to room temperature.

Control and experimental formulations were prepared using the same procedure. However, lycopene was incorporated only into the test formulation, whereas the control formulation contained an additional 0.03 g of distilled water to compensate for the absence of lycopene.[5][6]

Preparation of gel

The gel base was prepared by dispersing Carbopol 940 (1 g) in distilled water at room temperature using a mechanical stirrer operated at 1000 rpm. Stirring was continued until a uniform dispersion was obtained and all visible lumps of Carbopol were completely eliminated.

Following complete hydration and dispersion of Carbopol 940, triethanolamine was added dropwise with continuous stirring. The pH of the dispersion was monitored after each addition, and the process was continued until the pH reached the desired range of 6.0–6.5, resulting in the formation of a homogeneous gel.[7]

Incorporation of emulsion into gel

The prepared emulsion was slowly incorporated into the gel base in a 1:1 ratio under continuous stirring using a mechanical stirrer at 1000 rpm for 15 min, resulting in the formation of a uniform emulgel. The same method was employed for the preparation of both the control and lycopene-loaded formulations.
For formulation optimization, twenty-five different emulgel formulations were developed by varying the concentrations of components in the emulsion phase, including liquid paraffin and Span 20, as well as the concentration of Carbopol 940 in the gel phase. All formulations were subjected to accelerated stability testing at 40°C, 40°C ± 75% RH, and 50°C for 30 days. Following the stability study, the formulations were evaluated for physical appearance and phase separation. The formulation that demonstrated the greatest stability and satisfactory organoleptic characteristics was selected as the optimized formulation and used for further preparation of the control and test emulgels.[8]

Stability studies:
Accelerated stability studies were conducted on both the control and lycopene-loaded emulgel formulations over a period of six months. The study aimed to assess the physical stability of the formulations as well as the stability of the incorporated lycopene during storage. Stability evaluations were carried out under various temperature and humidity conditions, including 8°C, 25°C, 40°C, 40°C ± 75% relative humidity (RH), and 50°C. The formulations were periodically examined to determine any changes in their physicochemical properties and lycopene content throughout the study period.For organoleptic evaluation, emulgel was physically analyzed for any change in color, smell, liquefaction and appearance of microbial culture.[9]
Following the preparation of the emulgel formulations, centrifugation studies were performed to assess phase separation. The test was conducted initially and subsequently at predetermined sampling intervals during storage. For this purpose, 5 g of emulgel was transferred into a disposable stoppered centrifuge tube and centrifuged at 5000 rpm for 10 min at 25°C. The formulations were then examined for any signs of phase separation.[10]
The pH of freshly prepared formulations as well as samples stored under different stability conditions was measured using a calibrated digital pH meter.
A standard calibration curve of lycopene in n-hexane was constructed by measuring absorbance at 472 nm using a UV–Visible spectrophotometer. The developed analytical method was subsequently employed to determine the lycopene content in freshly prepared formulations and in samples stored under various conditions, thereby evaluating the stability of the active ingredient within the emulgel system.[11]
All evaluations were carried out on freshly prepared formulations and at predefined sampling intervals of 24 h, 48 h, 3, 7, 14, 21, 30, 45, 60, 90, 120, 150, and 180 days following storage under the specified conditions. Each measurement was performed in triplicate, and the results were expressed as mean values.[12]

Development of analytical techniques to measure lycopene in emulgel formulation
An analytical technique based on UV-visible spectroscopy was created to measure the amount of lycopene in the emulgel formulation.
The method's linear range of lycopene, precision, accuracy, specificity, and sensitivity were all confirmed. The following method was modified for the aforementioned purpose: 1 g of test emulgel was placed in a beaker, and 10 ml of ethanol was added while the gel structure was continuously stirred for 10 minutes using a magnetic stirrer. The lycopene was then extracted from the emulgel by adding 70 ml of n-hexane and stirring for an additional 10 minutes.[13] The n-hexane phase was separated, filtered, and its volume was increased to 100 milliliters using n-hexane.The n-hexane phase was separated, filtered, and its volume was increased to 100 milliliters using n-hexane. At 472 nm, the final solution's absorbance was measured. The concentration of lycopene was determined using the conventional straight line equation, and a blank solution was made in the same manner using 1 g of the control formulation.One gram of the emulgel should be weighed and combined with one hundred milliliters of an appropriate solvent, according to the author of the mentioned reference. Lastly, using a linear line equation to determine the active ingredient's concentration.[14]
The optimal course of action in this experiment was to rupture the gel structure and then extract the medication using a solvent extraction technique before determining the concentration.[14][15]

Result

3.1. Lycopene extraction

Since synthetic drugs may have a variety of adverse effects, screening plants for significant antioxidant phytoconstituents may direct the investigation of molecules with greater safety and efficacy.The process of extracting certain chemical compounds from natural sources is crucial and has been applied extensively.The process of extracting certain chemical compounds from natural sources is crucial and has been applied extensively. A large range of chemical compounds are obtained from natural products and are widely utilized in various industries, even though there are numerous synthetic processes available to prepare compounds. For example, lycopene, which is extracted from tomato fruit, is widely used as an antioxidant.The extraction process is primarily modified to extract specific substances from natural sources. For the extraction of lycopene, an economical extraction technique with a similar yield was effectively established. The stated goal was confirmed by the results.[16]

3.2. Lycopene analysis, both qualitative and quantitative

The identification test for carotenoids, kmax measurement, FTIR spectrum determination, and comparison with the spectrum of standard lycopene powder and literature were all part of the qualitative analysis of lycopene.
The identification test for carotenoids was carried out, and the outcome was stated as the color of the lycopene solution in acetone disappearing when sodium nitrate and sulfuric acid solutions were added one after the other.[17]
A kmax value of 471.8 nm was obtained from the UV scan of the lycopene solution in n-hexane.The FTIR spectra of the extracted and standard lycopene are shown in Figs. 1 and 2, respectively. Table 1 displays the prominent peaks of the functional groups of the extracted lycopene, standard lycopene. FTIR analysis was used to confirm the presence of the main functional groups in the extracted lycopene. Lycopene was authenticated by comparing the transmittance against wave number FTIR spectra of the extracted product with the spectrum obtained by scanning standard.[18] The solvent or trace amounts of any impurities could be the cause of several additional tiny peaks.Beer Lambert Law and mass measurement of the product, lycopene, were used to calculate the amount of lycopene per kilogram of tomato fruit. The results were 154.83 mg/Kg of tomato fruit (equivalent to 1.44 ~ 10^6 mol/L of lycopene in solution) and 149.8 mg/Kg of tomato fruit, respectively.
Experiments conducted both within and between days (n = 3) revealed that the yield, using both techniques, fell between 145 and 156 mg/kg of tomato.[19]

3.3. Emulgel development

Lycopene cream is available, however the main ongoing issue is the formulation's stability. Temperature stability is a major stability issue. As the temperature rises, the cream's viscosity decreases. Phase separation, creaming, and coalescence issues brought on by this decrease in viscosity result in formulation deterioration and active moiety degradation. Additionally, an increase in fluidity may lead to chemical and physical interactions between chemicals, changes in the product's appearance that make it unappealing to consumers, etc.Because lycopene is soluble in organic compounds but insoluble in water, the problem of lycopene liberation and subsequent absorption prevents the production of gel formulations.
In order to get the greatest results, a topical lycopene dose form with high stability and equivalent lycopene liberation had to be developed. The stated problem was the focus of the current study, and the findings provided evidence to address the issue. Additionally, the newly created stable emulgel was anticipated to be highly beneficial for topical lycopene delivery in the future.[20]

3.4. Lycopene emulgel stability assessed in vitro

3.4.1. Thermal stability and organoleptic assessment

With the exception of the formulation kept at 50°C, neither color nor odor had changed since the last observation. On the 60th day, the test formulation at 50 °C showed a minor color shift from orange to yellowish orange, but no particular odor was detected in any of the samples. Furthermore, until the end of the study period, no microbe growth, liquefaction, or phase separation was noted in any sample. These findings showed that the formulation remained stable even at high humidity and temperatures of 40°C and 75%, respectively.Lycopene degradation at high temperatures was shown by a color shift at 50 degrees Celsius. The formulated emulgel did not exhibit liquefaction, which is a sign of mild phase separation. In contrast to the cream formulations, which exhibit a shift in viscosity and physical state from semisolid to liquid, the centrifugation test showed a high degree of stability even at high temperatures.Phase separation brought on by the two phases' differing densities under the effect of gravity causes creaming.
By decreasing the globule size, creaming can be minimized or avoided. During the 180-day study period, no phase separation was seen in either of the control and test emulgel samples maintained at specified conditions. It demonstrates that, when creaming was taken into account as a stability criterion, all formulas were stable under all storage circumstances. It can be explained by a variety of stability variables, chief among them being the appropriate agitation speed, which reduced coalescence and stopped the formulation's components from breaking and separating.[21]

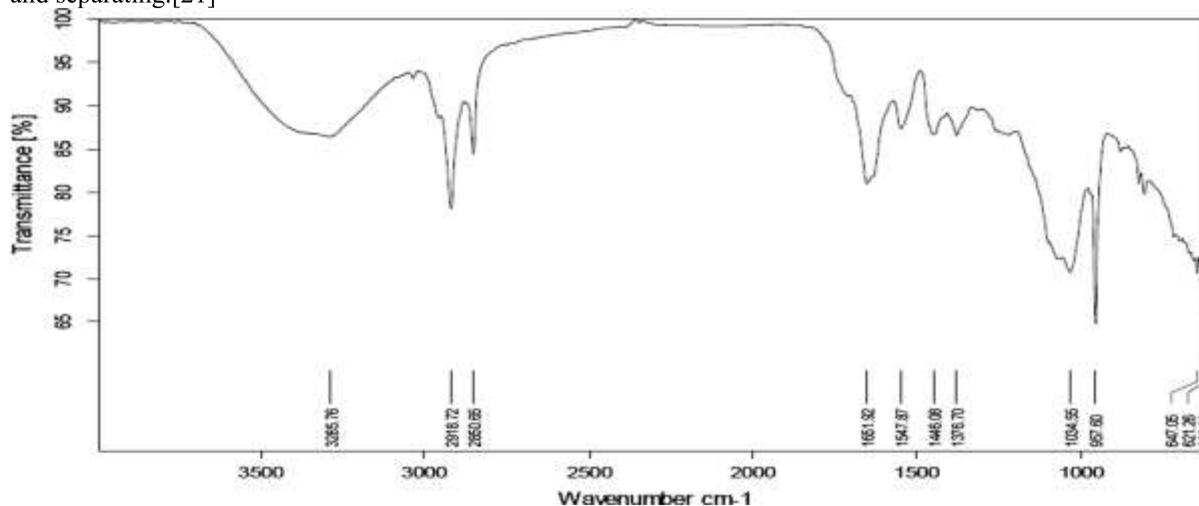

Fig.1. FTIR spectrum of standard lycopene.

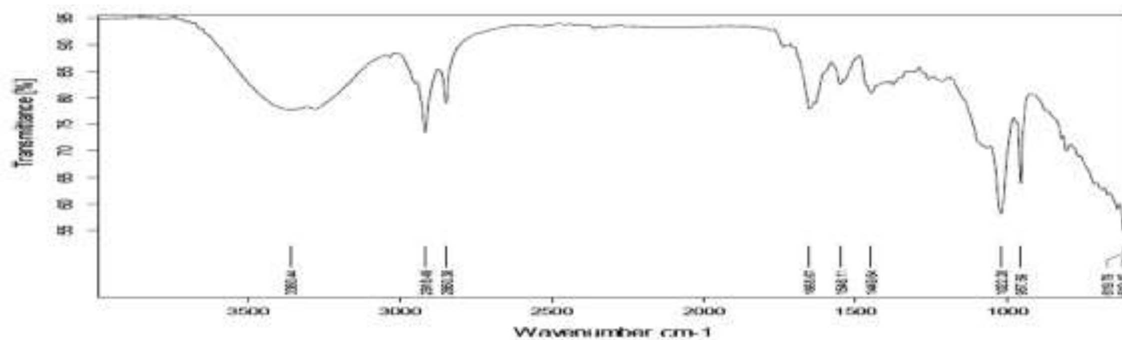

Fig.2. FTIR spectrum of extracted lycopene.

Tabel.1 Interpretation of FTIR spectra of lycopene from different sources.

Reported by Kamil et al.	**Reported by Lopez-Cervantes et al.**	**Standard Lycopene**	**Extracted Lycopene**	**Functional Group Determination**
3200–3450 cm^{-1}	3356 cm^{-1}	3285 cm^{-1}	3360 cm^{-1}	CH (Stretching)
2918 cm^{-1}	2922 cm^{-1}	2918 cm^{-1}	2918 cm^{-1}	CH (Stretching)
1643 cm^{-1}	1652 cm^{-1}	1652 cm^{-1}	1656 cm^{-1}	C=C (Stretching)
1101.07 cm^{-1}	1088 cm^{-1}	1035 cm^{-1}	1022 cm^{-1}	CH (Trans)
960 cm^{-1}	965 cm^{-1}	958 cm^{-1}	958 cm^{-1}	R–CH=CH–R

3.4.2. Test for centrifugation

An essential method for assessing and forecasting the shelf life of semisolid compositions like emulsion is centrifugation. The physical stability of semisolid formulations held at various temperatures in terms of phase separation is typically assessed using the centrifugation test (also known as stress testing) Phase separation was not evident in any of the formulations during the research period. Additionally, it served as a stability check.3.4.3. Changes in the emulgel formulation's physical properties in relation to temperature and time

The pH values of all control and test formulations, maintained at various storage settings (e.g., 8 C, 25 C, 40 C, 40 C + 75% RH, and 50 C), were monitored at varied reading intervals and plotted against time in relation to stability studies, as seen in Figs. 3 and 4.

The pH of both formulations varied between 5.49 and 5.9 over the course of the trial, according to the results.Furthermore, variation was greater at 50 °C after 45 days, and this shift was slightly greater in the test formulation than in the control, but it was still within the specified range because skin pH values vary widely, ranging from 4.0 to 7.0 (Lambers et al., 2006). This was thought to be caused by the oxidation phenomenon. In summary, the pH was not considerably impacted by the presence of lycopene.[22]

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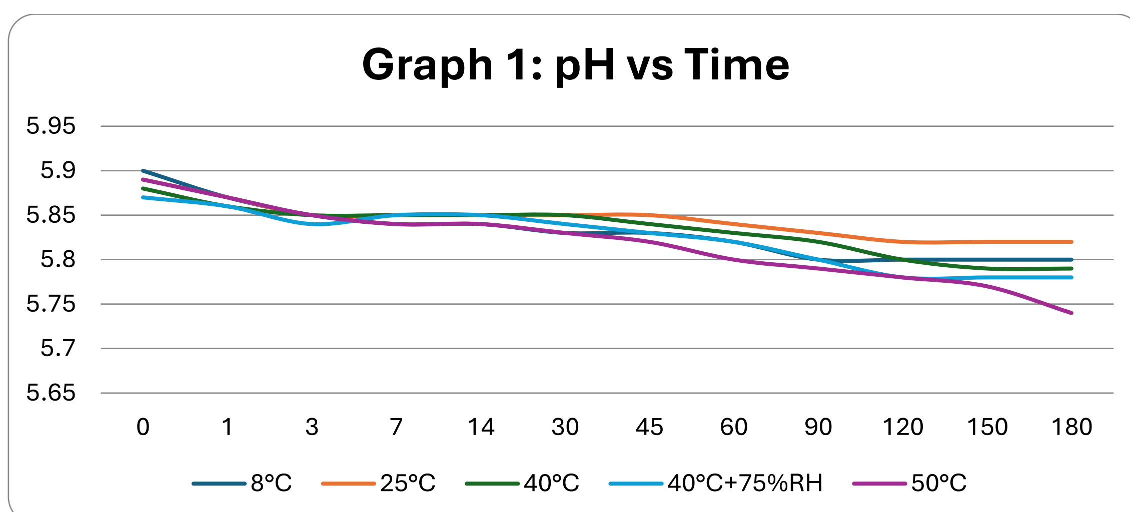

Fig. 3. Variation of pH of control formulation with respect to time kept at different storage conditions.

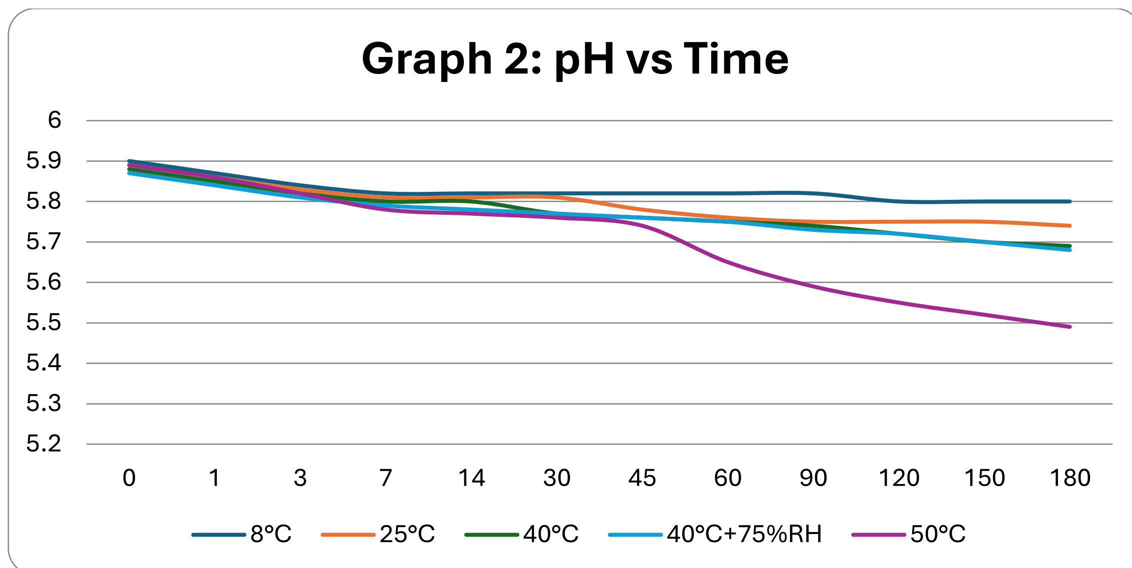

Fig. 4. Variation of pH of test formulation with respect to time kept at different storage conditions

3.5. Development of analytical methods

The lycopene standard curve, which was created at a kmax of 472 nm using n-hexane as the solvent, is displayed in Fig. 5.

The approach was validated by determining the linear range of lycopene, precision, accuracy, and sensitivity. The assay's linear lycopene range was 1–10 mg/mL. LOD (limit of detection) and LOQ (limit of quantification) were computed in order to determine the sensitivity.

LOD and LOQ formulas were 3.3 SD/b and 10 SD/b, respectively. In this case, b is the slope of the linear straight line and SD is the standard deviation of the response (absorbance). LOQ was 0.34 mg/mL and LOD was 0.11 mg/mL.

Interference studies were used to assess the method's specificity. Common metal ions and emulsion and gel formulation excipients were added to the lycopene solution as interfering agents for this purpose. Liquid paraffin, span 20, tween 20, propylene glycol, methyl paraben, carbopol 940, and triethanolamine were utilized as excipients, and Na+, K+, Ca2+, Mg2+, Al3+, Ag2+, Fe2+, Fe3+, Cu2+, and Zn2+ were frequently employed as metal ions. In every instance, the percentage change in absorbance following the addition of an interfering substance was less than 5%. Additionally, tolerated amounts of metal ions were many times higher than their anticipated concentration as contaminants, and all excipients were taken in much higher doses than those found in the formulation.As indicated in Table 2, the relative standard deviation percentage and the percentage of recovery, both intraday and interday, were calculated at the n = 3 level. All recoveries in this experiment fell between 94 and 105%, and the RSD percentage for all measurements was approximately 5.36.[23]

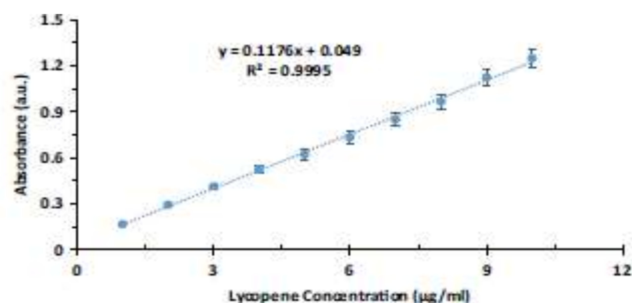

Fig. 5. Standard lycopene curve at 472 nm.

3.6. Effect of storage condtions on concentration of Lycopene in formulation.

The change in lycopene concentration in emulgel over time under various humidity and temperature settings is shown in Fig. 6.

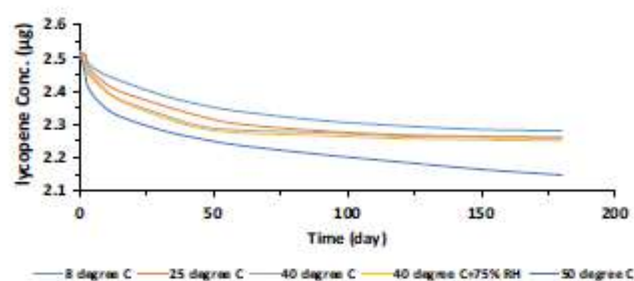

Fig. 6. Variation in lycopene concentration in emulgel with respect to time at various storage conditions.

Table 2.Precision and accuracy of the method for determination of lycopene concentration in emulgel (n = 3).

Concentration of Lycopene (μg/mL)	Interday Mean Recovery (%) ± SEM	Interday RSD (%)	Intraday Mean Recovery (%) ± SEM	Intraday RSD (%)
2	101 ± 1.15	1.99	98 ± 0.5	0.86
5	99 ± 0.47	0.80	101 ± 0.2	0.39
8	102 ± 1.5	2.56	103 ± 1.5	2.1

The figure makes it evident that the lycopene concentration variation range was narrow under all storage settings, with the exception of 50 C at day 60 and beyond. Since lycopene oxidation is temperature dependent, oxidation was expected to occur at this high temperature. Compounds that absorb light in a particular wavelength range—more precisely, the ultraviolet and visible regions—are frequently analyzed using UV analysis. Additionally, it is both a quantitative and qualitative method. Similar to other substances, a diluted lycopene solution in n-hexane exhibited maximum absorbance at 472 nm when scanned across a range of wavelengths between 200 and 800 nm. The method was designed with the intention of determining the lycopene concentration in the emulgel formulation.In order to confirm the stability of the developed product, emulgel, with respect to lycopene, the product's active ingredient, the previously indicated procedure was necessary. The effects of various environmental factors on the product's lycopene content were also investigated. The method provided a valuable capability for the specified purpose that will be beneficial for other objects with similar features.Cosmetic compositions seem stable when created, however during storage, temperature and time-dependent changes take place. All dispersed systems have the same causes of emulgel physical instability, including creaming (or sedimentation), reversible aggregation (flocculation), and/or irreversible aggregation (coalescence) of oil droplets.A product's final acceptability is contingent upon its formulation's stability and attractiveness. There is currently no sensitive and fast way to identify possible instability in an emulgel.
Stress conditions, such as temperature and centrifugation, are typically used to assess the product's stability. It is also possible to forecast stabilities using other techniques, such as rheological characteristics, electrical conductivity measurements, and/or pH testing.[24]

Null hypothesis(μo)
I. Temperature, humidity, time, and accelerated storage conditions all considerably affect the pH values of the control and test formulation.
II. The mean pH values of the control and test formulations held under accelerated temperature and humidity conditions differ significantly over time.
III. The amount of lycopene in the formulation varies greatly depending on the accelerated storage conditions and duration.
IV. The outcomes of experiments conducted both within and between days in relation to the development of analytical methods vary significantly.

Alternative hypothesis (μo)
I. With regard to storage conditions and time, there is no discernible difference in the pH values of the control and test formulations.
II. With regard to storage conditions and duration, the mean pH values of the test and control formulations do not differ appreciably.
III. At various storage temperatures and high humidity levels, the lycopene concentration in the formulation does not change noticeably over time.
IV. The results of both intra-day and inter-day studies do not differ significantly.
Two-way ANOVA (analysis of variance) and Post Hoc tools at the 5% significance level were used to test the first statement of the null hypothesis, which states that the pH values of the control and test formulation differ significantly with respect to accelerated storage conditions, temperature and humidity, and time. With the exception of 50 °C and time periods of the 60th day and later, where a significant fluctuation in pH values was found for the lycopene formulation, "P-values" for both control and test formulations with regard to storage conditions and time were <0.05.The mean pH values of the control and test formulations held at various accelerated conditions of temperature and humidity with respect to time were compared using a paired sample t-test at the 5% level of significance. It was the second null hypothesis statement.[25]
With the exception of 50 °C and time points on the 60th day and beyond, the "P-values" computed for various levels of these parameters varied, although the change was not statistically significant as $p < 0.05$.This confirmed that, save from 50 C at time points 60 days and later, the inclusion of lycopene had no discernible impact on the formulation's pH.
The stability of the active moiety in emulgel was the subject of the third null hypothesis assertion. For this, two-way ANOVA and post-hoc tests were run at a 5% significant level. The results of the statistical analysis confirmed that, with the exception of the time level of the 60th day and subsequent at 50 C, there is no significant difference in the lycopene content in the formulation at nearly all levels of all parameters, $p < 0.05$.These findings demonstrated that the lycopene formulation remained entirely stable over the course of the six-month trial period, even at 40°C and 75% relative humidity. LSD calculations showed $p > 0.05$ for temperatures of 50 °C at time points 60 days and later. Therefore, at these levels, the lycopene concentration in emulgel varied considerably.

The t-test was used to examine the final claim of the null hypothesis at a 95% confidence level. The fourth assertion of the null hypothesis was rejected since the p-values were less than 0.05, confirming that there were no significant differences between the findings of the intra-day and inter-day studies.[26]

4. Conclusion

The analysis's findings showed that the extraction method was straightforward in terms of tools, processes, labor, and time. Additionally, the yield was equivalent to existing approaches and it was cost-effective. This effort resulted in the effective development of an O/W based stable emulgel that contained lycopene, a carotenoid derived from Solanum lycopersicum L. Even at a high temperature of 40 °C and a relative humidity of 75%, the produced formulation remained stable in terms of phase separation and active moiety. Additionally, the analytical technique created for lycopene emulgel analysis was sensitive, repeatable, and specific enough to be used to determine the amount of lycopene in emulgel formulation

Conflict of interest:

Neither this experiment nor any aspect of this publication contains any conflicts of interest.

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