Permeability Enhancement To Treat Atopic Dermatitis: A Research

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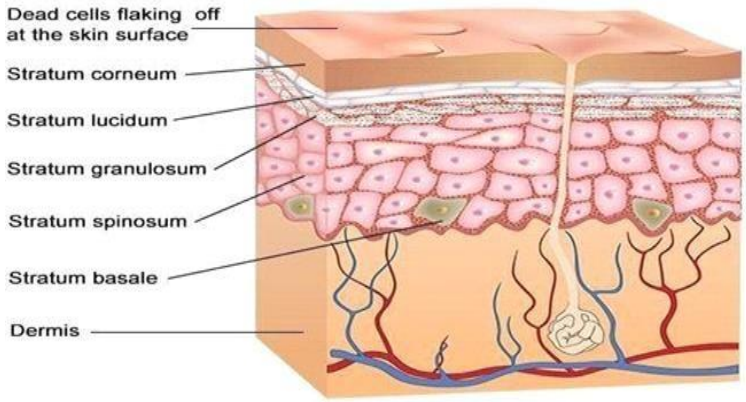
Abstract:
The skin's main function is to protect the body from the environment. Nonetheless, the skin might become inflamed as a result of a variety of reasons such as hereditary, environmental or hormonal effects when the body's defensive mechanisms are overtaxed, it produces more Skin conditions have been associated with reactive oxygen species (ROS) and pro- inflammatory mediators. Studies on several organic compounds with antioxidant and anti- inflammatory properties are being studied right now as possible therapies for various inflammatory disorders. Nature offers a plethora of materials for the development of various pharmacological medicines.Topical therapies are often prioritised in the treatment of skin problems administration in order to minimise systemic adverse effects and limit medication degradation via first-pass metabolism. As a result, the characteristics of medication delivery vehicles might either help or hinder drug penetration through the skin. In light of the skin's hydrophobicity, Nanoparticles made of lipids called nanostructured lipid carriers (NLC), are a viable method for improving medication administration into the dermal layers. This review's objective is to introduce NLC as a beneficial tool for improving the delivery of natural anti-inflammatory compounds to the skin.

Keyword: natural products, ROS, degradation, hydrophobic, strategy, nanostructured lipid carriers

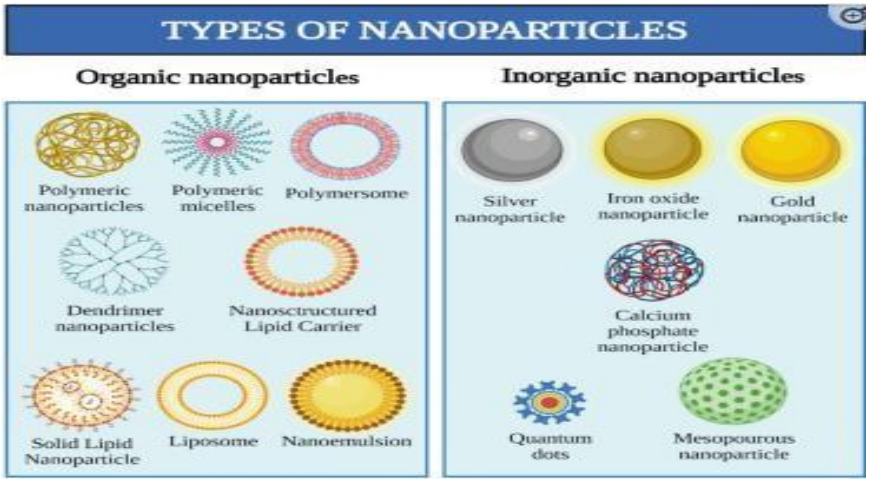
Introduction:
Skin illnesses are common across the globe, affecting a huge percentage of the population. They include disorders such as melanoma, intraepithelial carcinoma, Squamous cell cancer (SCC), in addition to basal cell carcinoma (BCC) [1]. Skin tumours, in particular, has become a frequent malignancy in the United States, with studies showing that one in every five Americans may during the course of their lifespan [2, 3]. When compared to other kinds of skin cancer, melanoma, the most serious form, has a fatality rate of 1.62% [4]. According to the American Cancer Society, there will be around 100,350 new cases of melanoma in the nation in 2020, with 6,850 deaths [5]. Despite being the most common kind of skin cancer, BCC often has a favourable prognosis and places a major burden on healthcare systems [6]. Fortunately, skin cancer may lead to early identification and treatment in an approximate 14%improvement in five-year survival [7]. However, correctly detecting skin problems is a challenging task since it requires considering a range of visual cues, such as the presence of lesions, their location on the body, colour, scale, and arrangement [8].

The skin is made up of the epidermis, dermis, and hypodermis, the body's biggest organ. It has three basic functions: protection, sensibility, and thermoregulation, making it an effective environmental defence. The stratum corneum, the epidermis's outermost layer, serves as an optically neutral protective barrier of varied thickness. It is made up of keratinocytes, which create the protein keratin, which is important for improving skin protection. On the skin, the stratum corneum scatters incident light. Melanin, the pigment that gives the skin its tan or brown colour, is produced by Melanocytes are found in the epidermis' basal layer. Melanocytes multiply and act as filters their production of melanin in response to sun exposure, shielding the skin from UV rays. The amount of UV absorption relies on the quantity of melanocytes, but aberrant melanocyte growth may lead to melanoma. The dermis, or top layer of skin, is composed of collagen fibres, sensory receptors, blood vessels, and nerve endings, which provide flexibility and durability to the skin [9].



Patients with atopic dermatitis have a weakened skin barrier, making them more vulnerable to xerosis as well as environmental allergens and irritants. These variables may cause inflammation, itching, and other atopic dermatitis-related symptoms. Reduced ceramide levels may contribute to the skin barrier deficit. Sphingolipids called ceramides, which are found in the stratum corneum, are crucial for maintaining limiting excessive transepidermal water loss and protecting the skin's barrier properties. Inflammators and allergens may penetrate the skin due to this compromised barrier, which results in an overactive Th2 response in acute lesions (as shown by increased IL-4 and IL-5 cytokines) and a Th1 response in chronic lesions (as indicated by IFN-gamma and IL-12). Keratinocytes release as a consequence of scratching the skin, TNF-alpha, IL-1, and IL-6 are among the inflammatory cytokines that are produced. The high frequency of Staphylococcus aureus colonisation, which is observed in over 90% of atopic dermatitis patients, is also attributed to lower levels of antimicrobial peptides including The epidermis of atopic patients contains human beta- defensins and cathelicidins. Secondary infections may be brought on by S. aureus and impetiginization in atopic dermatitis lesions [10-11].

Nanosystems have become more diverse during the last century. To create superior formulations, organic and inorganic nanomaterials are often mixed for the administration of targeted medications. The picture depicts some of the well-known nanoparticles classified by their basic elements [12]. Regardless of the material used, there are several general benefits associated with better drug solubility, targeted drug delivery, increased bioavailability, protection against deterioration and decreased activation of the reticuloendothelial system, and nanoencapsulation of substances are all benefits [13].



Nanostructured Lipid Carriers

In contrast to SLNs, nanostructured lipid carriers are made of lipid blends, which combine a liquid and a solid lipid, and do not crystallize upon cooling. 70:30 to 99:0.14 is the ideal lipid mix ratio. This mixture, like SLNs, has a solid state at body temperature **[14].** In comparison to SLNs, NLCs may be advantageous since their drug-loading capacity is improved because to the incorporation of liquid lipids, which results in an uneven crystal structure. This seals the container, keeping the drugs within.

It is possible to improve the solubility of hydrophobic drugs in NLCs due to the fact that a greater quantity of the medication is soluble in a liquid lipid as opposed to a solid lipid **[15-16].**

The stratum corneum may keep close contact with the lipid particles due to their tiny size, which enhances the entry of medicine into the mucosa or skin as well as the hydration and flexibility of the skin.

Quercetin, a flavonoid with the greatest antioxidant activity, is well-known for its extraordinary antioxidant capabilities. It also has other pharmacological properties, including anti-inflammatory potential [17]. In a 2012 research, Chen-Yu

and colleagues found that The quantity of quercetin retained in the skin and skin permeability may be increased by nanostructured lipid carriers (NLC) as compared to a quercetin solution containing polyethylene glycol. Particularly, the NLC system improved the anti-inflammatory benefits of quercetin, suggesting that it might be used to treat inflammatory diseases [17].Bose and Michniak-Kohn produced another combination incorporating NLC and quercetin in 2013, with encouraging results for topical administration. When compared to Solid Lipid Nanoparticle (SLN) formulations, the NLC system delivered the most quercetin, as measured by the amount of quercetin retained in the skin.**[18]**.

Methods and materials:

List of chemicals used:

Materials used in the study were purchased in India only.

OttoChemie Pvt. Ltd., Mumbai, India, provided the quercetin.

Quercetin, Soya lecithin, Premium Spanish extra virginolive oil,Cholesterol, Carbopol934, Dialysis membrane, Deionized water, Triethanolamine.

List of instruments: Brook field viscometer, Refrigerator, Dissolution apparatus, pH-meter, Probesonicators, Homogenizer mixture, Thermometer, Centrifuge, UV-visible Spectrophotometer, Digital Weighing balance, Magneticstirrer, Zeta Sizer.

Methods Preformulation studies

Preformulation studies are essential to produce dosage forms that are safe, effective, and stable. Some pre-formulation parameters were evaluated to gather preliminary information about Quercetin.

Calibration Curve

To prepare the standard stock solution, In 100 cc of acetonitrile, 100 milligrammes of quercetin were dissolved. To get samples, this solution was diluted repeatedly. of 2g, 4g, 6g, 8g, and 10g concentration. At 364 nm, materials were spectrophotometrically analysed. The operation was repeated three times.

Partition coefficient

The dispersion of the medication is measured by the partition coefficient between organic and aqueous phases, was calculated using an n-octanol: water system by shake flask method. The difference between initial drug additions and organic phase concentration was used to calculate the aqueous phase concentration**[19]**.

PO/W= Concentration in the organic phase/Concentration in the aqueous phase

Log Po/w=$\log(\text{Solute}_{octanol}/\text{Solute}_{water})$

Screening of Ingredient

Excessive medicine is added to 100 µl of lipid/oil mixtures, vortexed, shaken, centrifuged, and spectrophotometrically examined at 364 nm.

Procedure for the method of preparation of Quercetin NLC

Previously, a batch as large as 20 mL was made and then scaled up to a 500 mL batch. In response to particle size, NLC was optimized regarding lipid quantity, surfactant concentration, and stirring rate. Here is a brief explanation of the optimized batch approach. Separate preparations for the aqueous and lipid phases were made.

Drug content

The NLC gel solution was centrifuged at 14000 revolutions per minute for 30 minutes after being filtered using a 0.22 µm membrane filter. The NLC gel was then diluted with water (X100), and the mixture's drug content was ascertained. Utilising a UV-Vis spectrophotometer, the medication combination was identified by calculating the 364 nm absorbance and contrasting it with the standard curve built for the actual drug concentration. **[20]**.

Evaluation of polydispersity index and particle size

The drug concentration of the resulting combination was then assessed after the NLC gel had been diluted with water. A UV-Vis spectrophotometer measured the absorbance at 364 nm and compared the results to determine the drug combination it to a standard curve produced for the actual drug concentration**[21]**.

Entrapment efficiency

By using the dialysis method to separate un-entrapped pharmaceuticals, encapsulation efficiency (EE) was calculated. Removing the clear supernatant, A Remi R-8C centrifuge wasused to spin 1 mL of newly manufactured NLC dispersion at 13000 rpm for 20 minutes, and quercetin concentration was determined using a UV spectrophotometer. The EE of NLCs inside of NLCs was calculated using the following formulae.**[22].**

$$\% \text{ Entrapment efficiency} = (\text{Total drug} - \text{Free drug}/\text{Total drug}) \times 100$$

Transformation of Quercetin loaded NLC in to gel
Dispersing 1.0% w/w Carbopol 934 into optimised Nanostructured Lipid Carrier (NLC) formulations resulted in the optimised gel. The dispersion was stirred at 500 rpm for 30 minutes using a mechanical stirrer (Remi IKAR W20), then gradually neutralised with triethanolamine (TEA) [23]. The gel was then placed in a refrigerator for later usage after being sonicated (Lark DP120) and centrifuged at 5000 rpm (Remi R-C8) for 10 minutes to remove air bubbles that had been trapped.**[24].**

Characterization of gel formulationPhysical examination
The prepared gel formulation was inspected visually for its colour, transparency,and smoothness.

Transparency & smoothness
The test tube was filled with the gel (5 g), which was then visually inspected to determine its transparency. To check the homogeneity, roughness, and smoothness of thegel, it was rubbed between the fingers.

Determination of pH
A 10% (w/v) NLC gel solution in distilled water was produced for pH testing. The pH values were determined using a digital pH metre that has previously been calibrated using buffer solutions with pH values of 4, 7, and 10. The pH measurements were carried out three times at 25°C.

Viscosity Measurement
The R/S Plus rheometer was used to determine the Quercetin-loaded NLC gel's degree of viscosity (Brookfield Engineering laboratories). The experimental formulation's viscosity was evaluated at a temperature of 25±1 ºC at a speed of ten revolutions per minute with spindle number C75. All of the measurements were carried out three times.

Texture examination
The texture of the optimized gel was analyzed using the TA10 and TA15 100 TABT kit by the automated CT3 Texture Analyzer (Brookfield Engineering Laboratories, USA) [25]. Evaluations were made of the topical gel's stiffness, spreadability, and adhesiveness. using the suggested characteristics listed elsewhere in the supplemental materials. The texture analysis was performed three times.

***In-vitro* drug release study**
Using a USP dissolving test equipment fitted with Using a 200 mL glass jar and enhancer cell, the in-vitro release of quercetin from drug solution and NLCs gel was studied. In enhancer cells, Quercetin solution and NLC Gel containing 2 mg of Quercetin were packed via a dialysis membrane with a MWCO of 12 KDa. After the enhancer cell had been built, it was placed into the jar and agitated constantly at 50 rpm and 32.5 °C.

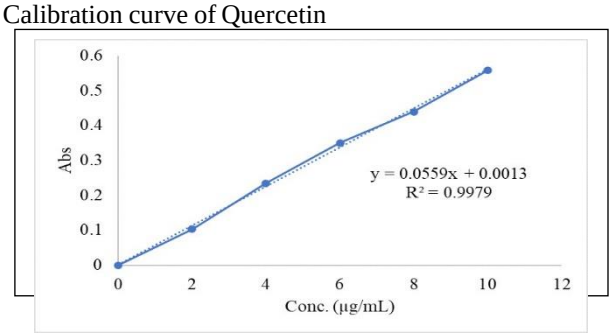
Stability Study
An accelerated stability study was done over the course of 3 months to find out how environmental stress affects the physical properties of a gel made from Quercetin NLC. The pH, viscosity, texture, and appearance of the NLC gel were measured at 25°C ± 2°C/60% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH to see how stable it was.

RESULTS:
To produce a Quercetin-loaded nano-structured lipid carriers-based topical gel for atopic dermatitis, the emulsification-cum-solidification process was utilized. This gel has beendescribed and pharmaceutically evaluated in terms of the *in-vitro* procedure.

Pre-formulation study
Calibration curve

Table 9. Calibration curve of Quercetin

Conc. (µg/mL)	Abs
0	0
2	0.1031
4	0.2347
6	0.3501
8	0.4395
10	0.5578



Partition coefficient:

Reported Log P	Observed Log P
1.82(at 25ºC)	1.79±0.14(at 25ºC)

(Values are reported as mean ± S.D., n=3)

Preparation of NLCs:

Soy lecithin and Olive oil (1:2/solid lipid and liquid lipid) (10% w/w), cholesterol (5% w/w) (stabilizer), and Quercetin (2% w/w) were dissolved in 1 mL of a 1:1 mixture of chloroform and methanol. Methylparaben (0.1 % w/w) was added to double-distilled water. The water phase was then added drop by drop to the organic phase and mixed for 20 minutes at 12000 rpm (IKA T25 digital Ultra Turrax, India) with a high-speed homogenizer. The mixing vessel was kept at a cooler temperature (almost 25 °C) using an ice jacket. NLC was discovered as amixture of particles that were placed in a jar with a tight lid for further investigation.

Characterization of the developed NLCs

High-speed homogenization has been used to prepare NLCs of Quercetin, which have been well-studied for size, surface charge, homogeneity, effectiveness in loading and entrapping, and stability. The NLC that was made was found to have a particle size of 213.9±17.28 nm and a PDI of 0.311±0.071. This demonstrates that the carriers are of the same size and are in the nanometer range. NLC dispersion in water has a refractive index at 25 degrees of1.3328±0.03 and a viscosity of 0.7698±0.076 cPs. A smaller PDI means that the particles are all the same size, which is needed for the particles to be more stable when stored. These carriers' spectrum of nanosizes is ideal for topical application.

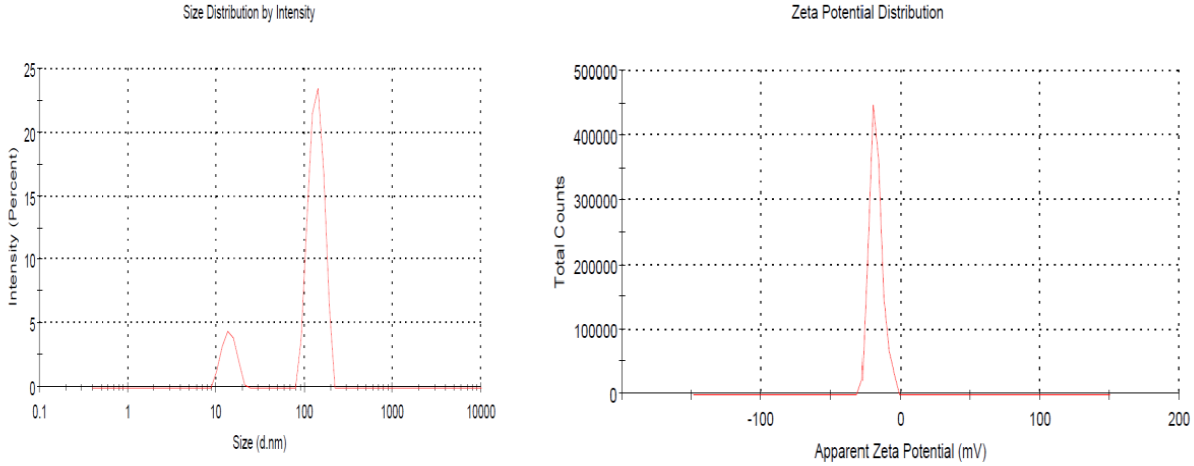


Figure 8: Particle size distribution of NLCs& Zeta potential of NLCs Characterization of formulation:

S.No	Parameter	Result
1-	Entrapment Efficiency	88.33±0.33 %
2-	Viscosity analysis	5011.33 ± 39.6
3-	pH	5.30 ±0.32
4-	*In-vitro* drug releasePure drug	89.90± 2.96 % in 12 hours 23.90±1.98 %

Development of NLC gel

Table: Optimization of NLC gel batches

Batches	Carbopol-934	TEA	consistency
1	0.8	0.3	Loose
2	0.8	0.4	Loose
3	0.8	0.3	Loose
4	1.0	0.4	Loose
5	1.0	0.3	Loose
6	1.0	0.4	Good
7	**1.2**	**0.3**	**Good**
8	1.2	0.4	hard
9	1.4	0.4	hard
10	1.4	0.4	hard

Parameters	Results (Mean±SD)

Hardness/Firmness (g)	32.56 ± 1.53
Spreadability (mJ)	2.20 ± 0.02
Extrudability (mJ)	115.4 ± 2.55
Adhesiveness (mJ)	2.01 ± 0.17

Table: Texture data of Quercetin NLC gel with the help of CT3 Brookfield Texture Analyzer

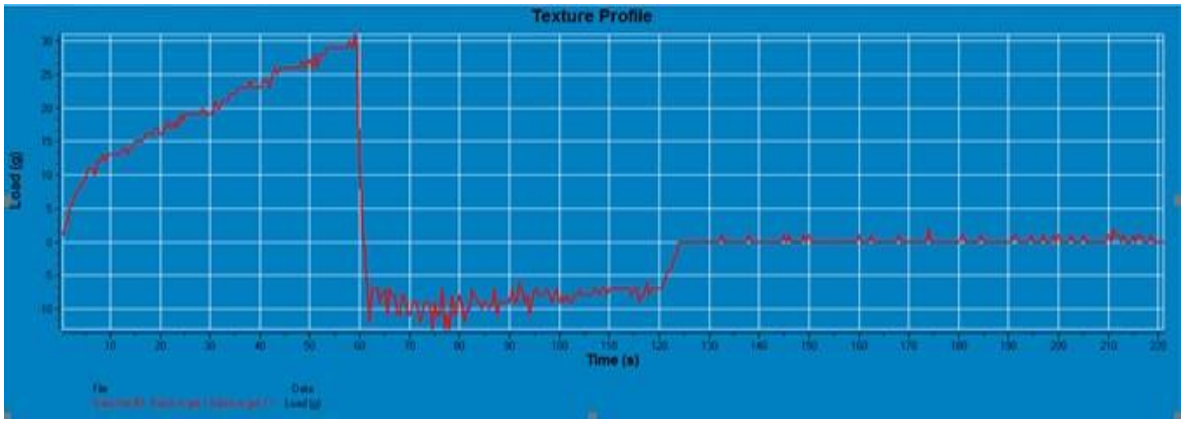


Figure 10: Texture profile of Quercetin NLC gel as hardness/firmness, adhesiveness, adhesive force

Stability Study

Table 13. Stability Study data of different formulations.

Parameters		**25°C±2°C/60%RH± 5%RH**				**40°C±2°C/75% RH±5%RH**			
		0thday	30thday	60thday	90thday	0thday	30thday	60thday	90thday
pH		5.30 ±0.32	5.39±0.18	5.38±0.28	5.39±0.12	5.36±0.28	5.37±0.18	5.39±0.27	5.31±0.1
Viscosity(CP)		5056.33 ±56.60	5095.66 ±15.82	5095± 71.84	5046.66 ±81.14	5056.33 ±56.6	5011± 98.5	5065± 56.31	5048± 54.68
Spread-ability(g)		2.20±0.02	2.21±0.2	2.19±0.15	2.18±0.15	2.20±0.02	2.21±0.1	2.21±0.05	2.22±0.55
Visual appearance	Colour	+++	+++	+++	+++	+++	+++	+++	+++
	Odour	+++	+++	+++	+++	+++	+++	+++	+++
	Texture	+++	+++	+++	+++	+++	+++	++	+++

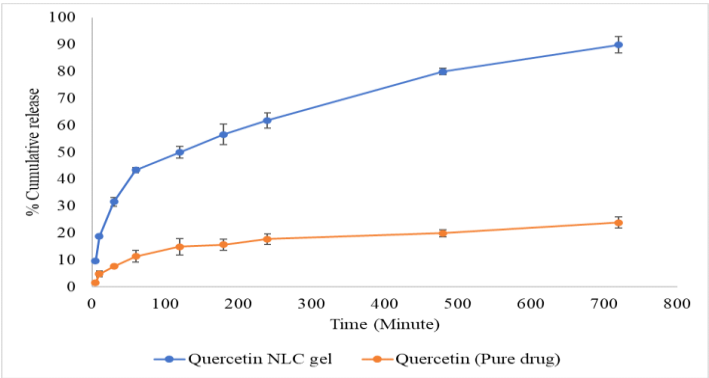


Figure 11: *In-vitro* release of Quercetin-loaded NLC gel

Conclusion:

Traditional medication delivery techniques are significantly outperformed by nanostructured quercetin particles. These enhanced delivery formulations not only solve issues relating to quercetin's low solubility, bioavailability, and stability, but also overcome the skin's strong barrier in the context of topical application. The use of nanomedicine and nano-drug delivery devices is a popular development. Because of its protective qualities against oxidative stress and inflammation, quercetin, an important endogenous antioxidant, is widely used in cosmetics, nutraceuticals, and pharmaceutical products for skincare and therapeutic reasons. Despite quercetin's intrinsic poor hydrophilicity and restricted percutaneous absorption, novel techniques to overcome these limitations have been devised. Among the many approaches, nanoparticle-based drug delivery systems have received considerable attention and exhibited several benefits for topically delivering a hydrophobic molecule such as quercetin. Human trials, however, are required for these formulations to be clinically useful. In-depth research and dosage optimisation may pave the path for new

quercetin carriersystems developed for topical treatment to be commercially successful.

I have shown in my project how quercetin can improve skin permeability by testing different parameters like pH, calibration curve, stability, entrapment efficiency, viscosity etc.

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