

Original Article

Development and characterization of ethosomes based gel formulation for enhanced topical delivery

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ABSTRACT

The present research work aims at development and evaluation of diclofenac loaded ethosomal gel. The innumerable adverse effects due to oral use of diclofenac impelled to develop topical drug loaded ethosomal gel for better patient compliance and reduced adverse effects. The ethosomes were prepared by hot method using different ratios of polymers, ethanol and propylene glycol. The prepared ethosomes were characterized by morphological studies, entrapment efficiency and zeta potential. The vesicle size and entrapment efficiency varied from 180 to 930 nm and 38% to 90% respectively. The gel was prepared by incorporating ethosomal dispersions containing drug into the Carbopol gel under mechanical stirring. Gel formulations were characterized for pH determination, spreadability, drug content, viscosity measurement, and *in vitro* drug diffusion studies. FT-IR and stability studies of the prepared formulation were also investigated. FT-IR studies confirmed that there was no chemical interaction between drug and excipients used in the formulation. Formulation F8 with maximum drug content of 9.98 % and drug release of 82.13 % was selected as best formulation. From this research work it can be deduced that topical ethosomal gel formulation is a promising system for excellent percutaneous absorption of drug.

1. INTRODUCTION

Diclofenac sodium is a nonsteroidal anti-inflammatory drug (NSAIDs) used in treatment of rheumatoid arthritis [1]. It has a short half-life in plasma (2 hrs) and only 50% of the drug reaches the circulation. Similar to other NSAIDs, oral administration of this drug is also associated with severe gastrointestinal side effects like ulceration and gastro intestinal bleeding [2]. The above problem can be resolved by transdermal delivery of NSAIDs. Although transdermal route has high patient compliance due to non-invasive method of application, constant systemic drug level, but penetration through transdermal route always remained an area of concern [3, 4].

Ethosomes are a relative novel approach, which is specially associated with the advancement in the field of transdermal drug delivery systems [5]. Skin, a barrier for topical/transdermal drug

delivery systems offer various advantages but because of its low drug diffusion rate across the stratum corneum, it forms a major obstacle in designing the transdermal drug delivery systems [6]. In this context, Ethosomes plays an important role in delivering the drug's to the targeted site by permeating through skin.

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation [7]. Ethosomes are soft lipid vesicles containing phospholipids, alcohol in relatively high concentration and water. They are elastic in nature due to the presence of ethanol in the bilayer structure [8].

The objective of the present study was to develop diclofenac loaded ethosomal gels for better anti-inflammatory activity by improving permeation and sustaining the drug release.

2. EXPERIMENTAL

Diclofenac sodium was received as gift sample from Ranbaxy. Soy phosphatidylcholine was purchased from American lecithin, Mumbai. Ethanol, Sodium bi carbonate, Hydrochloric acid was purchased from Rankam laboratory, Mumbai. All the materials used in this study were of analytical and pharmaceutical grade.

2.1 Drug excipient compatibility studies

The compatibility between drug and polymers was detected by IR spectra. FTIR analysis was carried out on Corporation, Japan in the region of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and the data was analysed [9-11].

Table 1. Variable composition of Ethosomal formulations

Formulation Code	Ethanol	Polymer Carbopol	Propylene Glycol	Phospho lipid	Soya Phosphate dlycholine	Drug	Distilled water (v/v)
F1	20	2	10	2	2	1	q.s
F2	30	3	10	3	2	1	q.s
F3	40	4	10	4	3	1	q.s
F4	20	2	10	2	2	1	q.s
F5	30	4	10	3	2	1	q.s
F6	40	1	10	4	3	1	q.s
F7	20	2	10	2	3	1	q.s
F8	30	1	10	3	2	1	q.s
F9	40	2	10	4	2	1	q.s

2.3 Characterization of Ethosomes

2.3.1 Vesicular shape and surface morphology

Surface morphology of ethosomes was determined by using Scanning Electron Microscopy (Hitachi S-3700N). SEM gives a three dimensional image of the globules [15, 16].

2.3.2 Drug entrapment efficiency

The total volume of the ethosomal suspension was measured. 5 ml of this formulation was diluted with distilled water up to 8 ml and centrifuged at 15,000 rpm for 45 min at 40 °C using a cooling centrifuge. After centrifugation, the supernatant and sediment were recovered, their volume was measured. Then sediment was analysed using n- propanol and filtered through a 0.45 μm nylon disk filter. The concentration of Diclofenac in the supernatant and sediment was analyzed by UV- spectroscopic method 274 nm [17, 18].

2.3.3 Zeta Potential

The zeta potential of the ethosomes was determined using zeta sizer (HORIBA SZ-100). Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

2.2 Preparation of Diclofenac ethosomal gel

Ethosomes were prepared by hot method using different lipids like soya phosphatidyl choline and phospholipid. Lipids and cholesterol in different ratios as shown in Table 1 were accurately weighed and dissolved in water and kept for stirring using magnetic stirrer for 30 min with heating at 40°C. Organic phase comprising of specified amount of Etoricoxib added to ethanol followed addition of propylene glycol was kept for stirring separately. Then, lipid solution was added drop by drop to the organic phase with continuous stirring on a magnetic stirrer for 1 hr. The solution was subjected to sonication using probe sonicator for 15 min to reduce the vesicle size. The gel formulations were prepared incorporating vesicular dispersions containing drug into the Carbopol gel under mechanical stirring [12-14].

2.4 Evaluation of ethosomal gels

2.4.1 Physical evaluation

All the formulations of diclofenac sodium were evaluated for organoleptic characteristics, occlusiveness and wash ability [19].

2.4.2 Measurement of pH

The pH of the formulated gels were determined using digital pH meter. The electrode was immersed in the gel and readings were recorded from pH meter [20].

2.4.3 Viscosity study

Viscosity measurements were done on Brookfield viscometer (Model-RVT, serial no-107392) by selecting suitable spindle number and rpm. 50 gm of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor. The procedure was repeated three times and observations are recorded as mean [21, 22].

2.4.4 Spread ability

A sample of 0.1 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. Diameters of spreader circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations [23].

2.4.5 Homogeneity and grittiness

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates. Also, the homogeneity can be detected when a small quantity of the gel is rubbed on the skin of the back of the hand. The grittiness of prepared gel is also observed in the same manner.

2.4.6 Extrudability study

The extrudability of gel formulations were determined by filling gel in the collapsible tubes. The extrudability was determined in terms of weight in grams required to extrude a 0.5 cm. ribbon of gel

2.4.7 Drug content

A specific quantity (100 mg) of developed gel and marketed gel were taken and dissolved in 100ml of phosphate buffer of pH 6.8. The volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at 276.0 nm using phosphate buffer (pH 6.8) as blank.

2.4.8 In-vitro drug release studies

In-vitro release studies were carried out using Franz diffusion cell containing two compartments (cells). Upper one is donor (diffusion) cell, consisting of two open ends and lower one is receptor cell, with one open end. One end of the donor compartment was covered with Himedia dialysis membrane, which was previously soaked in warm water and placed on the receptor compartment. The receptor cell contained a small magnetic bead and was rotated at a constant speed and the temperature was adjusted to 37 ± 0.5 °C. Samples were withdrawn at periodic intervals for 8 hours and replaced with fresh buffer solution to maintain sink conditions. The drug content was analyzed using UV-Visible spectrophotometer using phosphate buffer (pH 7.4) as blank at 276 nm [24, 25].

2.4.9 Stability studies

Stability of the optimized gel was performed at $5 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$ and $25 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ at different time intervals (0–3 months). The gel was observed after each week for possible change in colour, odour, consistency, pH, viscosity, spreadability and extrudability.

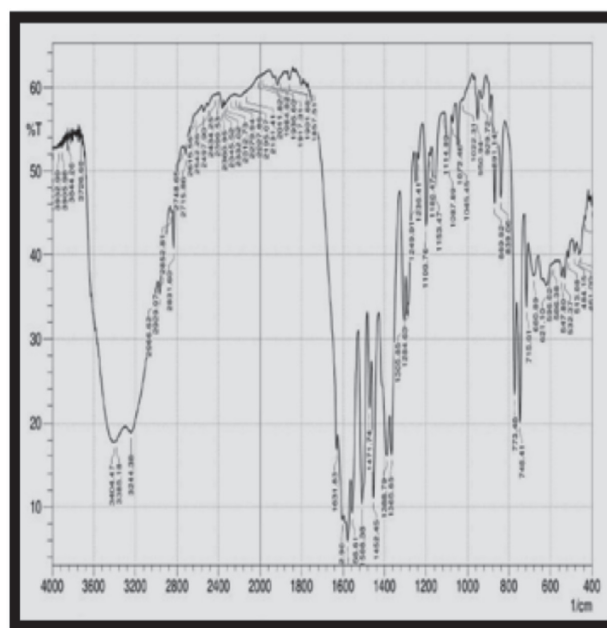
3. RESULT AND DISCUSSION

3.1 Drug polymer interaction studies

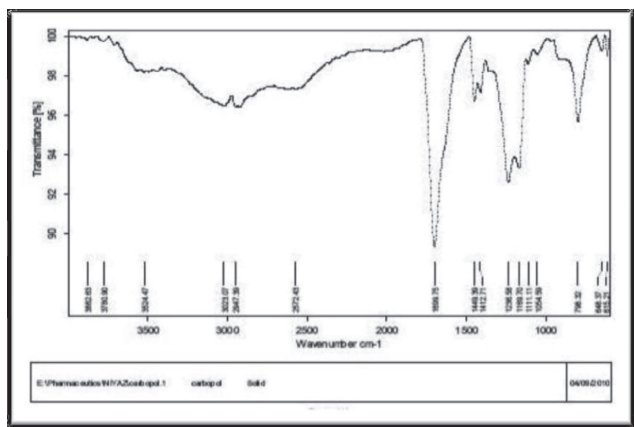
The FTIR Spectrum of Diclofenac sample recorded by FTIR spectrum is shown in Figure 1(a) which was compared with standard functional group. Group frequencies of Diclofenac are shown in Table 2. These characteristics peaks are useful in identification of drug. FTIR of propylene paraben and physical mixture in Figure 1 (b) and (c) respectively. There was no significant change in the peaks of pure drug and drug polymer mixture. Therefore, it can be inferred that there is no specific interaction was observed between the drug and the polymers used in the formulations.

Table 2. Comparison between peaks obtained in drug and in a mixture

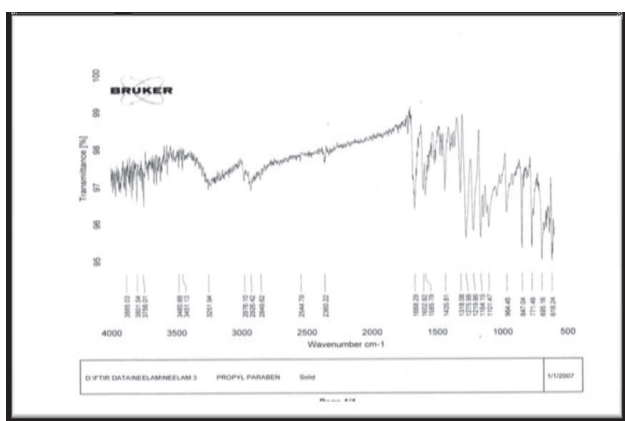
Peak obtained in drug (frequency cm^{-1})	Functional group	Peak obtained in mixture (frequency cm^{-1})
3424.38	OH stretching	3421.36
2817.36	CH_2 stretching	2880.41
3013.20	CH (Aromatic stretching)	3298.95
1616.15	C=N Stretching	1616.21
1456.80	CH (Aromatic binding)	1456.20
868.75	C–F stretch	876.49



(A)



(B)



(C)

Fig. 1. FT-IR spectra: (A) Drug (B) Propylene paraben and (C) Physical Mixture

3.2 Characterization of Ethosomes

3.2.1 Vesicular shape and surface morphology

Figure 2 depicts spherical, uni-lamellar vesicles with smooth surface were observed under scanning electron microscopy (SEM).

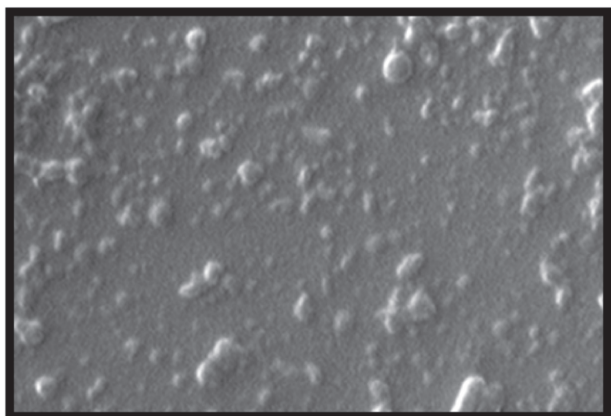


Fig. 2. Scanning electron microscope image of diclofenac ethosomes

3.2.2 Drug entrapment efficiency

Entrapment efficiency of Diclofenac ethosomal formulation was high and independent of processing parameters. Under different phospholipids and ethanol added, the entrapment efficiency was between 38% to 90% (Table 3). Therefore, based on high % entrapment efficiency batch F8 as compare to other batches were chosen for the further studies.

3.2.3 Zeta potential of optimized formulation

Figure 3 depicts the Zeta potential graph of the ethosomal gel dispersion. The value was found to be -4.61 which indicate that ethosomes were stable and presence of the drug cause diminution of surface charge of the investigation sample because probably a share of drug is situated on the lipid particle.

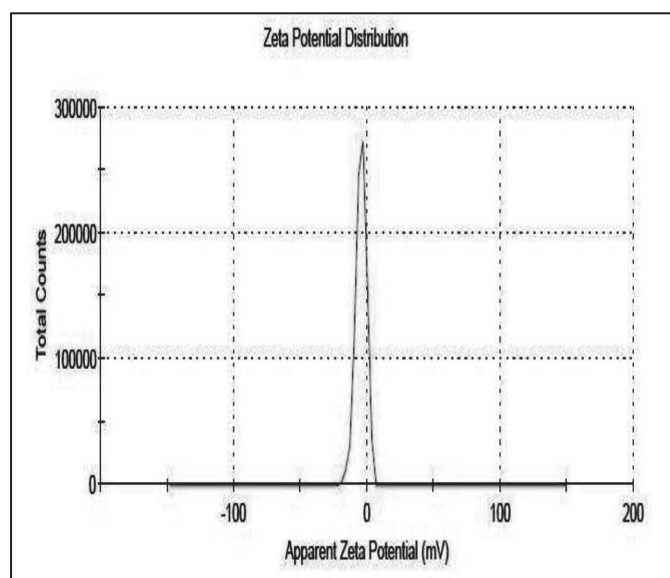


Fig. 3. Zeta potential of optimized formulation

Measurement result Zeta potential: -4.61(mv), Dopfer:4.7, Mobility:3.638e-005(cm²/vs), Base Fequency: 235, Conductivity: 0.8715 (ms/cm)

3.2.4 Particle size

Figure 4 depicts the size of Ethosomal formulation were found tens to a few hundreds of nano-meters. The milky dispersion typically had PDI values above 0.25 indicative of broad size distributions and typical broad size distribution of the ethosomal nanoparticles with diameters ranging from 180 to 930 nm. The mean diameter of the cubic nanoparticles varied within the range of 650-750 nm. However, phospholipid and ethanol ratio seemed to have little effect on particle size. The dependence of vesicle size on phospholipid content was determined for ethosomes containing 20% to 40% ethanol and phospholipid concentration ranging from 0.3% to 0.9%. Ethosome vesicle size ranging from 181 nm to 933 nm. Ethosomal vesicle size varies

with concentration of phospholipid. A three-fold increase in phospholipid concentration (from 0.3% to 0.9%) resulted in two to three fold increase ethosome size (from 224 nm to 724 nm).

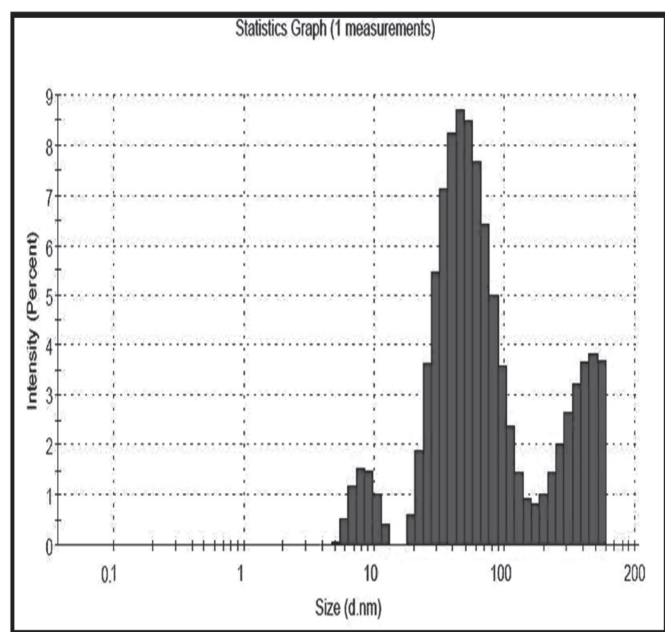


Fig. 4. Particle size of diclofenac loaded ethosomes

3.3 Evaluation of Ethosomal gel

The prepared formulations had a smooth and homogeneous appearance. Viscosity is an important physical property of topical formulations, which affects the rate of drug release; in general, an increase of the viscosity vehicles would cause a more rigid structure with a consequent decrease of the rate of drug release. The viscosity of carbopol 934 gel base and ethosomal gel by brook-field viscometer was found to be 8000 and 98,000 cps (centipoise). The pH values ranged from 7.54 to 8.98, which are considered acceptable to avoid the risk of irritation after skin application. The spreadability of ethosomal gel was found to be 4.2.cm². The spreadability results showed that ethosomal gel was most effective i.e. F8 formulation showed best result for spreadability. The extrudability of ethosomal gel was found to be positive. The extrusion of gel an important parameter during its application gel with high consistency may not extrude from the tube where as low viscous gel may flow quickly. Hence suitable consistency is required to extrude a gel from the tube. Ethosomal gel was found to be homogeneous and no grittiness was noted. Drug content ranges from 9.5 to 9.98%. F8 formulation had maximum drug content. Table 3 display the values of pH, viscosity and drug content for each gel.

Table 3. Evaluation parameters of ethosomal gel

Formulation	Spreadability	pH	Drug content	Colour	Extrudability	Viscosity (centipoise)	Entrapment Efficiency (%)	% cumulative release
F1	4.5	7.95	9.55	White	+	96,000	38	81.21±1.12
F2	4.5	7.71	9.71	Colourless	+	8000	46	78.19±0.89
F3	4.5	8.08	9.99	White	+	98,000	58	72.35±0.68
F4	4.5	8.78	9.78	White	-	95,000	65	87.90±1.11
F5	4.5	8.07	9.79	White	-	96,000	72	81.45±1.09
F6	4.5	7.54	9.93	White	+	98,000	78	77.32±0.88
F7	4.5	8.17	9.76	White	+	96,000	68	88.55±0.33
F8	5.0	8.98	9.98	White	+	93,000	90	82.13±1.34
F9	4.5	7.89	9.89	Milky	+	96,000	62	77.11±1.11

3.3.1 In-vitro drug release

In-vitro drug release from diffusion medium and release drug profile of Diclofenac gel containing different concentration of carbopol, propylene glycol and soya phospholipid, are shown in Table 3 and Figure 5. Release profile of Diclofenac gel in various gel formulation the drug release decrease with increase in concentration of the gelling agent. The drug release

values were also found lower for the formulation in which polymer concentration was kept high. In addition, viscosity increased (from 8000 cPs to 98,000 cPs in Table 3) as polymer concentration increased. Viscosity is inversely related to the release of active substance from formulations and its penetration through the diffusion barriers. The decrease in the release could be attributed to increased micro viscosity of the gel by increasing

polymer concentration. Thus, both high concentration of polymer and high viscosity compete each other in decreasing the release of active substance from the formulation. In our study, the finding that higher polymer concentration resulted in lower drug release from the vehicles is in agreement with Lauffer's molecular diffusion theory of polymer gels, which states that the diffusion coefficient of a solute is inversely proportional to the volume fraction occupied by the gel-forming agent. It may be observed that release of drug occurred fast initially then after some time release become slower. From graph it was observed that cumulative release of Diclofenac was more from ethosomal formulation than marketed gel over a period for 8 hrs. Ethosomal formulation was designed to achieve high permeability and ultimately increase the bioavailability of the drug. Figure 6 represents comparative release profile of marketed and experimental formulation.

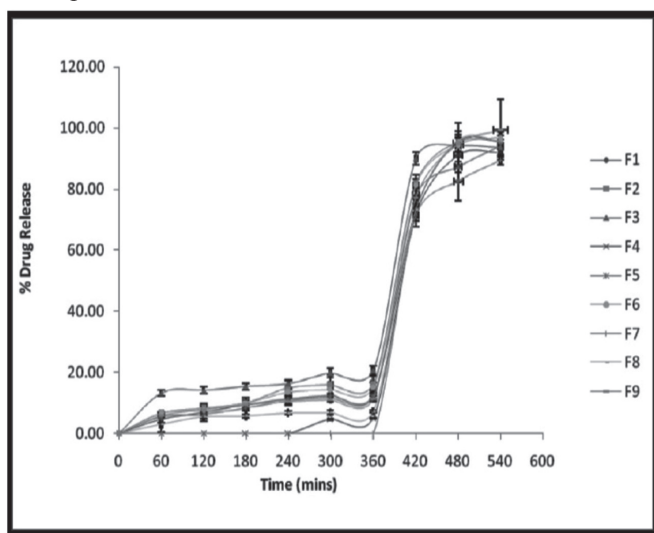


Fig. 5. Cumulative % drug release from Diclofenac gel at different time interval

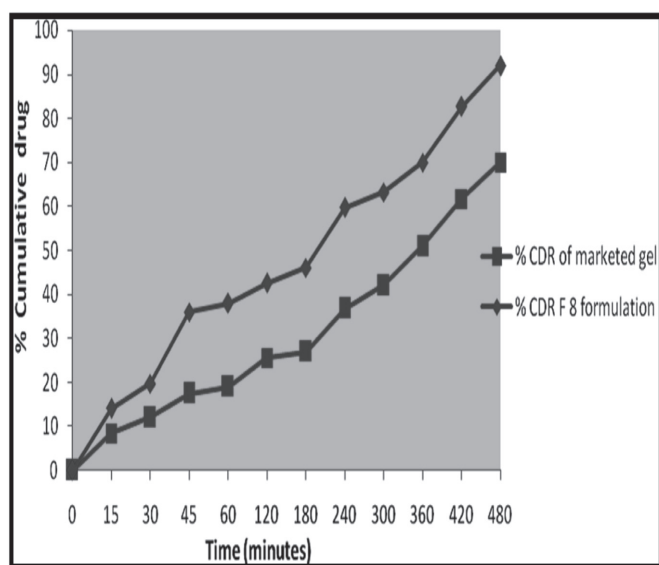


Fig. 6. Comparative release profile of marketed and experimental formulation

4. CONCLUSION

The present work on the preparation of topical ethosomal gel containing Diclofenac is an attempt to utilize the immense potential of ethosomes as a carrier to increase the permeability. It can be concluded that ethosomes can provide better skin permeation than liposomes. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Application of ethosomes provides the advantages such as improved permeation through skin and targeting to deeper skin layers for various skin diseases. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

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