Design, Formulation, and Evaluation of Self Micro-Emulsifying Drug Delivery System of Dipyridamole

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Abstract

Self-microemulsifying drug delivery system (SMEDDS) is an important strategy to enhance solubility and bioavailability of drugs having low aqueous solubility. Dipyridamole is BCS class II drug which suffers from poor solubility and bioavailability. The present work was undertaken to improve the solubility of dipyridamole by formulating SMEDDS. The solubility of dipyridamole was estimated in various oils, surfactants, and cosurfactants. Pseudoternary phase diagram was developed by water titration method to select the surfactant to cosurfactant ratio (S_{mix} ratio). Liquid SMEDDS was prepared using selected combination of oil, surfactant, and cosurfactant and different parameters were evaluated to select optimized combination. Results revealed that castor oil in which drug solubility was greater and 1:2 S_{mix} ratio was used for the development of various liquid SMEDDS formulation. Thus, due to the fast emulsification, higher drug content, thermodynamic stability, and percent transmittance S5 formulation containing 60% castor oil and 40 % S_{mix} (1:2 ratio) was selected optimized and further investigated. The average droplet size of the optimized S5 formulation after dilution with distilled water was obtained 160.2±3.6 nm and displayed Gaussian distribution. Moreover, lower value of PDI that is 0.324 indicated uniformity of the globule size in the microemulsion formed. The S5 formulation possessed zeta potential of -23.1 mv indicating the stability of the formulation. The results of transmission electron microscopy revealed spherical shape of microemulsion droplets. Hence, liquid SMEDDS of dipyridamole can be employed to modify the solubility and bioavailability of dipyridamole having inadequate water solubility.

Keywords: Castor oil, dipyridamole, SMEDDS, solubility, TEM

Introduction

The limited aqueous solubility of drugs is well documented issue which restrict therapeutic application of many drugs owing to decreased bioavailability. The major obstacle faced by formulation scientist is desired concentration of drugs at target site [1]. Therefore, formulation of SMEDDS to improve the solubility and bioavailability of poorly water-soluble drugs is an attractive alternative. SMEDDS are “isotropic mixtures of oil, surfactant, co-surfactant, and drug substance”. After gentle mixing with water or aqueous media, microemulsion can be produced quickly [2-4]. The formation of microemulsion is spontaneous process because there is collective action of the particular pharmaceutical excipients having less free energy. The

microemulsion droplets provide vast surface area after dispersion in digestive tract which lead to quick release of the drug substance present in dissolved form [5].

Dipyridamole is a platelet aggregation inhibiter BCS class-II drug which induces vasodilation used to cure and prevent strokes [6]. Chemically, dipyridamole is a weak base having pKa value 6.4, practically insoluble in water which display pH dependant solubility. Its reported intrinsic solubility at 37 °C is 8 mgL^{-1}. Moreover, oral bioavailability of dipyridamole varies from 37-44 % [7-8]. Hence, to enhance the solubility and bioavailability of dipyridamole various methods have been used such as cocrystal formation, solid dispersion, and liquid SMEDDS [9-11]. The objective of this work was to design, prepare and optimize SMEDDS of the poorly water-soluble drug dipyridamole to modify the oral bioavailability.

Materials

Dipyridamole was purchased from the BLDpharmatech, (India) Pvt. Ltd. The different oils, surfactant, and co-surfactant were purchased from SD fine chemicals Ltd. Mumbai, India. The remaining chemicals and solvents employed in this work were of reagent grade.

Methods

Determination of drug solubility in different vehicles

In order to select the appropriate oil, surfactant, and cosurfactant for the formulation of SMEDDS solubility study was conducted in these components. In 10 mL stoppered vials, 5 mL of selected oils, surfactants, and co-surfactants was taken. After adding excess quantity of drug, the vial was mixed by a vortex mixer. These mixtures after shaking for 72 hrs at ambient condition using magnetic stirrer kept aside (24 hrs) to achieve equilibrium. After reaching equilibrium, samples were centrifuged at 3000 rpm for 15 minutes and filtered through Whatman filter paper. The resulting filtrates were diluted as required and solubility of drug was estimated by UV spectrophotometer at 289 nm [12].

Construction of pseudoternary phase diagram with different ratio of surfactant, co-surfactant, and oil

A pseudo-ternary phase diagram, including oil, surfactants and co-surfactants (S_{mix}) in different ratio and water was fabricated to find out the microemulsion formation region. Six different S_{mix} ratio (Tween 80:Transcutol H) that is 1:1, 1:2, 1:3, 2:1, 3:1, 4:1 were taken to construct pseudo-ternary phase diagram. The phase diagram was created by mixing oil and specific S_{mix} ratio ranging from 1:9 to 9:1. The ability of these mixture to form microemulsion spontaneously was observed after mixing thoroughly on a magnetic stirrer and titration against distilled water until the cloudy system was obtained.

Preparation of liquid SMEDDS using selected combination of oil, surfactant, and co-surfactant

Eight liquid SMEDDS formulations were designed using castor oil and S_{mix} (1:2 ratio) containing Tween 80 and transcutol H. The amount of drug in each formulation was constant that is 10%. An accurate amount of dipyridamole was taken in glass vial and oil, surfactant and cosurfactant were added. This mixture was further mixed slowly by vortex mixing to completely dissolve the drug. The prepared formulations stored at ambient conditions in screw capped glass tubes until further use. The composition of various SMEDDS formulation given in Table 1.

Table 1: Composition of designed SMEDDS formulations

Components (% w/w)	**Batches**								
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	
Dipyridamole	10	10	10	10	10	10	10	10	
Castor oil	50	52.5	55	57.5	60	62.5	65	67.5	
S_{mix} ratio	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	
Tween 80	16.66	15.83	15	14.16	13.33	12.5	11.66	10.83	
Transcutol H	33.34	31.66	30	28.34	26.67	25	23.34	21.67	

Evaluation of Liquid SMEDDS

Emulsification time, % transmittance and drug content

The self-emulsification of formulations was evaluated by a standard USP dissolution apparatus II (Paddle) (Lab India, Mumbai). The formulation comprising single dose of drug was added in 200 mL of water maintained at 37±2 °C and 50 rpm to impart gentle agitation. The emulsification of the formulations was observed visually.

Liquid SMEDDS formulation (1 ml) was diluted with 100 mL of distilled water at 37 ± 0.2 °C by agitating slowly using magnetic stirrer and the percentage transmittance was measured at 560 nm by UV spectrophotometer (Shimadzu 1900i) against distilled water as blank.

Drug content was estimated by taking liquid SMEDDS formulation equivalent to single dose of drug and dissolved in methanol with continuous stirring. This solution was subjected to filtration through 0.2 µm filter and drug content determined by UV spectrophotometer at 289 nm. Percentage drug content was determined by following formula

$$\% \text{ Drug Content} = \frac{\text{Practical Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Determination of viscosity, cloud point, and refractive index

The viscosity of the SMEDDS formulation was determined by digital rotational viscometer (Labman LMDV-100). Abbe's refractometer was employed for the determination of refractive index of the SMEDDS formulation. The cloud point was evaluated by adding formulation to 250 ml of distilled water and heated until solution turned turbid. The temperature at which formulation becomes turbid was noted as cloud point.

Thermodynamic stability study

Heating cooling cycle: The formulations were stored at 4 °C (refrigerator temperature) and 45 °C for not less than 48 hrs (six cycles). In case of centrifugation formulations were centrifuged at 5000 rpm for 30 min. Formulations were subjected to three freeze thaw cycles between -10 °C and 25 °C for not less than 48 hrs. Thermodynamic stability of formulations determined as pass/failed.

Droplet Size, Zeta Potential and Polydispersity Index (PDI) of optimized formulation

The selected formulation was further subjected to evaluation by determining Droplet Size, Zeta Potential and Polydispersity Index. The droplet size of selected SMEDDS formulation was estimated by diluting 100 µL of formulation to 250 mL in a beaker and slowly mixed with aid of glass rod. All these parameters were evaluated by using Zetasizer Nanoseries (Malvern Instrument Ltd. UK).

Transmission electron microscopy (TEM)

The optimized liquid SMEDDS formulation was subjected to TEM investigation for morphological analysis. Selected SMEDDS formulation was diluted with distilled water (1:25) and agitated slightly to mix properly. One drop of diluted sample was placed on copper grids and excess sample removed with filter paper.

Results and discussion

Determination of drug solubility in different vehicles

Self-emulsifying formulations comprising oils, surfactants, co-surfactants, and drug, should be a clear and monophasic liquid after introduction into aqueous phase at ambient temperature. Moreover, these formulations should have adequate solvent capacity to convert the drug into solution. The excipients utilized for formulation are widely used in cosmetics and food industry. In pharmaceutical formulations these are used for oral, parenteral, and topical administration. These are essentially biologically safe excipients relatively nontoxic and nonirritant. Among the different oils investigated maximum solubility (0.45±0.049) was obtained with castor oil. Hence, castor oil was selected for further formulation development. Moreover, dipyridamole exhibited greater solubility in the surfactant Tween 80 and cosurfactant ethanol which were selected for further studies. The solubility of dipyridamole in different vehicles is shown in Figure 1. Therefore, based on the solubility of drug castor oil, tween 80 and Transcutol H was selected for the development of SMEDDS.

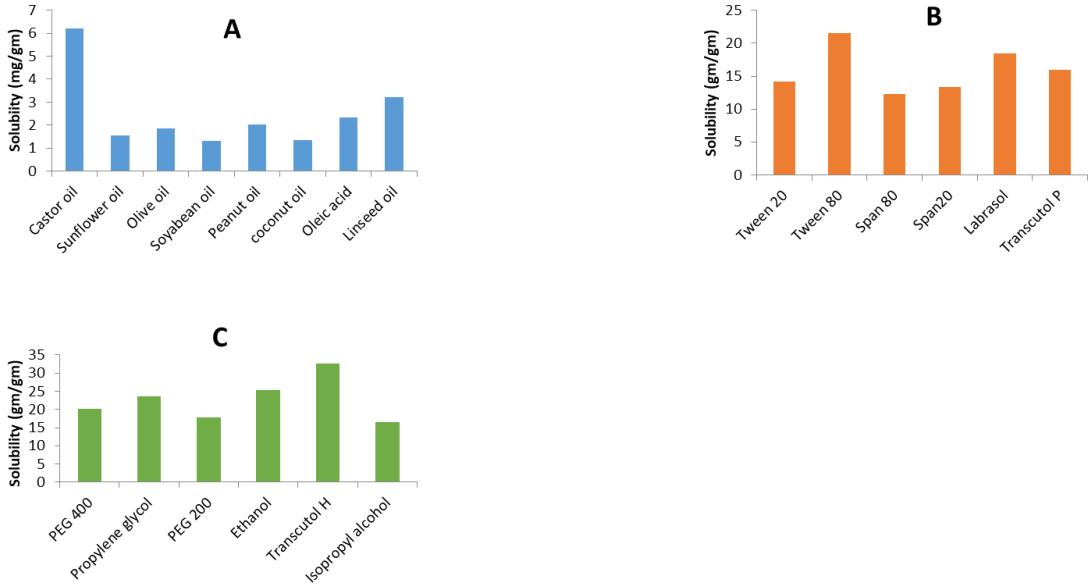

Figure 1: Solubility of dipyridamole in A] oils B] Surfactant C] Cosurfactant

Construction of pseudoternary phase diagram with different ratio of surfactant, co-surfactant, and oil

Self-microemulsifying systems when added into aqueous media form oil in water emulsion after gentle shaking. Surfactant and cosurfactant decrease interfacial energy and coalescence due to their adsorption at the interface. This in turn leads to formation of emulsion which is thermodynamic stable. Hence, appropriate selection of oil, surfactant, and cosurfactant along with the ratio of oil to S_{mix} is critical for the microemulsion formation. In this investigation, castor oil along with tween 80 and Transcutol H (S_{mix}) was employed in different ratio.

The pseudo-ternary diagram is mainly constructed to recognise the self-microemulsifying region and phase behaviour of vehicles used in the formualtion. The clear and homogeneous mixture obtained through gentle stirring is the self-microemulsifying region denoted by shaded portion. Pseudoternary phase diagrams were fabricated separately for each S_{mix} ratio and results revealed that S_{mix} ratio of 1:2 displayed wider microemulsion region. Moreover, 2:1 and 3:1 S_{mix} ratio showed comparable microemulsion region which can be ascribed to increase in the S_{mix} ratio. But, S_{mix} ratio of 1:2 which displayed greater microemulsion region was selected for further investigation. Figure 2 shows series of pseudoternary phase diagrams with various ratio of surfactant/cosurfactant.

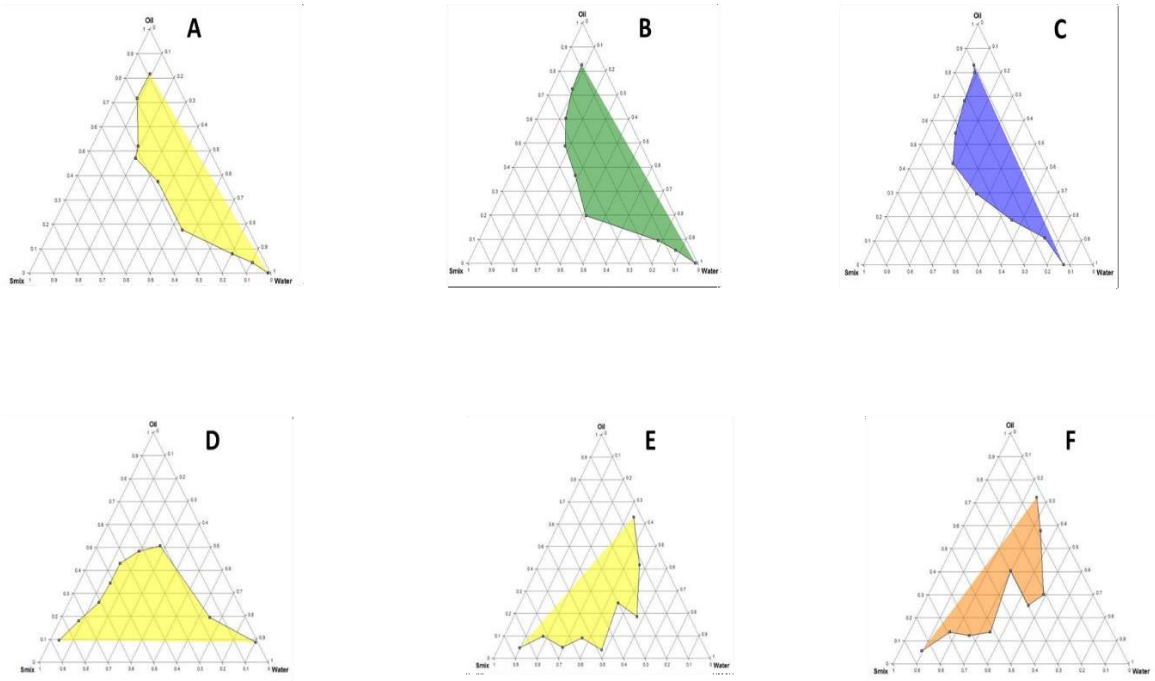

Figure 2: Pseudoternary phase diagrams with different surfactant/cosurfactant (S_{mix}) ratio A) 1:1 B) 1:2 C) 1:3 D) 2:1 E) 3:1 F) 4:1

Preparation and evaluation of liquid SMEDDS using selected combination of oil, surfactant, and co-surfactant

Total eight Liquid SMEDD formulations were designed and prepared using various proportions of castor oil and 1:2 S_{mix} ratio (Tween 80 as surfactant and Transcutol H as cosurfactant). The amount of drug added in each formulation was 10% and kept constant in all the formulations.

Emulsification time, % transmittance and drug content

In order to select the optimized formulation, each formulation was subjected to evaluation with respect to different parameters. The results of the emulsification time (Sec), % transmittance, and drug content are presented in Table 2.

The emulsification efficacy is generally evaluated by determining the emulsification rate. In general, when the SMEDDS formulation when diluted with water under mild agitation should disperse rapidly and completely. The formulation S5 exhibited minimum emulsification time (23.67 ± 1.15 sec) and appearance was also clear transparent. Moreover, % transmittance was estimated which provides idea about the strength of the SMEDDS to circumvent drug precipitation upon dilution. A high value of transmittance is desired which signals transparent solutions that is optical clarity. Besides, this validate drug does not undergo microprecipitation. Formulation S5 displayed maximum transmittance of 98.35±1.32% which is closer to the % transmittance of the water indicating the optical clarity. Additionally, the drug content of all the formulations was estimated and observed in the range 91.36±0.79 - 98.76±0.49 %. Each formulation revealed good drug content but formulation S5 showed highest drug content followed by S4 formulation. Overall, the formulation S5 was promising with respect to parameters determined.

Table 2: Emulsification time, % transmittance and drug content of the prepared formulations

Formulation	Emulsification time (Sec)	% Transmittance	Drug content
S1	32.40±0.85	90.80±0.85	93.46±0.97
S2	37.12±1.04	87.20±0.78	95.26±0.78
S3	32.52±1.21	92.6±1.25	92.86±0.48
S4	27.65±0.92	90.55±0.65	96.21±0.67
S5	23.67 ± 1.15	98.35±1.32	98.76±0.49
S6	60.50 ± 0.95	88.45±0.56	95.89±0.60
S7	81.50 ± 1.24	75.36±0.85	91.36±0.79
S8	103 ± 1.46	66.90±0.85	94.36±1.35

Determination of viscosity, cloud point, and refractive index

The temperature beyond which turbidity appears in an aqueous solution of water-soluble non-ionic surfactant is the cloud point. The formation of a stable microemulsion is indicated by the cloud point and temperature exceeding this led to irreversible separation of phase. The appearance of cloudiness above this temperature can be attributed to the dehydration of polyethylene oxide of the Tween. Generally, the cloud point for SMEDDS should be greater than 37 °C to prevent phase separation which may occur in the gastrointestinal tract. However, the developed formulations displayed cloud point above 70° indicting the stability of the formulation at body temperature.

Viscosity of the SMEDDS formulations was evaluated in order to examine the physical stability of the system. The formulations exhibited viscosity from 85.5 to 104 cps but S5 formulation possessed viscosity of 85.5±1.7 cps which could be due to the optimum concentration of oil. Additionally, refractive index of the formulations was also estimated which provide idea about transparency and isotropic nature. The refractive index for entire formulations was between 1.445-1.482. The results for the viscosity, cloud point, and refractive index of each formulation are given in the Table 3.

Table 3: Viscosity, cloud point and refractive index of developed formulations

Formulation	Viscosity (cps)	Cloud point (°C)	Refractive Index
S1	92±2	72.5±1.15	1.467
S2	101±1.7	71.5±0.89	1.471
S3	89±1.5	74±1.06	1.454
S4	98±0.5	76±1.25	1.462
S5	85.5±1	79±0.85	1.475
S6	91±1.2	73.5±0.98	1.482
S7	99±2.08	70±0.46	1.445
S8	104±1.35	71±0.67	1.453

Thermodynamic stability study

Thermodynamic stability of the SMEDDS formulation was investigated using distinct approaches *viz.* centrifugation, heating-cooling cycle, and freeze-thaw cycle tests. The result revealed the stability of all the formulations containing various proportion of oil, surfactant, and cosurfactant. After investigation of SMEDDS formulation, no phase separation, precipitation, creaming, and cracking detected. Moreover, dispersibility test already indicated formation microemulsion in less than 1 minute for most of the formulations.

Selection of optimized formulation

Considering the various evaluation parameters optimized batch was selected. The emulsification time for S5 formulation was minimum that is 23.67±1.15 sec. Furthermore, S5 batch displayed % transmittance and drug content of 98.35±1.32 and 98.76±0.49% respectively. Apart from these evaluation parameters, viscosity, cloud point, and refractive index of this formulation was acceptable and optimum. Moreover, this formulation was detected stable after thermodynamic stability study. Thus, due to the fast emulsification, higher drug content, thermodynamic stability, and percent transmittance S5 formulation containing 60% castor oil and 40 % S_{mix} (1:2 ratio) was selected optimized and further investigated.

Droplet Size, Zeta Potential and Polydispersity Index (PDI) of optimized formulation

The crucial parameter for SMEDDS formulation which is most relevant to the absorption of drug is the droplet size distribution. As we know smaller droplet size provides greater interfacial area and thereby more absorption. Besides, smaller droplet size lead quick drug diffusion into aqueous phase and in turn enhanced dissolution of the drug. The average droplet size of the optimized S5 formulation after dilution with distilled water was obtained 160.2±3.6 nm and displayed Gaussian distribution. Moreover, lower value of PDI that is 0.324 indicated uniformity of the globule size in the microemulsion formed. The non-uniformity of the globule size results in higher value of the PDI. Hence, the optimized S5 formulation exhibited desired globule size and PDI required for better performance. Figure 3 shows the globule size distribution of the optimized SMEDDS formulation.

The coalescence of microemulsion globules depends on the electrostatic repulsive forces between them and can be prevented with increase in these electrostatic repulsive forces. In contrast, phase separation would result if there is decrease in the electrostatic repulsive forces between the globules. Hence, magnitude of surface charge that is zeta potential of the microemulsion droplets is important for stability of the SMEDDS formulation. In general,

microemulsion formulation should have zeta potential between -30 to +30 mV for adequate stability. However, the S5 formulation possessed zeta potential of -23.1 mv indicating the stability of the optimized formulation. Zeta potential of the optimized S5 formulation given in Figure 3.

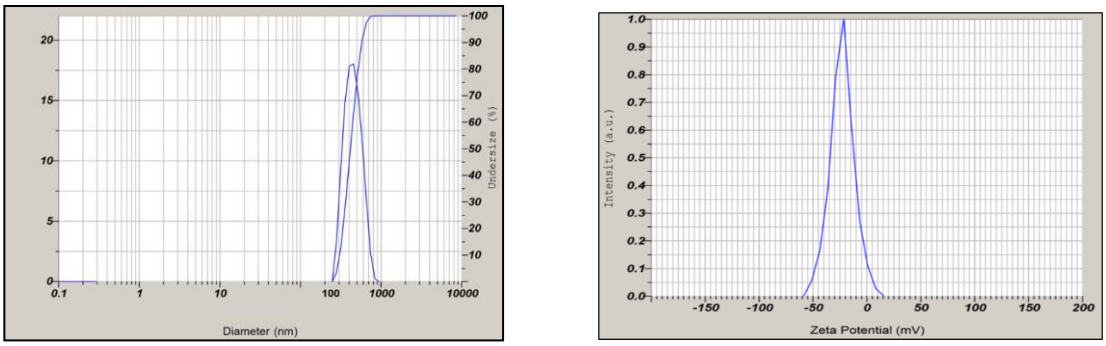

Figure 3: Globule size distribution and zeta potential of optimized SMEDDS formulation

TEM of optimized liquid SMEDDS formulation

Optimized formulation was subjected to morphological characterization through TEM and its image is displayed in Figure 4. The results revealed that microemulsion droplets were spherical in shape.

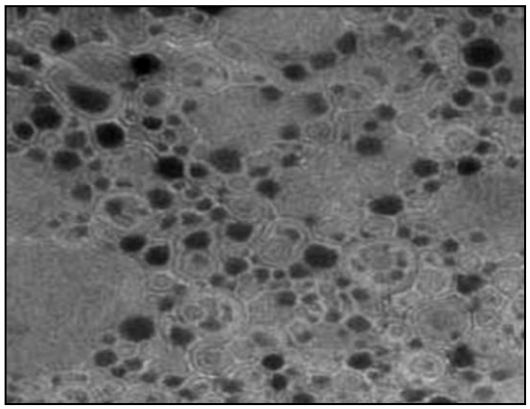

Figure 4: TEM image of optimized SMEDDS formulation

Conclusion

An optimized formulation containing castor oil, tween 80 and transcutol H (S_{mix} ratio 1:2) has been developed having enhanced solubility and dissolution thereby leading to greater absorption. The various vehicles were selected by determining solubility of dipyridamole in different oils, surfactant, and cosurfactant. Considering several evaluation parameters S5 formulation containing 60% castor oil and 40 % S_{mix} was selected optimized. Moreover, S5 formulation exhibited 160.2±3.6 nm average globule size, lower PDI 0.324, desirable zeta potential -23.1 mv and spherical microemulsion droplets. Hence, the developed SMEDDS formulation could be employed to improve the oral bioavailability of the dipyridamole.

Acknowledgement

Not Applicable

Conflict of Interest

Authors declare no conflict of interest.

Funding Source

Nil

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