Combined Effect of Cosolvent and Cyclodextrin on Solubilization of Nonpolar Drugs

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Abstract □ Solubility enhancement has broad implications in parenteral formulation design. A simple mathematical model has been developed to describe the combined effect of cosolvency and complexation on nonpolar drug solubilization. The total drug solubility is determined by the summation of three drug species present in the solution: free drug [D], drug–ligand binary complex [DL], and drug–ligand–cosolvent ternary complex [DLC]. The proposed model established the dependencies of these three species upon the intrinsic drug solubility, $[D_u]$, the cosolvent solubilizing power, σ, the binary and ternary intrinsic complexation constants, K_b^{int} and K_t^{int}, and the cosolvent destabilizing powers for the binary and the ternary complexes, ρ_b and ρ_t. A nonpolar solute, Fluasterone, is used to evaluate the newly generated equation. The model explains the decline in drug solubility produced by low cosolvent concentrations as well as the increase in the solubility produced by high cosolvent concentrations that are observed at all cyclodextrin concentrations.

Introduction

Solubility enhancement has broad implications in parenteral formulation design. This is especially true for poorly water-soluble drugs, as it is often necessary to deliver the desired dose in a specified volume of aqueous liquid. Over the years a variety of solubilization techniques have been studied and widely used including pH adjustment, cosolvent addition, surfactant addition, and cyclodextrin addition.[1−5]

Among these techniques, cosolvent and cyclodextrin addition are highly effective for nonpolar solutes. As a water-miscible or partially miscible organic solvent, the cosolvent reduces strong water−water interactions and thereby reduces the ability of water to squeeze out nonpolar solute.[2] Cosolvency is often considered at early stages due to its huge solubilization potential. Because of their safety, cosolvents are employed in approximately 10% of FDA approved parenteral products.[3] Cyclodextrins are cyclic oligomers of dextrose or its derivatives joined by α-1,4-linkages. They increase drug solubility by forming an inclusion complex with the nonpolar region of the drug molecule (guest) being inserted into the cavity of the cyclodextrin molecule (host).[3−6] Such a drug−ligand complex has a rigid structure and a definite stoichiometry, usually one-to-one at low ligand concentrations.[4−6] It is of note, however, that there exist clinical limitations to these aforementioned methods.[2,3] For example, high concentrations of cosolvent have high viscosity and high tonicity, and phlebitis can result from precipitation of the solubilized drug upon iv injection.[7,8] In fact, ethanol in concentrations greater than 10% may well produce significant pain.[7,8] Some cyclodextrins have been reported to have significant renal toxicity.[3,8,9]

Recently, the combined use of cosolvency and complexation has drawn particular interest.[10−14] Zung et al. observed synergistic effects of cosolvency and complexation in solubilizing pyrene by using a series of alcohols.[14] The complexation constants of both pyrene/β-cyclodextrin and pyrene/γ-cyclodextrin were found to be much greater in the presence of an alcohol than in pure water. It was suggested that the cosolvent act as a space-regulating molecule so that the drug molecule can better fit into the cyclodextrin cavity. In other studies, it was found that the presence of cosolvents decreases the formation of drug−ligand complex. Pitha et al. reported that the complexation constant of testosterone with hydroxy propyl-β-cyclodextrin (HPβCD) is 10 000-fold lower in 80% ethanol than in water.[11] They reasoned that the cosolvent may act by competing with the drug for entry into the cyclodextrin cavity or by reducing the solvent polarity. A similar antagonistic cosolvent effect was observed for ibuprofen in a HPβCD−propylene glycol−water system.[10]

Given the fact that both cosolvency and complexation have been well studied and understood, it is of interest to explore the mechanisms of the combined effect of the two techniques on nonpolar drug solubilization and to explore the dynamics among the solute, cosolvent, and the cyclodextrin. The knowledge gained in this study may shed light for possible synergistic effect out of this combined technique and be useful in future parenteral formulation design.

This paper aims at constructing a simple mathematical model to explain the combined effect of cosolvency and complexation on nonpolar drug solubilization. The model will be evaluated by using Fluasterone as nonpolar solute, ethanol (EtOH) as cosolvent, and hydroxy propyl-β-cyclodextrin (HPβCD) as complexing ligand. Fluasterone (16α-fluoro-5-androsten-17-one) is a structural analogue of dehydroepiandosterone that is being developed for cancer chemoprevention.[12]

Theoretical Background

Assumptions—The proposed model is based upon the following assumption: the complex formed is either a drug−ligand (cyclodextrin) binary complex or a drug−

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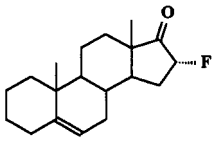

Fluasterone

ligand–cosolvent ternary complex with the stoichiometries assumed as 1:1 and 1:1:1, respectively.

Basics—For a given complexant solution there is an equilibrium between the free drug and the drug–ligand binary complex. When a cosolvent is introduced into the solution, it not only changes the concentration of the free drug [D] and the binary complex [DL], but it also may be involved in the formation of a drug-bearing ternary species, DLC.[14–16] In the presence of a binary 1:1 complex and a ternary 1:1:1 complex, the total solubility of the drug [D^{tot}] is:

$$[D^{tot}] = [D] + [DL] + [DLC] \quad (1)$$

Free Drug—The concentration of free (i.e., uncomplexed) drug [D] is related to intrinsic drug solubility [D_u] and cosolvent concentration [C] by:[2,8,17]

$$[D] = [D_u] \times 10^{\sigma[C]} \quad (2)$$

where σ is the cosolvent solubilizing power. The value of σ depends on the polarity of both the solute and the solvent.[2,8] Here it is assumed that complexation ligand has a negligible effect on the solubilizing power. Similar assumptions have been made in some other studies.[2,8] Equation 2 indicates that the logarithm of solubility in a mixed solvent increases linearly with cosolvent composition, i.e., an increase in [C] will produce an exponential increase in [D]. It has been validated on hundreds of nonpolar solutes in ethanol, propylene glycol, and other cosolvents.[2,8,17]

Binary Complex—The concentration of drug–ligand binary complex [DL] is related to the concentration of free drug [D], the total concentration of ligand [L], and the apparent binary complexation constant, K_b^{app}, by:[18]

$$[DL] = K_b^{app}[D][L] \quad (3)$$

in which K_b^{app} is a function of cosolvent concentration.[11,18,20] The apparent complexation constant, K_b^{app}, in a cosolvent–water solution is empirically related to the cosolvent concentration and the intrinsic complexation constant K_b^{int}, i.e., the complexation constant in water, by:

$$K_b^{app} = K_b^{int} \times 10^{-\rho_b[C]} \quad (4)$$

where ρ_b is the destabilizing power of the cosolvent for the binary complex. The value of ρ_b depends on the polarity deference between the solute and the cosolvent, the steric factors between the solute and the complexing ligand. By incorporating eqs 2 and 4 into eq 3, the binary complex can be expressed as:

$$[DL] = K_b^{int} \times 10^{-\rho_b[C]}[D_u] \times 10^{\sigma[C]}[L] = [D_u][L]K_b^{int} \times 10^{(\sigma - \rho_b)[C]} \quad (5)$$

which shows that [DL] is linearly dependent upon the ligand concentration and exponentially dependent upon the cosolvent concentration. If $\sigma > \rho_b$, an increase in [C] will give rise to an exponential increase in [DL]. If $\sigma < \rho_b$, an increase in [C] will give rise to an exponential decrease in [DL]. If $\sigma = \rho_b$, [C] will have no effect upon [DL].

Ternary Complex—The concentration of the 1:1:1 ternary complex [DLC] is related to the free drug concentration [D], the ligand concentration [L], the cosolvent concentration [C], and apparent ternary complexation constant, K_t^{app}, by:

$$[DLC] = K_t^{app}[D][L][C] \quad (6)$$

By analogy to eq 4, K_t^{app} is related to the cosolvent concentration and the intrinsic ternary complexation constant, K_t^{int}, by:

$$K_t^{app} = K_t^{int} \times 10^{-\rho_t[C]} \quad (7)$$

where ρ_t is the cosolvent destabilizing power for the ternary complex. The value of ρ_t depends on the polarity deference between the solute and the cosolvent, the steric factors between the solute and the complexing ligand. Note that K_t^{app} approaches K_t^{int} as the cosolvent concentration approaches zero. The concentration of the ternary complex can be expressed by inserting eqs 2 and 7 into eq 6. This gives:

$$[DLC] = K_t^{int} \times 10^{-\rho_t[C]}[D_u] \times 10^{\sigma[C]}[L][C] = [D_u][L][C]K_t^{int} \times 10^{(\sigma - \rho_t)[C]} \quad (8)$$

which indicates that [DLC] has a linear dependency upon the ligand concentration and a complex dependency upon the cosolvent concentration. If $\sigma > \rho_t$, an increase in [C] will produce an increase in [DLC]. If $\sigma < \rho_t$, an increase in [C] will increase [DLC] only when $[C] > 10^{(\sigma - \rho_t)[C]}$; when $[C] < 10^{(\sigma - \rho_t)[C]}$, an increase in [C] will decrease [DLC]. If $\alpha = \rho_t$, an increase in [C] will increase [DLC] linearly.

Total Solubility—In the presence of both cosolvent and complexant the drug's total solubility is determined by the summation of three solution components: free drug [D], drug–ligand binary complex [DL], and drug–ligand–cosolvent ternary complex [DLC]. Inserting eqs 2, 5, and 8 into eq 1 gives:

$$[D^{tot}] = [D_u] \times 10^{\sigma[C]} + [D_u]K_b^{int} \times 10^{(\sigma - \rho_b)[C]}[L] + [D_u]K_t^{int} \times 10^{(\sigma - \rho_t)[C]}[L][C] \quad (9)$$

where the total solubility is related to the parameters: [D_u], K_b^{int}, K_t^{int}, σ, ρ_b, and ρ_t, which have been described in the preceding sections. In the following sections, the combined effect of cosolvent and complexation on drug solubilization described by eq 9 is confirmed by solubilization of a very nonpolar compound, Fluasterone, in a water–EtOH–HPβCD system.

Methods

Solubility Determination—Fluasterone was added to vials containing certain percentages of both hydroxy propyl-β-cyclodextrin (HPβCD) and ethanol (EtOH). HPβCD concentration ranges from 0 to 20% and ethanol concentration ranges from 0 to 75% were investigated. The sample vials were rotated using an end-over-end mechanical rotator at 20 rpm (Glas-Col Laboratory Rotator, Terre Haute, IN) at 25 °C for 6 days (preliminary data indicate that Fluasterone is stable for 50 days under these conditions). Samples with drug crystals present were considered to have reached their equilibrium solubility and were removed from the rotator, passed through a 0.45-μm filter, and analyzed by HPLC. All samples were prepared in duplicate.

HPLC Analysis of Fluasterone—A Pinnacle octylamine column (150 cm × 4.6 mm, Restek, Bellefonte, PA) was used with a mobile phase composed of 75% acetonitrile in water. The flow rate was controlled at 1.1 mL/min (125 Solvent Module, Beckman,

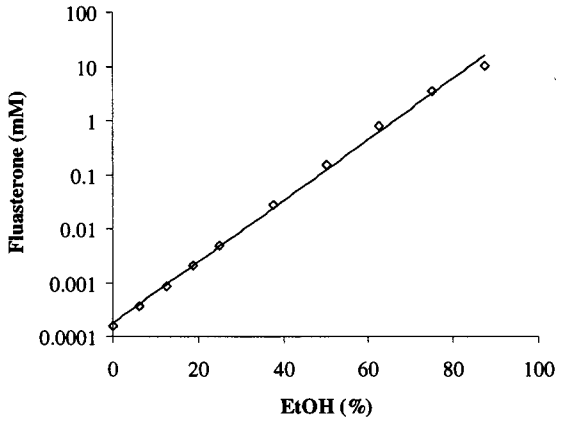

Figure 1—Fluasterone solubility as a function of EtOH concentrations.

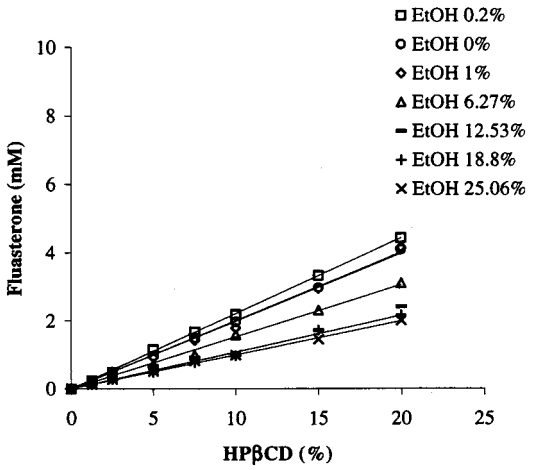

Figure 2—Solubility of Fluasterone as a function of HPβCD and EtOH concentrations.

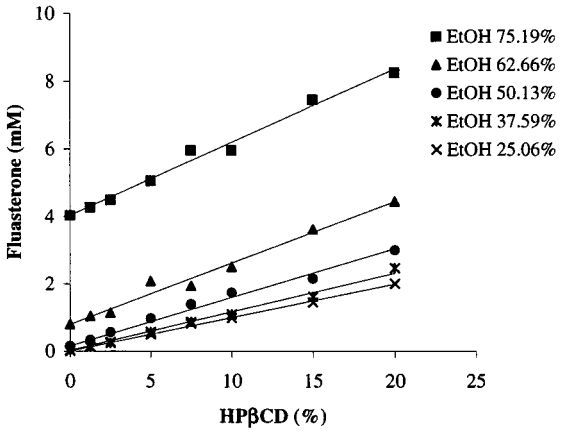

Figure 3—Solubility of Fluasterone as a function of HPβCD and EtOH concentrations.

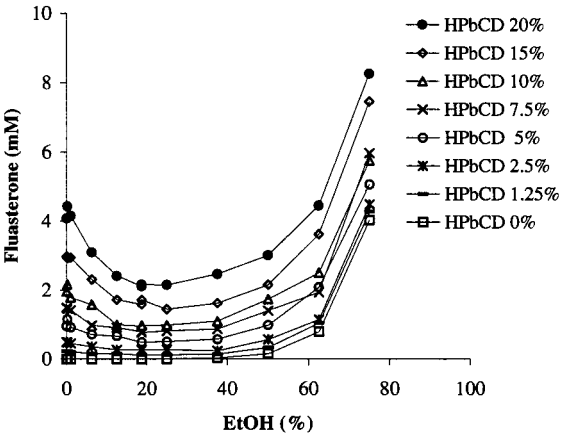

Figure 4—Solubility of Fluasterone as a function of EtOH concentration in different HPβCD concentrations.

Fullerton, CA). The column effluent was monitored at a wavelength of 220 nm (168 detector, Beckman, Fullerton, CA). The retention time of a 100 μL sample was 6.3 min. The evaluation of the assay was made by using Fluasterone standard solutions at concentrations ranging from 0.001 to 0.1 mg/mL, intraday and interday, coupled with different solubilizing agents. The relative standard deviation was 1.05%. None of the solubilizing agents interfere with the assay.

Results and Discussion

Solubilization by Ethanol Alone—The intrinsic solubility [D_u] of Fluasterone was determined to be 0.045 μg/mL (0.000155 mM). Figure 1 shows the dependency of the drug's total solubility [D^{tot}] upon ethanol concentration [C]. The exponential solubility increase is described by eq 2, with a cosolvent solubilizing power (σ) of 0.3401.

Solubilization by HPβCD Alone—The aqueous solubility of Fluasterone increases linearly with HPβCD concentration [L] up to 20% as shown by the open circles in Figure 2. Incorporating the slope into eq 3 indicates the formation of a 1:1 reversible drug−ligand complex with an intrinsic complexation constant (K_b^{int}) of 1.80×10^5 M^{-1}.

Solubilization by Combined Use of Ethanol and HPβCD—Figure 2 shows that at every HPβCD concentration investigated the Fluasterone solubility is slightly higher in the presence of 0.2% ethanol (open squares in Figure 2) than in pure water. An ethanol concentration of 0.2% is not enough to function as a cosolvent to affect drug complexation.[14−16,21] Nevertheless, it produces a consistent increase in drug solubility. This suggests that ethanol must increase Fluasterone solubility by some other mechanism, such as the formation of a complex that contains drug, ligand, and cosolvent. Equation 9 shows that the formation of a ternary complex would be responsible for the increased Fluasterone solubility in the HPβCD solutions containing 0.2% EtOH. Here, as in other studies,[15,16] it is assumed that the ternary species responsible for the increased drug solubility is a 1:1:1 complex. Extrapolation of K_t^{app} ($= K_t^{int} \times 10^{-\rho_t[C]}$) to zero cosolvent concentration by eq 9 gives a K_t^{int} of 1.42×10^4 M^{-1}.

The solubility of Fluasterone increases linearly with HPβCD concentration at all ethanol concentrations. It is interesting to note that the slope of the solubilization curve varies with ethanol concentrations [C]. The slope decreases when [C] increases from 0.2% to 25.06% as shown in Figure 2, but increases when [C] increases from 25.06% to 75.19% as shown in Figure 3. This can be seen more clearly from cross sections of the data of Figures 2 and 3 at equal HPβCD concentrations that are shown in Figure 4. The figure also indicates a minimum total drug concentration at about 25% ethanol. Nearly 60 total solubility [D^{tot}] data points from Figure 4 and known values of [D_u], σ, K_b, K_t, [L], and [C] were used to calculate ρ_b and ρ_t by means of nonlinear regression analysis. Their values were found to be 0.515 and 0.340, respectively. As the cosolvent concentration increases, the magnitude of ρ_b[C] and ρ_t[C] increases, resulting in a greater destabilizing power for both the binary and ternary complexes. This is because the increased cosolvent concentration reduces solvent polarity so that nonpolar molecules are more likely to stay out of the cyclodextrin cavity.

The concentration of binary drug−ligand complex [DL] is proportional to the product of the apparent complexation

Table 1—Estimation of Solubilization Parameters

parameter	symbol	value
intrinsic binary complexation constant	K_b^{int} (M^{-1})	1.80×10^5
intrinsic ternary complexation constant	K_t^{int} (M^{-1})	1.42×10^4
cosolvent solubilizing power	σ	3.40×10^{-1}
cosolvent destabilizing power on binary complex	ρ_b	5.15×10^{-1}
cosolvent destabilizing power on ternary complex	ρ_t	3.40×10^{-1}

constant and the concentration of the free drug, i.e., K_b^{app}-[D], which equals $[D_u]K_b^{int} \times 10^{(\sigma - \rho_b)[C]}$. An increase in the cosolvent concentration simultaneously produces an exponential increase in [D] ($= [D_u] \times 10^{\sigma[C]}$) and an exponential decrease in K_b^{app} ($= K_b^{int} \times 10^{-\rho_b[C]}$). As a result, [DL] is dependent upon the difference between the solubilizing power (σ) and the destabilizing power (ρ_b) of the cosolvent for [DL]. Since $\sigma = 0.340$ and $\rho_b = 0.515$, this difference is negative. An increase in [C] leads to an exponential decrease in [DL], and the concentration of the binary complex becomes negligible when [C] reaches 50%.

The concentration of the ternary complex depends on the cosolvent concentration [C] and the difference between the cosolvent solubilizing power (σ) and the cosolvent destabilizing power (ρ_t) for the ternary complex. Nonlinear regression analysis of the solubility data indicates that the difference between σ and ρ_t for Fluasterone is negligible (see Table 1). Consequently, the exponential term in eq 8 is constant and an increase in ethanol concentration produces a linear increase in the ternary complex concentration [DLC].

With the values of $[D_u]$, K_b^{int}, K_t^{int}, ρ_b, and ρ_t given in the table, we can calculate [D], [DL], [DLC], and $[D^{tot}]$ at any given combination of EtOH and HPβCD concentration by using eqs 2, 5, 8, and 9, respectively. Figure 5a shows the calculated values of [D], [DL], [DLC], and $[D^{tot}]$ at 20% HPβCD under different EtOH concentrations. Both the exponential increase and the exponential decrease can be seen more directly as straight lines on the semilogarithmic scale of Figure 5b. The figure also shows that the calculated $[D^{tot}]$ decreases initially and approaches a minimum where [C] is approximately at 25%. Such a decrease is due to the fact that the decrease in [DL] outweighs the increase in [D] and [DLC] resulting from the addition of ethanol. After 25%, $[D^{tot}]$ starts to increase due to the increase in both [D] and [DLC]. Note that [DLC] is greater than [DL] even though K_b^{int} is approximately 10-fold greater than K_t^{int}. This finding is consistent with testosterone studies[11] in which [DL] was diminished in a solution containing HPβCD and 60% ethanol. However, the solid produced by evaporating the aqueous solvent contained a small amount of ethanol. Since a 1:1:1 testosterone–HPβCD–ethanol complex would contain only 2.7% ethanol, this observation might be explained by the existence of the ternary complex [DLC], such as described above.

Validation of the Proposed Model—Calculated total drug solubilities (solid lines) using eq 9 are compared in Figure 6 with the experimental solubility data (symbols) at different concentrations of HPβCD. The strong agreement between the predicted and the observed solubility data supports the validity of the proposed model. Note that as the cyclodextrin concentration approaches zero, the total solubility approaches the log-linear relationship commonly observed in a simple cosolvent–water system.

Conclusion

An equation is developed to describe the combined effect of ethanol and HPβCD upon Fluasterone solubility. The equation is validated with respect to the intrinsic drug solubility, $[D_u]$, the cosolvent solubilizing power, σ, the binary and ternary intrinsic complexation constants, K_b^{int} and K_t^{int}, and the cosolvent destabilizing powers for the binary and the ternary complexes, ρ_b and ρ_t. This equation can be used to explain the linear dependence of nonpolar solute solubility upon cyclodextrin concentration that is observed at all ethanol concentrations. It also can be used to describe the decline in the solubility produced by low cosolvent concentrations as well as the increase in the solubility produced by high cosolvent concentrations that are observed at all cyclodextrin concentrations. Thus it provides a theoretical background for understanding the dynamics of the combined cosolvent–complexant technique in the solubilization of nonpolar drugs.

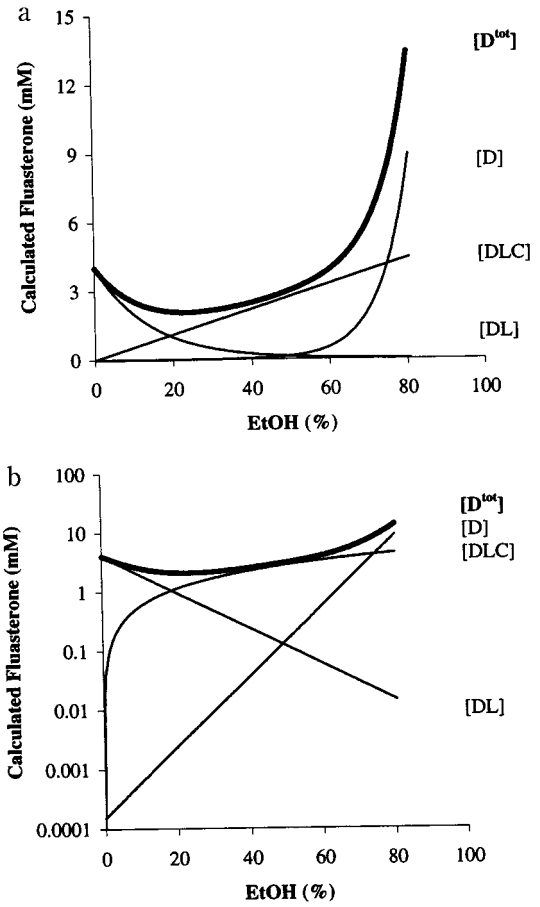

Figure 5—Calculated solubility in 20% HPβCD.

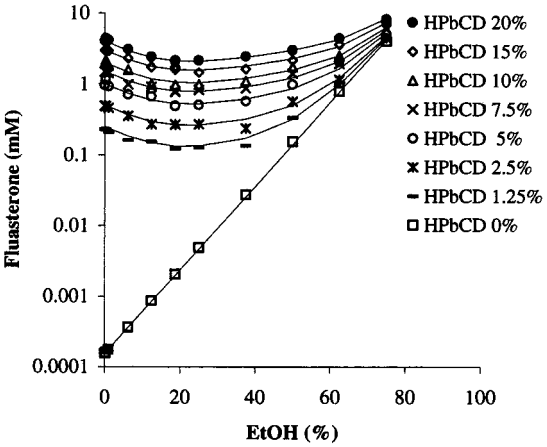

Figure 6—Calculated total drug solubilities (solid lines) versus the experimental solubility data (symbols) at different HPβCD concentrations.

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