

IMPROVEMENT IN BIOAVAILABILITY OF CLASS-III DRUG: PHYTOLIPID DELIVERY SYSTEM

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ABSTRACT

Over the past century, phytochemical and phyto pharmacological sciences established the compositions that are biological active and health promoting. Most of the biologically active constituents of plants are polar or water soluble molecules. However, water soluble phyto-constituents (like flavonoids, tannins, terpenoids, etc.) are poorly absorbed either due to their large molecular size which cannot absorb by passive diffusion, or due to their poor lipid solubility; severely limiting their ability to pass across the lipid-rich biological membranes, resulting poor bioavailability. These types of drugs are then classified into biopharmaceutical classification system classes: high solubility/low permeability (Class III). It has been observed that complexation with certain other clinically useful nutrients substantially improves the bioavailability of such extracts and their individual constituents. The nutrients so helpful for enhancing the absorption are the phospholipids. Phytosome is a patented technology developed by a leading manufacturer of drugs and nutraceuticals, to incorporate standardized plant extracts or water soluble phyto-constituents into phospholipids to produce lipid compatible molecular complexes, called as phytosomes and so vastly improve their absorption and bioavailability.

Keywords: Phytosomes, Biopharmaceutical classification system

INTRODUCTION

The Biopharmaceutics Classification System (BCS) was first developed by Amidon et al [1] and was designed to correlate a drug's solubility and permeability with the rate and extent of oral drug absorption. As recently reviewed by Wu and Benet [2] a drug is considered to have high solubility when the highest dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1 to 7.5 at 37°C. A drug is considered to be highly permeable when the extent of absorption (bioavailability) is at least 90%. Drugs are then classified into four BCS classes: high solubility/high permeability (Class I); low solubility/high permeability (Class II); high solubility/low permeability (Class III); and low solubility/low permeability (Class IV). The present review is mainly considered into BCS class III phytomedicine for improving their bioavailability by new trend that is phytosomes.

The term "phyto" means plant while "some" means cell-like. The phytosomes technology produces a little cell, whereby the plant extract or its active constituent is protected from destruction by gastric secretions and gut bacteria owing to the gastro-protective property of phosphatidylcholine [3]. Most of the bioactive constituents of phytomedicine are water-soluble molecules (e.g. phenolics, glycosides, and flavonoids). However, water-soluble phyto-constituents are limited in their effectiveness because they are poorly absorbed [4] when taken orally or when applied topically. Many approaches have been developed to improve the oral bioavailability, such as inclusion of solubility and bioavailability enhancers, structural modification and entrapment with the lipophilic carriers [5-7]. Water-soluble phyto-constituent molecules (mainly polyphenols) can be converted into lipid-compatible molecular complexes, which are called phytosomes. This phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, and without compromising nutrient safety [8]. They have improved pharmacokinetic and pharmacological parameters which are advantageous in the treatment of acute diseases as well as in pharmaceutical and cosmetic compositions [9].

Phospholipids are complex molecules that are used in all known life forms to make cell membranes. They are cell membrane building blocks, making up the matrix into which fit a large variety of proteins that are enzymes, transport proteins, receptors, and other biological energy converters. In humans and other higher animals the phospholipids are also employed as natural digestive

aids and as carriers for both fat-miscible and water-miscible nutrients. They are miscible both in water and in oil/ lipid environments, and are well absorbed orally. Phospholipids are small lipid molecules in which the glycerol is bonded only to two fatty acids, instead of three as in triglycerides, with the remaining site occupied by a phosphate group [10]. The phospholipids mainly employed to make phytosomes, is phosphatidylcholine, derived from soybean (*Glycine max*) [11].

The phytosome process has been applied successfully too many popular herbal extracts including *Ginkgo biloba*, grape seed, hawthorn, milk thistle (*Silybum marianum*), green tea (*Thea sinensis*) and ginseng (*Panax ginseng*). The flavonoids and terpenoids components of these herbal extracts are able to directly bind to phosphatidylcholine.

Various Properties of Phytosomes

Chemical

A phytosomes is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex results from the reaction of stoichiometric amounts of phospholipids with the selected polyphenol (like simple flavonoids) in a nonpolar solvent [8]. On the basis of their physicochemical and spectroscopic data, it has been shown that the main phospholipid-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functional groups of the substrate. They are lipophilic substances with a clear melting point, freely soluble in nonpolar solvents, and moderately soluble in fats. When treated with water, phytosomes assume a micellar shape forming liposomal-like structures. In liposomes the active principle is dissolved in an internal pocket or floats in the layer membrane, while in phytosomes the active principle is anchored to the polar head of phospholipids, becoming an integral part of the membrane [12, 9, 13]. Molecules are anchored through chemical bonds to the polar head of the phospholipids, as can be demonstrated by specific spectroscopic techniques [14-15].

Biological

Phytosome are advanced forms of herbal products that are better absorbed and utilized to produce better results than conventional herbal extracts. The increased bioavailability of the phytosomes over the non complexed botanical derivatives has been

demonstrated by pharmacokinetics studies or by pharmacodynamic tests in experimental animals and in human subjects [16].

General method for preparation of phytosomes

Phytosomes are prepared by reacting 3–2 moles (preferably with one mole) of a natural or synthetic phospholipid, such as phosphatidylcholine, phosphatidylethanolamine or phosphatidylserine, with one mole of phyto-constituents either alone or in the natural mixture in an aprotic solvent, such as dioxane or acetone, in a 1:2 or 1:1 ratio [17]. The optimum ratio of phospholipid to phytoconstituent is 1:1. The complex thus formed can be isolated by precipitation with an aliphatic hydrocarbon or lyophilization or spray drying [9]. Some liposomal drug complexes operate in the presence of water or buffer solution where the phytosomes interact with a solvent with a reduced dielectric constant. The common stages for the preparation of phytosomes [18] are charted in Fig. 1. Mareno and Lampertico [17], Jiang et al [19], Maiti et al [20] have described the methods used for phytosome preparation.

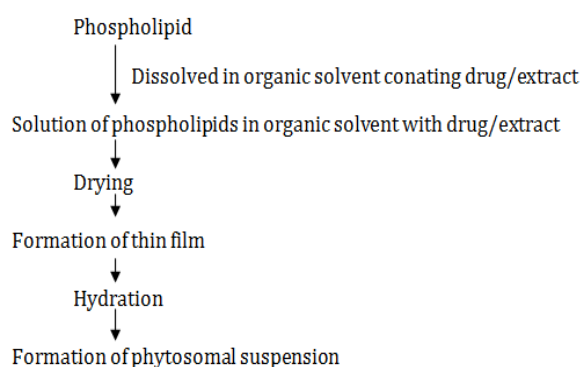


Fig. 1: Common stage for preparation of phytosome [18]

Mechanism of phytospholipid Complex formation

The poor absorption of flavonoid nutrients is likely due to two main factors. First, these are multiple ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion, nor does the intestinal lining actively absorb them, as occurs with some vitamins and minerals. Second, flavonoid molecules typically have poor miscibility with oils and other lipids. This severely limits their ability to pass across the lipid-rich outer membranes of the enterocytes, the cells that line the small intestine. The phytosome technology meets this challenge. Phytosomes results from the reaction of a stoichiometric amount of the phospholipid (phosphatidylcholine) with the standardized extract or polyphenolic constituents (like simple flavonoids) in a non polar solvent. Phosphatidylcholine is a bifunctional compound, the phosphatidyl moiety being lipophilic and the choline moiety being hydrophilic in nature. Specifically the choline head of the phosphatidylcholine molecule binds to these compounds while the lipid soluble phosphatidyl portion comprising the body and tail which then envelopes the choline bound material. Hence, the phytoconstituents produce a lipid compatible molecular complex with phospholipids, also called as phytospholipid complex. Molecules are anchored through chemical bonds to the polar choline head of the phospholipids, as can be demonstrated by specific spectroscopic techniques. Precise chemical analysis indicates the unit phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine molecule. The result is a little microsphere or cell is produced [16].

Physico-chemical characterization of phytosomes

The behavior of phytosomes in both physical and biological system is governed by the factors such as physical size, membrane permeability; percent entrapped solutes, chemical composition as well as the quantity and purity of the starting materials. Therefore,

the phytosomes are evaluated for their organoleptic properties i.e. shape, size, its distribution and physico-chemically characterized by UV, IR, NMR, DSC, SEM etc. Percentage drug entrapment, percentage drug release profile are also studied accordingly [21].

Visualization

Visualization of phytosomes can be achieved using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) [22].

Vesicle size and Zeta potential

The particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS) [23]. Their sizes vary between 50 nm to a few hundred μm .

Entrapment efficiency

The entrapment efficiency of a drug by phytosomes can be measured by the ultracentrifugation technique [24].

Transition temperature

The transition temperature of the vesicular lipid systems can be determined by differential scanning calorimetry [25].

Surface tension activity measurement

The surface tension activity of the drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer [26].

Vesicle stability

The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. The mean size is measured by DLS and structural changes are monitored by TEM [27].

Drug content

The amount of drug can be quantified by a modified high performance liquid chromatographic method or by a suitable spectroscopic method [28].

Spectroscopic evaluations

To confirm the formation of a complex or to study the reciprocal interaction between the phyto-constituent and the phospholipids, the following spectroscopic methods are used [29].

¹H-NMR

Bombardelli et al studied the NMR spectra of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine [30]. In nonpolar solvents, there is a marked change of the ¹H-NMR signal originating from the atoms involved in the formation of the complex, without any summation of the signal peculiar to the individual molecules. The signals from the protons belonging to the flavonoids are to be broadened that the proton cannot be relieved. In phospholipids, there is broadening of all the signals while the singlet corresponding to the N-(CH₃)₃ of choline undergo an uplift shift. Heating the sample to 60 results in the appearance of some new broad bands, which correspond mainly to the resonance of the flavonoid moiety.

¹³C-NMR

In the spectrum of (+)-catechin and its stoichiometric complex with distearoylphosphatidylcholine, particularly when recorded in C₆D₆ at room temperature, all the flavonoid carbons are clearly invisible. The signals corresponding to the glycerol and choline portion of the lipid (between 60–80 ppm) are broadened and some are shifted, while most of the resonances of the fatty acid chains retain their original sharp line shape. After heating to 60 the signals belonging to the flavonoid moieties reappear, although they are still very broad and partially overlapping.

FTIR

The formation of the complex can be also be confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectroscopy is also a useful tool for the control of the stability of phytosomes when micro-dispersed in water or when incorporated in very simple cosmetic gels. From a practical point of view, the stability can be confirmed by comparing the spectrum of the complex in solid form (phytosomes) with the spectrum of its micro-dispersion in water after lyophilization, at different times. In the case of simple formulations, it is necessary to subtract the spectrum of the excipients (blank) from the spectrum of the cosmetic form at different times, comparing the remaining spectrum of the complex itself.

In-vitro and in-vivo evaluations

Models of *in-vitro* and *in-vivo* evaluations are selected on the basis of the expected therapeutic activity of the biologically active phyto-constituents present in the phytosomes [29]. For example, *in-vitro* antihepatotoxic activity can be assessed by the antioxidant and free radical scavenging activity of the phytosomes. For assessing antihepatotoxic activity *in-vivo*, the effect of prepared phytosomes on animals against thioacetamide, paracetamol or alcohol induced hepatotoxicity can be examined [31-32]. Skin sensitization and tolerability studies of glycyrrhethinic acid-Phytosome® ointment, a commercial product, describe the *in vivo* safety evaluation methodology [33].

Formulating with Phytosome® complex: [33]

Phytosome® complexes can be formulated both orally and topically. In order to obtain the best performances of this technological innovation both in terms of formulating manageability and enhanced bioavailability (as appropriate disintegration and dissolution time of oral forms, for instance) Indena suggests the most appropriate manufacturing procedures to obtain effective formulations.

Soft gelatin capsules

Soft gelatin capsules represent an ideal solution to formulate Phytosome® complexes. The Phytosome® complex can be dispersed in oily vehicles to obtain suspensions to be filled in soft gelatin capsules. Vegetable or semi-synthetic oils can be used to this purpose. Indena recommend a granulometry of 100% <200 µm to best perform capsule production. According to Indena experience, not all the Phytosome® complexes behave in the same way when dispersed in oily vehicles and when the oily suspension is filled in the soft gelatin capsules; for this reasons preliminary feasibility trials should be performed to select the most suitable vehicle.

Table 1: Soft gelatin capsule formulation with Phytosome® complex [33]

Composition	Amount
Ginkgoselect® Phytosome®	180.0 mg
Soybean oil	250.0 mg
Soy lecithin	q.s.
Glycerylmonostearate	q.s.

Hard gelatin capsules

The Phytosome® complex can be formulated in hard gelatin capsules as well. A direct volumetric filling process (without pre compression) can be applied, even if the apparently low density of the Phytosome® complex seems to limit the maximum amount of powder that can be filled into a capsule (usually not more than 300 mg for a size 0 capsule). With a piston tamp capsule filling process; however, it is possible to increase the amount of powder which can be filled in a capsule, but pre compression might affect the disintegration time. Indena recommend to carefully monitoring the related parameters during product/process development. A

preliminary dry granulation process is advisable define the best manufacturing process.

Table 2: Hard gelatin capsule formulation with Phytosome® complex: Dry granulation process [33]

Composition	Amount
Ginkgoselect® Phytosome®	180.0 mg
Dicalcium phosphate dehydrate	196.0 mg
Silicified microcrystalline cellulose	47.0 mg
Croscarmellose sodium	23.0 mg
Talc	2.0 mg
Magnesium stearate	2.0 mg

Tablets

Dry granulation represents the ideal manufacturing process to obtain tablets with higher unitary doses and with suitable technological and biopharmaceutical properties. However, due to the limited flowability, potential stickiness and low apparent density of the Phytosome® complex, a direct compression process can be applied only for low unitary doses; note that whenever a direct compression process is applied, the Phytosome® complex should be diluted with 60-70% of excipients to optimize its technological properties and to obtain tablets with appropriate technological and biopharmaceutical characteristics. On the other hand, wet granulation should be avoided due to the negative effect of water and heat (granulation/drying) on the stability of the phospholipid complex.

Table 3: Tablet formulation with Phytosome® complex: Dry granulation process [33]

Composition	Amount
Leucoselect® Phytosome®	100.0 mg
Soy polysaccharides	138.0 mg
Corn starch	100.0 mg
Silicon dioxide	6.0 mg
Talc	3.0 mg
Magnesium stearate	3.0 mg

Topical dosage form

The Phytosome® complex can be formulated topically as well. The ideal process to incorporate the Phytosome® complex in emulsion is to disperse the phospholipidic complex in a small amount of the lipidic phase and add it to the already created emulsion at low temperatures (not higher than 40°C). The Phytosome® complexes are dispersible in the main lipidic solvents employed in topical formulations. In case of formulations containing a limited amount of lipids, the Phytosome® complex might also be dispersed into the watery phase, and again added to the final formulation at temperature lower than 40°C [33].

Table 4: O/W emulsion with Phytosome® complex [33]

Composition	Amount
Escin/β-Sytosterol Phytosome®	1.50 g
Glycyrrhethinic acid Phytosome®	1.50 g
Isopropyl miristate	5.00 g
Hydrogenated lanolin	5.00 g
Cetyl palmitate	3.00 g
Polysorbate 20	2.20 g
Polyacrylamide (and)	2.00 g
C13-14 isoparaffin and laureth-7	
Phenoxyethanol	1.00 g
Dimethicone 350 cps	0.50 g
Imidazolidinyl urea	0.30 g
Tocopherol	0.20 g
Preservatives	0.30 g
Ascorbyl palmitate	0.10 g

Disodium edentate	0.10 g
Purified water	q.s. to 100 g

Advantage of phytosomes [34-37]

- It enhances the absorption of lipid insoluble polar phyto-constituents through oral as well as topical route showing better bioavailability, hence significantly greater therapeutic benefit.
- Appreciable drug entrapment.
- As the absorption of active constituent(s) is improved, its dose requirement is also reduced.
- Phosphatidylcholine used in preparation of phytosomes, besides acting as a carrier also acts as a hepatoprotective, hence giving the synergistic effect when hepatoprotective substances are employed.
- Chemical bonds are formed between phosphatidylcholine molecule and phyto-constituent, so the phytosomes show better stability profile.
- Application of phyto-constituents in form of phytosome improve their percutaneous absorption and act as functional cosmetics.

Application of phytosome

Recent research shows improved absorption and bioavailability with phytosomes as compared to the conventional means. Most of the phytosomal studies are focused to *Silybum marianum* (milk thistle) which contains premier liver-protectant flavonoids. Yanyu et al. prepared the silymarin phytosome and studied its pharmacokinetics in rats. In the study the bioavailability of silybin in rats was increased remarkably after oral administration of prepared silybin-phospholipid complex due to an impressive improvement of the lipophilic property of silybin-phospholipid complex and improvement of the biological effect of silybin [38].

Grape seed phytosome is composed of oligomeric polyphenols (grape proanthocyanidins or procyanidins from grape seed extract, *Vitis vinifera*) of varying molecular size, complexed with phospholipids. The main properties of procyanidin flavonoids of grape seed are an increase in total antioxidant capacity and stimulation of physiological antioxidant defenses of plasma, protection against ischemia/reperfusion induced damages in the heart, protective effects against atherosclerosis thereby offering marked protection for the cardiovascular system and other organs through a network of mechanisms that extend beyond their great antioxidant potency [39].

Maiti et al. developed the quercetin-phospholipid phytosomal complex by a simple and reproducible method and also showed that the formulation exerted better therapeutic efficacy than the molecule in rat liver injury induced by carbon tetrachloride [40].

Hesperetin is a potent phyto-molecule abundant in citrus fruits, such as grapefruit and oranges. In spite of several therapeutic benefits viz. antioxidant, lipid-lowering, anti-carcinogenic activities their shorter half life and lower clearance from the body restricts its use. To overcome this limitation, recently Mukerjee et al. developed a novel hesperetin phytosome by complexing hesperetin with hydrogenated phosphatidylcholine. This complex was then evaluated for antioxidant activity in CCl₄-intoxicated rats along with pharmacokinetic studies. It was found that the phytosome had a sustained release property for over 24 h and enhanced antioxidant activity. Pharmacokinetic study revealed that the phytosome had higher relative bioavailability than that of parent molecule at the same dose level [41].

Maiti et al. (2006) developed phytosomes of curcumin and naringenin in two different studies [20, 42]. In the first study, phytosomes of curcumin were developed to overcome the limitation of absorption and to investigate the protective effect of curcumin-phospholipid complex on carbon tetrachloride-induced acute liver damage in rats. The complex showed enhanced aqueous and n-octanol solubility. The antioxidant activity of the complex was significantly higher than that of pure curcumin at all dose levels tested. In the other study, the developed phytosomes of naringenin exhibited better antioxidant activity than the free compound with a prolonged duration of action, which may be helpful in reducing the rapid elimination of the molecule from the body.

Green tea extract generally contains a totally standardized polyphenolic fraction (not less than 66.5 %, containing epigallocatechin and its derivatives) obtained from green tea leaves (*Thea sinensis*) and mainly characterized by the presence of epigallocatechin 3-O-gallate, the key compound. These compounds are potent modulators of several biochemical processes linked to the breakdown of homeostasis in major chronic-degenerative diseases such as cancer and atherosclerosis. Green tea has got several long term beneficial activities such as antioxidant, anticarcinogenic, antimutagenic, antiatherosclerotic, hypocholesterolemic, cardioprotective, antibacterial and anticariogenic effects. Despite such potential actions green tea polyphenols have very poor oral bioavailability from conventional extracts. The complexation of green tea polyphenols with phospholipids strongly improves their poor oral bioavailability. A study on absorption of phytosomal preparations was performed in healthy human volunteers along with non complexed green tea extract following oral administration. Over the study period of 6 hours the plasma concentration of total flavonoids was more than doubled when coming from the phytosomal versus the non-phytosomal extract. Antioxidant capacity was measured as TRAP (Total Radical-trapping Antioxidant Parameter). The peak antioxidant effect was a 20% enhancement and it showed that the phytosome formulation had about double the total antioxidant effect [43 - 44].

Table 5: Marketed formulations of phytolipid delivery system [43,45,46]

Brand Name	Medicinal Plant	Indication	Extract/Active Constituent
SILIPHOS™	<i>Silybum marianum</i>	liver protectant	Silybin
Ginkgoselect®	<i>Ginkgo biloba</i>	Vasokinetic	Ginkgoflavonglucosides, ginkgolides, bilobalide
Leucoselect®	Grape seed	cardiovascular system	Grape procyanidins
Greenselect®	Green tea leaves	cancer and atherosclerosis	Polyphenols
Mirtoselect®	Bilberry	potent antioxidants	Anthocyanosides
Sabalselect®	Saw palmetto berries	non-cancerous prostate enlargement	Extract
Lymphaselect™	<i>Melilotus officinalis</i>	chronic venous insufficiency of the lower limbs	Standardized extract
Polinacea™	<i>Echinacea angustifolia</i>	enhances immune function	Echinacosides
18β-glycyrrhetic acid Phytosome®	Licorice	Soothing	18β-glycyrrhetic acid
Centella Phytosome®	<i>Centella asiatica</i>	Cicatrizing, trophodermic	Triterpenes
Crataegus Phytosome®	Hawthorn	Antioxidant	Vitexin-2"-O-rhamnoside

Escin β -sitosterol Phytosome® Ginselect® Phytosome®	Chestnut <i>Panax ginseng</i>	Anti-oedema Skin elasticity improver, Adaptogenic	Escin β -sitosterol Ginsenosides
Ginkgo biloba Terpenes Phytosome® Ginkgo biloba Dimeric Flavonoids Phytosome®	<i>Ginkgo biloba</i> <i>Ginkgo biloba</i>	Soothing Lipolytic, vasokinetic	Ginkgolides and bilobalide Dimeric flavonoids
PA2 Phytosome®	Horse chestnut	Anti-wrinkles, UV protectant	Proanthocyanidin A2
Sericoside Phytosome®	<i>Terminalia sericea</i>	Anti-wrinkles	Sericoside
Visnadex®	<i>Amni visnaga umbel</i>	Vasokinetic	Visnadin

Table 6: List of flavanoids for phytolipid drug delivery system [47]

Source	Composition	Uses
Citrus flavanoids	Rutin, hesperidin and naringin	Antioxidants and sometimes anti-inflammatory
Blueberries	Anthocyanins	Anti-inflammatory
Green tea	EpiGalloCatechin Galleate	Antioxidant
Soy beans	Genistein, glycitein and daidzein	Inhibits tyrosine kinases involved in tumorigenesis
Asparagus	Rutin	Strengthens capillary walls
• Red onions, buckwheat, red grapes and green tea	Quercetin	Antioxidant
• Highest in apple skins		
Milk thistle	Silymarin	Antioxidant, anti-carcinogenic
Tangerines	Tangeretin	Stopping cancer cell proliferation
Pomegranate	Punicalagin	Antioxidant
Strawberries	Ellagic Acid	Reduces esophageal and colon cancers
Blueberries, tomatoes and bell peppers	Chlorogenic Acid	Antioxidant

CONCLUSION

Biopharmaceutical classification system which was originally developed for chemical entity may also be helpful for active constituents of herbal plants. Based on this system there are four types of class of drug from I-IV. In the medicinal product there are wide variety of flavanoid and terpene which are helpful for human being but they are difficult to produce the desirable effect due to high water solubility and lipid impermeability. A simple and most promising approach for delivery of such herbal extract is development of phytosome. Phytosomes are novel formulations which offer improved bioavailability of hydrophilic flavonoids and other similar compounds through the skin or gastrointestinal tract. As far as the potential of phytosome technology is concerned, it has a great future for use in formulation technology and applications of hydrophilic plant compounds.

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