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NANOSUSPENSION: AN ATTEMPT TO ENHANCE BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

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ABSTRACT

Most of the new chemical entities coming out from High-throughput screening in drug discovery process are failing due to their poor solubility in the water. Poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the critical problems associated with poorly soluble drugs is too low bioavailability. The problem is even more complex for drugs belonging to BCS CLASS II category, as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. There are number of formulation approaches to resolve the problems of low solubility and low bioavailability. These techniques for solubility enhancement have some limitations and hence have limited utility in solubility enhancement. Nanotechnology can be used to resolve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} meters. The present article describes the details about nanosuspensions. Nanosuspensions consist of the pure poorly water-soluble drug without any matrix material suspended in dispersion. The review article includes the methods of preparation with their advantages and disadvantages, characterization and evaluation parameters and pharmaceutical applications. A nanosuspension not only solves the problems of poor solubility and bioavailability but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy.

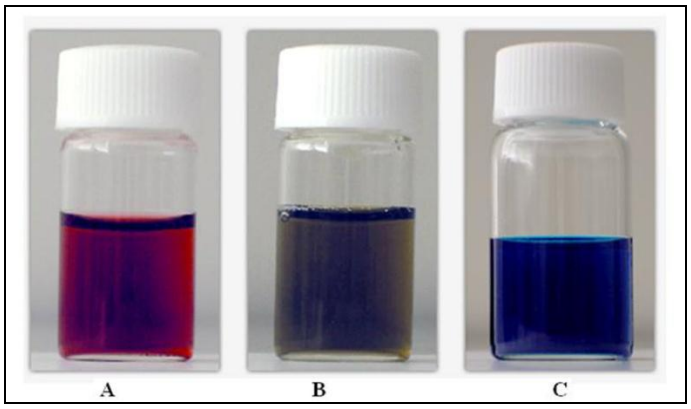
INTRODUCTION: Most of the new chemical entities (about 40%) coming out from High-throughput screening in drug discovery process are failing due to their poor solubility in the water [1]. As per a recent report [2], 46% of the total New Drug Applications (NDA) filed between 1995 and 2002 were BCS class IV, while only 9% were BCS class I drugs, revealing that a majority of the approved new drugs were water insoluble Because of their poor solubility it will become more complicated to incorporate them into the conventional dosage forms and thus decreasing the bioavailability of the drugs [3].

The problem is even more complex for drugs such as Glibenclamide (belonging to BCS CLASS II) as classified by BCS System [4] as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. For class II drugs, the rate limiting factor in their intestinal absorption is dissolution/solubility and thus the performance of these drugs is dissolution rate-limited and is affected by the fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are greatly affected by the shape as well as the particle size of the drug. Therefore decrease in particle size results in an increase in dissolution rate [5]. There are number of formulation approaches that can be used to resolve the problems associated with the low solubility and low bioavailability of these class II drugs. Some of the approaches to increase solubility include micronization [6], solubilisation using co-solvents, use of permeation enhancers, oily solutions, surfactant dispersions [6], salt formation [7] and precipitation techniques [8-9].

Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement like microspheres, emulsions, microemulsions [10], liposomes [11], super critical processing, solid-dispersions [12] and inclusion complexes using Cyclodextrins [13] show reasonable success but they lack in universal applicability to all drugs. These techniques are not applicable to the drugs, which are not soluble in both aqueous and organic Media.

However, there still remains an unmet need to equip the pharmaceutical industry with particle engineering technologies capable of formulating the poorly soluble drugs to improve their efficacy and to optimize therapy with respect to pharmacoeconomics. One such novel technology is nanosuspension technology. Nanosuspensions are sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants [14]. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion [15]. These can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster.

This is one of the unique advantages that it has over other approaches for enhancing solubility. This approach is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries. The suspensions can also be lyophilised and into a solid matrix. Apart from these advantages it is also having the advantages of liquid formulations over others. In the present review we are mainly focussing on the different methods of preparation, critical parameters and evaluation of the nanosuspension. Fig. 1 shows some of the nanosuspensions [16].



A- Gold nanosuspension in water, **B** -Silver nanosuspension in water, **C** -VOPc (vanadyl phthalocyanine) nanosuspension in water

FIGURE 1: FEW TYPES OF NANOSUSPENSIONS.

Nanosuspensions differ from nanoparticles [17] which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid-lipid nanoparticles [18] (SLN), which are lipidic carriers of drug. The potential benefits of nanoparticles over conventional technologies are described in **Table 1** [19].

TABLE 1: POTENTIAL BENEFITS OF NANOSUSPENSION TECHNOLOGY

ROUTE OF ADMINISTRATION	POTENTIAL BENEFITS
Oral	• Rapid dissolution and • High bioavailability • Reduced fed/fasted ratio
Intravenous (I.V)	• Tissue targeting • Rapid dissolution • Longer duration of retention in systemic circulation
Ocular	• Higher bioavailability • Less irritation • More consistent dosing
Inhalation	• Higher bioavailability • More consistent dosing
Subcutaneous/ intramuscular	• Higher bioavailability • Rapid onset • Reduced tissue irritation

Preparation of Nanosuspensions: Preparation of nanosuspensions were reported to be a more cost effective and technically more simpler alternative than liposomes and other conventional colloidal drug carriers, particularly for poorly soluble drugs and yield a physically more stable product. The simplest method of preparation of nanosuspensions is micronization by colloid or jet milling [20], which improves the dissolution rate but is not having any effect on saturation solubility. Nanosuspension engineering processes currently used are preparation by precipitation, high pressure homogenization, emulsion and milling techniques. These techniques and the obtained compounds are summarized in **Table 2** and are briefly described in the following sections. Mainly there are two methods for preparation of nanosuspensions. The conventional methods of precipitation are called 'Bottom Up technology'. The 'Top Down Technologies' are the disintegration methods and are preferred over the precipitation methods. These include Media Milling (Nanocrystals), High Pressure Homogenization in water (Dissocubes), High Pressure Homogenization in nonaqueous media (Nanopure) and combination of Precipitation and High-Pressure Homogenization (Nanoedege). Few other techniques used for preparing nanosuspensions are emulsion as templates, microemulsion as templates etc.

- **Precipitation:** The most common method of precipitation used is anti solvent addition method in which the drug is dissolved in an organic solvent and this solution is mixed with a miscible antisolvent. Mixing processes vary considerably. Precipitation has also been coupled with high shear processing. The NANOEDGE process (is a registered trademark of Baxter International Inc. and its subsidiaries) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy [32].

TABLE 2: SUMMARY OF THE NANOSUSPENSION FORMATION TECHNOLOGIES

Technology	Advantage	Disadvantage	Drug
Precipitation	Simple process. Ease of scale up. Economical production.	Growing of crystals needs to be limit by surfactant addition. Drug must be soluble at least in one solvent.	Carbamazepine [8] Cyclosporine [21] Griseofulvin [22]
Emulsion/Microemulsion template	High drug solubilization. Long shelf life. Ease of manufacture.	Use of high amount of surfactant and stabilizers. Use of hazardous solvent in production.	Breviscapine [23] Griseofulvin [24]
High pressure Homogenization	Applicable to most of the drugs Very dilute as well as highly concentrate nanosuspension can be prepared. Aseptic production possible.	High number of homogenization cycles. Drug should be in micronized state. Possible contamination could occur from metal ions coming off from the walls.	Albendazole [25] Aphidicolin [26] Azithromycin [27] Fenofibrate [28]
Milling methods • Media milling	Applicable to the drugs that are poorly soluble in both aqueous and organic media. Little batch to batch variation. High flexibility in handling large quantities of drugs.	Time consuming. Difficult to scale up. Prolonged milling may induce the formation of amorphous & instability.	Cilostazol [29] Danazol [3] Naproxen [3]
• Dry Co-grinding	Easy process and no organic solvent required. Require short grinding time.	Generation of residue of milling media.	Clarithromycin [30] Glibenclamide [31]

This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Rapid addition of a drug solution to an antisolvent leads to sudden super saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded. The success of drug nanosuspensions prepared by precipitation techniques has been reported in some journals [32-33].

- **Lipid Emulsion/Microemulsion Template:** Lipid emulsions as templates are applicable for drugs that are soluble in either volatile organic solvents or partially water miscible solvents. In this method the drug will be dissolved in the suitable organic solvent and then emulsified in aqueous phase using suitable surfactants. Then the organic solvent will be slowly evaporated under reduced pressure to form drug particles precipitating in the aqueous phase forming the aqueous suspension of the drug in the required particle size. Then the suspension formed can be diluted suitably to get nanosuspensions [34]. Moreover, microemulsions as templates can produce nanosuspensions. Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids such as oil and

water stabilized by an interfacial film of surfactant and co-surfactant. The drug can be either loaded into the internal phase or the pre-formed microemulsion can be saturated with the drug by intimate mixing. Suitable dilution of the microemulsion yields the drug nanosuspension [34]. An example of this technique is the griseofulvin nanosuspension which is prepared by the microemulsion [34]. The advantages of lipid emulsions as templates for nanosuspension formation are that they easy to produce by controlling the emulsion droplet and easy for scale-up. However, the use of organic solvents affects the environment and large amounts of surfactant or stabilizer are required.

- **High Pressure Homogenization:** It is the most widely used method for the preparation of the nanosuspensions of many poorly water soluble drugs [35-37]. Different methods developed based on this principle for preparation of nanosuspensions are *Dissocubes, Nanopure, Nanoedge, Nanojet technology.* In the high pressure homogenization method, the suspension of a drug and surfactant is forced under pressure through a nanosized aperture valve of a high pressure homogenizer.

The principle of this method is based on cavitation in the aqueous phase. The particles cavitations forces are sufficiently high to convert the drug microparticles into nanoparticles. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required [38-39]. Figure 2 gives the schematic representation of the high-pressure homogenization process

  - DissoCubes technology is an example of this technology developed by R.H. Müller using a piston-gap-type high pressure homogenizer, which was recently released as a patent owned by SkyePharm plc [34]. Scholer *et al*. prepared atovaquone nanosuspensions using this technique.
  - Nanopure is suspensions homogenized in water-free media or water mixtures.
  - Nanoedge is combination of precipitation and homogenization techniques resulting in smaller particle size and better stability in a shorter time.
  - *Nanojet technology*, also called as opposite stream, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure.

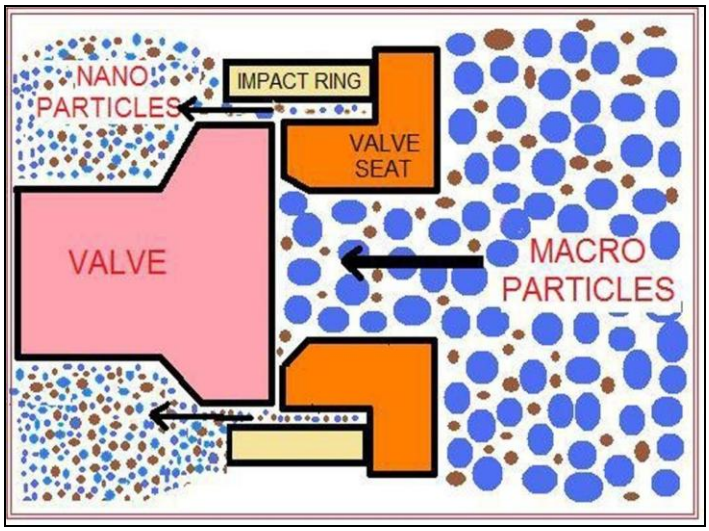


FIGURE 2: SCHEMATIC CARTOON OF THE HIGH-PRESSURE HOMOGENIZATION PROCESS

- **Milling Techniques:**
  - **Media milling:** Media milling is a further technique used to prepare nanosuspensions [24, 40]. This patent-protected technology was developed by Liversidge et al. [41]. Formerly, the technology was owned by the company NanoSystems but recently it has been acquired by Elan Drug Delivery. In this technique, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. The drug nanoparticles are obtained by subjecting the drug to media milling. High energy and shear

forces generated as a result of impaction of the milling media with the drug provide the necessary energy input to disintegrate the microparticulate drug into nanosized particles. The milling medium is usually composed of glass, zirconium oxide or highly cross-linked polystyrene resin. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters <200nm is 30–60 min. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then milling media or pearls are rotated at a very high shear rate.

- **Dry Co-Grinding:** Recently, nanosuspensions can be obtained by dry milling techniques. Successful work in preparing stable nanosuspensions using dry-grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media has been reported [42]. Itoh *et al* [35] reported the colloidal particles formation of many poorly water soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinylpyrrolidone (PVP) and sodium dodecylsulfate (SDS).

Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used [43]. Physicochemical properties and dissolution of poorly water soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug [44]. Dry co-grinding can be carried out easily and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable. Table 3 shows some drugs and their status in market.

TABLE 3: SOME DRUGS AND THEIR STATUS IN MARKET

Drug	Category	Route of Administration	Status
Fenofibrate	Anticancer	Oral	Phase I
Rapamuane	Antiemetic	Oral	Marketed
Emend	Antiasthamatic	Oral	Marketed
Thymectacin	Antidiabetic	I.V.	Phase I/II
Silver	Eczema, Atopic dermatitis	Topical	Phase I
Busulfan	Hypolidemic	Intrathecal	Phase I
Paclitaxel	Anticancer	I. V.	Phase IV
Insulin	Antidiabetic	Oral	Phase I
Budesonide	Anticancer	Pulmonary	Phase I

Physical, Chemical and Biological Properties of Nanosuspensions: Nanosuspension formulation increases the saturation solubility as well as dissolution rate. Basically the saturation solubility is a compound specific constant which is temperature dependent. The saturation solubility also depends on the polymorphism of the drug as different polymorphs have different solubilities. It is also dependent on the particle size. This size-depend encycomes only into effect for particles having a size below approximately 1 µm. Another marked property is the adhesiveness generally described for nanoparticles [45].

As the particle size decreases the adhesive properties of the particles will be improved and thus improved oral delivery of poorly soluble drugs. Improved bioavailability, improved dose proportionality, reduced fed/fasted variability, reduced inter-subject variability and enhanced absorption rate (both human and animal data) [46] are some of the main effects observed on oral administration. These data have been acquired *in vivo* in animals but also in humans as reported by the company Nano Systems. A drastically remarkable report is that of the increase in bioavailability for danazole from 5 % (as macrosuspension) to 82% (as nanosuspension) [46]. The application of high

pressures during the production of nanosuspensions was found to promote the amorphous state [47]. The degree of particle fineness and the fraction of amorphous particles in the nanosuspensions were found to be dependent on production pressure number of cycles of homogenisation and hardness of drug. The increase in the amorphous fraction leads to a further increase of the saturation solubility. The homogenization process (giving uniform particle size) was able to overcome Ostwald ripening [48] which means physical long-term stability as an aqueous suspension [49].

In oral drug administration, the bioavailability mainly depends upon the solubility of the drug, highly active compounds have failed in the past because their poor solubility has limited *in vivo* absorption and did not lead to effective therapeutic concentrations. As an example, Atovaquone is given orally three times 750 mg daily, because of the low absorption of only 10–15%. Oral administration of nanosuspensions can overcome this problem because of the high adhesiveness of drug particles sticking on biological surfaces and prolonging the absorption time.

Evaluation of Nanosuspensions [50-51]: The characterisation of the nanosuspensions is also similar to that of the suspensions such as colour, odour, presence of impurities and other important characteristics as mentioned below.

- ***In-Vitro* Evaluations:**
    - **Particle size and size distribution**
    - **Particle charge (Zeta Potential)**
    - **Crystalline state and morphology**
    - **Saturation solubility and dissolution velocity**
    - **Stability**
- ***In-vivo* evaluation:**
- ***In-Vitro* Evaluations:**
    - **Particle size and size distribution:** It is the most important parameter in the evaluation of the suspensions as it is having the direct effect on the solubility and dissolution rate and the physical stability of the formulation. The mean particle size and the width of particle size can be determined by Photon Correlation Spectroscopy (PCS) [52], laser diffraction and coulter current multisizer. Particle size and polydispersity index (PI) governs the saturation solubility, dissolution velocity and biological performance. PCS measures the particle size in the range of 3nm-3 µm only. PI governs the physical stability of nanosuspension and should be as low as possible for long-term stability (Should be close to zero). LD measures volume size distribution and measures particles ranging from 0.05- 80µm upto 2000µm. Atomic Force Microscopy is used for visualization of particle shape [53]. For IV use, particles should be less than 5 µm, considering that the smallest size of the capillaries is 5-6 µm and hence a higher particle size can lead to capillary blockade and embolism.
    - **Particle charge (Zeta Potential):** The particle charge is of importance in the study of the stability of the suspensions. Usually the zeta potential of more than ±40mV will be considered to be required for the stabilisation of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ±20mV of zeta potential is required.
    - **Crystalline Sate and Particle Morphology:** It is of importance as there are chances of the polymorphism during the storage of the nanosuspensions. Hence it is necessary to study the crystal morphology of the drug in suspension. Differential Scanning Calorimetry

(DSC) is most commonly used for such studies [54]. When nanosuspensions are prepared drug particles may get converted to amorphous form hence it is essential to measure the extent of amorphous drug generated during the production of nanosuspensions. The X-Ray Diffraction (XRD) is commonly used for determining change in crystallinity and the extent of the amorphous form of drug [55].

- **Saturation solubility and Dissolution Velocity:** The main advantage associated with the nanosuspensions is improved saturation solubility as well as dissolution velocity. These are studied in different physiological solutions at different pH. Kelvin equation and the Ostwald-Freundlich equations can explain increase in saturation solubility. Determination of these parameters is useful to assess *in vivo* performance of the formulation.

- **Stability of Nanosuspensions:** Stability of the suspensions is dependent on the particle size. As the particle size reduces to the nanosize the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening and improving the stability of the suspension by providing a steric or ionic barrier. Typical examples of stabilizers used in nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones. Lecithin may be preferred in developing parenteral nanosuspensions [40].
- ***In vivo* evaluation:** The *in vivo* evaluation of the nanosuspensions is specific to drug and route of administration. Most commonly the formulation was given by required route of administration and the plasma drug levels were estimated using HPLC-UV visible Spectrophotometry. Other parameters which are generally evaluated *in vivo* are
  - Surface hydrophilicity/hydrophobicity (determines interaction with cells prior to phagocytosis)
  - Adhesion properties
  - Interaction with body proteins

APPLICATIONS: Formulating the drug as nanosuspensions increases the saturable concentration, dissolution rate as well as bioavailability of the drug. These nanosuspensions are having application in different routes of administrations like oral, parenteral, topical, ophthalmic, mucoadhesive, pulmonary and targeted drug delivery. Oral administration of nanosuspensions is a drug delivery strategy, not only to improve bioavailability, but also to target gastrointestinal bacterial and parasitic infections because of improved adhesion properties. Nanosuspension technology is considered as suitable new colon delivery systems for the treatment of colon cancer, helminth infections, gastrointestinal inflammation or GIT associated diseases like sprue (zoeliaki).

Infections like tuberculosis, listeriosis, leishmaniasis, and toxoplasmosis are caused by parasites residing the macrophages of the MPS, thus being relatively easily accessible by I.V. injected particles. The I.V. injected particles are heavily and quickly taken up by the MPS cells in case they absorb uptake promoting proteins like apolipoproteins. However, some parasites do also reside in the brain (CNS). The brain-localized parasite mostly leads to relapsing infections if not cured. Therefore, it would be of importance to target drug nanoparticles via surface modification to the brain. A successful targeting of the peptide, dalargin, to the brain using Tween 80® surface modified polyisobutylcyanoacrylates nanoparticles has been reported by Kreuter et al. [56]. A nanosuspension of Amphotericin B developed by Kayser *et al.* showed a significant improvement in its oral absorption in comparison with the

conventional commercial formulations [57]. In case of I.V administration the particle size less than 5µm is preferred. The particle size in nano range will favour the passage of the drug particles into the small capillaries in the body without any blockade. A stable intravenously injectable formulation of omeprazole has been prepared to prevent the degradation of orally administered omeprazole [37].

Aqueous suspensions of the drug can be easily nebulised and given by pulmonary route as the particle size is very less. Different types of nebulisers are available for the administration of liquid formulations. Some of the drugs successfully tried with pulmonary route are budesonide, ketotifen, ibuprofen, indomethacin, nifedipine, itraconazole, interleukin-2, p53 gene, leuprolide, doxorubicin etc. [58] Nanosuspensions can be used for targeted delivery also as the surface of the particle can be suitably modified to make them target specific. Kayser formulated a nanosuspension of Aphidicolin to improve drug targeting against leishmania-infected macrophages [26]. Scholer et al. Prapeared a nanosuspension formulation of Atovaquone and showed an improved drug targeting to the brain in the treatment of toxoplasmic encephalitis in a new murine model infected with Toxoplasma gondii [55].

CONCLUSIONS: Nanosuspensions are chiefly seen as vehicles for administering poorly water soluble drugs have been largely solved the dissolution problems to improve drug absorption and bioavailability. Nanosuspension technology can be combined with traditional dosage forms: tablets, capsules, pellets, and can be used for parenteral products. They have recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents or diagnostic agents. Because of their submicron size they are easily targeted to the tumour area. Moreover the possibility of surface functionalization with a targeting moiety has open new avenues for targeted delivery of drugs, genes, photosensitizers and other molecules to the desired area. To take advantage of nanosuspension drug delivery, simple formation technologies and variety applications, nanosuspensions will continue to be of interest as oral formulations and non-oral administration develop in the future. It is expected that future research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicle.

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