Vitamin A Microencapsulation Within Poly(methyl methacrylate)-*g*-Polyethylenimine Microspheres: Localized Proton Buffering Effect on Vitamin A Stability

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ABSTRACT: To stabilize vitamin A in a cosmetic/dermatological formulation, we present here a new encapsulation method based on polymer microspheres having a localized "proton-buffering" capacity. Poly(methyl methacrylate)-*g*-polyethylenimine (PMMA-*g*-PEI) was prepared by direct condensation grafting of PEI onto poly(methyl methacrylate-*co*-methyl acrylic acid). The reaction was confirmed by FT-IR analysis showing the amide vibration at 1,550 cm^{-1}. Elemental analysis indicated that the weight content of the grafted PEI was 1.6% (w/w). Vitamin A was encapsulated into PMMA-*g*-PEI microspheres by using an oil-in-water (O/W) single emulsion method. The presence of PEI moiety dramatically improved the chemical stability of vitamin A in microspheres. Vitamin A encapsulated within PMMA-*g*-PEI microspheres maintained 91% of its initial activity after 30-day incubation at 40°C, while only maintaining 60% within plain PMMA microspheres. This study demonstrates that proton-buffering within hydrophobic polymer matrix is a useful strategy for stabilizing "acid-labile" active ingredients. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 517–522, 2004

Key words: graft copolymers; microencapsulation; stabilization; functionalization of polymers; polyimines

INTRODUCTION

Vitamin A has been widely utilized as a popular active ingredient for antiaging dermatological treatments. It has been established that retinoids can act as important regulators in the proliferation and differentiation of cells. Topical application of retinoids is effective for stimulating collagen synthesis in the dermis and for treating skin diseases such as acne and psoriasis.[1–3] However, the use of vitamin A has been restricted because of high potency of skin irritation and intrinsic chemical instability.[4–6]

Conventional cosmetic and pharmaceutical formulations usually have a large amount of water, which often mediates the degradation reaction of vitamin A. For that reason, recent research for stabilizing vitamin A has focused on isolating it from aqueous media by using various microencapsulation techniques, such as polymer microparticles,[7–10] liposomes,[6] solid-lipid nanoparticles (SLN),[11] etc. Although the chemical stability can be partially improved by these physical encapsulation techniques, vitamin A still has an instability problem in a water-based formulation for long-term storage. Therefore, a new technique is necessary to improve its chemical stability in commercial products.

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The structural degradation of vitamin A can happen via various pathways in the presence of water, light, oxygen, high temperature, lipid peroxides, etc. Generally, isomerization is induced by high temperature (all-*trans* form to 13-*cis* form) and ultraviolet light (all-*trans* form to 9-*cis* form), decomposition by oxygen and lipid peroxide, and dehydration by water.[5] In particular, dehydration is accelerated at low pH because the terminal hydroxyl group of vitamin A is highly susceptible to proton-mediated hydrolysis. In our previous study, it was found that vitamin A mainly degrades to anhydro-vitamin A, an inactive form, via acid hydrolysis in a conventional emulsion cream.[12] Therefore, it is conceivable that vitamin A can be stabilized, if the accessability of proton molecules to vitamin A is minimized.

In this contribution, we propose a new stabilization strategy for vitamin A, which is based on polymer microspheres having a locally alkaline microclimate. A similar concept was found in encapsulating therapeutic proteins in biodegradable poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) microspheres or implants, where acidification of the inner polymer environment is a critical issue for the protein instability problem.[13–17] An acidic microclimate within PLGA matrix, which is suspected as one of main sources of protein inactivation, can be induced as polyester hydrolysis produces acidic degradation products. To overcome this problem, Schwendeman and colleagues coincorporated an antacid, $Mg(OH)_2$, into PLGA polymer,

which increased microclimate pH and prevented protein inactivation.[15–17]

It was expected that this stabilization strategy would be available for vitamin A stabilization. However, simple addition of basic salt additives into polymer matrix was not effective for stabilizing vitamin A, since the salts were easily hydrated and released to external phase as the incubation proceeded. It should be noted that the storage and delivery period of conventional cosmetic/dermatological commercial products often lasts 2 years. To deal with this issue, we adopt here a polymer encapsulation technique combined with a chemical immobilization of antacid molecules onto a hydrophobic polymer backbone. For this purpose, poly(methyl methacrylate)-*g*-polyethylenimine (PMMA-*g*-PEI) was synthesized by a simple coupling reaction and used for the encapsulation of vitamin A. Vitamin A was loaded into the polymer microspheres and the integrity of vitamin A was monitored at 40°C.

Experimental

Materials

Poly(methyl methacrylate-*co*-methacrylic acid [poly(MMA-*co*-MAA)] was obtained from Aldrich Chemical Co (Milwaukee, WI). It has a weight average molecular weight (M_w) of 34 kDa and a MMA to MAA molar ratio of 1 : 0.016. Polyethylenimine (PEI, linear type, degree of polymerization = 10), 1,3-dicyclohexylcarbodimide (DCC), and *N*-hydroxy succinimide (NHS) were also obtained from Aldrich Chemical Co. Retinol 50C (1 : 1 mixture of all-*trans* vitamin A and Tween 80) was purchased from BASF (Ludwigshafen, Germany) and Salcare SC95, a polyquaternium-based thickner, from Ciba Specialty Chemicals Inc. (Basel, Switzerland). Polyvinylalcohol (PVA, 88% hydrolyzed, M_w 25,000) was purchased from Polysciences (Warrington, PA). All other reagents were of analytical grade.

Polymer synthesis

Poly(MMA-*co*-MAA) (0.15 mmol), DCC (7 mmol), and NHS (7 mmol) were dissolved in anhydrous dioxane (100 mL). The solution then was slowly added to PEI (0.02 mmol) dissolved in 100 mL anhydrous DMF. The conjugation reaction was carried out for 10 h at room temperature under nitrogen atmosphere. The insoluble dicyclohexylurea was removed through filtration (Millipore 0.45 μm, HVLP 04700). The polymer product was obtained by precipitation into excess methanol under vigorous agitation. The resultant PMMA-*g*-PEI was dried *in vacuo* and stored at 4°C before use. Infrared (IR) spectra were obtained by the KBr tablet method with a Bio-Rad infrared spectrometer (model FTS-40, Cambridge, MA). Nitrogen content was determined by elemental anlaysis (PE-2400, Perkin–Elmer) and used for the calculation of PEI grafting efficiency, as calculated from eqs. (1) and (2), respectively.

$$N = \frac{\text{Weight of nitrogen}}{\text{total weight of polymer}} = \frac{nx_{EI}M_n}{M_b + nx_{EI}M_{EI}} = \frac{140\,n}{15{,}000 + 430\,n} \quad (1)$$

$$\text{Grafting efficiency} = \frac{n}{n_{MAA}} \times 100 \quad (2)$$

where N represents the nitrogen content, which can be measured from elemental analysis, n is the number of PEI grafted to backbone, x_{EI} is the degree of polymerization of PEI ($\approx$ 10), M_b is the molecular weight of backbone polymer ($\approx$ 15,000 g/mol), M_{EI} is the molecular weight of ethylenimine (= 43 g/mol), M_n is the molecular weight of nitrogen ($\approx$ 14 g/mol), and n_{MMA} is the number of MAA in the backbone.

Vitamin A encapsulation

Vitamin A–loaded polymer microspheres were prepared by an oil-in-water (O/W) single emulsification and solvent evaporation technique. Four grams of polymer (PMMA or PMMA-*g*-PEI) and 0.5 g of Retinol 50C were dissolved in 20 mL of CH_2Cl_2. The resulting homogeneous solution was emulsified in 200 mL of aqueous solution [0.5% (w/v) PVA and 10% NaCl] for 3 min by agitation at 2,000 rpm and subsequently stirred magnetically for 12 h at room temperature to remove CH_2Cl_2 via evaporation. The hardened microspheres were collected by centrifugation at 8,000 rpm for 20 min and washed twice with 1 L of deionized water.

Characterization

Morphologies of the obtained microspheres were examined on an optical microscope (OM; Olympus BX50, Olympus Optical Co. Ltd, Tokyo, Japan) and a scanning electron microscope (SEM; S-4300, Hitachi, Japan). Vacuum-dried microspheres were mounted on an aluminum stub covered with a carbon adhesive tape and then coated with gold. Size average and distribution of microspheres was observed by using a Mastersizer X (Malvern, UK). The internal distribution of vitamin A in polymer microspheres was observed by using confocal laser scanning microscopy (CLSM). The microspheres were dispersed in 50% (v/v) glycerol aqueous solution and imaged under the fluorescence confocal microscope (Radiance 2000/MP, Bio-

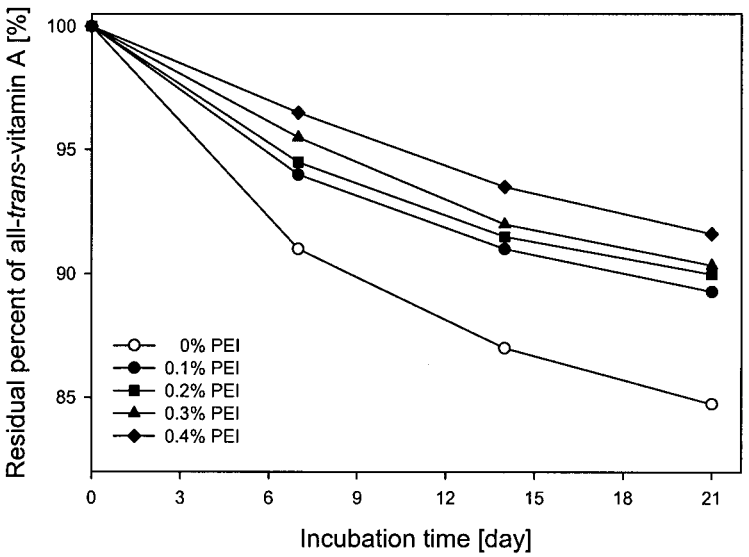

Figure 1 Stability profiles of vitamin A dissolved in ethanol solution with different PEI concentrations. Each point represents the average value of at least three experiments.

Rad, UK) equipped with an Ar/Kr laser and Nikkon eclipse TE300. The specific surface area was analyzed using an ASAP 2010 analyzer. Sorption measurements were performed using ultrapure nitrogen gas as the adsorbate and liquid nitrogen as a coolant. Surface area was calculated by the Brunauer–Emmett–Teller (BET) method.[18]

Preparation of a cosmetic cream

Polydecene (14.5 g), cetostearyl alcohol (2.0 g), and polysorbate 60 (1.5 g) were heated to 70 ± 5°C and then added into distilled deionized water (77 mL) at 70°C under homogenization at 7,000 rpm for 10 min. They were then cooled to around 50°C. Vitamin A microspheres was suspended in butylene glycol (3.0 g) and then immediately added to the emulsion at room temperature. After another homogenization for 2 min, the emulsions were stabilized sterically with Salcare SC95. All experimental procedures were carried out under dark conditions due to the light sensitivity of vitamin A. In addition, as a degassing process, the prepared cream was placed under vacuum for at least 10 min. The emulsion cream was then transferred into an aluminum tube with nitrogen blows and stored at 4°C until use.

Quantitative analysis of vitamin A

To measure the loading amount of intact vitamin A within the microspheres, vitamin A was extracted from the microspheres by methanol with a bath sonication for 30 min at room temperature. Vitamin A was quantitatively analyzed by high-performance liquid chromatography (HPLC) composed of HP 1100 series (Hewlett–Packard, Palo Alto, CA) and a C_{18} reversed-phase column (Nova-Pak C18, 3.9 × 150 mm, Waters, Milford, MA). Methanol was eluted as an isocratic mobile phase at a rate of 1.0 mL/min. The eluate was monitored by UV absorption measurement at 325 nm. All-*trans*-retinol (Sigma Chem. Co., St. Louis, MO) was used as a standard compound.

RESULTS AND DISCUSSION

The "proton-buffering" effect of PEI on the stability of vitamin A was examined by monitoring the residual amount of all-*trans*-vitamin A at 40°C under darkness. Figure 1 shows that PEI effectively improved the chemical stability of vitamin A in anhydrous ethanol solution. In this case, HPLC analysis revealed that the formation of anhydro-vitamin A was gradually decreased as the concentration of PEI increased. In addition, other organic bases, such as triethylamine and methyl glucamine, showed the same effect on vitamin A stability (data not shown).

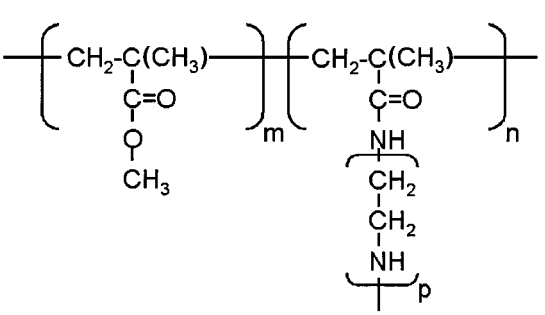

Figure 2 Chemical structure of PMMA-*g*-PEI.

However, the direct addition of organic bases into emulsion formulations was practically improper, because organic bases destabilized the ordered structures of emulsions. It should be noted that organic bases can rearrange to the oil/water interface via hydrophobic and/or electrostatic forces, interact with other ingredients (e.g., surfactants, thickeners, etc.), and ultimately affect the colloidal stability of cosmetic formulations. For this reason, we have tried to combine a polymer-based microencapsulation with a chemical conjugation, which was expected to preclude the diffusion-out and contact of the organic bases to an external aqueous phase. In this study, PEI was chosen because it has a high proton-buffering capacity and a chemical structure adequate for versatile modification.

PEI was grafted onto poly(MMA-*co*-MAA) via a coupling reaction. Poly(MMA-*co*-MAA) was first esterified with NHS by using DCC to activate the carboxylic acid groups and then reacted with the terminal primary amines of PEI (see Fig. 2).[19] To suppress a crosslinking reaction, excess amounts of PEI were used and unreacted PEI was removed by rinsing with excess methanol. The FT-IR spectrum indicated that a new amide vibration peak appeared in the range of 1,540–1,560 cm^{-1}, indicating the successful introduction of PEI. PEI content was estimated as 1.6% and the grafting efficiency was 67.3%, as determined by elemental analysis.

Polymer microspheres loaded with vitamin A were prepared by an O/W emulsification and solvent evaporation method. The average sizes of microspheres were estimated as 49.1 μm for PMMA microspheres and 38.6 μm for PMMA-*g*-PEI microspheres, respectively. The relatively smaller size of the PMMA-*g*-PEI microspheres might be due to the amphiphilic molecular structure of the polymer.[19] Encapsulation efficiency of vitamin A was 86.0% for PMMA microspheres and 100.0% for PMMA-*g*-PEI microspheres, respectively. It was not clearly understood why the PMMA-*g*-PEI microspheres produced the highest loading efficiency. However, it was reproducibly observed in several trials. One possible explanation is that PVA, an emulsifying agent, formed micelle-like aggregates, solubilized vitamin A into their micellar cores, and was washed out concomitantly with vitamin A during the rinsing step in excess water. PVA is likely to be adsorbed from the aqueous phase onto the microsphere/water interfaces during the formation of microspheres.[20–22] Although it is very difficult to discuss here the precise mechanism, it is expected that the presence of PEI at the polymer/water interfaces might affect the adsorption/desorption behavior of PVA.

The internal morphology of the microspheres is highly influenced by many process parameters, including polymer molecular weight and solubility, volume ratio of organic/aqueous phases, rate of solvent evaporation, loading content of drugs or additives, etc.[23] Higher density of microspheres is advantageous for stability of vitamin A because water molecules expedite the acid hydrolysis reaction of vitamin A. To prepare nonporous and dense particles, O/W emulsions were produced by the agitation of two practically immiscible liquids: methylene chloride and water. Methylene chloride can be easily eliminated via evaporation because it has a relatively low boiling point, 39.8°C, and a low solubility in water, 1.32% (w/w), at 20–25°C. However, it should be noted that the density of polymer microspheres prepared from the O/W emulsion method can be decreased due to the water partitioning into the oil phase: about 0.2% (w/w) of water can be dissolved in methylene chloride[24] and small molecules such as water are expected

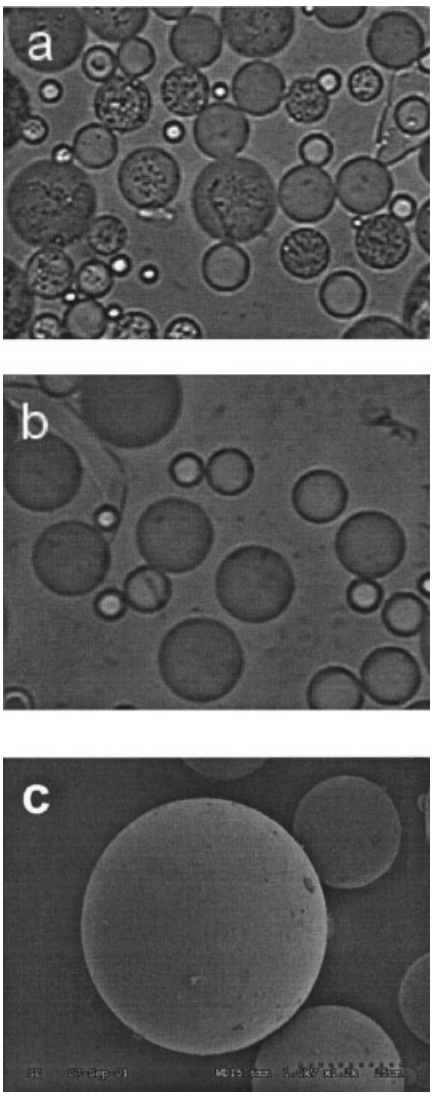

Figure 3 Optical microscopic images of PMMA-*g*-PEI microspheres prepared (a) without NaCl and (b) with the addition of 10% (w/v) NaCl into external aqueous phase during the particle formation (magnification ×200). (c) The SEM version of (b).

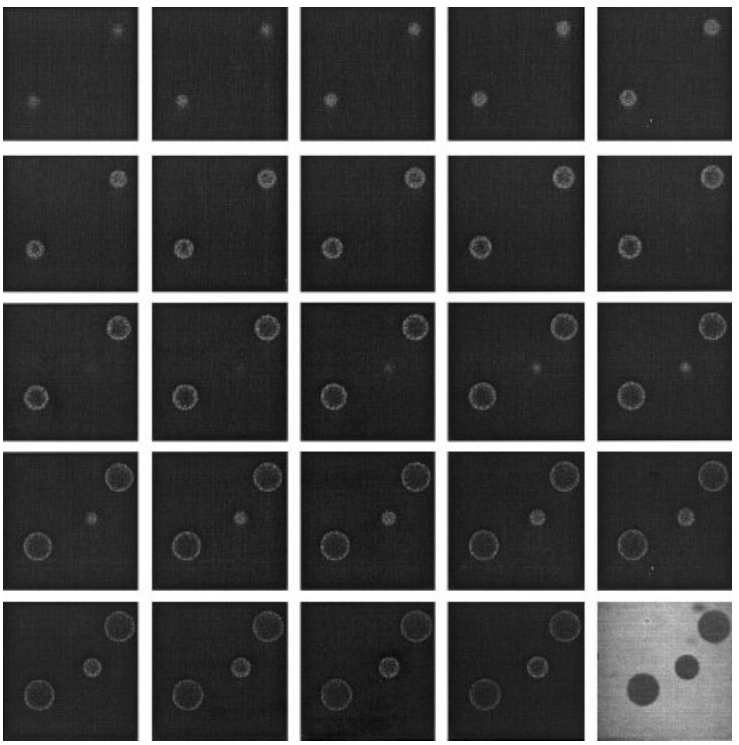

Figure 4 CLSM images of vitamin A–loaded PMMA-*g*-PEI microspheres. The images, at planes separated by 1.5 mm, were collected from top (upper left) to bottom (lower right) at a wavelength of 488 nm. The last image was obtained on a confocal transmission microscope.

to penetrate into the inner phase. Particularly, as the solvent evaporates and the polymer concentration starts to increase, a concentrated polymer skin-layer can be produced in the polymer/water interfacial region.[25] The formation of a dense, viscous polymer layer is analogous to the development of a concentrated top layer detected during the initial stages of solvent evaporation while casting a polymer film.[26] This skin-layer formation can entrap water permanently within the inner phase of microspheres. This is why the microspheres prepared by a single O/W emulsion method often have a highly porous structure surrounded by a dense polymer skin coating, as previously reported.[27] As for vitamin A encapsulation, porous morphology induced by water infiltration is disadvantageous, as both water and air can accelerate the degradation reaction of vitamin A.

To preclude the water infiltration inward, 10% (w/v) of NaCl was added to the external water phase. The addition of NaCl into the dispersing aqueous phase can induce a water out-flow from the internal phase through the organic layer, which acts as a diffusion barrier between the two phases.[28,29] This strategy is very useful to increase the density of microspheres as far as the added salts do not affect the activity of encapsulated active compounds. Figure 3 shows the OM and SEM pictures of the prepared microspheres. While small water droplets were entrapped within the polymer microspheres prepared without NaCl, transparent single-phase microspheres having smooth and nonporous surface were successfully produced with 10% NaCl addition. BET analysis revealed that the surface area of PMMA-*g*-PEI microspheres was dramatically decreased from 2.40 to 0.61 m^2/g by using 10% NaCl. The distribution of vitamin A within polymer microspheres was examined by using CLSM, as shown in Figure 4. Vitamin A was homogeneously dispersed throughout the whole microsphere, although it seems that fluorescence intensity was slightly brighter in the peripheral region than in the central region. This might be because the density of polymer microspheres is higher in the outer domain of the microspheres, due to the reason mentioned above.

Figure 5 represents the stability profiles of vitamin A loaded within polymer microspheres in an emulsion cream formulation. It was clearly demonstrated that the stability of vitamin A in PMMA-*g*-PEI microspheres is superior to that in PMMA microspheres. Vitamin A encapsulated within PMMA-*g*-PEI microspheres maintained 91% of its initial activity after 30-day incubation at 40°C, while only 60% within plain PMMA microspheres. Reversed-phase HPLC analysis indicated that the amount of anhydro-vitamin A in PMMA-*g*-PEI microspheres was significantly reduced (data not shown). Accordingly, this means that

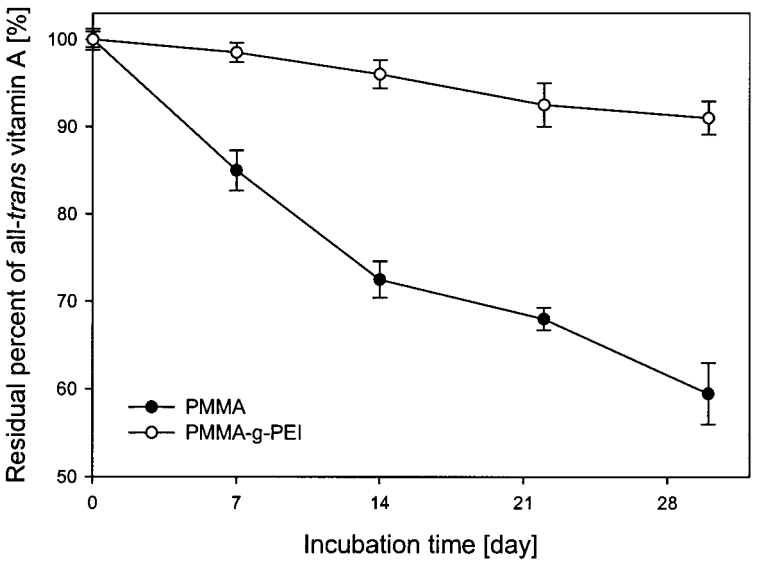

Figure 5 Stability profiles of vitamin A in an emulsion cream as a function of incubation time: (●) PMMA and (○) PMMA-*g*-PEI microspheres. The cream was preserved in an aluminum tube at 40°C.

the stabilization effect resulted from the decrease of dehydration reaction of a terminal hydroxyl group of vitamin A.

In conclusion, we propose a new stabilization strategy of vitamin A based on microencapsulation with a functionalized polymer, PMMA-*g*-PEI. To maintain a high local pH within microspheres, PMMA-*g*-PEI was used for vitamin A encapsulation. It was demonstrated that the proton-buffering effect of PEI moiety successfully stabilized vitamin A within hydrophobic polymer matrix by reducing the dehydration reaction. This microclimate-modified encapsulation technique will provide a very useful approach for the stabilization of active compounds in commercial products for long storage.

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