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## Antioxidant CMK-5 mesoporous carbon covalently functionalized with gallic acid and ascorbic acid

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### Abstract

Mesoporous carbon CMK-5 was synthesized and subsequently functionalized via covalent grafting with two potent natural antioxidants, ascorbic acid (AA) and gallic acid (GA), to develop novel antioxidant-active hybrid materials. The successful covalent attachment was confirmed through comprehensive characterization techniques, including fourier-transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), and transmission electron microscopy (TEM). The antioxidant performance was systematically compared using two complementary assays: the DPPH radical scavenging assay and the ferric reducing antioxidant power (FRAP) test. The results indicated that functionalization effectively enhances the antioxidant activity of CMK-5. The DPPH scavenging capacity increased from less than 42.3% for pristine CMK-5 to 61.5% for CMK-5-AA, and 73.7% for CMK-5-GA. Similarly, in the FRAP assay, CMK-5-GA exhibited the highest reducing power. A consistent trend of CMK-5-GA > CMK-5-AA > CMK-5 was observed in both methods, unequivocally proving the superior efficacy of GA, attributed to its higher number of phenolic hydroxyl groups facilitating more efficient hydrogen atom transfer and electron donation. This work presented a suitable strategy for creating advanced antioxidant carbon-based hybrids for potential applications in biomedicine, food preservation, or catalytic systems.

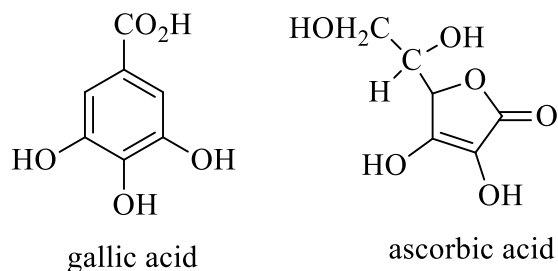
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## Introduction

Antioxidants are of paramount scientific and industrial importance across diverse sectors, including human health, food preservation, and polymer stabilization (Shahidi and Zhong, 2010; Hussain *et al.*, 2014; Halliwell and Gutteridge, 2015). Within the food industry, antioxidants are crucial additives that prevent oxidative rancidity, a major cause of nutrient loss, off-flavor development, and shortened shelf-life in lipid-rich products (Nerin *et al.*, 2008; Carocho *et al.*, 2014). Similarly, in polymer science, antioxidants are incorporated to retard oxidative degradation, thereby extending the material's service life (Marturano *et al.*, 2023).

Among the diverse array of studied natural antioxidants, gallic acid (GA) and ascorbic acid (AA) (Fig. 1) have garnered significant and sustained scientific interest.



**Fig 1.** Chemical structure of gallic acid and ascorbic acid.

GA is a trihydroxybenzoic acid renowned for its potent antioxidant activity, primarily attributed to its ability to donate hydrogen atoms (HAT mechanism) from its phenolic hydroxyl groups and to transfer electrons (SET mechanism). GA is widely utilized as a raw material in the production of inks and paints, and serves as a potent antioxidant in the food, cosmetic, and pharmaceutical industries (Spizzirri *et al.*, 2009; Rajan and Muraleedharan, 2017; Saini *et al.*, 2025). AA, a vital water-soluble vitamin, functions predominantly as a single electron transfer agent, playing crucial roles in collagen synthesis and immune function (Nimse and Pal, 2015; Akbari, 2016). Their applications span from therapeutic and nutraceutical agents to preservatives in the food and cosmetic industries (Ain, 2024). Despite their widespread utility, the practical application of molecular antioxidants, particularly natural polyphenols, is constrained by several intrinsic limitations that compromise their efficacy and longevity. First, in applications, such as food

packaging or polymer stabilization, the leaching and/or volatilization (migration) of the antioxidant from the host matrix is a major concern (Wrona *et al.*, 2017, Pedro *et al.*, 2022). Second, insufficient thermal stability limits the use of antioxidant during common industrial thermal processes like pasteurization, sterilization, or polymer extrusion (Deligiannakis *et al.*, 2012; Wu *et al.*, 2024). Third, phenolic antioxidants like GA are prone to rapid autoxidation and subsequent polymerization under ambient aerobic conditions, leading to deactivation and phenomena such as enzymatic browning (Drosos *et al.*, 2011). These fundamental challenges highlight the critical need for advanced stabilization strategies to harness the full potential of these bioactive compounds in advanced materials. To overcome these fundamental challenges, a robust and widely adopted strategy involves the immobilization of antioxidants onto solid supports (Lucente-Schultz *et al.*, 2009; Cirillo *et al.*, 2011, Deligiannakis *et al.*, 2012, Morais *et al.*, 2020, Novais *et al.*, 2023). Covalent immobilization concurrently enhances chemical stability, prevents leaching/migration and improved thermal stability, thereby establishing a robust platform for next-generation functional materials. The selection of the solid matrix is pivotal to the success of this strategy. Mesoporous carbon materials like CMK-type have garnered significant academic and industrial interest due to their outstanding physicochemical properties, including high surface area, large pore volume, excellent thermal stability, and enhanced mass transport. These unique attributes have established them as versatile platforms for diverse applications, ranging from energy storage and catalysis to biomedicine and environmental remediation (Eftekhari and Fan, 2017; Rahman *et al.*, 2021, Mehdipour-Ataei and Aram, 2023).

Herein, we synthesized ordered mesoporous carbon (CMK-5) and functionalized its surface via covalent grafting with two potent natural antioxidants, GA and AA. The successful immobilization was confirmed through a suite of physicochemical characterization techniques. The central objective of this work was to conduct a comparative evaluation of the antioxidant efficacy of the synthesized materials using the pluronic P123,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and the ferric reducing antioxidant power test. This approach aims to elucidate structure activity relationships and

assess the potential of these functionalized carbons for advanced applications, particularly in active packaging systems where durable, non-migratory antioxidant performance is critical.

## Materials and Methods

DPPH, AA, GA, sodium nitroprusside, tetraethyl orthosilicate, and furfuryl alcohol were purchased from Sigma-Aldrich chemical company, USA. Aluminium chloride ( $\text{AlCl}_3$ ), potassium ferricyanide, sulfanilamide, and ferric chloride ( $\text{FeCl}_3$ ) were purchased from Merck company, Germany.

Infrared spectra were recorded by Bruker spectrophotometer. Transmission electron microscopy (TEM) images were obtained using a Philips instrument. Thermogravimetric analysis was carried out using a Du Pont TGA 951 instrument.

### Synthesis of CMK-5 mesoporous carbon

The ordered mesoporous carbon CMK-5 was synthesized using a nanocasting procedure with aluminum-doped SBA-15 as a hard template, adapted from a reported method (Alinezhad and Zare, 2013). Briefly, the Al-SBA-15 template was first prepared by dissolving 12 g of Pluronic P123 in a solution of 375.6 g deionized water and 74.4 g concentrated HCl (37%) at 38°C. To this, 31.5 g of tetraethyl orthosilicate (TEOS) and 16.5 g of KCl were added under stirring. The mixture was maintained at 38°C for 24 hours, then transferred to an autoclave and hydrothermally treated at 130°C for another 24 hours. The solid product was filtered, washed with water, and calcined at 550°C in air for 5 hours to obtain SBA-15. Aluminum was incorporated by impregnating the calcined SBA-15 with an aqueous  $\text{AlCl}_3$  solution (Si/Al molar ratio = 20), followed by drying and calcination to yield Al-SBA-15. Subsequently, the Al-SBA-15 template was impregnated with furfuryl alcohol (FA) as a carbon precursor at room temperature. The FA-filled composite was then heated at 80°C for 16 hours to polymerize the FA within the mesopores. After polymerization, the composite was filtered and washed with ethanol and acetone to remove excess FA. The obtained material was carbonized under vacuum by heating to 850°C at a ramp rate of 10°C/minute, holding for 3 hours. Finally, the silica template was removed by etching the carbon-silica composite with a 10% HF solution in

a 1:1 (v/v) ethanol/water mixture. The resulting carbon material was washed thoroughly with water and ethanol, dried at 100°C, and denoted as CMK-5.

### Synthesis of AA and GA immobilized on cmk-5

#### Oxidation of CMK-5 to CMK-5-COOH

0.5 g of CMK-5 was dispersed in 20 ml of 1 M nitric acid and stirred magnetically at 80°C for 3 hours. Following oxidation, the mixture was filtered, and the solid residue was washed extensively with deionized water and ethanol until the filtrate reached a neutral pH. The resulting carboxyl-functionalized carbon, denoted as CMK-5-COOH, was dried in an oven (Bazuła *et al.*, 2008).

#### Preparation of CMK-5-COCl

0.4 g of CMK-5-COOH was reacted with 15 ml of thionyl chloride at 50°C for 24 hours under continuous stirring. The product was then filtered and washed thoroughly with tetrahydrofuran until the filtrate became colorless. The obtained acyl chloride-functionalized carbon, denoted as CMK-5-COCl, was dried in an oven.

#### Immobilization of antioxidants on the carbon substrate

For covalent grafting, 0.2 g of CMK-5-COCl was separately reacted with 2 g of either AA or GA in xylene. Each mixture was stirred at 80°C for 24 hours. After the reaction, the solid product was filtered and washed extensively with water and acetone to remove any unreacted antioxidant. The final functionalized materials, designated as CMK-5-AA and CMK-5-GA, were dried under vacuum at 50°C.

#### DPPH radical scavenging assay

The free radical scavenging activity of CMK-5, CMK-5-GA, and CMK-5-AA was evaluated using the 2,2-diphenyl-1-picrylhydrazyl assay. Briefly, 2 mg of each sample (CMK-5, CMK-5-GA, or CMK-5-AA) was dispersed in 4 ml of a 100  $\mu\text{M}$  ethanolic DPPH solution. The mixture was stirred and then kept in the dark at room temperature for 1 hour. After incubation, the mixture was centrifuged or filtered, and the absorbance of the supernatant was measured at 517 nm (Cirillo *et al.*, 2011). A

control sample containing all reaction components except the carbon material was used to determine the initial absorbance ( $A_0$ ). The same procedure was performed in triplicate for each sample. The radical scavenging activity, expressed as a percentage, was calculated using the following formula:

$$\text{Inhibition \%} = \frac{A_0 - A_1}{A_0} \times 100$$

Where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the sample.

### Ferric reducing antioxidant power assay

The reducing power was evaluated according to methods reported in the literature (Nabavi *et al.*, 2012). In this procedure, 2 mg of the sample (CMK-5, CMK-5-GA, or CMK-5-AA) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 30 minutes. The reaction was then stopped by adding 2.5 ml of a 10% trichloroacetic acid (TCA) solution. The mixture was centrifuged at 3000 rpm for 10 minutes. Subsequently, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of a 0.1% ferric chloride solution. The absorbance of the resulting solution was measured at 700 nm against a reagent blank prepared similarly but without the carbon sample.

### Statistical analysis

All experiments were performed in three independent replicates. Results for the DPPH assay were expressed as percentage inhibition. For the FRAP assay, the ferric reducing activity was presented as the mean absorbance at 700 nm. Data for each assay were analyzed separately using one-way analysis of variance (ANOVA) in SPSS 16 (SPSS Inc., USA). Where ANOVA indicated significant differences ( $p < 0.05$ ), group means were compared using Duncan's post-hoc test.

## Results

### Characterization of CMK-5 materials

The functionalized CMK-5 hybrids (CMK-5-GA and CMK-5-AA) were synthesized via covalent grafting of GA and AA onto the pristine CMK-5

surface. FT-IR spectra of CMK-5, CMK-5-AA, and CMK-5-GA were presented in Fig. 2. The FT-IR spectrum of CMK-5 displayed characteristic broad bands in the range of 1100–1300  $\text{cm}^{-1}$  and 1550–1600  $\text{cm}^{-1}$ . The bands between 1100 and 1300  $\text{cm}^{-1}$  were attributed to C–O stretching vibrations, while the peak near 1580  $\text{cm}^{-1}$  corresponded to aromatic C=C stretching modes of the carbon framework. After covalent functionalization with AA and GA, significant spectral changes were observed. The appearance and notable enhancement of a band around 1720  $\text{cm}^{-1}$  in both CMK-5-AA and CMK-5-GA spectra confirmed the C=O stretching vibrations of AA and GA. Furthermore, a broad band emerged around 3500–3300  $\text{cm}^{-1}$ , indicative of O–H stretching vibrations from the hydroxyl groups of the antioxidants. The increased intensity of the bands associated with C–O, C=C, C=O, and O–H vibrations in the functionalized samples, compared to the pristine CMK-5, provided clear evidence for the successful covalent attachment of the antioxidant molecules to the carbon surface.

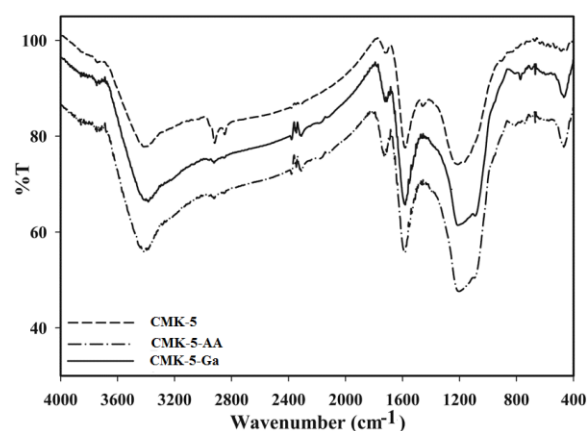


Fig. 2. FT-IR spectra of CMK-5, CMK-5-AA, and CMK-5-GA.

Figs. 3 and 4 showed the TEM of CMK-5 carbons before and after immobilization of GA and AA. The dark lines in Figs. 3 and 4 were the interconnected carbon nano-rods. The two kinds of pore systems in CMK-5 were visible in Figs. 3 and 4. The white lines between the dark lines were the projection of the mesopores generated in the space previously occupied by the walls of the SBA-15 template. The white parts in the dark lines can be interpreted as the projection of mesopores derived from the incomplete filling of silica pores. According to Figs. 3 and 4, the hexagonal structure of CMK-5 has been preserved during the various stages.

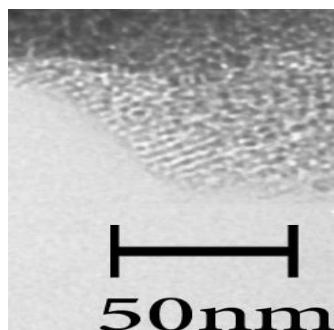


Fig. 3. TEM image of CMK-5.

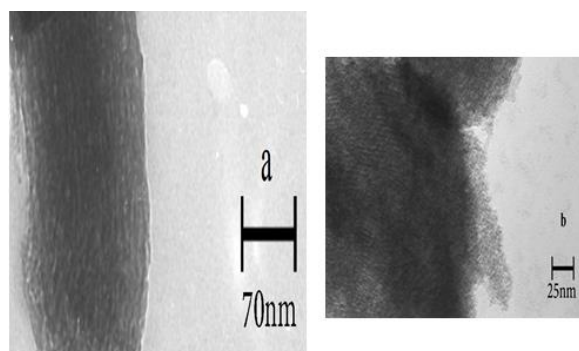


Fig. 4. TEM images of (a) CMK-5-GA and (b) CMK-5-AA.

The results of thermogravimetric analysis of the carbon samples were shown in Fig. 5. In the temperature range between 100 and 150°C, the adsorbed water was lost from the samples. For the CMK-5-GA and CMK-5-AA samples, the greatest mass loss occurred in the range of approximately 200-600°C, which was related to the decomposition of the attached groups, indicating the covalent bonding of organic groups (AA and GA) on the surface of the nanoporous carbon. The amount of weight lost for both combinations was about 17%.

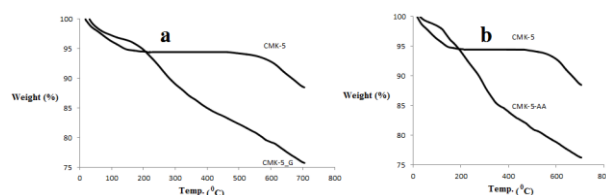


Fig. 5. a) TGA curves of the CMK-5 and CMK-5-GA. b) TGA curves of the CMK-5 and CMK-5-AA.

### DPPH free radical scavenging activity

The free radical scavenging capacity of the CMK-5 materials was evaluated using the stable

DPPH radical as a probe. Following the DPPH procedure, the characteristic purple color of the DPPH solution faded upon reaction with the antioxidants. The absorbance of each sample was measured at 517 nm, and the percentage of radical scavenging activity was calculated for each concentration. CMK-5s scavenging ability was evaluated by DPPH method and expressed as inhibition (%). The inhibition values for CMK-5, CMK-5-AA, and CMK-5-GA were statistically significant ( $42.3 \pm 2$ ,  $61.5 \pm 2$  and  $73.7 \pm 0.4$  respectively;  $p < 0.05$ ). As shown in Table 1, the results demonstrated that all CMK-5-based materials possessed the ability to scavenge DPPH radicals. Notably, CMK-5-GA exhibited superior radical scavenging activity compared to both pristine CMK-5 and CMK-5-AA, indicating a more effective interaction with the free radical.

Table 1. DPPH radical scavenging activity (%) of the mesoporous carbons.

Sample	DPPH scavenging activity (%)
CMK-5	$42.3 \pm 2^c$
CMK-5-GA	$73.7 \pm 0.4^a$
CMK-5-AA	$61.5 \pm 2^b$

### Evaluation of ferric reducing power

Following the procedure FRP test, the yellow color of the reaction mixture changed to various shades of bluish-green in the presence of the samples, depending on their respective reducing capacities. The reducing power of the CMK-5 samples was quantified by measuring the absorbance of the resulting solutions at 700 nm, and the results showed statistically significant differences ( $p < 0.05$ ) (Fig. 6). Increasing absorbance at 700 nm indicated an increase in reducing power ability. CMK-5-GA had better electron donating activity than tested samples.

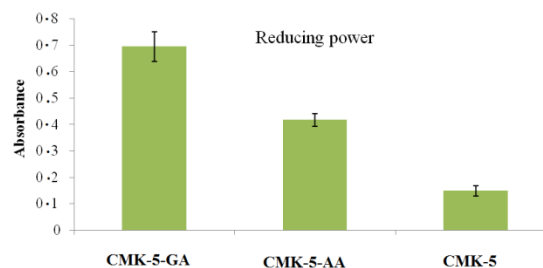


Fig. 6. Reducing power of the CMK-5 samples

### Discussion

This study successfully demonstrated the covalent functionalization of CMK-5 with two natural antioxidants, GA and AA, resulting in novel

hybrid materials with significantly enhanced antioxidant properties. CMK-5 is an ideal scaffold material due to its exceptional textural properties, including a high surface area and ordered porosity. These characteristics, which are crucial for high loading capacities and accessibility of active sites, are well-documented for nanocasted carbons. (Lucente-Schultz *et al.*, 2009; Cirillo *et al.*, 2011). In this work, CMK-5 was functionalized via a three-step synthetic strategy: (i) surface oxidation, (ii) acyl chloride activation, and (iii) esterification (Fig. 7).

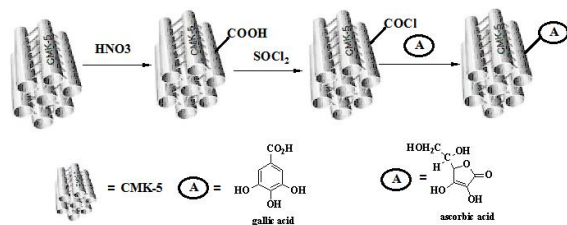


Fig. 7. Grafting of CMK-5 with antioxidant molecules.

FT-IR spectroscopy revealed the emergence of C=O stretching vibrations at  $1720\text{ cm}^{-1}$  in the functionalized samples, which can be characteristic of the grafted antioxidant molecules and newly formed ester linkages. TGA analysis provided supporting evidence for the presence of organic moieties on the carbon surface. The significant weight loss observed between  $200\text{--}600^\circ\text{C}$  for CMK-5-GA and CMK-5-AA, which is absent in pristine CMK-5, corresponds to the decomposition of the immobilized antioxidant molecules. The amount of antioxidants in these two hybrids is almost the same ( $\sim 17\%$ ). Furthermore, TEM analysis confirmed a critical finding: the highly ordered hexagonal mesostructure of CMK-5 remained intact throughout the multi-step chemical functionalization. This structural preservation underscores the robustness of the CMK-5 framework and ensures that its advantageous porous network, essential for mass transport and high surface area, is retained in the final functional materials. The DPPH assay, which probes HAT capability, clearly established the activity trend: CMK-5-GA > CMK-5-AA > CMK-5. This result is consistent with the antioxidant properties of the grafted molecules. GA possesses a phenolic structure that enables radical scavenging via the HAT mechanism (Spizzirri *et al.*, 2009; Rajan and Muraleedharan, 2017). AA, while a potent reductant, primarily operates through a single

electron transfer mechanism and its enediol structure is less efficient in direct HAT compared to phenols (Spizzirri *et al.*, 2009; Akbari, 2016; Rajan and Muraleedharan, 2017). The FRAP assay, measuring reducing power, further validated the activity trend and provided a more complete picture. The significantly higher absorbance for CMK-5-GA indicates its superior electron-donating ability. This can be attributed to the higher amount of oxidizable hydroxyl groups of GA molecule compared to AA. Our findings are consistent with literature reports where polyphenol-grafted surfaces exhibit strong antioxidant effect (Cirillo *et al.*, 2011, Deligiannakis *et al.*, 2012, Morais *et al.*, 2020, Novais *et al.*, 2023). The observed radical scavenging activities of free CMK-5, though modest compared to functionalized CMK-5s, presents an intriguing point of discussion. This inherent activity can be contextualized within the broader framework of carbon nanostructure chemistry. It is well-established that curved  $\text{sp}^2$  carbon networks, such as those in C60 fullerenes and carbon nanotubes (CNTs), exhibit notable radical scavenging properties (Galano, 2009). The CMK-5 structure, synthesized via nanocasting of SBA-15, possesses a unique architecture consisting of interconnected, partially curved carbon nanorods. Unlike perfectly flat graphite, these curved domains create local strain and enhance chemical reactivity. Therefore, the baseline DPPH scavenging capacity of pristine CMK-5 can be plausibly attributed to this nanoscale curvature.

From an application perspective, particularly for active food packaging, these results have profound implications. The covalent linkage directly addresses the major industrial challenges of antioxidant leaching and migration into food simulants, a key regulatory hurdle for packaging safety. By permanently anchoring the antioxidant, our hybrids offer a non-migratory active system. Moreover, the preserved mesoporosity (as seen in TEM) is vital for potential functionalities such as the synergistic scavenging of oxygen within the pore network. The superior and broad-spectrum activity of CMK-5-GA makes it a particularly promising candidate for protecting oxygen- and light-sensitive food products rich in unsaturated lipids.

In this study, we successfully developed and characterized a novel class of antioxidant hybrid materials based on the covalent functionalization

of ordered mesoporous carbon CMK-5 with two essential natural antioxidants, GA and AA. Through a systematic synthetic route involving surface oxidation, activation, and coupling, stable CMK-5-GA and CMK-5-AA hybrids were fabricated. Comprehensive characterization via FT-IR and TGA provided conclusive evidence for the formation of covalent linkages, while TEM confirmed the preservation of the ordered hexagonal mesostructure throughout the chemical modification process. The results from DPPH and FRAP assays established a consistent and clear hierarchy: CMK-5-GA > CMK-5-AA > CMK-5. This definitive trend underscores that the chemical nature of the grafted molecule is the primary determinant of the hybrid's antioxidant performance. This covalent grafting strategy effectively addresses the critical limitations hindering the direct use of natural antioxidants by permanently anchoring them to a robust, high-surface-area solid support. This makes the developed hybrids, particularly CMK-5-GA, promising candidates for advanced applications, especially in active food packaging, where non-migratory, long-lasting protection against oxidative rancidity is paramount.

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## Conflict of Interest

There are no conflicts to declare.

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