Resolution of Ascorbic Acid in The Presence of Uric Acid by The Montmorillonite K_{10}-clay/SDS Modified Carbon Paste Electrode

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Abstract:- **Montmorillonite K_{10}-clay/SDS modified carbon paste electrode was developed for the simultaneous detection of Ascorbic acid in the presence of Uric acid in 0.1 M phosphate buffer solution of pH 7.0. The modified electrode has exhibited a stable and sensitive current response towards the Ascorbic acid and was utilized for the simultaneous detection of Ascorbic acid and Uric acid. When compared with a bare CPE, the modified electrode exhibited a remarkable shift in the oxidation potentials of Ascorbic acid in the cathodic direction. The anodic peak potentials for the Ascorbic acid is 0.299 V in 0.1 M phosphate buffer solution (PBS) at pH 7.0, at modified carbon paste electrode, it was -0.020 V. The Montmorillonite K_{10}-clay/SDS modified CPE was also effective for the simultaneous determination of Ascorbic acid and Uric acid in a mixture and resolved the overlapping anodic peaks of these two species into two well defined voltammetry peaks in differential pulse voltammetry. The proposed method showed an excellent stability, reproducibility and is well suited for the analysis of real samples.**

Keywords- **Montmorillonite K_{10}-clay; Ascorbic acid; Uric acid; Cyclic voltammetry; Differential pulse voltammetry.**

I. INTRODUCTION

Ascorbic acid, Uric acid and Dopamine are physiologically important components which are widely distributed in the body of many mammals and exhibits message transfer in the brain and defense against the disease among which the Ascorbic acid is a water soluble vitamin and also a compound which takes part in many important life processes. Due to its antioxidant and pH regulator properties, this vitamin is present or added to a variety of food products and pharmaceuticals. Ascorbic acid takes part in several biological reactions. It prevents and treats the scurvy, common cold, mental illness, infertility and cancer. Ascorbic acid plays a unique redox and electrochemical role [1]. Similarly Uric acid (2,6,8–trihydroxypurine, UA) also a major nitrogenous compound in urine, and also a product of purine metabolism in human body and its higher levels leads to many clinical disorders [2]. High levels of UA in the blood (hyperuricemia or Lesch-Nyhan syndrome) are linked with the body disorders like gout, kidney and cardiac problems. Many epidemiological studies have suggested that the elevated serum UA is also a risk factor for cardiovascular disease [3-8]. In the extracellular fluid of the central nervous system, AA and UA are present in very high concentration, while the DA level is over 3 orders of smaller magnitude (< 100 nM) [9].

The simultaneous determination of such compounds in real biological samples is difficult because of redox reactions of both substances at the bare carbon paste electrodes regularly take place at very similar potentials [10] and often suffer from a pronounced fouling effect, which results in rather poor reproducibility and sensitivity. Hence, to explore a simple, accurate and reliable method for the simultaneous determination of uric acid and ascorbic acid became an urgent need. During the last decades, new electrodes using organic molecules as sensing elements have been developed. Due to their ease of construction and low cost, the modified electrodes are most widely used. Also the modification of these electrodes is simple because it only requires mixing the modifier with the carbon paste. In this way, it is possible to add a wide variety of products (inorganic or organic substances [11,12], biomolecules, animal and plant tissues [13].

The traditional analytical techniques like various chromatographic methods are applied for simultaneous determination of UA and AA. In which HPLC with UV [14], MS detection [15,16], GC [17] and HPCE [18] are preferred. Similarly numerous HPLC methods have been developed for the simultaneous determination of AA and UA in serum, plasma, urine and tissues [19,20], but these methods are required costlier instruments, well equipped laboratory and also trainees.

Consequently, it has attracted much interest of electrochemist to develop voltammetric sensors for the detection of AA and UA in the extracellular fluid. However, in the assay of AA, the electrochemical methods

suffer from inferior selectivity because of the presence of Uric acid (UA) which coexists in physiological fluids and whose oxidation potentials are always close to that of AA. Therefore, there has been a significant attempt to separate the oxidation peak potentials of AA and UA and many electrochemical approaches have been used to implement the above goal.

Modified carbon paste electrode can be prepared by adding different types of modifiers. Modification can be done by grinding in an agate mortar [21-23], by electropolymerisation [24-26] and also by immobilization method [27,28]. Similarly carbon nanotubes also play a major role in carbon paste electrode modification, hence several authors have reported the excellent electrocatalytic properties of carbon nanotubes in the redox behavior of different biomolecules. [29-31]. Immobilization of polymers on carbon paste electrode was a good approach by electro polymerization, in which some of the immobilized Polymer-modified electrodes was also applied for the selective determination of bio active molecules [32-34].

In the present study, we have fabricated a Montmorillonite K_{10}-clay modified carbon paste electrode (MMCPE) by bulk modification method. The same electrode was used for the selective detection of AA in the presence of UA and also we have examined the mass transport characteristics of the neurotransmitter Ascorbic acid (AA) in Montmorillonite K_{10}- clay modified carbon paste electrode. The Montmorillonite K_{10}-clay modified carbon paste electrode showed an excellent electocatalytical activity towards the selective and sensitive detection of AA in the presence of UA in 0.1 M of phosphate buffer solution at pH 7.0. The peak to peak separation between AA and UA was 20 mV and 340 mV respectively. The potential difference in between AA and UA was 360 mV which is quite sufficient to identify the AA and UA simultaneously.

II. MATERIALS AND METHODS

A. Apparatus

The electrochemical experiments were carried out with a CH-Instruments Model No. CHI610D Electrochemical work station with a connection to a personal computer for the electrochemical measurements and treating of data. All the experiments were carried out in a conventional three-electrode system. The electrode system contained a working carbon paste electrode, homemade cavity of 3 mm diameter, a platinum wire as counter electrode and saturated calomel electrode as reference electrode. Bare carbon paste electrode was prepared by grinding 70% of graphite powder and 30% of silicon oil in an agate mortar by hand mixing for about 30 min to get a homogenous paste. The paste was packed into the cavity of CPE and smoothened on weighing paper.

B. Chemicals

Analytical grade Ascorbic acid, Uric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate and silicon oil were procured from Himedia Chemicals. Fine graphite powder (particle size <20µm) was supplied by Sigma-Aldrich chemicals. All chemicals were used as supplied without further purification. All chemicals were of analytical grade and were used without further purification. Uric acid stock solution was prepared by dissolving known quantity of it in 0.1 M sodium hydroxide solution and Ascorbic Acid in double distilled water. Phosphate buffer (pH 7.0) was prepared as per the literature with 0.1 M NaH_2PO_4 and Na_2HPO_4 solution in double distilled water.

C. Preparation of bare carbon paste electrode and modified carbon Paste electrode

The bare carbon paste electrode was prepared by hand mixing of 70% graphite Powder with 30% silicon oil in an agate mortar to produce a homogenous carbon paste. The modified carbon paste electrode was prepared by taking different weights of Montmorillonite K_{10}-clay (10, 20, 30 and 40 mg) in silicon oil (30%) and graphite (70%). The prepared modified carbon paste and bare carbon paste was packed into a homemade Teflon cavity and polished using the soft paper [35].

III. Results and discussion

A. Electrochemical investigation of AA at Montmorillonite K_{10}-clay modified carbon paste Electrode (MMCPE)

The effect of Montmorillonite K_{10}-clay concentration in the carbon paste electrode was investigated through bulk modification (direct mixing) method for 0.1 mM AA in 0.1 M phosphate buffer solution of pH 7.0 by Cyclic Voltammetric method. The modified carbon paste electrode with 20 mg of Montmorillonite K_{10}-clay had showed high anodic peak current when compared with the bare CPE. The electrochemical response of 10, 20, 30 and 40 mg of Montmorillonite K_{10}- clay MMCPE is shown in Figure. 1.

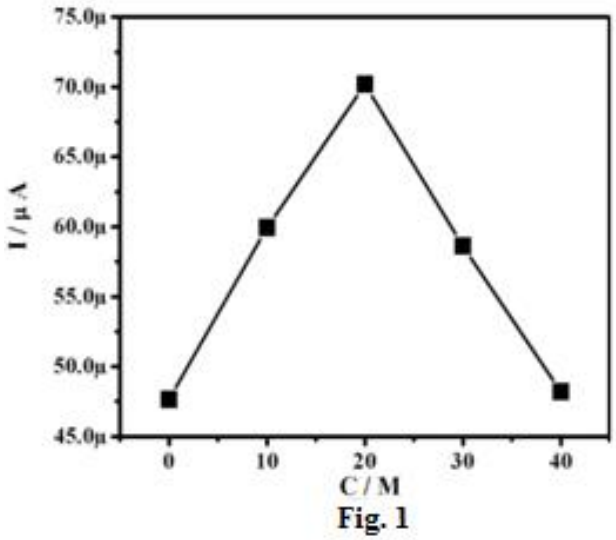

Figure. 1 Effect of concentration of Montmorillonite K_{10}-clay on anodic peak current (Ipa) in 0.1 mM AA and 0.1 M phosphate buffer solution Scan Rate: 50 mVs^{-1}

B. The response of AA at the bare CPE and MMCPE

Figure. 2 shows the electrochemical response of 0.1 mM AA in 0.1 M phosphate buffer solution of pH 7.0 at the bare CPE and the MMCPE with a scan rate of 50 mV/s. Compared with the bare CPE there was a remarkable four folds enhancement in the peak current and also there was a reduction in the over potential which showed the electrocatalytic efficiency of the Montmorillonite K_{10}-clay. The mechanism could be explained as follows; under these conditions, the Montmorillonite K_{10}-clay layered lattice structure could strongly incorporate the cations by ion exchange process [36].

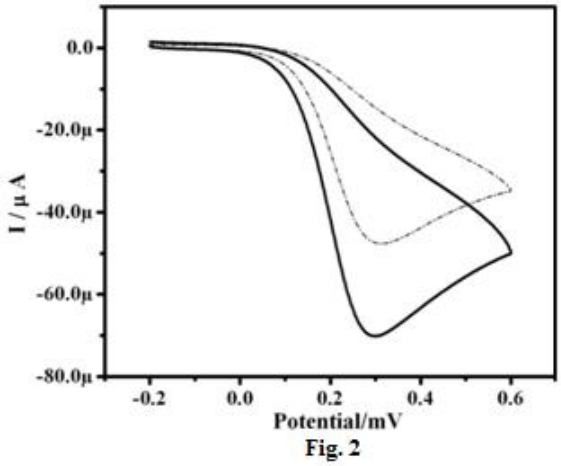

Figure. 2 Cyclic voltammogram of 0.1 mM AA at solid line for modified carbon paste electrode prepared with Montmorillonite K_{10}-clay (MMCPE) and at dotted line for bare CPE.

C. Effect of scan rate

The effect of scan rate for 0.1 mM AA in 0.1 M PBS at pH 7.0 was studied by CV at MMCPE.

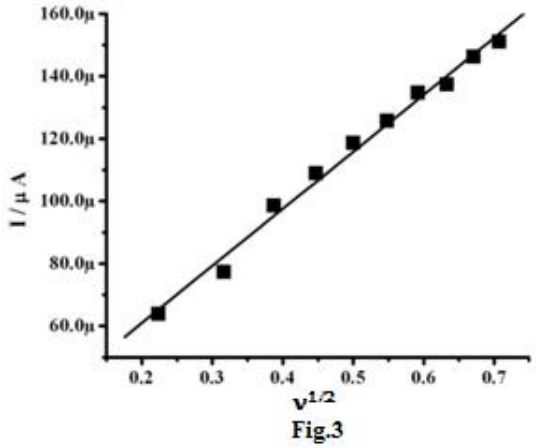

Figure. 3 Graph of anodic peak current Vs square root of scan rate (ν1/2) for 0.1 mM AA in PH 7.0, 0.1 M phosphate buffer solution at MMCPE.

MMCPE showed an increase in the redox peak currents with an increase in the scan rate (50 to 500 mV$\mathbf{s^{-1}}$). The graph of redox peak current (Ipa) *vs.* square root of scan rate ($v^{1/2}$) was plotted. The graph obtained had a good linearity between scan rates and peak current as shown in Figure.3, in the range from 50–500 mV$\mathbf{s^{-1}}$. The redox peak currents were proportional to $v^{1/2}$ the correlation coefficient (r^2) was 0.9952, which indicate that the electrode reaction was a diffusion controlled process.

D. Effect of pH value on the determination of AA at the MMCPE

The suitable pH of the supporting electrolyte had a significant influence on the determination of AA by electro catalysis of MMCPE by affecting both peak currents and peak potentials. The effect of pH value on the determination of AA in the phosphate buffer solution at MMCPE was carefully investigated in a wide pH range of 5.5 to 8.0. Figure. 4 illustrates the dependency of the AA anodic peak current and formal potential [E (V)] on the pH of buffer solution. It could be seen that the anodic peak current of AA increases with increasing pH value until it reaches 7.0, (shown with (-□-) square symbol) and then there was a decrease in the peak current of AA until it reaches 8.0. The formal potential of AA shifts towards lower potential with the increase of the pH value of solution and depends linearly on the pH value in the range of 5.5–8.0 with a slope of 0.07298 V/pH. (R^2 = 0.981) (Shown with closed circles (-●-)).

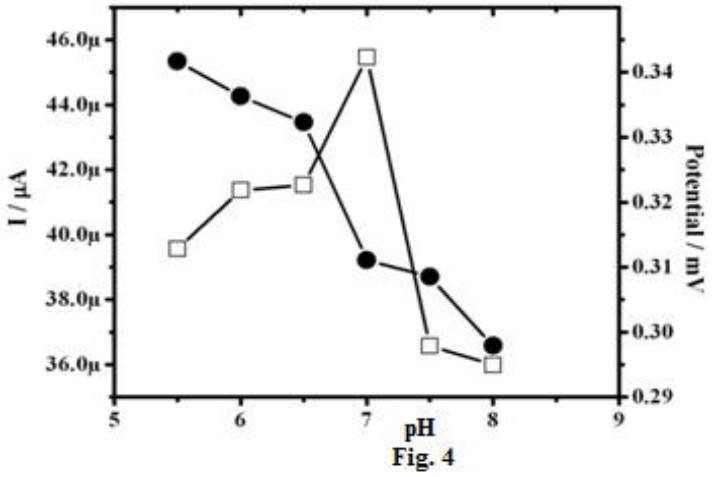

Figure. 4 Effect of pH on anodic peak current (Ipa) (-□-), anodic peak potential (Epa (-●-) of 0.1 mM AA in 0.1 M phosphate buffer solution.

E. Concentration effect of AA

The differential pulse voltammetric technique was used for the analysis of AA concentration which was varied from 4.1 µM to 513 µM and is shown in Figure. 5 for the MMCPE. By increasing the concentration of AA from 4.1 µM to 513 µM, the graph of Ipa versus concentration of AA showed an increase in anodic peak current has shown with the linear regression equation as Ipa (µA) =0.0055(C/µM) + 0.53µA (n=20,R^2=0.9995) shown in Figure. 6.

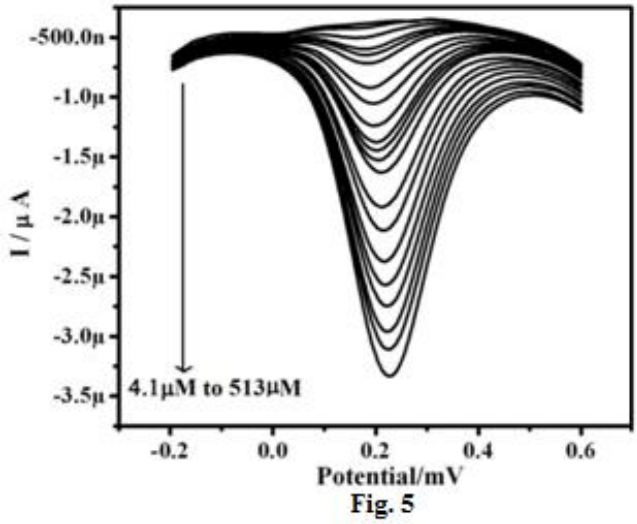

Figure. 5 Series of differential pulse voltammograms obtained for AA at MMCPE in 0.1 M (pH 7.0) phosphate buffer solution.

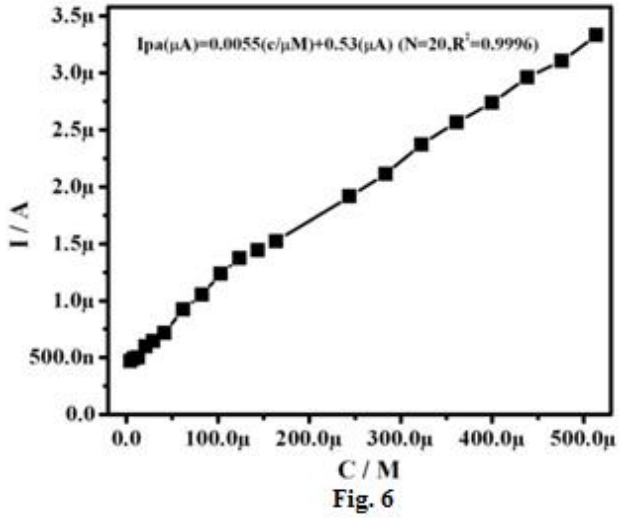

Figure. 6 Graph of anodic peak current Vs concentration of Ascorbic acid at MMCPE in 0.1 M phosphate buffer solution.

Determination of detection limit (DL) and quantification limit (QL) were carried only by using the following equations 1 and 2 [28, 35-38].

$$DL = 3S_b/S \quad \longrightarrow 1$$
$$QL = 10S_b/S \quad \longrightarrow 2$$

Where S_b is the standard deviation, S is slope of the working curve DL is the detection limit, QL is the quantification limit. The determined detection limit and quantification limit were 41 µM and 136 µM respectively.

F. Resolution of AA in the presence of UA by cyclic voltammetry

The simultaneous resolution of AA and UA was studied by cyclic voltammetric method. The obtained resulting cyclic voltammograms for the electrochemical response of AA (1 mM) and UA (0.4 mM) at bare CPE (curve a), the MMCPE (curve b) and MMCPE/immobilized SDS (curve c) in 0.1 M phosphate buffer solution of pH 7.0 at the scan rate of 50 mVs^{-1} is shown in Figure. 7. At the bare CPE, a well redox peak potential of AA and UA was not observed and with MMCPE there was a well resolution between AA and DA with well recognizable difference in their peak potential and showed a significant enhancement in redox peak currents for both AA and UA in the presence of MMCPE/immobilized SDS.

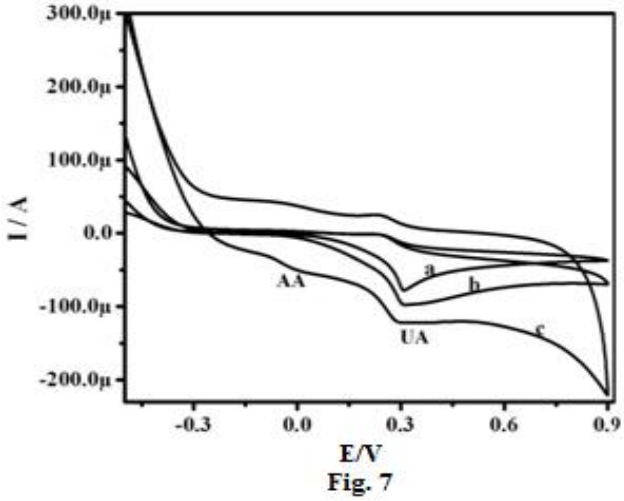

Fig. 7

Figure. 7 Cyclic voltammograms obtained for the electrochemical response of 1mM of AA and UA

G. Effect of various concentration changes of UA in the presence AA and AA in the presence of UA

The differential pulse voltammetric study of various Uric acid concentrations in the presence of constant concentration of AA (1 mM) at the MMCPE/immobilized SDS modified electrode for 0.1 M phosphate buffer was studied and is shown in Figure. 8 A. The anodic peak current of UA increases with increase in the concentration from 0.4 mM to 2.6 mM, while the anodic peak current of AA keeps constant due to its constant concentration in the experiment. Furthermore, it was observed that even in the presence of high concentration of AA it did not interfere with the determination of low concentration of UA. Therefore the MMCPE/immobilized SDS modified electrode showed its good selectivity and sensitivity in the electrochemical detection of UA in the presence of AA.

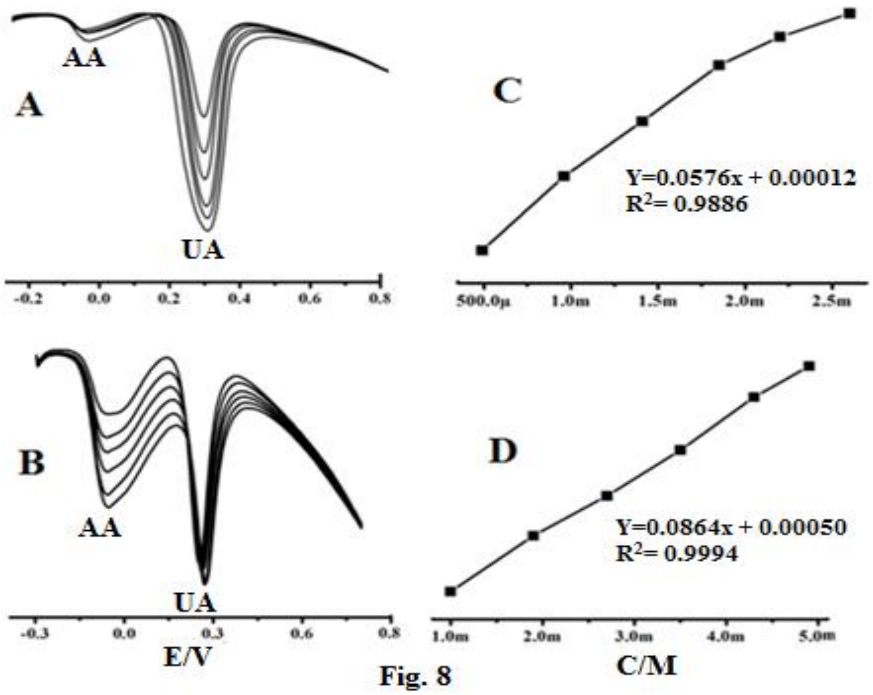

Fig. 8

Figure. 8 (**A**) UA concentration variation at constant AA concentration, (**B**) AA concentration variation at constant UA concentration, (**C**) the linear relationship between the anodic peak current and concentration variation of UA, (**D**) the linear relationship between the anodic peak current and concentration variation of AA

The corresponding graph of anodic peak current versus concentration of UA showed a linear regression Ipa (mA) =0.05767 Cm M L^{-1} + 1.26 (N=6, R^2=0.9886) shown in Figure. 8. C. The detection limit and quantification limit of UA in the presence of constant concentration of AA (1 mM) was found to be 0.40 mM and 1.3 mM respectively. Similarly the Ascorbic acid concentration variations in the presence of constant

concentration of UA (0.4 mM) at the MMCPE/immobilized SDS modified electrode in 0.1 M phosphate buffer was also studied and is shown in Figure. 8. B. The anodic peak current of AA increases with increase in the concentration from 1 mM to 4 mM, while the anodic peak current of UA keeps constant due to its constant concentration in the experiment. Furthermore, it was observed that even in the presence of high concentration of UA it did not interfere with the determination of low concentration of AA. Therefore the MMCPE/immobilized SDS modified electrode showed its good selectivity and sensitivity in the electrochemical detection of AA in the presence of UA. The corresponding graph of anodic peak current versus concentration of UA showed a linear regression (Ipa (mA) =0.08646 Cm M L^{-1} + 0.505 (N=6, R^2=0.9994) and is shown in Figure. 8. D. The detection limit and quantification limit of AA in the presence of constant concentration of UA (0.4 mM) was found to be 1.6 mM and 5.5 mM respectively.

IV. Conclusion

The present study involves the development of MMCPE/immobilized SDS modified carbon paste electrode by bulk modification (direct mixing) of carbon paste followed by immobilization of SDS on the surface of MMCPE for the simultaneous study of AA and UA. The developed MMCPE/immobilized SDS is more sensitive and selective than the bare carbon paste electrode and MMCPE. The selected supporting electrolyte medium i.e 0.1 M phosphate buffer at pH 7.0 plays a crucial role in the determination of UA and AA simultaneously at lower levels. The simultaneous determination of AA and UA by differential pulse voltammetric study at MMCPE showed a poor sensitivity and selectivity. Hence the present developed method of MMCPE/immobilized SDS modified carbon paste electrode was used as chemical sensor for the simultaneous determination of biological active compounds likes UA and AA. The present developed method could be applied for the analysis of AA and UA in real samples.

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