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Using thermal and spectroscopic data to investigate the thermal behavior of epinephrine

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ABSTRACT

Epinephrine is a neurotransmitter of the catecholamine class that acts in the mammalian central nervous system. The TG-DTA curves of epinephrine showed that the anhydrous compound starts decomposition at 165 °C, under the conditions used in this work. The reflectance FTIR spectra and X-ray powder diffraction patterns of epinephrine before and after heating up to 210 °C, as well as the TG-FTIR spectra of sample heated between 30 and 600 °C, were obtained and reveled that after heating, structural changes occurred in the sample. At temperatures higher than 205 °C the thermal decomposition took place with elimination of methylamine in agreement with the first mass loss observed in the TG curve in both air and N_2 atmospheres (TG = 17.0%, calcd. = 17.0%). The melting was observed at 205 °C (DTA) or 203 °C (DSC) but this process occurred overlapped with decomposition characteristic of an incongruent melting process.
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1. Introduction

Epinephrine (EP, adrenaline, Fig. 1) is a neurotransmitter of the catecholamine class that acts in the mammalian central nervous system [1]. Cathecolamines control the nervous system in a series of biological reactions and chemical processes [2].

Many diseases are related to changes of EP concentration in living systems. It also serves as a chemical mediator for converting the nerve pulse to different organs. Many phenomena are related to the EP concentration in blood as well as in urine. The normal amount of epinephrine in a healthy person serum stays at nmol L^{-1} level. Due to its importance in living systems and common use in emergency medicine [3], epinephrine has attracted much attention of the scientists working on life science and medicine fields [4].

There are many methods applied in the determination of EP in aqueous solutions, such as high performance liquid chromatography (HPLC) [5,6], capillary electrophoresis [7,8], flow injection [9,10], chemiluminescence [11,12], fluorimetry [13] and spectrophotometry [14,15]. As an electroactive molecule, it has also been determined via electrochemical techniques and some reports showed the electrochemical response of EP at different kind of electrodes, such as electrochemically pretreated glassy carbon electrode [16], carbon fiber microelectrode [17], polymer film modified glassy carbon electrode [18,19] and self-assembled monolayer modified electrode [20,21], among others.

Previous works reported the EP melting at 211–212 °C or 215 °C (when rapidly heated), but in this case accomplished by decomposition [22]. Lee and Burton [23] described studies concerning the thermal stability of several EP-derivative formulations since these compounds use to be both light and thermally unstable. These authors submitted epinephrine maleate, fumarate, hydrochloride and bitatrate salts to heating at 95 °C during 20 days and concluded that the stability is related to the melting of the salt. Any previous report employing thermoanalytical techniques in the evaluation of the thermal behavior of epinephrine was found in the literature.

Thus this work aims to contribute with a better understanding of the physical chemical properties of EP. Thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) were used to evaluate the thermal behavior of EP in solid state. Volatile decomposition products were characterized by TG-FTIR coupled analysis while the solid residues were investigated using FTIR and mass spectrometry.

2. Experimental

Epinephrine (Sigma, minimum 98.0% purity) was submitted to thermal analysis as received. Simultaneous thermogravimetry and differential thermal analysis (TG-DTA) were performed in a

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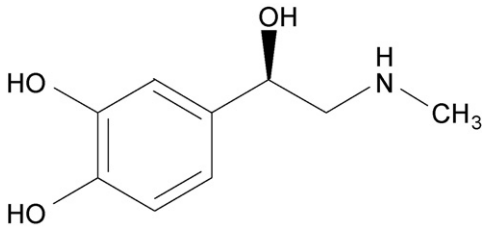


Fig. 1. Structure of epinephrine.

SDT Q600 apparatus from TA Instruments. All the TG-DTA curves were obtained under air and N_2 dynamic atmospheres (gas flow of 100 mL min^{-1}) and at a heating rate of 10 °C min^{-1}. The sample masses were about 8 mg. Alumina crucibles were used in the TG-DTA experiments. The simultaneous TG-DTA modulus was calibrated with aluminum (99.99+ %) for temperature.

DSC curves were recorded using a DSC Q10 modulus (TA instruments) under an air flow of 100 mL min^{-1} and at a heating rate of 10 °C min^{-1}. The sample masses were about 3 mg and covered aluminum crucibles with a pin hole (ϕ = 0.7 mm) in the center of the lid were employed during the analysis. DSC modulus was calibrated using indium metal (99.99+ %) for temperature and enthalpy.

Epinephrine samples were heated up to 205 °C in the thermobalance and the residue collected after cooling. This residue was submitted to mass spectrometry analysis in a Brüker ULTROTOF-Q with electron spray ionization. Samples were dissolved in methanol and introduced in an infusion pump at a 100 μL h^{-1} flow. The capillary was heated at 150 °C with a nebulizing gas flow of 4 L min^{-1} and 4 kV.

X-ray diffraction powder patterns were obtained using a D-5000 X-ray diffractometer (Siemens), with Cu Kα radiation (λ = 1.544 Å) and a setting of 40 kV and 20 mA. A 2θ range from 5° to 70° was used.

Coupled TG-IR analysis was performed in a Nicolet iS10 spectrophotometer (Thermo Scientific) coupled to the gas exhaust of a TGA/SDTA 851 Mettler Toledo.

Reflectance FTIR spectra of epinephrine and its decomposition product obtained at 205 °C were recorded in a Nicolet iS10 spectrophotometer (Thermo Scientific).

3. Results and discussion

The TG-DTA curves of epinephrine are shown in Figs. 2 and 3. These curves show that, under the experimental conditions used in this work, the anhydrous compound is stable up to 165 °C and above this temperature the thermal decomposition occurs in three (N_2) or four (air) consecutive and/or overlapping steps between 165 and 450 °C (N_2) or 165 and 600 °C (air) and thermal events corresponding to these losses.

In both atmospheres, the first step occurs between 165 and 209 °C with loss of 17%, corresponding to a sharp endothermic peak at 205 °C. For the N_2 atmosphere the last two overlapping steps observed between 220 and 450 °C with loss of 47%, the thermal decomposition occur with the formation of carbonaceous residue. Any thermal event corresponding to these losses is observed in the DTA curve, probably due to the heat involved in these steps is insuf-

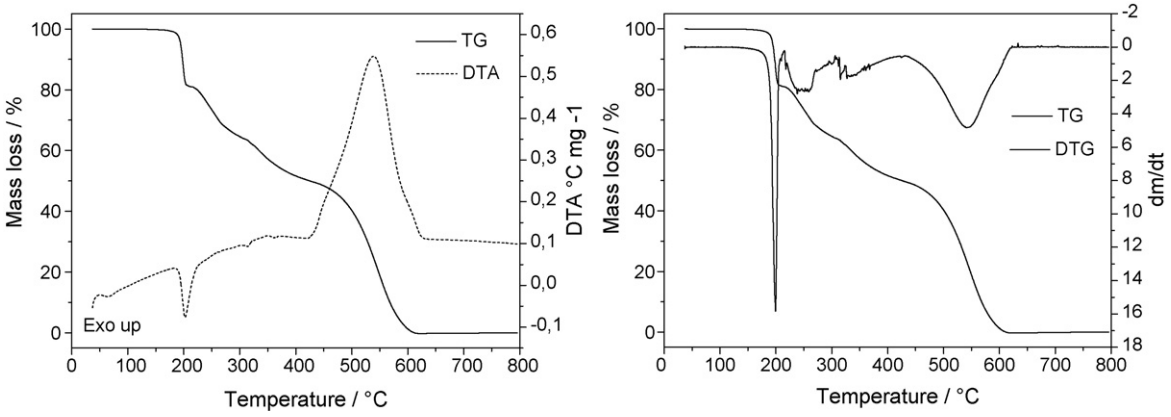


Fig. 2. TG-DTA and TG/DTG curves of epinephrine (m_i = 7.641 mg), under air atmosphere.

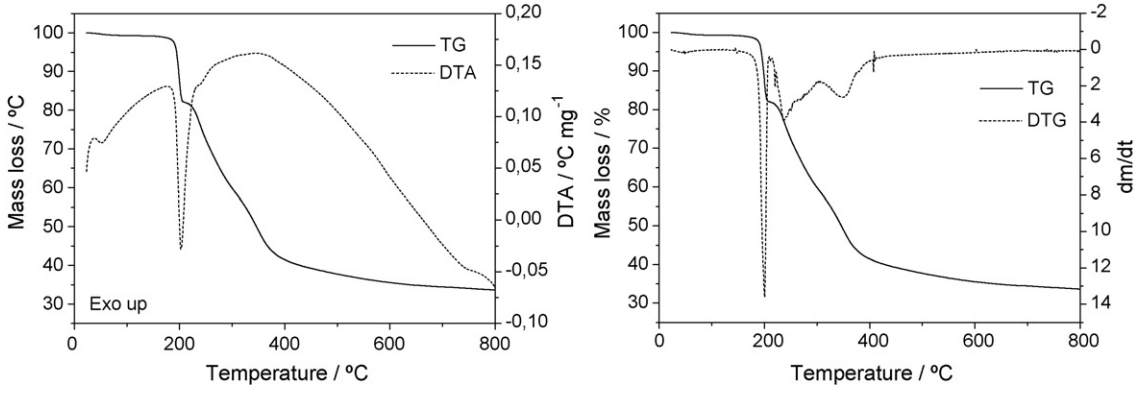


Fig. 3. TG-DTA and TG/DTG curves of epinephrine (m_i = 8.768 mg), under N_2 atmosphere.

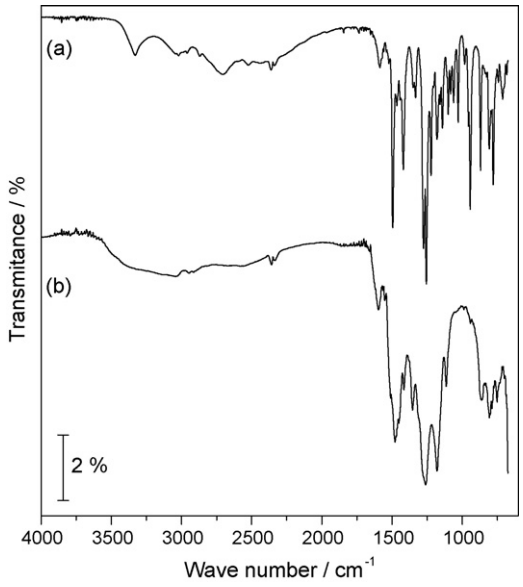


Fig. 4. Reflectance infrared spectra of: (a) epinephrine at room temperature; (b) epinephrine heated up to 205 °C.

ficient to reach the minimum equipment sensitivity and causes the registration of the thermal event.

A total mass loss was observed during the last three steps observed between 220 and 620 °C. Any thermal event was observed corresponding to the second mass loss, but small endothermic peak at 315 °C and a large exothermic one at 530 °C corresponding to the last two steps are attributed to the thermal decomposition and oxidation of evolved product, respectively.

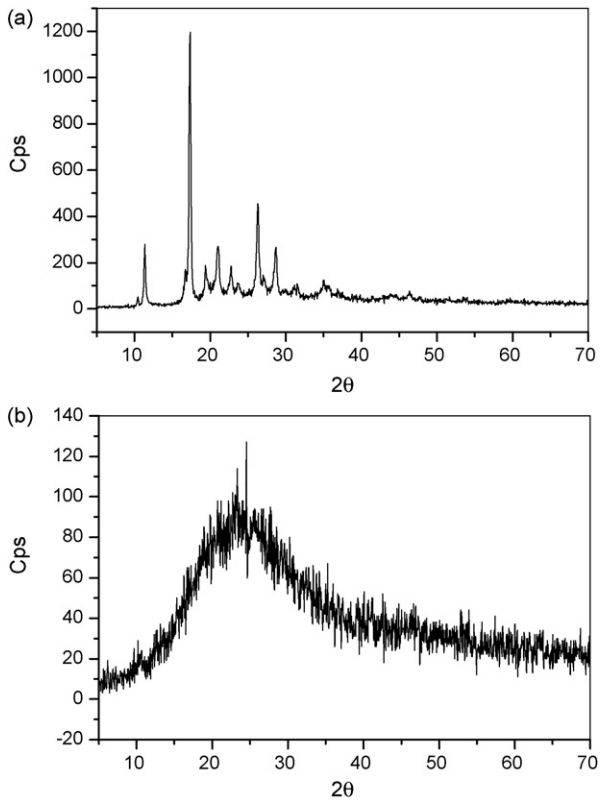


Fig. 5. X-ray diffractograms of: epinephrine (a) at room temperature; (b) heated up to 205 °C.

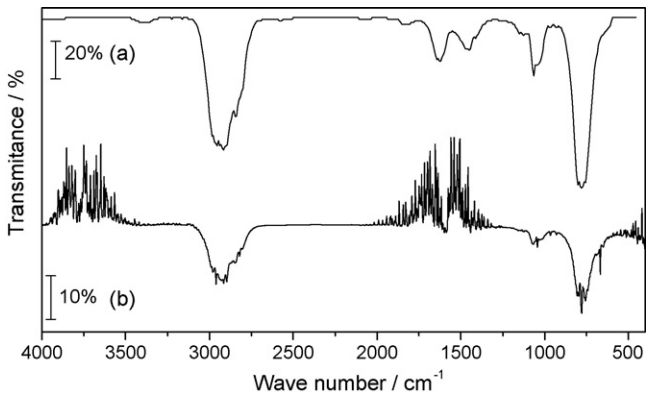


Fig. 6. TGA-FTIR infrared spectra of: (a) standard methylamine; (b) methylamine collected up to 200 °C, in gas phase, from the Grand-Schmitt plot.

The reflectance FTIR spectra and X-ray powder diffraction patterns of epinephrine before and after heating up to 210 °C, as well as the TG-FTIR spectra of sample heated between 30 and 600 °C, are presented in Figs. 4–7, respectively. These results showed that after heating up to 210 °C, structural changes occur in the sample (Figs. 4 and 5) and up to *ca.* 205 °C the thermal decomposition occurs with elimination of methylamine (Figs. 6 and 7), in agreement with the first mass loss observed in the TG curve in both atmospheres (TG = 17.0%, calcd. = 17.0%).

The loss of methylamine should result in benzophenone as the decomposition product (Eq. (1)). However, the mass spectra of the residue obtained at 205 °C suggested that there is a mixture of compounds in the crucible at such temperature (Fig. 8). The peak at z/m 300.1223 must be related to a compound with formula $C_{16}H_{12}O_6$ (MM = 300.28 g mol^{-1}) suggesting a condensation of two benzophenone molecules (MM = 152.05 g mol^{-1} each), with loss of two hydrogen atoms.

The mass spectrum of the residue also showed the presence of peaks with m/z = 323.1381, suggesting the presence of a condensate with formula $C_{16}H_7O_6N_2$ (MM = 323.23 g mol^{-1}). Two other compounds with m/z = 189.1007 and 176.0699, corresponding to the loss of $C_7H_3O_3$ and $C_8H_4O_3$, respectively, can be observed. However, considering the mass loss observed in the TG curve, we believe that these are not products of the thermal decomposition, but resulted from the fragmentation of the $C_{16}H_7O_6N_2$.

Thus, the first mass loss in the thermal decomposition of the EP could be represented by Eqs. (1) and (2):

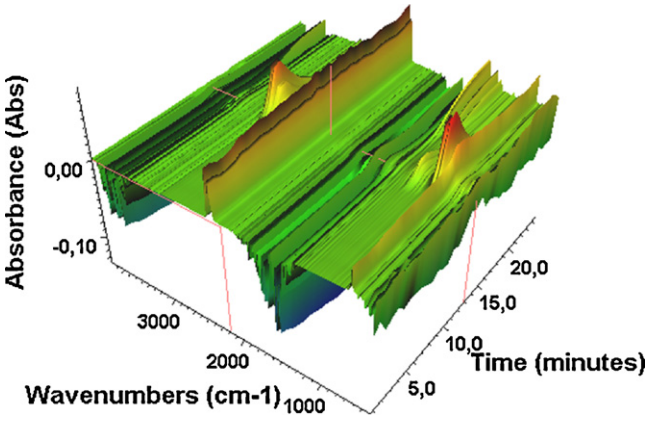


Fig. 7. TGA-FTIR infrared spectra Grand-Schmitt plots.

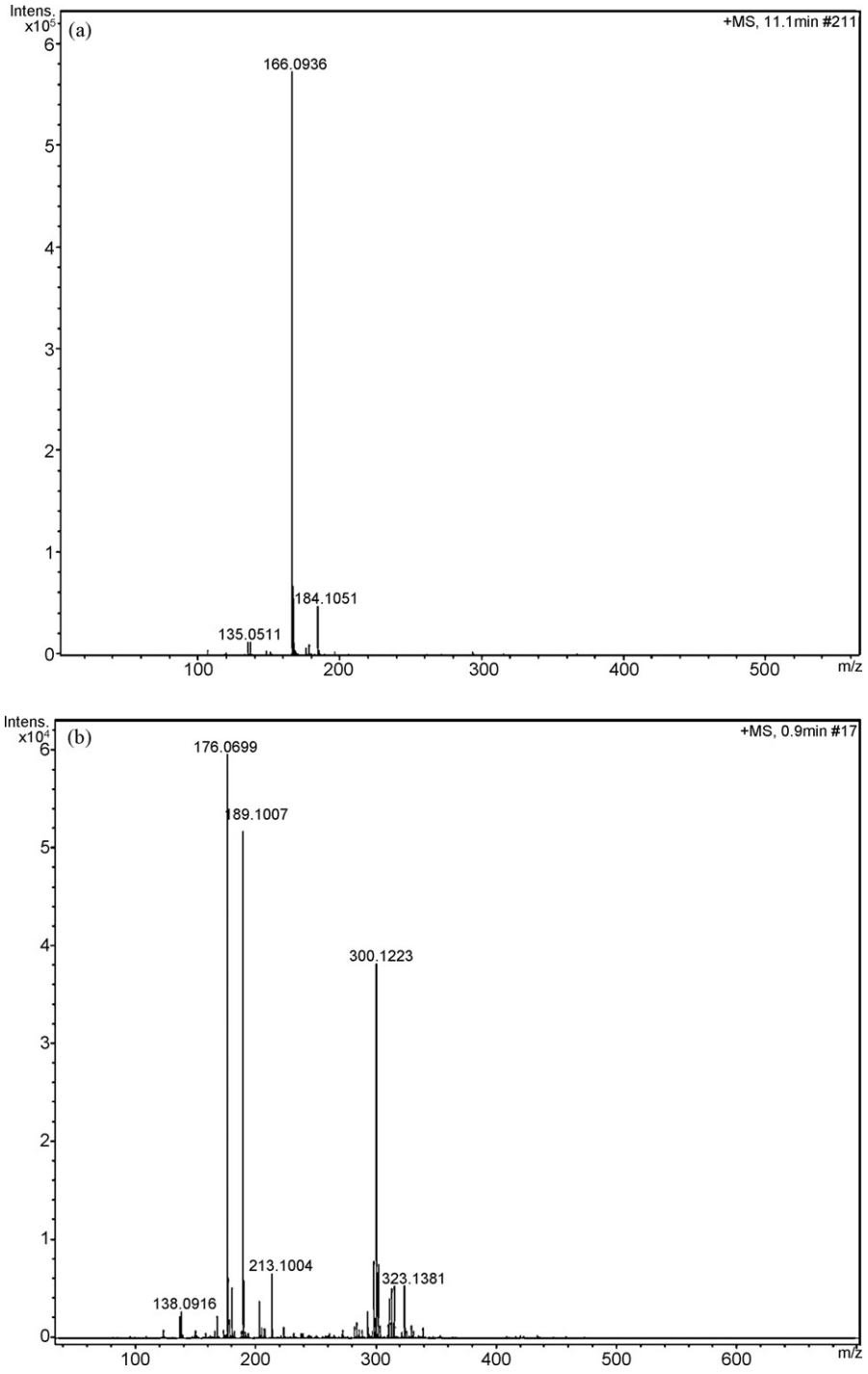
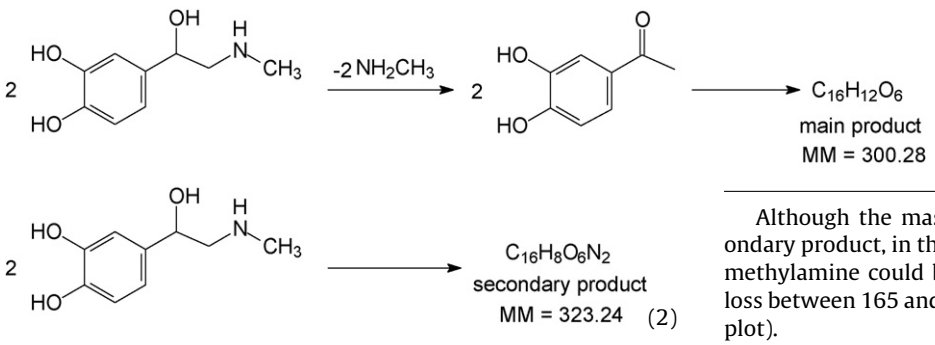


Fig. 8. Mass spectra: (a) standard epinephrine; (b) epinephrine heated up to 205 °C.



Although the mass spectra revealed the presence of the secondary product, in the evolved gas analysis using TG-FTIR only the methylamine could be positively identified during the first mass loss between 165 and 209 °C, as presented in Fig. 7 (Grand-Schmitt plot).

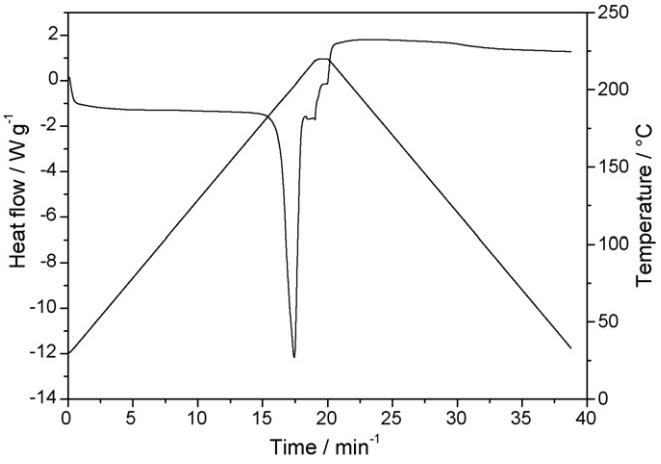


Fig. 9. DSC curve of epinephrine (m_i = 3.10 mg), under N_2 atmosphere.

The second step where the final mass loss occurs is observed between 209 and 618 °C with losses of 82.9% corresponding to the exothermic peaks at 540 °C was attributed to the oxidation of the organic matter. As additional information, TG-FTIR revealed that benzene is released from the sample at around 600 °C. Under N_2 atmosphere above 209 °C only the formation of a carbonaceous material is observed.

The DSC curve of epinephrine is shown in Fig. 9. The sharp endothermic peak at 203 °C, corresponding to the first mass loss of TG curve and the endothermic peak at 205 °C in the DTA curve is attributed to the overlapped melting and thermal decomposition of the epinephrine. The melting of the compound was confirmed by heating the sample in a glass tube. So such event can be attributed to an incongruent melting of epinephrine, the property exhibited when a solid does not simply melt, but reacts and decomposed to form another solid plus liquid [24].

The melting temperature that occurs with decomposition, 205 °C (DTA) or 203 °C (DSC) is in disagreement with the temperature 211–212 °C, described in the literature [22]. This disagreement undoubtedly is due to the different conditions used to determine the melting temperature, mainly when one considers the incongruent melting as above.

4. Conclusion

The TG-DTA and DSC curves and the heating of the sample in a glass tube showed that an incongruent melting of the epinephrine occurs. TG-FTIR data also showed that the first mass loss of the TG curve is due to the elimination of methylamine and the main intermediate is a compound with formula $C_6H_{12}O_6$, with lower amount of $C_{16}H_8O_6N_2$.

The TG-DTA, DSC and TG-FTIR provided previously unreported information concernedly the thermal behavior of this compound.

Acknowledgements

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