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Synthesis and Characterization of PVA-Enzyme/GA/PPy/ PVC-KTpClPB-o-NPOE Indicator Electrodes, XRD Analysis, FTIR and Variable Signal Analysis

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Abstract: This study aims to synthesize and characterize PVA-Enzyme/GA/PPy/PVC-KTpClPB-o-NPOE indicator indicator electrodes, XRD and FTIR analysis and variable signal analysis for urea sensors. The method used is biosensor potentiometry with urease enzyme immobilization technique which analytes urea. Modify 6 mg of enzyme from the amount of 1 drop and 3 drops in 0.5 mL (50% water: 50% ethanol) in PVA solution and o-NPOE plasticizer in PVC-KTpClPB. The indicator electrode is designed in multi-membrane, namely PVA-Enzyme/GA/PPy/PVC-KTpClPB-o-NPOE. PPy was dissolved in H2SO4 at a concentration of 8 M. Modification of PVA-Enzyme/(GA 2.9%)/PVC-KTpClPB-(o-NPOE 61%) was carried out in one layer each. The number of enzyme drops to see the difference in the intensity of the XRD diffraction spectrum and the difference in the transmittance of the FTIR spectrum from the indicator electrode with multi membrane modified PPy with H_2SO_4 denoted H_2SO_4-1 and H_2SO_4-3, respectively. The best results were obtained on the indicator electrode with the notation H_2SO_4-1

Keywords: Multi-membrane; Enzyme modification; PVA-Enzyme/GA/PPy/PVC-KTpClPB-o-NPOE; H_2SO_4; Potentiometer cell.

Introduction

It has been researched that PVA-Enzyme membrane coated with Glutaraldehyde (GA) of variation (2.6 - 3.0)% can increase the width of the absorbance peak, the best is at 2.9% GA. The increase in the width of the absorbance peak is closely related to the increase in the sensor detection range. Modification of this membrane to increase peak width and increase peak absorbance. The addition of GA to the PVA-Enzyme/GA/PVC-KTpClPB electrode had an effect on decreasing the absorbance peak (Hakim, 2021). The increase in the absorbance peak is related to the potentiometric cell voltage and the sensitivity of the biosensor. The PVA-Enzyme/GA/PVC-KTpClPB indicator electrode has the best sensitivity of one layer in each layer (Hakim, 2021) of 49.69 mV/decade detection range 10^{-4} - 10^{-2} M. The peak increase was made by adding o-NPOE to the PVC-KTpClPB solution and modifying the number of drops of 0.5 mL urease enzyme from 50% water and 50% alcohol in PVA solution. According to (Hakim, 2022) FTIR analysis of PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE membrane, the best composition was o-NPOE 61%, and XRD diffraction spectrum pattern analysis showed a change in activity.

Modification of the number of drops of 6 mg of urease enzyme in 10 mL (50% water: 50% ethanol) in PVA solution, can be seen in Figure 2, the difference in signal activity from the potentiometer cell is very small.

Based on the addition of o-NPOE to the PVC-KTpClPB solution and the number of enzyme droplets in PVA, the researchers designed and characterized the PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrode for XRD analysis as shown in Figure 1.

The GA-crosslinked composite increased ionic conductivity (Hsu, 2022), the phase purity of the PVA/GA and PVA/ZIF-8/GA composites was determined using X-ray diffraction. The chemical structure of the sample was determined using Fourier transform infrared spectroscopy.

Enzymes are proteins that act as powerful catalysts to convert substrates into products that generate

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electrons. Direct electrical activation of enzymes is a common approach to stimulate the oxidation (or reduction) of enzyme substrates. The immobilization technique involves interaction with a solid carrier. Binding to the carrier can be done through physical or chemical interactions between the enzyme and the carrier, namely hydrogen bonds, van der Waals forces and hydrophobic interactions, (Bié, 2022). As well as carrier porosity, including pore size distribution, structure and volume of chemical carriers and mechanical resistance of operating conditions.

The immobilization technique for biosensor construction involves urease enzyme into PVA polymer coated with GA coated with PVC-KTpClPB-o-NPOE for urea bioser (Hakim, 2021). There are several guidelines for the material composition of membrane-based sensors: A) prepared by dissolving ion association complex (2 mg), plasticizer o-NPOE, 133 mg), and PVC (66 mg) in THF (3 mL), (Elbehery et al., 2019); B) PVC composition: o-NPOE: DPA: NaTPB as 33:61:3:3 (w/w) as the optimum composition with the closest Nernstian response (Kaur, Chhibber and Mittal, 2017); C) Composition 30:65.5:0.5 mg PVC:o‐NPOE:KTpClPB (Badakhshan et al., 2019), regarding the composition of PVC, plasticizers and lipophilic additives. Ionophores (I) 1.10% (w/w), lipophilic salt (KTpClPB) 0.25% (w/w), plasticizer (o-NPOE) 65.65% (w/w), and PVC 33.00 % (w/w) dissolved in tetrahydrofuran (Lenar, 2020).

Electrodes formulated with membranes containing 1 wt.% ionophore, 66 wt.% o-NPOE, 33 wt.% PVC (plasticizer : PVC = 2:1) and a lipophilic cationic derivative (35 mol%) (Vlascici et al., 2008). The poly vinyl chloride (PVC) membrane consists of a plasticizer, o-nitrophenyloctylether (o-NPOE), a lipophilic salt, and sodium tetraphenylborate (NaTPB), and the required amount of ionophore (Harpreet Kaur, 2017).

The best composition of the enzyme urease 6 mg 10 mL (50% water: 50% alcohol), 35 mg PVA, 50 mg, 35 mg PVC, 50 mg KTpClPB, plasticizer (o-NPOE) 61% (Hakim, 2022). The following is the analysis of the PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrodes. The urease enzyme in PVA is designed in two forms, namely the number of drops taken from 6 mg 0.5 mL of (50% water: 50% alcohol). Observing (Abd-Rabboh et al., 2022), the two modifications of Ionophores I, II or III (2.0%), KTFPB (1.0%), PVC (32.0%), and plasticizer (65%) 15 L of membrane solution were added with drop-casting. On the basis of this ion modification, a modification of the number of enzyme droplets in PVA was used.

Methods

The material for making PVA-Enzyme/GA/PPy/PVC-KTpClPB-o-NPOE indicator electrodes is tungsten with a diameter of 1.0 mm 99.99% Aldrich 267562, PVA[-CH_2CHOH-]n, enzyme EC 3.5.1.5 (Urease) U4002, 50 - 100 ku type ix, glutaraldehyde (GA), PVC $(CH_2CHCl)n$, potassium tetrakis (4-chlorophenyl) borate (KTClPB), o-nitrophenyloctylether (o-NPOE), Tetrahydrofuran C_4H_8O; Phosphate Buffer KH_2PO_4, KCl, urea standard 56180, from Sigma-Aldrich.

The potentiometer cell is equipped with an Ag/AgCl MF-2052 RE-5B reference electrode with a PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode connected to a microcomputer (ADI Powerlab instrument, Australia), a magnetic stirrer, and flow injection (FIA). PPy was dissolved with H_2SO_4 at the lowest concentration soluble at 8 M. The indicator electrodes were sequentially coated by PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE.
The number of drops of urease enzyme in the PVA solution in two variations, namely one drop and three drops was denoted H_2SO_4-1 and H_2SO_4-3, respectively.

The PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode can be analyzed by XRD, FTIR and variable signal analysis. Aims to obtain a good indicator electrode.

Result and Discussion

Results of Characterization Analysis of Variable Signals PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE

On the PVA-Enzyme/GA/PVC-KTpClPB indicator electrode, the data records of the variable signal analysis in Figure 1 are symmetrical up and down but left and right are not symmetrical, indicating that the oxidation and reduction processes have not been good. On the PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrodes in Figures 2 and 3, the analysis of the upper and lower variable signals, as well as the symmetrical right and left shows good oxidation processes. As well as the signal response of the PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode, at a scale of 1:1 in a time of 5.33 minutes with the same scale in Figure 3, much better than the PVA-Enzyme/GA indicator electrode /PVC-KTpClPB-o-NPOE Figure 2.

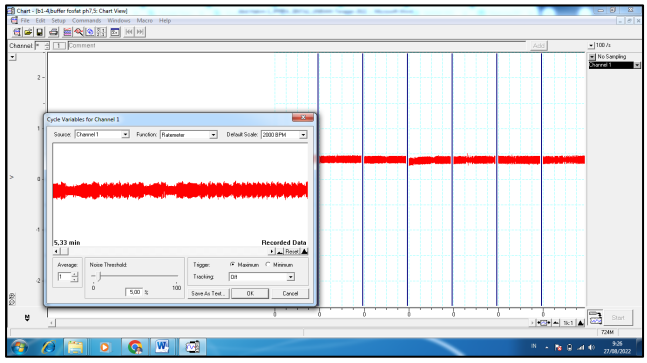

Figure 1. Analysis of Variable Signals PVA-Enzyme/GA/PVC-KTpClPB

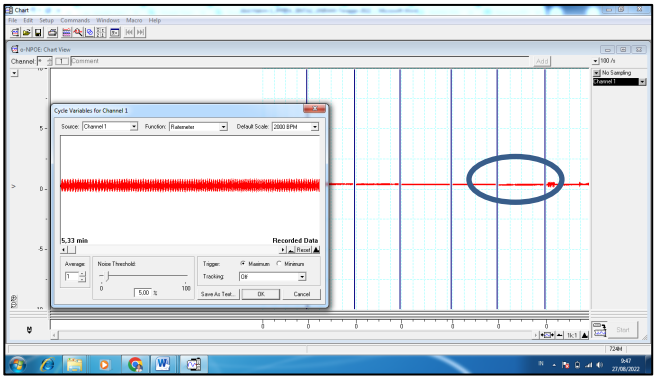

Figure 2. Analysis of Variable Signals PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE, 1 drop of enzyme

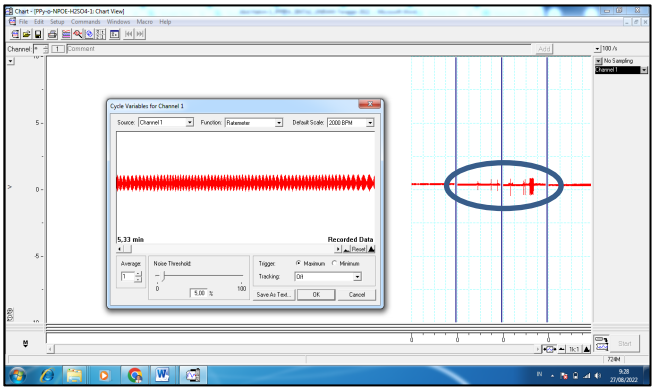

Figure 3. Analysis of Variable Signals PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE, 1 drop of enzyme

The sign of the activity response can be seen in Figures 2 and 3 in the form of a blue circle. More activity with the addition of the number of drops of the urease enzyme. There is a different response in Figure 1 and Figure 2, one drop of 6 mg of urease enzyme in 10 mL (50% water: 50% alcohol). In Figure 3, 6 mg of the enzyme urease in 0.5 mL (50% water: 50% alcohol), on the contrary in Figure 4 does not show any signal response. Signal symmetry and signal response are needed for analysis to study the time response of the potentiometer cell from voltage to time.

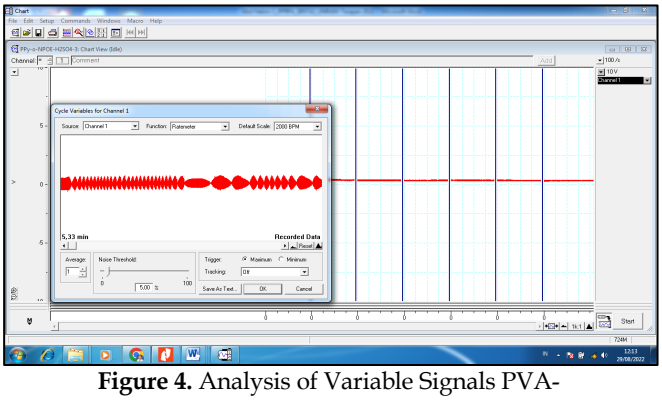

Figure 4. Analysis of Variable Signals PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE, 3 drops of the enzyme

Characterization results of XRD analysis of PVA-Enzyme/GA/PPy/PVC-KTpClPB-o-NPOE indicator electrodes

The results of the analysis of the PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrodes and the PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrodes are denoted by H_2SO_4-1 and H_2SO_4-3 in Figure 5.

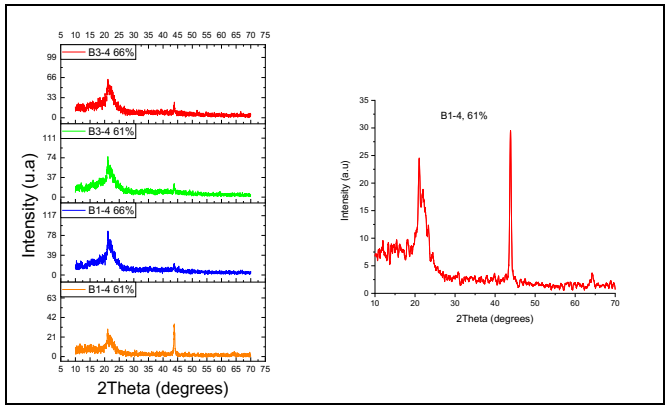

Figure 5. XRD Diffraction Spectrum Pattern PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrode

Table 1. PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE . indicator electrodes

2Theta	Intensity(a.u)
43.82	28.89
43.84	29.56
43.86	29.54

XRD analysis results Figure 4 XRD diffraction spectrum pattern PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrode, forming amorphous and crystalline spectrum patterns. After the addition of GA, there was a change in the crystal spectrum pattern. Followed by the addition of GA and o-NPOE on the indicator electrode layer (Hakim, 2021) formed an amorphous and crystalline spectrum pattern in Figure 4 with one drop of 6 mg urease enzyme in 10 mL in PVA.

According to the intensity spectrum pattern against the 2Theta XRD angle, there is an increase in the intensity (a.u) of 8 M H_2SO_4 with the amount of 1 drop to 3 drops in Figure 5, also followed by differences in signal response in Figures 2 and 3.

It turns out that the concentration of urease enzyme can increase the activity of the indicator electrode from one drop to three drops. Figure 5. In addition to concentration, molarity can increase the activity of the urease enzyme, see Figure 4 of the PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrode, and Figure 5 from the indicator electrode PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE.

According to Figure 4 and Table 2, the peak intensity of 29.56 (a.u) is located at an angle of 2Theta 43.84 degrees. When analyzed from the membrane layer pattern of PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE. The difference in soluble properties of PVA-Enzyme/GA/soluble in water, /PPy/soluble in H_2SO_4 acid, PVC-KTpClPB-o-NPOE is soluble in THF. The difference in solubility properties showed that the enzyme immobilized on PVA did not die as shown in Figure 6.

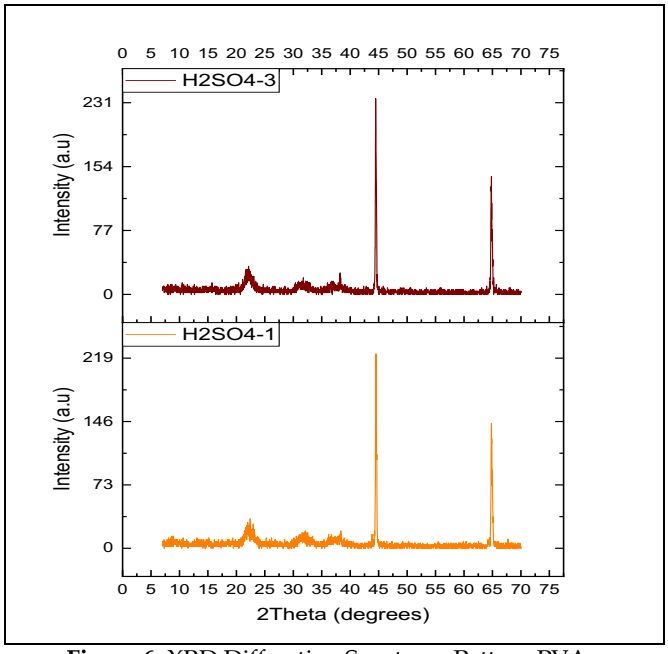

Figure 6. XRD Diffraction Spectrum Pattern PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrodes

According to table 2, the peak intensity of the PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode increased quite large in units of hundreds with the notation (1) H_2SO_4-1 peak intensitya 224 (a.u) at a diffraction angle of 44.54 degrees; (2) H_2SO_4-3 peak intensitya 236(a.u) at a diffraction angle of 44.48 degrees from the enzyme urease 6 mg in 0.5 mL (50% water: 50% alcohol).

When compared PVA-Enzyme/GA/PVC-KTpClPB-o-NPOE indicator electrode with one drop of enzyme from 6 mg in 10 mL (50% water: 50% alcohol). The peak intensity of 29.55 (a.u) at a diffraction angle of 43.84 degrees decreases in tens, see table 1.

This means that the urease enzyme contributed to increasing the peak intensity with respect to the 2theta angle and dampening the amorphous spectral pattern of the PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode, after the addition of o-NPOE.

The symmetry of the signal (Hakim, et al 2021) is highly dependent on the incoming signal and outgoing signal from the redox process. The incoming signal is not the same as the outgoing signal with a total signal data of 400 signals/second (Kim et al., 2017). The symmetry of the signal (Basile et al., 2016) can be seen in Figure 1, top and bottom symmetrical, while Figures 2 and 3 are top and bottom symmetrical followed by left and right symmetry from variable signal analysis. The voltage between the oxidation and reduction peaks according to (Rahman et al, 2008) is theoretically 59 mV for a reversible reaction.

Table 2. PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrodes

Diffracted angle	Intensity (a.u) indicator electrode	
2Theta	H_2SO_4-1	H_2SO_4-3
44.48	166	236
44.54	224	172

Results of FTIR characterization of PVA-Enzyme/GA/PPy/PVC-KTpClPB-o-NPOE indicator electrodes

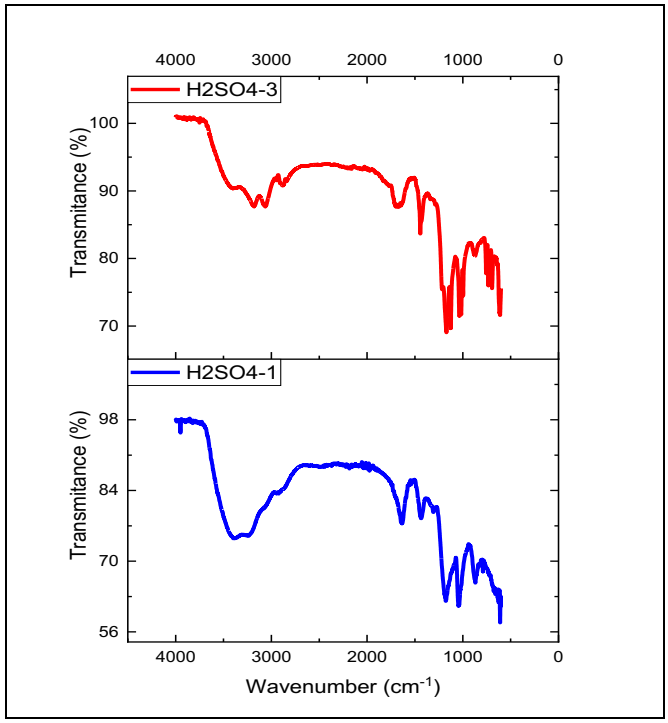

Figure 7. FTIR transmittance spectrum pattern of PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode (a) one drop, (b) three drops

On the basis of the symmetry of Figure 4 of the XRD spectrum pattern. It is stipulated to manufacture PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrodes as follows: PVA-Enzyme 1x coated with GA 1x coated PPy+H_2SO_4 1x and coated with PVC-KTpClPB-o-NPOE 1x, with the notation H_2SO_4 -1. The nature of the conduction polymer is shown in Figure 5, the XRD spectrum pattern and the

FTIR spectrum pattern in Figures 7 and 8. Besides the urease enzyme increasing the response and activity, it also increases the transmittance.

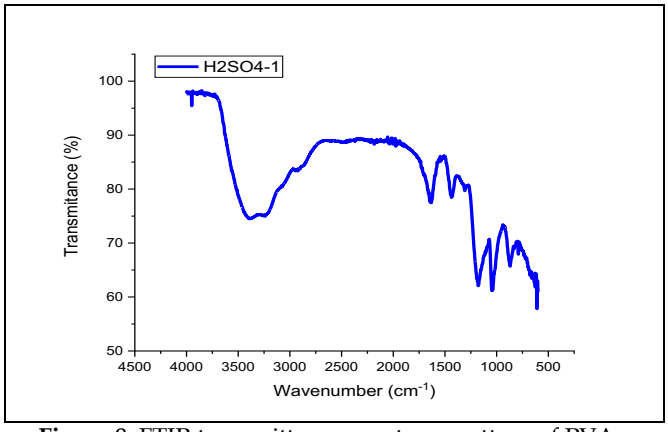

Figure 8. FTIR transmittance spectrum pattern of PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE indicator electrode one drop

Table 3. Transmittance range of H_2SO_4-1 and H_2SO_4-3 indicator electrodes

Wavenumber (cm)	Transmittance (%)	
	H_2SO_4-1	H_2SO_4-3
600	60.8895	75.5583
4000	97.9351	100.9955
Range	37.0456	25.4372

According to (Jaziri et al., 2022; Dacrory et al., 2022; He., 2022; Mohamed et al., 2022; Saeed et al., 2022) XRD analysis of increasing peak intensity and increasing transmittance FTIR analysis can be determined that the H_2SO_4-1 sample as the best indicator electrode sample is composed of PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE layers.

Conclusion

Based on the data above, the manufacture of tungsten indicator electrodes with membrane modifications was obtained in the H_2SO_4-1 sample. Composed of layers of PVA-Enzyme/GA/PPy+H_2SO_4/PVC-KTpClPB-o-NPOE

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