Nanoarchitectonics with NADPH Catalyst and Quantum Dots Copper Sulfide on Titanium Dioxide Nano-sheets Electrode for Electrochemical Biosensing of Sorbitol Detection

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Abstract: Sorbitol accumulation in the tissue is known to cause diabetic complications. Nanotechnology-enabled biosensor methods have high sensitivity, selectivity, and more rapid detection of an analytic for sorbitol which is used as a biomarker of diabetic complications. The biosensor used aldose reductase from serum blood to oxidize the NADPH by the enzymatic reaction and reduce glucose to sorbitol. Biosensors can be developed for diagnostic testing. Developing a simple, sensitive, and rapid method for sorbitol detection is significant for efficient monitoring of diabetic complications like neuropathy at the initial stages. This project synthesized quantum dots of copper sulfide (CuS QDs) to fabricate an Electrochemical sensor for the detection of sorbitol by the UV-irradiation technique. The crystal structure of CuS QDs was characterized using X-ray diffraction (XRD), which confirmed the synthesized sample’s hexagonal shape. The structure of the manufactured product was examined using energy-dispersive X-ray spectroscopy (EDX), and the result revealed just copper (Cu) and sulfide (S) elements, indicating that the synthetic material was pure. The morphology, optical properties, and particle size were investigated by scanning electron microscope (SEM), photoluminescence spectroscopy (PL), and transmission electron spectroscopy (TEM), respectively. The particle sizes of the CuS QDs were found to range between 5.4 to 9.1 nm. The CuS QDs will be dedicated to the conventional methods to synthesize the modified electrode functionalized with NADPH and covered with CuS QD (Ti-TiO₂/CuS/NADPH) demonstrated switchable interfacial properties. The electrochemical process was characterized by cyclic voltammetry (CV). The developed sensor was successfully tested to detect sorbitol in human serum samples. The high catalytic activity and the redox behavior of CuS QD make it an efficient matrix for the realization of sorbitol. These results indicate that CuS QD is a suitable candidate material for developing enzyme-based sorbitol biosensors.

Key words: biosensor, electrochemical deposition, sorbitol, NADPH, QDs CuS, aldose reductase

1 Introduction

A biosensor is analytical equipment that detects a chemical compound by combining a biological component with a physicochemical detector. The biomimetic component or sensitive biologically created material interacts with, binds to, or identifies the investigational analysis. As a result of the analytic’s interaction with the biological element, the transducer or detector element turns one signal into another, allowing easy quantitative measurement. The biosensor reader is connected to the associated electronics or information processors, which display the results user-friendly. A biosensor comprises three fundamental components: a transducer, a bio receptor, and a locator, and it is an electronic system that includes a signal amplifier utilized to identify essential metabolites, immunological atoms, and an assortment of other materials.
The electrochemical biosensor is also an excellent choice for monitoring biomarkers in human serum. It is low-cost, easy to operate, fast, environmentally friendly, and portable, which highly meets the requirement electrochemical biosensors have no interference from the particles or colors presented in samples. The analytical cyclic voltammetry (CV) Technique is a flexible electrochemical method for analyzing redox status in various mechanical and investigative settings. Typical applications incorporate drug performance assessment in pharmacological 

Nanomaterials in Biosensor Development Several materials are thought to be suitable for manufacturing nano biosensors. To be developed in large quantities (biosensing materials) must be biocompatible, reasonable, and reproducible. The main feature of nanomaterials is that they have at least one external dimension in the 1 - 100 nm range. The nanotechnology concept has opened up ways to observe, analyze, and manipulate nanoscale objects to fabricate high-performance materials and has seen successful advances such as bio-related technology. Nanotechnology is right now holding a colossal guarantee for electrochemical measurements. Due to their high surface area activity, quantum dot (QD) sensors may offer new avenues for early glucose diagnosis. Nanostructures have important functions in sensor manufacturing, including receiver stability, catalysis, enhanced electron transport, and participation in detecting reactions. The electrical attachment of redox proteins, particularly enzymes, to the electrode surface is crucial since a thick, non-conducting protein shell surrounds its active areas. Electron transfer between the electrode and the active site is blocked because the proteins are not electrically linked to the electrode. Nanoparticles increase electron transit between the electrode and the active center at the nanoscale.

Aldose Reductase is a cytosolic NADPH-dependent nicotinamide adenine dinucleotide phosphate hydrogen oxido-reductase that catalyzes the conversion of glucose to sorbitol. It is found in non-uniform levels in most mammals, with high levels in the eye (retina, cornea, and lens), peripheral nerves, kidney, myelin sheath tissues, and red blood cells frequently involved in diabetic patients complications. High glucose levels activate the polyol path production and increase susceptibility to intracellular oxidative stress, which leads to peripheral neuropathy.

According to a prior study, protocol detection in the presence of the cofactor NADPH is based on a redox pair that transforms protocol to a quinone/quinol that can be detected using simple electrochemistry. This method overcomes the problem of electrode fouling, which makes electrochemical propofol sensors unusable. The sensor has shown reasonable specificity in terms of potential interfering chemicals.

In our paper, we present an electrochemical Novel Biosensor for the Sorbitol detection based upon the cofactors NADPH immobilized alongside copper sulfide quantum dot upon the surface of a titanium/titanium dioxide electrode. Aldose reductase and NADPH are responsible for reducing glucose to sorbitol in the human body. Sorbitol is expected to be one biomarker of diabetic complications required to develop and test a rapid diagnosis.

2 Experimental

2.1 Chemicals and reagents

Titanium plate (Ti), sodium hydroxide (NaOH), ethanol (C2H5OH), copper acetate Cu(CH3COO)2, H2O, sodium sulfate (Na2S), urea (NH2CONH2), and phosphate buffer saline Na2HPO4, 12H2O were obtained from Sigma-Aldrich. All the solutions were prepared in deionized water and NADPH tetrasodium salt from CARL ROTH/Germany. Titanium/Titanium dioxide (Ti/TiO2) was the working electrode. The Platinum (Pt) was employed as a counter electrode, and the reference electrode was Calomel (Hg2Cl2) for electrochemical measurements.

2.2 New Synthesis of Quantum dots Copper sulfide (QDs CuS)

QDs CuS was synthesized by a photolysis method. The photo-cell comprises a 125 Watt of UV source (λ max of 365 nm) and an ice bath-cooled pyrex tube that prevents temperature rise caused by UV radiation (Fig. 1). In a typical experimental procedure, copper acetate (0.01 mole, 1.95 g) was dissolved in 100 mL of de-ionized water with a magnetically stirred. Then, 50 mL, 0.02 mole of urea was slowly added (drop by drop) to the copper solution and stirred for 1/2 h. The mixture solution was irradiated in a
Fabrication of CuS QDs was made from 0.5 g CuSO₄ added to 20 mL of 0.1 M H₂SO₄ and left to dry at room temperature (29.2, 31.8, 32.7, 48.0, 52.7, and 59.2°) attributed to hexagonal CuS crystal facets[102], [103], [006], [110], [108], and [116][34]. The measured peaks, which lacked any excess peaks(inclusions), were closely matched with the Copper sulfide powder, with peaks at 2θ = 29.2, 31.8, 32.7, 48.0, 52.7, and 59.2° attributed to hexagonal CuS according to the standard JCPDS card No. 06-0464, indicating that the CuS product has only one phase. The Debye-Scherrer formula was used to estimate the crystal structure of QDs CuS[35-36]:

\[ D = k \lambda / \beta \cos \theta \]

Where D = crystal size, k = 0.9 (Scherrer constant), \( \lambda = 0.154 \text{ nm} \) (wavelength of Cu-K radiations), \( \beta \) is the complete width at half maximum, and theta(θ) is the angle calculated from two values matching the XRD pattern. The

2.3 Electrodeposition of Copper sulfide quantum dot on the (Ti/TiO₂) Nanosheets electrode

Pure rutile TiO₂ nanosheets were synthesized by the electrolysis method according to the preschool[30]. A deposition device power supply 1502TD was used to deposit Copper sulfide quantum dots on the Titanium/Titanium dioxide Nanosheets electrode. The working electrode (Ti/TiO₂), and the titanium sheet as a counter electrode, were placed in an electrolyte. Electrolyte for electrodeposition was made from 0.5% of CuS QD(0.1 g/20 mL ethanol). Fabrication of CuS QD on Ti/TiO₂ was done by applying a direct (DC) voltage of 7.2 V with a current density of 1.9 × 10⁻⁶ mA/cm² at room temperature for 10 min, as shown in Fig. 2. After deposition, the coated (Ti/TiO₂) was rinsed with DW and left to dry at room temperature, as presented in the authors’ earlier work[30]. The optimization of the nanoparticle deposition process aimed to obtain a composite with the highest conductivity.

2.4 Immobilization of NADPH on CuS QDs Modified Ti/TiO₂ Electrodes

A 50 mg of the NADPH was dissolved in 100 mL of phosphate buffer saline (pH = 7). NADPH co-factor immobilized on activated surface of CuS QDs Modified Ti/TiO₂ Electrodes using drop-casting method incubation for 4-6 hours at room temperature to ensure attachment of NADPH with the surface of modified electrode.

2.5 Equipment and measurement

Electrochemical measurements were performed with an instrument: Potentiostat / Galvanostat / EIS Model: Vertex One EIS – Netherlands Applied Scan voltage: ± 10 V Output Voltage: 21 V Current Compliance: 100 mA Current range: ± 100 pA to ± 100 mA Scan rate: 30 mV/s Potential range: −2000 to 2000 mV Reference Electrode: Calomel (0.241V) Counter Electrode: Platinum (Area = 1 cm²). Several devices determined QDs CuS powder X-ray diffraction powder (XRD) was achieved using (Model D-5000) with a λ of 0.154 nm. Field emission scanning electron microscope (FE-SEM) model Jeol JSM-6010LV was used to determine the morphology and topography of the prepared sample. Energy-dispersive X-ray spectroscopy (EDX) was used to determine the chemical composition of CuS. Transmission electron microscopy (TEM) type JEOL JEM-2100 was used to investigate the shape and size of QDs CuS powder. Photoluminescence measurements (PL) emissions measured were used to determine the optical properties of QDs CuS.

2.6 Statistics

The statistical impacts of the study were estimated by (The SPSS version 26 ANOVA statistical software) and utilized to calculate the mean standard deviation (SD) from the data reported.

3 Results and Discussion

3.1 Characterization of QDs CuS

XRD analysis was used to determine the crystal structure of the produced sample. Figure 3 shows the XRD pattern of the Copper sulfide powder, with peaks at 2θ = 29.2, 31.8, 32.7, 48.0, 52.7, and 59.2° attributed to hexagonal CuS crystal facets[102], [103], [006], [110], [108], and [116][34]. The measured peaks, which lacked any excess peaks(inclusions), were closely matched with the Copper sulfide in phase CuS according to the standard JCPDS card (No. 06-0464), indicating that the CuS product has only one phase. The Debye-Scherrer formula was used to estimate the crystal structure of QDs CuS[35-36]:

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Where D = crystal size, k = 0.9 (Scherrer constant), \( \lambda = 0.154 \text{ nm} \) (wavelength of Cu-K radiations), \( \beta \) is the complete width at half maximum, and theta(θ) is the angle calculated from two values matching the XRD pattern. The
crystallite size was 9.14 nm.

The EDX technique was used to determine the chemical composition of CuS. Figure 4 shows the resulting spectrum, demonstrating that only Cu and S were present. The percentage amount of Cu and S was shown in the inner table. The two elements' molar ratio was 1:1, consistent with the theoretical CuS ratio. The discovery confirmed that the CuS crystal phase was present in the sample produced.

The corresponding EDX elemental mapping pictures of Cu and S with a 1:1 ratio are shown in Fig. 5. The Cu and S components can be uniformly scattered on the sample's surface, supporting the dispersion of the catalyst elements.

TEM images studied the shape and size of the CuS sample. For comparison, the CuS sample (Fig. 6). This TEM image absence of aggregates due to urea as a surfactant and the efficiency of the photolysis method. Interestingly, the CuS sample contains uniform quantum dots (QDs) with narrow particle size ranges that are well-dispersed (5.4 to 9.1 nm). The hexagonal shape is apparent in the images. This was very similar to the XRD result.

The surface shape and particle size of the CuS QDs produced were examined using SEM experiments. Figure 7 shows the typical SEM for this sample. The micrographs show that the samples have a good crystalline structure, as evidenced by the prominent display of lattice fringes. The QDs were well dispersed, spherical, and monodisperse (zero-dimensional) without aggregation (all dimensions are less than 100 nm). The SEM test demonstrates that the QDs have a spherical morphology. A TEM examination was
conducted to record TEM images of samples to understand the particle size of QDs better.

The charge carrier dynamics of QDs CuS were investigated using the photoluminescence (PL) quenching spectra. CuS QDs contain two peaks in their PL spectra, at 353 and 728 nm (Fig. 8). They recombine the sulfur-vacancy-related donor and the valence band peaks at 353 nm. The CuS trap state emission, related to copper’s origin vacancy, is responsible for the emission peak of 728 nm. Meanwhile, CuS has a lower PL intensity than other samples in the literature\(^4\), preventing light-excited charges from recombination and speeding up transport. The spectra show blue peaks that are notably different from those found in bulk samples, indicating a quantum size effect. The quantum dots exhibit quantum-confined effects and size-tuned optical properties due to this property. The lifetime of photo-generated electrons is increased. CuS can efficiently minimize photo-excited charge carrier recombination, according to PL experiments\(^4\).

![TEM image of QDs CuS.](image1)

![SEM images of QDs CuS.](image2)

![PL spectrum of QDs CuS.](image3)
3.2 Electrochemical characterization

The Aldose Reductase is the enzyme immobilized on the anode electrode CuS-QDs/Ti/TiO₂ to oxidation of NADPH to NADP⁺, catalyzing glucose reduction to sorbitol as shown in equations:

\[
\text{NADPH}^{\text{+}} + e^- \rightarrow \text{NADP}^{\text{+}} + \text{H}^+ \quad (1)
\]

\[
\text{glucose} + \text{NADP}^{\text{+}} \rightarrow \text{sorbitol} + \text{NADPH} + \text{H}^+ \quad (2)
\]

The cyclic voltammetric (CV) spectra of the various electrodes are shown in Fig. 9. Due to the absence of any redox couple in the electrolyte or the electrode with different measured potential (± 10 V) and scan rates from 20 to 200 mV/s, the Ti and Ti/TiO₂ electrodes do not offer any oxidation or reduction peaks with the presence of glucose from human blood serum in PBS solution (without mediator NADPH) as shown in Curve a and b in Fig. 9. The CuS-QD on the surface of the Ti/TiO₂ substrate (CuS-QD/Ti/TiO₂ electrode) is well defined; deficient oxidation and reduction peaks are observed in the presence of human blood serum using a PBS solution without mediator NADPH, and the CV spectra (curve c) shows weak redox peaks are present because CuS possesses a redox couple (Cu²⁺, Cu³⁺). Water molecules in PBS solution are highly adsorbed on the surface of CuS-QDs, where oxygen functions as a proton acceptor site for the water molecule via hydrogen bonding, resulting in the conversion of surface Cu²⁺ to Cu³⁺, which is the anodic peak owing to oxidation⁴⁴. Then cathodic peak -630.73 mV represents the reduction of Cu³⁺ to Cu²⁺, and a pair of weak redox peaks appear (curve c in Fig. 9).

Upon immobilization of NADPH onto the surface of the CuS-QD/Ti/TiO₂ electrode, The CV spectra (curve d in Fig. 9) of NADPH/CuS-QD/Ti/TiO₂ bioelectrode shows the anodic peak oxidation catalyzed through the presence of aldose reductase in the blood serum at (+ 422.1 mV) converting NADPH to NADP⁺ and a cathodic peak of reduction at (-359.3 mV) NADP⁺ catalyze glucose reduction to sorbitol.

Additionally, the scan rate on the current response was also investigated with different scan rates from 20 to 200 mV/s⁴⁵, and the maximum redox peaks, seen at 30 mV/s, also the highest electrocatalytic response was obtained at pH 8.5, in agreement with other reports based on electrocatalytic oxidation of NADPH⁴⁶. This indicates that NADPH is necessary to get high sensitivity and better electrocatalytic activity.

The cyclic voltammetry of NADPH/CuS-QD/Ti/TiO₂ bioelectrode of both anodic peak currents (Ipa) and cathodic peak currents (Ipc) increase linearly with glucose concentration serum diabetic patients, as shown in Table 1 and Fig. 10. The electrochemical characterization of the cyclic voltammetry sorbitol bio electrode was evaluated. The oxidation and reduction peaks of the transducer had a high response current (see Fig. 10 and Table 1). A quick electron transfer occurs due to the tiny spacing between the anodic and cathodic peaks. As a result, our sorbitol biosen-

![Fig. 9 Cyclic voltammetry of the Ti electrode (curve a), Ti/TiO₂ electrode (curve b), CuS-QD/Ti/TiO₂ electrode (curve c), NADPH/CuS-QD/Ti/TiO₂ bio electrode with presence of blood glucose serum in PBS buffer (curve d).](image-url)
The biosensor sensitivity was determined by the linear regression slope of this current response vs. glucose concentration plot. It was found that the response current increased with increasing concentration of glucose and the current reduction response was proportional to the glucose concentration from 0 to 5 mM.

The selectivity of the biosensor is always an important issue for the development of an electrochemical biosensor. The specificity of the binding mechanism has also been taken into account by comparing its response to the analyte with some similar molecular structures. In particular, fructose, mannitol, and uric acid are usually present in biological components such as blood samples or body fluids; as shown in Fig. 12, the electrodes are based on aldose reductase and measuring the release of sorbitol not affected by the presence of interfering. The biosensor

Table 1 The electrochemical characteristics of the bio electrode.

<table>
<thead>
<tr>
<th>Transducer</th>
<th>The hospital analysis</th>
<th>Oxidation</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose level</td>
<td>Ipa/mA</td>
<td>Epa/mV</td>
<td>Ipc/mA</td>
</tr>
<tr>
<td>a</td>
<td>93.00 mg/dl</td>
<td>+0.405</td>
<td>+242.45</td>
</tr>
<tr>
<td>b</td>
<td>169.00 mg/dl</td>
<td>+0.402</td>
<td>+280.48</td>
</tr>
<tr>
<td>c</td>
<td>181.00 mg/dl</td>
<td>+0.363</td>
<td>+301.89</td>
</tr>
<tr>
<td>mean</td>
<td>0.390</td>
<td>274.94</td>
<td>−0.477</td>
</tr>
<tr>
<td>SD</td>
<td>0.023</td>
<td>30.1</td>
<td>0.053</td>
</tr>
<tr>
<td>RSD</td>
<td>5.89</td>
<td>10.94</td>
<td>−11.11</td>
</tr>
</tbody>
</table>

Epa is the anodic peak potential.
Epc is the cathodic peak potential.
Ipa is the anodic peak currents.
Ipc is the cathodic peak currents.
ΔEp is the averaged peak potential separation between the anodic and cathodic.

Fig. 10 Cyclic voltammetry of bio electrode in PBS solution at pH 8.5 with 200 μL serum samples glucose level (a) 93.00 mg/dL. (b) 169.00 mg/dL. (c) 181.00 mg/dL.
shows a quick response for glucose. However, no noteworthy response was observed for adding fructose, mannitol, and glycerol. Thus, AR-NADPH/CuS-QD/Ti/TiO₂ is highly selective, and it can be applied for determination in human serum samples.

The reproducibility of the AR-NADPH/CuS/Ti-TiO₂ were also evaluated by measuring the current response $I_{pc}$ relative standard deviation ($\sigma_{RSD}$) from the standard deviation of $E_{pa}, E_{pc}, I_{pa}, I_{pc}$ and $\Delta E_{p}$ as shown in Table 1, which were found close to the result reported by CONGO and TAI amperometric transducer. Mónica Hernández-Cruz et al. observed that the mean peak potential separation between the anodic and cathodic peak ($\Delta E_{p}$) was similar to that of an ideal amperometric transducer.

The long-term stability of the biosensor was stored in PBS (pH 8.5) at 4°C and evaluated by measuring its response to glucose within 30 days and it retained approximately 93% of its original response recorded periodically by CV after 30 days indicating its very good stability. The intimate contact between CuS, NADPH, and aldose reductase enzyme in the serum catalase sensing sites, which results in a rapid response for sorbitol detection, is the fundamental component for attaining such a fast response. The possible mechanism of reduction of glucose is as follows: During AR biocatalysts that present in serum which are capable of catalyzing oxidation NADPH to NADP⁺ and reduction of glucose serum in PBS solution to sorbitol, the NADPH/CuS-QD/Ti/TiO₂ modified anode design was selected for further optimization.

A new design for dehydrogenase-based biosensors was established by immobilizing a NADP⁺-dependent dehydrogenase with salicylate hydroxylase (SHL) in front of a Clark electrode, compared to existing redox enzymes. The feasibility of the concept was verified using malic enzyme as the dehydrogenase, leading to creating an L-malate sensor. Effective re-oxidation of NADPH by SHL, generated an extended linear range from 0.01 to 1.2 mmol L-malate and dramatically reduced NADP⁺-requirement were some of the significant improvements over the biosensor techniques previously reported (0.025 mmol). Simultaneously, operational stability was extended to more than 30 days. Six samples yielded highly correlated results with the usual enzymatic technique. Furthermore, in the other research, the enzyme cytochrome P450 2B6 converts propofol to a quinone/quinol redox pair that can be detected using simple electrochemistry in the presence of the cofactor NADPH. This method overcomes the problem of electrode fouling, which makes electrochemical propofol sensors unusable. It was effectively proven in a serum-like solution, and the sensor displayed good specificity when it came to potential interfering chemicals. The NADPH serves as an electron supply for the enzyme, allowing it to catalyze propofol conversion. Unlike direct electrochemical oxidation, this method of converting propofol does not result in polymerization and so does not create electrode fouling. The redox pair can be monitored electrochemically, allowing for quick and easy detection of propofol concentration. Immobilizing the enzyme within yeast cells protects the enzyme’s stability, which can be difficult to achieve otherwise. Because chitosan is plentiful, biocompatible, and very porous, it was chosen as the immobilization material. The inclusion of gold nanoparticles helps sta-
bilize the enzyme even further. The necessity to regenerate NADP⁺ from NADPH is a significant issue when using NADP⁺ dependent enzymes. The enormous electrocatalytic activity of ferredoxin–NADP⁺ reductase (FNR) allows for direct oxidation of NADPH. Through linking to enzyme-catalyzed chemical reactions that utilise this cofactor system, the discovery leads to reversible electrochemistry of NADP⁺/NADPH and potentially endless prospects, either for selective synthesis or sensors. However, this frequently results in undesired electrode fouling. In the human body, aldose reductase enzyme with NADPH cofactor activated polyol route is employed to regenerate NADP⁺ by oxidizing catalysis and reducing glucose to sorbitol before changing the electrode surface to increase its specificity in the sample. We inserted decreased CuS QDs Modified Ti/TiO₂ onto the bio anode before changing the electrode surface to increase its specific surface area and improve the electrical conductivity between the enzyme and the electrode surface. To control the extent of glucose reduction in diabetic patients’ serum, the electrochemical reduction of glucose by cyclic voltammetry was employed.

4 Conclusion

In conclusion, quantum dots CuS has been successfully synthesized by photolysis method (UV-Irradiation method), which is considered a novelty. XRD patterns confirmed the production of covellite with hexagonal phases in structures. SEM and TEM studies demonstrated less polydispersity for particles with 5.4 to 9.1 nm diameter. CuS QDs PL curves show a large absorption band on the visible region between 353 and 728 nm, which has a higher intensity in the sample.

A novel non-enzymatic sorbitol sensor has been successfully fabricated via immobilization of NADPH on CuS QDs modified Ti/TiO₂ electrodes for easy evaluation to detect sorbitol in biological fluids, and diagnosis of diabetes complications was developed. The developed sorbitol biosensor was successfully tested in human serum samples to detect sorbitol. CuS QD’s high catalytic activity and redox behavior are responsible for electroactive site augmentation, adsorption energy modification, excellent charge transfer, and increased electrolyte penetration/ion diffusion. The sorbitol biosensor had a quick response, good contact between the redox site and the reaction, high stability, good sensitivity repeatability, and was inexpensive. The close contact between CuS, NADPH, and aldose reductase enzyme in the serum catalase sensing sites results in a rapid reaction for sorbitol detection during AR biocatalysts present in serum. The fundamental reason is that for achieving a fast response. As a result, this research could be very useful in developing a sensor that can detect sorbitol in analytical applications.

Author Contributions


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