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Short communication

A novel glucose sensor based on monodispersed Ni/Al layered double hydroxide and chitosan

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ABSTRACT

A novel cheap and simple amperometric glucose biosensor, based on the electrode modified with the Ni/Al layered double hydroxide (LDH) nanoflakes and chitosan (CHT), without glucose oxidase, is presented. The glucose biosensor based on monodispersed high active Ni/Al-LDH nanoflakes and CHT exhibits an appropriate linear range of 0.01–10 mM and good operational stability. The amperometric sensor shows a rapid response at the potential value 0.48 V. In addition, optimization of the biosensor construction, the effects of the applied potential, the scan rate as well as common interfering compounds on the amperometric response and human serum samples analysis of the sensor were investigated and discussed.

1. Introduction

Layered double hydroxides (LDH), well known as anionic or hydrotalcite-like clays consist of positively charged edge sharing octahedra forming brucite-like host layers. Net positive charges on the layers are balanced by exchangeable anions intercalated between the sheets (Miyata and Kumura, 1973; Allmann and Jepsn, 1969; Taylor, 1969; Radha et al., 2007). The layered structures, large surface areas and anion exchange properties of LDHs make them potentially attractive material as electrode surface modifications (Scavetta et al., 2007; Ballarin et al., 2005; Guadggnini et al., 2007).

Since 1987, many publications have reported on the use of synthetic LDHs for the modification of electrodes (Therias and Mousty, 1995). Among natural and synthetic clays, hydrotalcite-like compounds are particularly interesting as electrode surface modifiers, and have been proposed as amperometric or potentiometric sensors. The positive charged layers may be an attractive point to immobilize biomolecules depending on their isoelectric point (Shan et al., 2006). In addition, the reports on the synthetic lamellar material being used as immobilization matrix of enzymes for biosensors have become common in the past few years.

Chitosan (CHT) is a biocompatible, biodegradable and nontoxic natural biopolymer that exhibits excellent film forming ability and good adhesion (Han et al., 2007). Due to its rare combination of physicochemical properties, CHT is an attractive structural material and has also been studied as a support for immobilization of enzymes and the construction of amperometric biosensors (Shan et al., 2007). In addition, CHT aggregated with acetum could enhance biocompatibility, mechanical strength, and adhesion to the membrane on the glass carbon electrodes (GCE) (Xiang et al., 2007; Lin and Liu, 2005).

The determination of glucose is important because it is the product of the reactions catalyzed by a large number of oxidases and is of great significance in clinical chemistry, biochemistry, environmental and food chemistry.

In this context, we explore a composite system based on CHT and Ni/Al-LDH nanoflakes for the design of a glucose biosensor. Ni/Al-LDH nanoflakes can mix well with CHT aqueous solution to form a homogeneous solution, which can be readily immobilized onto the GCE surface and form a film after drying. The resulting organic/inorganic composite can combine the merits of the two components and provide a favorable microenvironment and a good conductive characteristic. Different from most of glucose biosensors, our biosensor detects glucose without glucose oxidase (GOD) due to the electro-oxidation mechanism of Ni ion in the Ni/Al-LDH nanoflakes. It is known that the detect efficiency and the operational stability of the GOD sensor strongly depend on the catalyses activity of the GOD, the value of the pH and the temperature limits. Whereas, the glucose sensor based on the synthetic monodispersed

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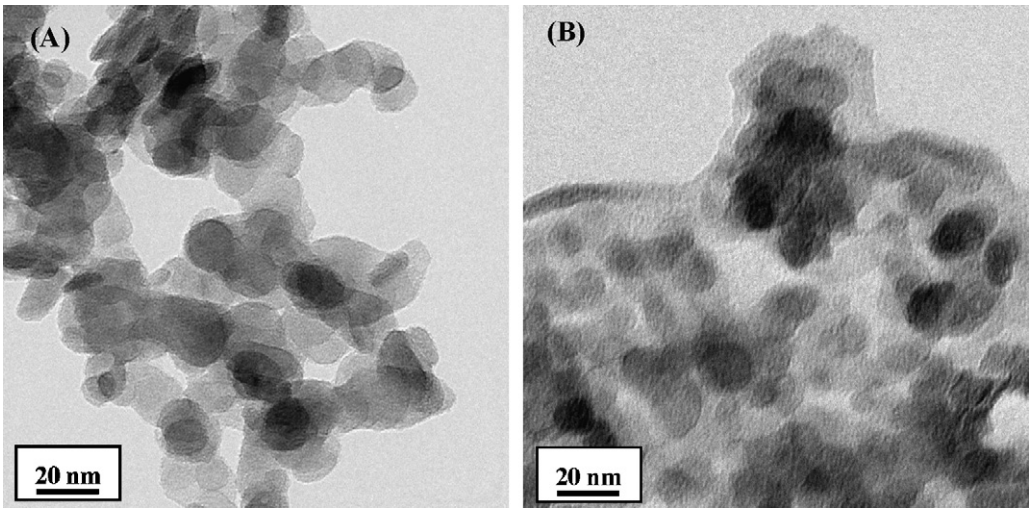

Fig. 1. TEM images of the Ni/Al-LDH nanoflakes (A) and the [Ni/Al-LDH]/CHT film (B).

and high active Ni/Al-LDH nanoflakes, without GOD, has fewer problems and a forseeable applied foreground.

2. Materials and methods

2.1. Reagents

CHT was purchased from Shanghai Boao Biotechnology Company Limited (China). Ni/Al-LDH:$Ni_6Al_2(OH)_{16}CO_3{\cdot}nH_2O$, was synthesized by the coprecipitation method. All the other reagents were of analytical grade and were used as received without further purification. All aqueous solutions were prepared in deionized distilled water. 100 mM glucose solution was daily prepared and stored overnight to reach mutarotational equilibrium before use.

2.2. Apparatus

The cyclic voltammograms (CV) were recorded with a Princeton Applied Research Model 660C Potentiostat/Galovanostate in a conventional three-electrode potentiostatic system, controlled by a personal computer via CHI instruments software. The working electrodes were modified GCEs (diameter 3 mm). The counter electrode was a Pt wire and the reference electrode was a saturate calomel electrode (SCE). Transmission electron microscope (TEM) was performed using a JEOL JEM-2010HT transmission electron microscope.

2.3. Preparation of modified electrodes

The Ni/Al-LDHs were prepared by titration of a mixed solution of the metallic ions with NaOH. 0.045 mol Ni(II) and 0.015 mol Al(III) dissolved in 300 ml carbonate-free water to form a mix solution. Then the mix solution was titrated with 0.12 mol sodium hydroxide and 0.0075 mol sodium carbonate for the formation of Ni/Al-LDH, and the Ni/Al molar ratio was about 3:1. After keeping stirring the solution at 60 °C for 24 h, the pure Ni/Al-LDH powder was obtained by washing the precipitate with distilled water and drying in vacuum for 2 days at 30 °C (sample L_1). The sample L_2 was obtained by washing the precipitate with distilled water, and kept it in the solution state with a concentration of 0.1 M.

A 2.0-wt% CHT solution was prepared by dissolving CHT flakes in 1.0 wt% acetic acid and then diluted into 0.2 wt% solution by water. The Ni/Al-LDH suspension was prepared by dispersing powder (sample L_1) in deionized water at a concentration with 0.1 M by stirring for 24 h. A defined amount of the aqueous mixture (for instance, containing 100 μl of L_1 or L_2 suspension, 0.1 M, and 200 μl of chitosan, 0.2 wt%) was spread on the surface of the GCE. The coating was then dried at 4 °C in a refrigerator.

3. Results and discussion

3.1. Morphology characterization of the Ni/Al-LDH

The TEM investigation shows that the Ni/Al-LDH nanoflakes are nanoscaled with a diameter of 20–40 nm and the thickness with several nanometers (Fig. 1(A)). The uniform-sized and monodispersed nanoflakes were suspended in the solution more than 6 months (L_2). After mixing Ni/Al-LDH with CHT, TEM image does not change in diameter and distribution of Ni/Al-LDH nanoflakes. The thin biocoating of CHT are observable in Fig. 1(B).

3.2. Electrochemical behaviour of electrodes modified with Ni/Al-LDHs

The [Ni/Al-LDH]/CHT-modified electrodes have been extensively studied by our group both in alkaline and neutral medium as amperometric sensors for the determination of glucose, through an electrocatalytic mechanism involving the Ni centers. In the potential range at which Ni centers can be involved in a redox process the material displays good conductivity which is due to the electronic and ionic transport (Scavetta et al., 2006). The best signals relevant to the couple Ni(III)/Ni(II) are observable in the pH range 11–13 and the electro-oxidation mechanism can be represented by the following reaction:

$$\text{LDH–Ni(II)} + \text{OH}_{\text{sol}}^{-} \rightleftarrows \text{LDH(OH}^{-}\text{)–Ni(III)} + \text{e}^{-} \quad (1)$$

$$\text{LDH(OH}^{-}\text{)–Ni(III)} + \text{Glucose} \rightarrow \text{LDH–Ni(II)} + \text{Glucolactone} \quad (2)$$

After injecting glucose into the solution, the current of deoxidization peak increased. We can describe that Ni(III) centers react as oxidant, the electrochemical oxidation of glucose is not similar to catalyzed reaction with enzyme (Zhao et al., 2007). The chemical reaction (2) involving the Ni(III) centers and the substrate molecules is slower than reaction (1) and the rate determining step of the electrocatalytic process is the diffusion of the substrate molecules toward the metal sites inside the LDH, where the OH^- diffusion–migration from the solution into the interlayers of the

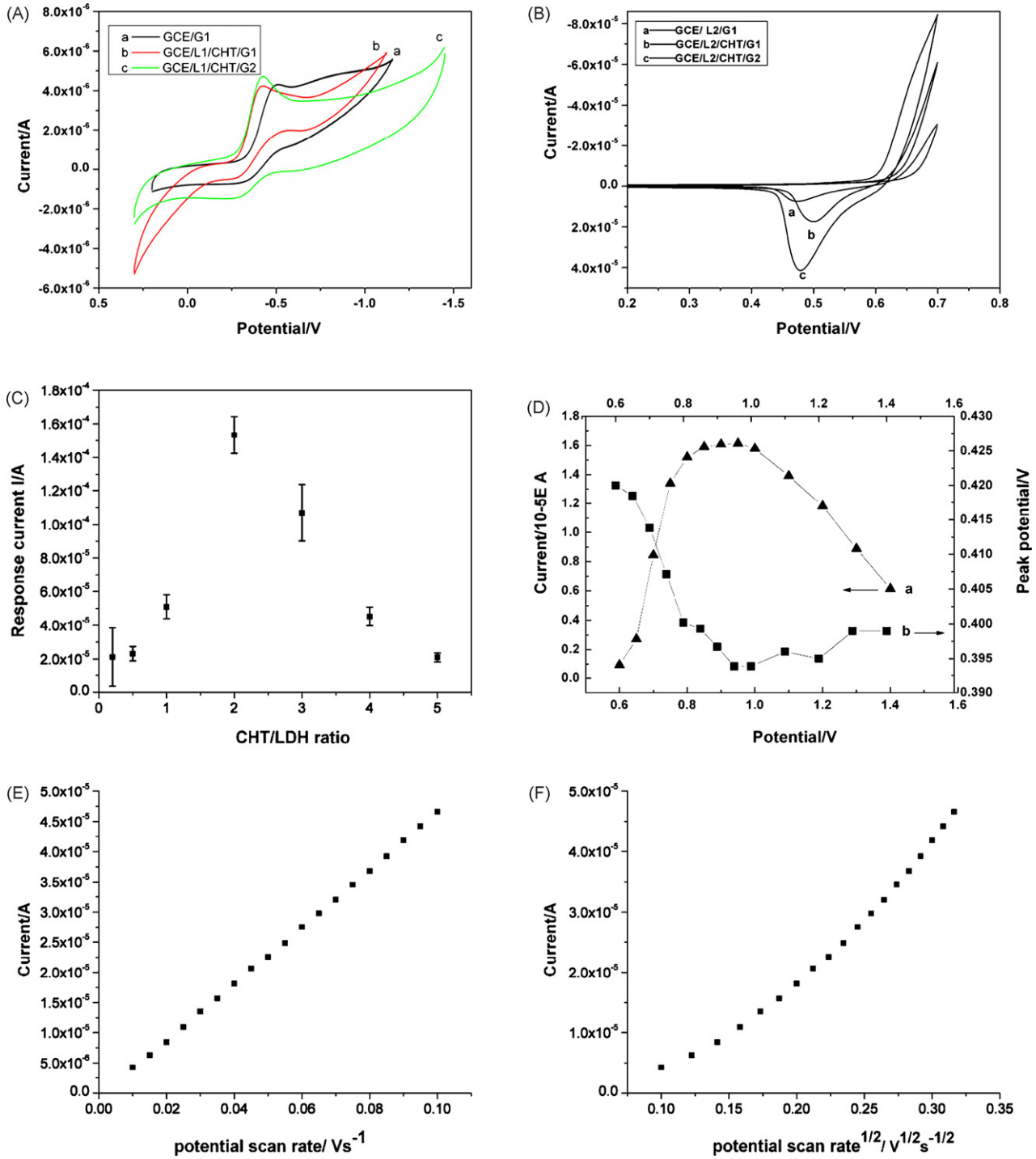

Fig. 2. CVs obtained from L_1/CHT (A) and L_2/CHT (B) modified electrodes in 0.1 NaOH solution containing 0.75 mM and 1 mM (G1, 0.75 mM; G2, 1 mM) glucose, potential scan rate: 0.05 V/s. (C) The influence of CHT/L_2 volume ratio on the biosensor sensitivity (E_{app} = 0.90 V, in 0.1 M NaOH containing 1 mM glucose, at 25 °C, concentration of the L_2 suspension is 0.1 M and the chitosan solution is 0.2 wt%). (D) The influence of applied potential on the L_2/CHT-modified electrode correspond to 1 mM glucose solution at 25 °C, symbol a, b is the response of peak current and the peak potential, respectively. Anodic peak current vs. potential scan rate (E) and the square root of the potential scan rate (F).

LDH. An anodic potential ensures the electroneutrality (Scavetta et al., 2001, 2006; Shan et al., 2006).

3.3. Optimization of [Ni/Al-LDH]/CHT biosensor

Fig. 2A and B shows the CVs of Ni/AL-LDH film and [Ni/AL-LDH]/CHT film in 0.1 M NaOH solution, at a scan rate of 0.05 V s^{-1}. The dependence of applied potentials for amperometric signal of the GCE/L_1 sensor was displayed in Fig. 2A. The maximum response was obtained at −0.48 V, while a small decrease in sensor response was observed for more negative potentials. The curve a in Fig. 2A was the electrochemical response of the electrode modified with L_1 in 0.75 mM glucose solution. The L_1 film on the electrode was destroyed in NaOH solution within 1 h, but the lifetime of the

electrode modified with L_1/CHT would be prolonged to 4 days. The curves b and c in Fig. 2A were the electrochemical response of the electrodes modified with L_1/CHT in 0.75 mM and 1 mM glucose solution, respectively. The current peak values would increase and applied potential would decrease after combining the CHT and Ni/Al-LDH. The chitosan was used as conductive media and immobilization materials here.

Substituting L_2 for L_1, we can see clearly that the peak potential, peak current and applied potential are different obviously (Fig. 2B). Because the sample L_1 is obtained by drying the Ni/Al-LDH suspension, the size of Ni/Al-LDH in L_1 will become bigger, and some Ni/Al-LDH nanoflakes will reunite after drying. Then, the uniform-sized (20–30 nm) and monodispersed Ni/Al-LDH nanoflakes directly from the suspension in sample L_2 have larger surface area and higher active than sample L_1. The peak current of L_2 is bigger and the applied potential is smaller than L_1 under the same conditions. Thus, the monodispersed Ni/Al-LDH nanoflake is a key for the response curve of the biosensor keeping linear.

Fig. 2C shows the influence of the coating composition exhibiting different CHT/L_2 volume ratio (0.2–5) on the biosensor sensitivity (determined as the slope of the linear part of the calibration curve. Concentration of the L_2 suspension is 0.1 M and the chitosan solution is 0.2 wt%). The I_0 was appointed as background current in the solution without glucose, I' as practice current in the glucose solution, I as response current, and $I = I' - I_0$. The sensitivity increases with CHT/L_2 volume ratio from 0.2 to 2 and decreases from 2 to 5. A proportion of 2:1 CHT/L_2 results in the best composition for this membrane (50 μl L_2/CHT mixture was spread on the GCE surface). With the CHT/L_2 volume ratio being fixed at 2, the effect film thickness on the biosensor sensitivity was easily examined by varying the amount of CHT/L_2 mixture deposited on the electrode surface. A maximal increase in response is observed up to 100 μl L_2/CHT mixture deposited on the electrode surface. Then, the biosensor response decreases with the increase of [Ni/Al-LDH]/CHT mixture volume more than 100 μl.

Fig. 2D shows the dependence of amperometric response of L_2/CHT-modified electrode on the operating potential. The peak current can be changed with the applied potential (symbol a). The dependence of the peak potential on the applied potential is shown in Fig. 2D (symbol b). Taking into account of the sensitivity of the biosensor and interference of easily oxidizable compounds present in buffer solution, 0.90 V was selected as the best compromise of applied potential in subsequent experiments (Roto and Villemure, 2002, 2007).

CV of the L_2/CHT-modified electrode at different scan rates is further investigated. The same to Hong' group, the peak currents from CVs exhibited a linear proportionality to potential sweep rates (Hong et al., 2002). Fig. 2E and F shows the dependence of anodic peak current on the potential scan rate (Fig. 2E) and the square root of the potential scan rate (Fig. 2F). A rapid diffusion-controlled charge transfer occurs only at slow scan rates. But anodic peak current began to deviate from linearity at higher scan rates above 0.045 V s^{-1}, due to rather slow electron-transfer reaction under fast potential scans (Pamidi and Wang, 1996). So we chose the 0.05 V s^{-1} as the scan rate. The effect of temperature on the biosensor response to 0.3 mM glucose was evaluated. As the response of the [Ni/Al-LDH]/CHT-modified electrode was about 8.2×10^{-9} A $°C^{-1}$, the influence of temperature to the biosensor was found to be negligible.

The reproducibility of the biosensor fabrication was evaluated via the comparison of the sensitivity of different electrodes. Eight different L_2/CHT-modified electrodes were tested independently for the glucose amperometric response, providing a relative standard deviation (R.S.D.) value of 7.1%. A reproducible current response with a R.S.D. of 3.7% was observed in 50 successive assays of 1 mM glucose. The stability of the CHT/LDH-modified electrode stored at 4 °C was investigated by recording periodically its current response to 8.6 mM glucose. The biosensor retained 90.6 and 49.3% of its glucose sensitivity after 4 and 21 days, respectively.

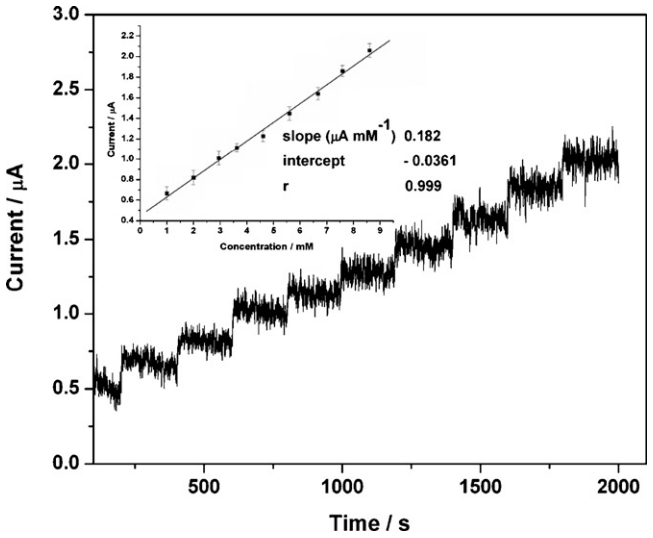

Fig. 3. Current time curve and calibration curve of L_2/CHT-modified electrode for glucose, under the optimum conditions (0.1 M NaOH at 25 °C, E_{app} = 0.90 V).

As an example, Fig. 3 reports the amperometric response recorded to subsequent additions of glucose, with the L_2/CHT-modified electrode, the corresponding calibration plot gives a linear result up to a concentration of 8.6 mM, which is shown in the inset. A low detection limit of 0.01 mM glucose was determined at the signal-to-noise ratio of 3. When considering clinically normal range of blood sugar (4–6 mM) in human blood serum, the analytical applicability of the [Ni/Al-LDH]/CHT biosensor developed in this work is promising.

3.4. The interference study

Because the electrochemical oxidation of Ni(III) to glucose has no special selective response, the effects of some possible interfering substances on the glucose biosensor were investigated at 0.7 V versus SCE in 0.1 M NaOH solution. The interference compounds taken into account were dopamine, ascorbic acid and urea acid in view of a possible application of the developed biosensor to the analysis of glucose in human serum. The current response value of ascorbic acid is 6.7% compared with the current response value of the same concentration glucose solution. The relative response obtained for other interfering species was found to be negligible. Since the physiological normal levels of glucose, ascorbic acid are 5.7 and 0.1 mM, respectively, our biosensor presents an acceptable anti-interferent ability to be applied for analysis of glucose in blood. So the L_2/CHT-modified electrode displays a sufficient selectivity to be used for the analysis of blood containing high or low glucose levels.

3.5. Application to human serum

The determination of glucose in human serum samples was performed by the developed biosensor, utilizing both calibration curve and the standard addition method. A standard colorimetric-enzymatic procedure was used as reference method for checking

Table 1
Determination of glucose in human blood serum

Serum samples	Measured (mmol L^{-1})	R.S.D. %($n = 5$)	Compared (mmol L^{-1})
1	6.16	4.8	5.86
2	6.25	2.2	6.11
3	15.37	7.5	14.22
4	6.68	2.1	6.82
5	5.77	4.5	5.51

the biosensor accuracy. Measurements on the samples obtained from the L_2/CHT-modified electrode were compared with those results from the spectrophotometric method using automatic biochemistry analyzer. The results obtained as mean from five determinations are shown in Table 1.

4. Conclusions

In this work, we gave the first successful example to detect glucose based on [Ni/Al-LDH]/CHT-modified electrode without GOD. The sensor has been applied to the determination of practical samples, the R.S.D. is less than 7.5% for the determination of glucose in human blood serum and the result is consistent with that from the spectrophotometric method. The preparation of the sensor is simple and also has a lower cost. In our future work, we attempt to increase sensitivity and stability of this kind of biosensors.

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