

Voltammetric detection of riboflavin based on ordered mesoporous carbon modified electrode

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Abstract The potential application of ordered mesoporous carbon (OMC)-modified glassy carbon electrode (OMC/GCE) in electrochemistry as a novel electrode material was investigated. X-ray diffraction, transmission electron micrographs, and cyclic voltammetry were used to characterize the structure and electrochemical behaviors of this material. Compared to GC electrode, the peak currents of potassium ferricyanide ($K_3[Fe(CN)_6]$) increase and the peak potential separation (ΔE_p) decreases at the OMC/GC electrode. These phenomena suggest that OMC-modified GC electrode possesses larger electrode area and faster electron transfer rate, as compared with bare GC electrode. Furthermore, riboflavin was detected using OMC/GC electrode in aqueous solutions. The results showed that, under an optimum condition (pH 7.0), the OMC/GC electrode exhibited excellent response performance to riboflavin in the concentration range of 4.0×10^{-7} to 1.0×10^{-6} M with a high sensitivity of $769 \mu A \text{ mM}^{-1}$. The detection limit was down to around 2×10^{-8} M. With good stability and reproducibility, the present OMC/GC electrode was applied in the determination of vitamin B₂ content in vitamin tablets, and satisfactory results were obtained.

Keywords Ordered mesoporous carbon · Riboflavin · Vitamin B₂ · Cyclic voltammetry

Introduction

Riboflavin (RF), often called vitamin B₂ (VB₂), belongs to the huge family of flavins [1–4] and plays an important role

as cofactor in enzymes [1]. Also, it is an important compound for general human health. Studies show that numerous diseases are related to the deficiency of riboflavin. Riboflavin is also a biochemical molecule widely existing in food and pharmaceutical products. These pharmaceutical products range in complexity from single-vitamin to multivitamin formulations. Therefore, it is important to examine riboflavin electrochemical behavior and to develop a quantitative method for studying its physiological function in clinical medicine. So, analytical methods for the determination of VB₂ need to be both sensitive and selective because of the presence of potential interferences and the low concentrations of analyses.

At present, various methods have been used to detect riboflavin, such as spectrophotometry [5, 6], high performance liquid chromatography [7–10], and capillary electrophoresis [11]. However, these analytical techniques for the detection of riboflavin are relatively time-consuming, lack sensitivity and selectivity, require expensive instruments, and are not available in most quality control laboratories. In contrast, the electroanalytical technique has many attractive features, such as favorable portability, easy operation procedures, high sensitivity, and selectivity to some extent, and therefore is very popular in the determination of electroactive compounds. However, there have been only few reports on applying electroanalytical methods in the determination of riboflavin [12–14], usually involving adsorptive stripping voltammetry at mercury electrodes under basic conditions. Riboflavin did not show significant adsorption on solid electrodes including carbon, gold, and platinum [15]. Electroanalytical determination of VB₂ at mercury film electrodes also suffered from rapid decomposition in alkaline medium under ambient light [13].

The immobilization of electron mediators onto electrode surfaces to produce chemically modified electrodes for use in electroanalysis has been widely investigated during the

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last two decades [16–19]. Ordered mesoporous carbons (OMCs), owing to their considerable properties, such as uniform and tailored pore structure, high specific surface areas, large pore volume, and chemical inertness [20–23], have potential applications such as molecule separation, adsorption, catalysis, sensors, energy storage, and capacitors [24–26]. A lot of research findings suggest that OMC, which can increase the electron transfer rate, has full potential applications based on the fabrication of chemically modified electrodes and was mainly used to construct sensing films of various biomolecules, including dopamine, L-cysteine, and so on [27–30]. In spite of such capability of ordered mesoporous carbons, there are no studies on the electroanalytical applications for the determination of riboflavin. The large surface area and the presence of edge plane-like defective sites on OMC are contributed immensely to the adsorption of riboflavin and improving sensitivity and lowering detection limit.

In this article, the OMC was utilized to make up the surface of a glassy carbon electrode, which was subsequently employed to sensitively determine riboflavin in pharmaceutical preparations. OMC can provide higher electrical conductivity, enlarged active surface area, and more coordinate sites to the interface so that it can enhance the detection sensitivity of riboflavin. Based on the strong enhancing effect of riboflavin, a new, rapid, simple, sensitive and inexpensive method is proposed for the determination of riboflavin.

Experimental

Reagents

RF was obtained from Shang Hai Reagent Co. (China) and stored in the shade. All other chemicals not mentioned here were of analytical reagent grade and were used as received. Double-distilled water was used throughout. Aqueous solutions were prepared with doubly distilled water and stored in the shade. Phosphate buffer solution (PBS, 0.05 M) was used as supporting electrolyte solution, which was made from K_2HPO_4 , KH_2PO_4 , and adjusting the pH with H_3PO_4 or KOH.

Apparatus

All the electrochemical experiments were performed with a CHI 830b electrochemical analyzer (CH Instruments, Shanghai Chenhua Instrument Corporation, China) in a conventional three-electrode cell. A conventional three-electrode cell was used, including an Ag/AgCl (KCl saturated) electrode as reference electrode, a platinum wire as the counter-electrode, and the glassy carbon electrode (GCE, geometric area 0.068 cm^2) modified with OMC as working electrode. All potentials in this paper were

measured and reported versus Ag/AgCl electrode. The sample solutions were purged with purified nitrogen for at least 15 min to remove oxygen prior to the beginning of a series of experiments. All measurements were carried out at room temperature. Small-angle X-ray diffraction (XRD) patterns were obtained on an X-ray Pmax 2200 PC (Rikagu, Japan) operating at 40 kV and 40 mA and using Cu $K\alpha$ radiation ($\lambda=0.15406\text{ nm}$). The transmission electron micrographs (TEM) were taken using an H-600 electron microscope (Hitachi).

Synthesis of OMC

OMC was synthesized according to the literature method [23]. Mesoporous silicates (SBA-15) were synthesized following a published hydrothermal procedure using Pluronic 123 (non-ionic triblock copolymer, $EO_{20}PO_{70}EO_{20}$) as the surfactant and tetraethoxysilane as the silica source [31]. The SBA-15 silicate material was then used as a template to synthesize ordered mesoporous carbon using sucrose as the carbon source according to a reported carbonization procedure [23]. The obtained mesoporous carbon material was denoted as OMC.

Electrode preparation and modification

Prior to the modification, the GC electrode of 3-mm diameter was polished with 1.0, 0.3, and 0.05 μm alumina slurry, respectively, and sonicated successively in 1:1 nitric acid, absolute alcohol, and double-distilled water. The electrode was then dried with high-purity nitrogen steam. Prior to modification, the electrode was treated electrochemically in 0.5 M H_2SO_4 solution by cycling the potential between -0.5 and 1.6 V at a scan rate of 100 mV s^{-1} until the cyclic voltammetry (CV) curves were stable (about 10 min). Finally, the electrode was thoroughly washed with double-distilled water. The specific procedure of preparation of modified electrode was as follows: 5 mg of the OMC was dispersed in 10 mL of *N,N*-dimethylformamide with the aid of ultrasonic oscillation to give a 0.5 mg mL^{-1} black suspension, then casting 5 μL of OMC suspension and the solvent evaporated under an infrared lamp to obtain the OMC/GC electrode.

Results and discussion

Characterization of OMC materials

Figure 1a shows XRD pattern of OMC material. It can be seen that OMC exhibits a well-ordered periodic structure as indicated by the presence of low angle correlation peaks assigned to (100), (110), and (200) diffractions. These

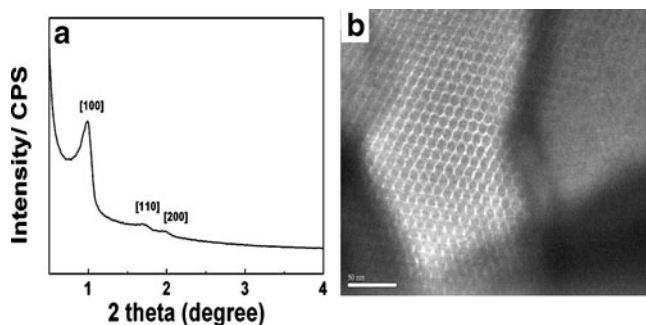


Fig. 1 a The small-angle XRD patterns of OMC, b TEM image of OMC

peaks are characteristic of hexagonal ordered structures [22], similar to the case of SBA-15. This indicates that the hexagonal order remains intact.

The morphology and the structure of OMC are also characterized using TEM. Figure 1b shows TEM image for the synthesized carbon materials. One can see from TEM image that the mesoporous materials have an ordered structure, an exact negative replica of SBA-15 with hexagonal arrangement of cylindrical tubes. A uniform pore size of about 4–4.5 nm can be observed, which is in agreement with the XRD results.

The electrochemical properties of OMC-modified GCE were investigated using potassium ferricyanide as electrochemical probe. Figure 2 shows CVs obtained at the OMC-modified electrode (Fig. 2, curve b) and bare GC electrode (Fig. 2, curve a) in the presence of 5 mM $K_3[Fe(CN)_6]$ /0.1 M KCl solution. The CVs show that the electrochemical response currents at OMC/GC electrode are higher than that of the GC electrode. Otherwise, the electroactive surface areas of OMC/GC and GC electrodes are obtained according to the Randles–Sevcik equation [32, 33]:

$$i_p = (2.69 \times 10^5)AD^{1/2}n^{3/2}\nu^{1/2}c^* \tag{1}$$

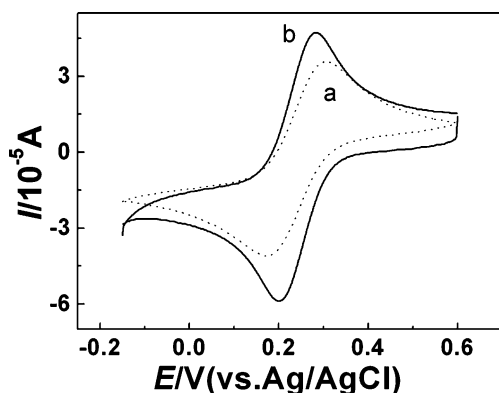


Fig. 2 Cyclic voltammograms obtained at the bare electrode (a) and OMC modified electrode (b) in 5 mM $K_3Fe(CN)_6$ /0.1 M KCl solution at a scan rate of 50 mV s^{-1}

where i_p is the peak current (A), n is the number of electrons participating in the redox reaction, A is the area of the electroactive surface area (cm^2), D is the diffusion coefficient of the molecule in solution ($\text{cm}^2\text{ s}^{-1}$), c^* corresponds to the bulk concentration of the redox probe (mol cm^{-3}), and ν is the scan rate (V s^{-1}). According to the equation, the average value of the electroactive surface area for OMC/GC electrode is 0.063 cm^2 , which is more than that of GC electrode (0.045 cm^2), suggesting that the OMC/GC electrode possesses larger effective surface area. In addition, the peak potential separation (ΔE_p) between the anodic and cathodic peaks is 78 mV for the OMC/GC electrode and 132 mV for GC electrode. The peak potential separation (ΔE_p) decreases, indicating that the OMC accelerates the electron transfer rate of ferricyanide. These results show that the OMC/GC electrode has relatively better electrochemical reacting ability. So, OMC may be used as a new material for the analytical application.

The electrochemical behavior of riboflavin at OMC/GC electrode

Figure 3 shows the CVs of $5.0 \times 10^{-4}\text{ M}$ RF in 0.05 M PBS (pH 7.0) at 50 mV s^{-1} on the bare GC (curve a) and OMC/GC electrode (curve b). At both electrodes, one pair of redox peaks is observed in the potential range of -0.90 to $+0.10\text{ V}$. But the peak currents are low, which is consistent with the reported references [16]. However, the electrochemical responses of RF at the OMC/GC electrode show great differences. Compared to the bare GC electrode, the OMC/GC electrode displays larger current signals with a 40-fold sensitivity augmentation. This implies that the electrochemical behavior of RF at the OMC/GC electrode is significantly improved. The significantly increased redox currents of RF at the OMC/GC electrode confirm that OMC shows highly effective enhancement to RF. The superiority in analytical performance of the OMC/GCE over the bare GCE may be

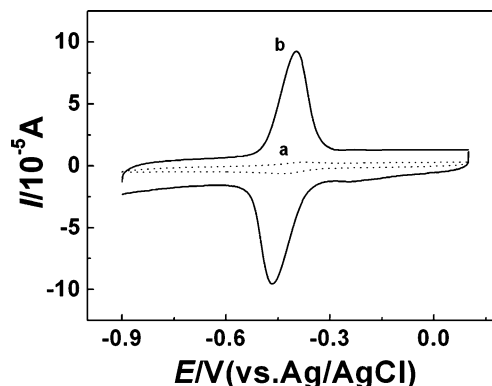


Fig. 3 CVs for $5.0 \times 10^{-4}\text{ M}$ RF at the GC electrode (a), OMC/GC electrode (b) in 0.05 M PBS (pH 7.0); scan rate: 50 mV s^{-1}

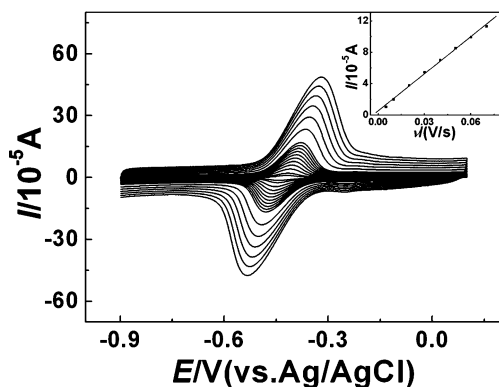


Fig. 4 CVs of OMC/GC electrode in pH 7.0 PBS containing 5.0×10^{-4} M RF at scan rates of $5\text{--}400$ mV s^{-1} . Inset the relationship between the peak currents and the scan rates ($5\text{--}70$ mV s^{-1})

principally ascribed to the unique properties of the OMC film (e.g., high specific surface areas, larger pore volume, and many edge-plane-like defective sites) that endow it the capability to accommodate higher amount of RF so as to enhance its surface concentration as well as enhanced kinetics.

The effect of scan rate on the voltammetric response of RF is shown in Fig. 4. At low scan rate, with increasing scan rate (ν), the anodic and cathodic peak currents (I_p) versus the potential scan rates are linear with little potential change as it is observed in the inset, suggesting the electrochemical reaction of RF on the OMC/GC electrode is a surface-controlled process. However, at high sweep rates, peak-to-peak separation becomes large, which indicates that the anodic and cathodic peak potentials of RF are dependent on scan rates (Fig. 5). It is found that E shifts as scan rate (ν) increases at high sweep rates, obeying the following equation:

$$E_{pa} = 0.568 - 0.0454 \ln \nu \quad (2)$$

$$E_{pc} = 0.350 + 0.0457 \ln \nu (E_p : \text{V}, \nu : \text{V/s}, r = 0.998) \quad (3)$$

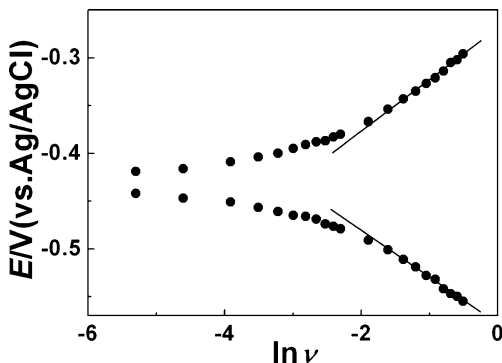


Fig. 5 Semilogarithmic dependence of cathodic peak potential and anodic peak potential, and scan rate for OMC/GC electrode in 0.05 M PBS (pH 7.0)

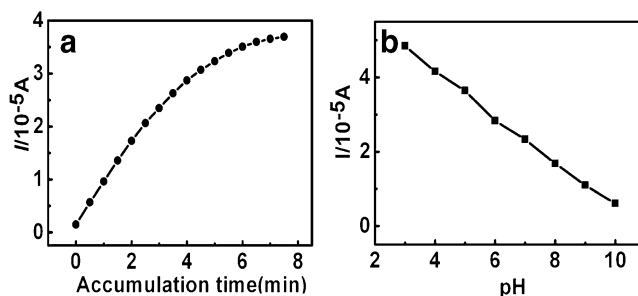


Fig. 6 **a** The influence of accumulation time on the anodic peak current. **b** The influence of adsorption pH on the anodic peak currents

According to the Laviron's equation [34], the electron transfer rate constant k_s of RF in OMC film is calculated to be 2.0 s^{-1} .

Optimization of the experimental parameters

Adsorption is important for the sensitive determination of RF on the OMC-modified GC electrode. Figure 6a shows the influence of accumulation time on the i_{pa} of RF at the OMC/GC electrode. When the accumulation time is below 4.0 min, the i_{pa} increases linearly with the accumulation time. However, the i_{pa} variety is not obvious when the accumulation time is longer than 4.0 min, suggesting that the amount of RF at the OMC/GC electrode surface tends to be saturated. Considering both sensitivity and working efficiency, an accumulation time of 4.0 min was employed for the determination of RF.

We also considered the influence of pH on the adsorption of RF at the OMC/GC electrode. Figure 6b shows the influence of pH on the adsorption capacity of RF on the OMC/GC electrode. An increase pH from 3.0 to 9.0 leads to a decrease in the peak current of RF. The reason might be due to the property of RF which is unstable and easily decomposed in basic solution. So, the adsorption capacity of RF gradually decreases with pH increasing and pH 3.0 as accumulation acidity.

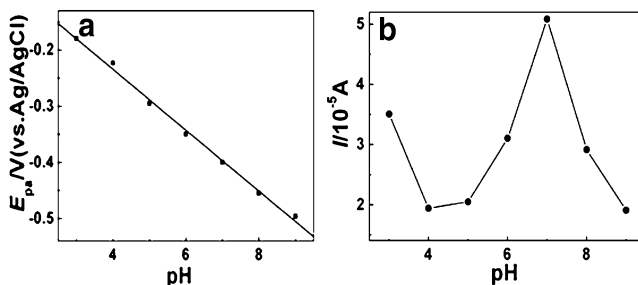


Fig. 7 **a** The influence of pH on the anodic peak potentials. **b** The influence of pH on the anodic peak currents

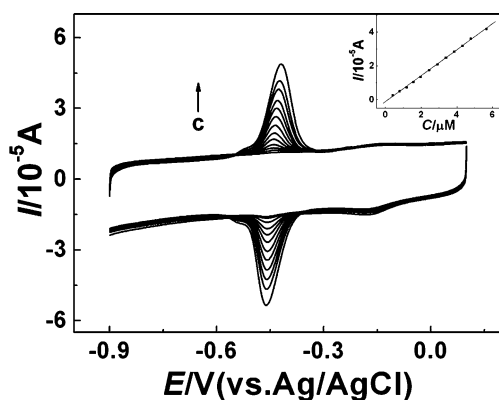
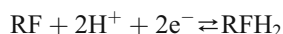


Fig. 8 Cyclic voltammograms of OMC/GC electrode in the presence of different concentrations of RF (from inner to outer, 1.0×10^{-7} – 1.0×10^{-4} M). Scan rate: 50 mV s^{-1} ; supporting electrolyte: phosphate buffer (0.05 M, pH 7.0)

It is generally accepted that RF undergoes a reversible process involving two electrons and two protons [35]. The electrochemical reaction of RF at a solid electrode may proceed by the following equation:



Therefore, the pH value of the solution influences the electrochemical behavior of RF at the OMC/GC electrode. The influence of buffer pH on the voltammetric response of RF is also investigated over the pH range of 3.0–9.0 in PBS containing 5.0×10^{-4} M RF. Before each detection, RF was adsorbed on the OMC/GC electrode at about 4 min of accumulation time in pH 3.0 solution. Figure 7 shows the influence of pH on the peak potentials and currents of RF redox reaction at the OMC/GC electrode. It is clear that the increase of the solution pH leads to the negative shift of peak potential (Fig. 7a). The data shows a linear variation of potential with pH in the range of 3.0–9.0 with a slope of 53 mV per pH unit, which is close to the theoretical one (58.6 mV/pH) at 22 °C for a reversible, two-proton coupled with two-electron redox reaction process. The pH value also affects the redox peak currents of RF (Fig. 7b). When pH increases from 4.0 to 7.0, the i_{pa} increases gradually. As the pH value is further increased from 7.0 to 9.0, the i_{pa} shows a slight decline. Obviously, the maximum peak currents are observed at pH value ~ 7.0 . Although the adsorption amount is the largest at pH 3.0, the electroactive of RF at pH 3.0 is lower than pH 7.0. So, pH 7.0 phosphate

buffer was employed for the determination of RF to achieve higher sensitivity.

Electrochemical determination of RF

The great stability of the OMC/GC electrode was observed by cycling the electrode many times in the PBS containing 5.0×10^{-4} M RF at a scan rate of 50 mV s^{-1} . The current level remained almost constant, indicating that the mediator does not leach from the electrode surface, even after several hours of use. This is very attractive for the development of new sensors with better stability.

As mentioned above, the OMC-modified electrode has a favorable and stable electrochemical behavior. It might be used as a chemically modified electrode to explore electroanalytical applications. The electrocatalytic properties of the OMC-modified electrode toward the riboflavin are evaluated. Figure 8 shows the RF voltammograms recorded after 4 min accumulation at pH 3.0 on the OMC/GC electrode. The peak current is measured for RF concentrations ranging from 1.0×10^{-7} to 1.0×10^{-5} M and is plotted as a function of the concentration of riboflavin in the aqueous solution. With the increase in the RF concentration, the currents of the redox peaks of RF are significantly increased. The inset of Fig. 8 shows the linear calibration curve for the detection of RF using the OMC/GC electrode. The current is proportional to the concentration of RF over the range from 4.0×10^{-7} to 1.0×10^{-6} M ($r=0.999$) and the sensitivity was calculated to be $769 \mu\text{A mM}^{-1}$. Otherwise, the limit of detection is also evaluated to be as low as 2×10^{-8} M. These results indicate that the OMC/GC electrode is more sensitive for the detection of RF than other reports in the literature, e.g., 1.3×10^{-5} M [36], 2.0×10^{-7} M [37], and 9.0×10^{-7} M [38]. These results demonstrate that the OMC/GC electrode is a good alternative for the determination of RF since it has a high sensitivity and good selectivity.

Reproducibility and stability

The OMC/GC electrode had favorable and stable electrochemical behaviors, and it might be used as a chemically modified electrode to explore electroanalytical applications. So the stability and reproducibility study of OMC/GC electrode was necessary. The five freshly prepared OMC/GC electrodes are used to measure RF in PBS. All three

Table 1 Results of determination of riboflavin in tablet samples ($n=5$)

	Found (μM)	Spike (μM)	After spike (μM)	RSD (%)	Mean recovery (%)
Vitamin B ₂	1.28	2.37	3.67	1.8	101
Multivitamin	0.70	1.10	1.84	2.2	104

electrodes exhibited similar current responses and a relative standard deviation (RSD) of 2.6% is obtained, indicating that the electrochemical behavior of the OMC/GC electrode is highly reproducible. To test the stability, the OMC/GC electrode towards the electrochemical response of RF has been examined as well. After 50 cycles of cyclic voltammetric sweep, the anodic and cathodic peak currents of RF remained at 87% of its initial current response. When the OMC/GC electrode was stored at 4 °C for 1 month, the oxidation peak current of RF remained at 92% of its original response. These results proved the excellent stability of OMC/GC electrode.

Determination of riboflavin in vitamin tablets

The application of OMC/GC electrode to determine the riboflavin content in vitamin tablets was studied using standard additions techniques. The vitamin tablets were grinded to a powder, and 10 mg of that powder was dissolved in 25 mL 0.05 M PBS as sample 1 and sample 2. Sample 1 and sample 2 were diluted to corresponding concentration in PBS before each measurement. A 5-mL aliquot of this test solution was placed in the electrochemical cell. The potential was controlled between -0.9 and $+0.1$ V for cyclic scans at 50 mV s^{-1} . The results are shown in Table 1. The recovered ratio was investigated and the value is between 101% and 104%. This result allowed us to use the proposed biosensor (OMC/GCE) in the determination of RF in the real sample.

Conclusions

In summary, we have demonstrated that riboflavin can be sensitively detected at an OMC-modified GCE by cyclic voltammetry. With this new detection scheme, a linear range of 4.0×10^{-7} to 1.0×10^{-6} M was achieved for CV. Furthermore, the detection limit was extremely lower (2×10^{-8} M), which was much smaller than the previously reported values. The excellent analytical performance of the OMC/GCE may be attributed to the unique properties of OMC that greatly improves the current response of riboflavin. In addition, the method has been successfully used to detect riboflavin in vitamin tablets with satisfying recoveries, which proves the method is feasible to be used for real samples.

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