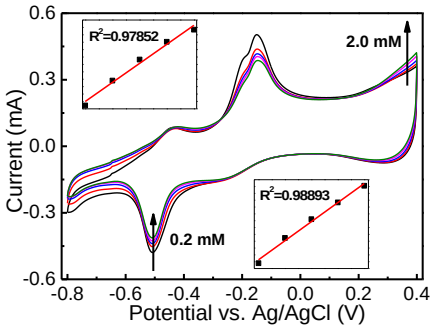

Fig. 3 CV responses of MXene/CTS/Cu_2O for simultaneous detection of glucose and cholesterol (adding 0.5 mM glucose and 0.05 mM cholesterol each time). The inset plots are corresponding peak currents fitted lines

2.3 Electrocatalytic mechanism

The obtained results demonstrate that the ternary MXene/CTS/Cu_2O electrode exhibits superior electrocatalytic performance towards glucose and cholesterol sensing and so has the potential for on-site and real-time multi-analyte monitoring. The irreversible oxidation of MXene upon exposure to an anodic potential over 0.2 V is overcome by the addition of CTS (Lorencova et al., 2017), which is attached to MXene layers through electrostatic and hydrogen-bonding interactions between its active amino groups and the OH, O and F groups on the surface of the MXene nanosheets (Song and Wang, 2020). The increased specific surface area ensures the reversible redox action of elemental Ti for glucose detection. Based on the results, we propose the following equation for the oxidation of glucose at peak VI:

$$2TiO_2+C_6H_{12}O_6(\text{glucose})+2H_2O \rightarrow 2Ti^{3+}+6OH^-+C_6H_{10}O_6\ (\text{glucolactone}) \quad (3)$$

For cholesterol sensing, Cu_2O nanoparticles act as the electrocatalyst and play vital roles in efficient electron transport. The predominance of transition metal species in electrocatalysts arises because of their unpaired d-electrons and unfilled d-orbitals which are available for forming bonds with absorbates, which can be considered as ligands to the central transition metal ion acting as the catalyst center (Pletcher, 1984). Moreover, the low redox potential for cholesterol sensing at peak I can greatly reduce the interference of other common species in biofluids, increasing the practicality of the biosensor for clinical application. There is published work supporting the view that hydroxyl radicals (.OH) can initiate the cholesterol auto-oxidation to produce oxysterols (Khaliq et al., 2020; Lee et al., 1997). Through the analysis of experimental phenomena (details provided in the supplementary material S10 and Fig. S8), the redox action can be deduced as follows:

$$Cu^{1+}+OH^-+e^- \rightarrow Cu+.OH \quad (4)$$

$$.OH+\text{Cholesterol} \rightarrow \text{Oxysterol} \quad (5)$$

In short, well-dispersed Cu_2O nanoparticles on a MXene/CTS substrate can provide abundant metal active edges and promote heterogeneous charge transfer and reactivity. With increasing concentrations of cholesterol, more Cu_2O particles are consumed, leading to a decreased signal. MXene/CTS film with high specific surface area supplies more channels for diffusion of ions and establishes easy access for glucose and cholesterol, accelerating the electron transfer rate and enhancing the conductivity of the composite. The positively charged CTS acts as an effective binder to enhance the interfacial interaction between the negatively charged MXene sheets, greatly improving the mechanical strength of the electrode (Fig. S9). The synergistic effect among these three components contributes to the particular electrocatalytic performance of the MXene/CTS/Cu_2O electrode. Table S1 shows the comparison of key performance indicators between our electrode and other biosensors. Evidently, the coupling and coordinated nanoarchitecture exhibits competitive electrocatalytic performance, and meets the need for non-enzymatic multi-analyte detection. The CV curves measured at different scan rates from 10 mV/s to 60 mV/s are shown in Fig. S10, illustrating the diffusion-controlled process for glucose and cholesterol detection.

2.4 Interference studies and practical applications

Interference tests were conducted to evaluate the selectivity of the MXene/CTS/Cu_2O towards glucose and cholesterol. The experiments were conducted with additions of 0.5 mM sucrose (SC), uric acid (UA), acetaminophen (APAP), lactose (LT), NaCl, ascorbic acid (AA) or l-cysteine (L-c). The amperometric response curves were recorded at peak VI and peak I (*vs.* Ag/AgCl) with 0.5 mM glucose and 0.05 mM cholesterol as the final addition, respectively. The peak current value of 1 M NaOH electrolyte was selected as the blank current. As shown in Fig. 4, considering the excessive concentration of

interfering species added, the current responses of glucose and cholesterol are still the most significant, demonstrating selectivity toward glucose and cholesterol detection.

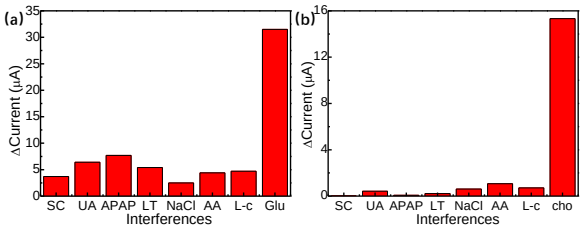

Fig. 4 Current responses of the MXene/CTS/Cu_2O electrode to the addition of interfering agents (0.5 mM): sucrose, uric acid, acetaminophen, lactose, NaCl, ascorbic acid and l-gystein at the potential for (a) glucose sensing (0.5 mM), (b) cholesterol sensing (0.05 mM)

The practical application of MXene/CTS/Cu_2O biosensor was investigated by testing the glucose and cholesterol concentrations in human serum samples which were provided by Zhongda Hospital in Nanjing, China. The normal concentrations of glucose and cholesterol in the human body are 5 mM and 5.2 mM respectively. The total cholesterol contains a mixture of free and esterified cholesterol in serum. Less than 10% of cholesterol exists in the free state (Shih et al., 2009; Raj et al., 2014) and it can be oxidated directly by the proposed non-enzymatic sensor without the need for cholesterol esterase and cholesterol oxidase (Huang et al., 2011; Dey and Raj, 2013; Patil et al., 2018). Therefore, the serum sample can be tested within the linear range at 20-fold dilution, ensuring minimal sample requirements. The spike and recovery method was employed and the analytical results are listed in Table 1. The results obtained suggest that the developed biosensor based on MXene/CTS/Cu_2O electrode exhibits suitability for simultaneous detection of glucose and cholesterol in actual samples and offers high potential for practical applications in clinical diagnosis.

Table 1 Simultaneous detection of glucose and cholesterol samples in 5% human serum with the MXene/CTS/Cu_2O electrochemical biosensor

Sample	Metabolites	[metabolites]$_{Added}$	[metabolites]$_{Measured}$	Recovery (%)
1	glucose	0.5 mM	0.50462107	100.92%
	choleaterol	0.1 mM	0.09803922	98.04%
2	glucose	0.5 mM	0.49353050	98.71%
	cholesterol	0.1 mM	0.10294118	102.94%

4 Conclusions

In summary, a free-standing MXene/CTS/Cu_2O electrode was formed through electrostatic interaction of MXene and CTS with opposite charges, followed by the electrodeposition of Cu_2O. Taking advantage of the synergistic function of MXene/CTS layers and Cu_2O nanoparticles, this ternary electrode exhibits excellent sensing capabilities for glucose and cholesterol with preferable linear ranges that can cover the full concentration range in clinical diagnosis. For glucose sensing, the sensitivity was 60.295 $\mu A \cdot mM^{-1}cm^{-2}$ with LOD to be 52.4 μM (S/N=3), while a sensitivity up to 215.71 $\mu A \cdot mM^{-1}cm^{-2}$ and LOD low to 49.8 μM (S/N=3) were achieved for cholesterol detection. Additionally, this biosensor possesses superior anti-interference ability and reproductivity, and thus exhibits great potential for genuine sample analysis. Accordingly, the as-prepared enzyme-free MXene/CTS/Cu_2O electrode acts as a biomimetic electrocatalyst with excellent performance for analysis of multi-metabolites, and overcomes the disadvantages of an enzyme-based biosensor. This work has proposed a versatile strategy for designed and fabricated self-assembled nanocomposite materials with tuned structural and functional properties. It is a first attempt which could be easily integrated into portable electrochemical devices, facilitating effective routine monitoring of blood metabolites and paving the way for commercialization and point-of-care testing.

Supporting Information

Supporting Information is available in the online version or from the author.

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Author contributions

Tao Hu designed the research. Man Zhang processed the corresponding data. Hui Dong and Tong Li participated in methodology and discussion. Xiaobei Zang provided the original technique of experiments and reviewing. Man Zhang

wrote the first draft of the manuscript. Zhonghua Ni and Xiao Li helped to organize the manuscript. Man Zhang and Xiao Li revised and edited the final version.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Institute of Process Engineering, Chinese Academy of Sciences China) and with the Helsinki Declaration of 1975, as revised in 2008(5). Informed consent was obtained from all patients for being included in the study.

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中文概要

题　目：自支撑 MXene/壳聚糖（CTS）/氧化亚铜（Cu_2O）电极:一种高效的同时测定葡萄糖和胆固醇的无酶生物传感器

目　的：研制低成本、高精度的多种代谢物同时检测生物传感器对医疗诊断具有重要意义。在本工作中，我们提出了一种基于 MXene/壳聚糖(CTS)/氧化亚铜(Cu_2O)纳米复合材料的独立无酶电极，用于同时高精度测定葡萄糖和胆固醇。

创新点：1. 利用 MXene、CTS 和 Cu_2O 的协同作用，通过电位分离，实现对葡萄糖和胆固醇的无酶同时检测；2.优化检测范围，可用于检测人体血液样本。

方　法：1.通过 MXene、CTS 和 Cu_2O 纳米材料的协同作

用形成了有效的界面结，促进了反应过程中的电荷转移，提高与待测物质的接触面积；2.分析电化学反应过程，构建电流信号与待测物浓度之间的关系，得到传感器的性能参数；

结　论： 1. MXene/CTS 薄膜具有较高的比表面积，为离子的扩散提供了更多的通道，同时 Cu_2O 纳米粒子可以提供丰富的金属活性边缘，促进了电荷转移，提高反应活性。2. Cu_2O 纳米颗粒在胆固醇检测中发挥了重要作用，而 MXene 同时作为导电基底和葡萄糖氧化剂，通过协同作用实现在不同电位上同时无酶检测葡萄糖和胆固醇。3. 在优化的电位范围-0.80 ~ 0.40 V 下，该传感器对葡萄糖和胆固醇具有良好的线性响应，灵敏度分别为 60.295 $\mu A \cdot mM^{-1}cm^{-2}$ 和 215.71 $\mu A \cdot mM^{-1}cm^{-2}$，检出限分别为 52.4 μM 和 49.8 μM。4. 通过对人血清样品的实时分析，验证了其良好的抗干扰能力和回收率(98.04-102.94%)，具有临床应用前景。

关键词： 无酶电极；多物质同时检测；胆固醇；葡萄糖；MXene

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