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Virus MIP-composites for SARS-CoV-2 detection in the aquatic environment

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ABSTRACT
SARS-CoV-2 is the virus responsible for causing the global COVID-19 pandemic. Identifying the presence of this virus in the environment could potentially improve the effectiveness of disease control measures. Environmental SARS-CoV-2 monitoring may become increasingly demanded in areas where the available testing methods are ineffective. In this study, we present an electrochemical polymer composites biosensor for measuring SARS-CoV-2 whole-virus particles in the environment. The sensitized layer was prepared from molecularly imprinted polymer (MIP) composites of inactivated SARS-CoV-2. Testing demonstrated increased sensor signaling with SARS-CoV-2 specifically, while lower responses were observed to the negative controls, H5N1 influenza A virus and non-imprinted polymers (NIPs). This sensor detected SARS-CoV-2 at concentrations as low as 0.1 fM in buffer and samples prepared from reservoir water with a 3 log-scale linearity.

1. Introduction
Since first reported in December 2019, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) that causes the coronavirus disease 2019 (COVID-19) has spread across the globe [1]. Reliable techniques such as real-time polymerase chain reaction (RT-PCR) [2], real-time loop-mediated isothermal amplification (RT-LAMP) [3] and antibody-based testing [4] have been developed for patient screening. However, health authorities and the general public alike are concerned about the presence of SARS-CoV-2 in the environment, particularly since it was found to be transmittable from asymptomatic infected persons, reflecting an obstacle to preventing its dispersal [5].

Researchers have carried out testing in several different environments and report that SARS-CoV-2 can be found in wastewater, reservoirs, and public spaces [6,7]. The virus identification methods employed in these works included nucleotide and antibody-based approaches; both of which could be suboptimal for samples obtained from natural environments [8]. Accordingly, the results of environmental SARS-CoV-2 testing performed with these protocols might not be accurate due to the presence of a high degree of background interference, and where the range of virus concentrations in the environment differ significantly from human samples [9]. To effectively implement adequate virus surveillance there is a need for optimizing existing techniques or using alternative approaches.

In the present study, we produced virus imprinted polymer composites as the SARS-CoV-2 electrochemical biosensor. The sensor is suited for virus monitoring in wastewater systems, where measurements can be performed onsite, without the requirement for any biomolecular reagents.

2. Materials and methods
2.1. SARS-CoV-2 MIPs preparation
We used four monomers with the optimized ratio (Fig. S1): acrylamide (AAM), methacrylic acid (MAA), methyl methacrylate (MMA), and N-vinylpyrrolidone (NVP). These components were combined in a 2:1:2:1 mol ratio and mixed in 300 µL dimethyl sulfoxide (DMSO) with 1.5 mg azobisisobutyronitrile (AIBN) and 47 mg N,N’-(1,2-dihydroxyethylene) bisacrylamide (DHEBA) as initiator and cross-linking agents,

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respectively. This mixture was stirred and heated to 65–70 °C until forming a gelatinous consistency, then 0.15 mg/mL graphene oxide (GO) was mixed in a 2:3 ratio. This polymer-GO composite was coated onto the working electrode (1 µL) and left for 15–20 min before applying a thin layer of SARS-CoV-2 (1 µL). The polymerization process was induced by placing this electrode in UV radiation for 3 hr then heating to 55 °C for 15 hr. The SARS-CoV-2 was removed from the polymer-GO composite layer by leaving in 1 M hydrochloric acid (HCl) for 1 hr then deionized water at 50 °C for 30 min. This SARS-CoV-2 MIP preparation scheme is illustrated in Fig. 1 a. Non-imprinted polymers (NIPs) were also prepared using the same polymers and protocol, although without imprinting the SARS-CoV-2 template.

2.2. Electrochemical measurement

The virus samples were prepared in 0.01 M phosphate buffered saline (PBS) pH 7.4 and wastewater. The wastewater, obtained from the chemistry building at Kasetsart University, was spiked with SARS-CoV-2 before centrifugation at 5000 rpm for 10 min. The SARS-CoV-2 sample was prepared as 0.01, 0.1, 1, 10 and 100 fM. Negative control was mimicked by the influenza A virus in PBS with the same concentrations as SARS-CoV-2. Reverse genetically engineered H5N1 (A/open-billed stork/Nakhonsawan/BBD0104F/04) was obtained from a biosafety level 3 laboratory of the Department of Microbiology, Mahidol University. The H5N1 virus was propagated in embryonated eggs, then inactivated with β-propiolactone.

All measurements were conducted using disposable screen-printed
In this work, SARS-CoV-2 detection was evaluated by measuring the redox-active peak at 0.2 V from CV graphs obtained from the electrochemical MIP composite sensor (Fig. 3a). At this potential, the signal obtained from the sensor increased with SARS-CoV-2 concentration, beginning with a 0.1 fM limit of detection (LOD) (Fig. 3b). This contrasts with corresponding signals observed from the NIP sensor, confirming that the imprinting procedure enhanced the current gain of the sensor in response to SARS-CoV-2. To examine the sensor selectivity to SARS-CoV-2, an experiment with the objective of detecting SARS-CoV-2 inside of wastewater. The results demonstrated a virus concentration-dependent response (Fig. 3b). Background interference from substances in wastewater was considered to be responsible for the signal level when SARS-CoV-2 concentration was below 0.1 fM. Practically, the signal increased in response to SARS-CoV-2 concentrations of ≥0.1 fM.

4. Conclusions

We have demonstrated that MIP-composites can be used as an alternative material to fabricate electrochemical sensors for SARS-CoV-2 detection in an aquatic environment. The sensor response to SARS-CoV-2 both in PBS and wastewater were higher than the negative controls such as NIP sensors and the influenza A H5N1 virus. From Fig. 3b, the sensitivity in term of the total relative signal change from 0.01 fM to 100 fM was 25%. The LOD was 0.1 fM in buffer and wastewater spiked with SARS-CoV-2. It should be noted that measurements are evaluated in molar unit, providing a representative value of the total number of SARS-CoV-2 particles in the sample, including both active and inactive viruses. This is typical of measurements taken from the environment, where it is challenging to dissociate whether viruses present are active or inactive. Nonetheless, the metric of overall virus content in aquatic environments provides a useful assessment for healthcare risk monitoring.

CRediT authorship contribution statement

Wannisa Sukjee: Conceptualization, Methodology, Validation, Investigation, Visualization, Writing – original draft, Writing – review & editing. Arunee Thitithanyanont: Investigation, Resources, Writing – original draft. Suwimon Manopwisedjaroen: Investigation, Resources, Writing – original draft. Supaphorn Seetaha: Methodology. Chutima Thepparit: Project administration, Supervision, Writing – original draft. Chak Sangma: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2022.131973.

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