

Supplemental information

Primary article title.

Electrochemical sensor with substitutional stripping voltammetry for highly sensitive endotoxin assay

Author's names.

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Abstract.

This supporting information intends to give information about the dependency of electrical charge to pAP concentration and the accumulate efficiency of oxidation current of pAP. Results of surface analysis of the deposition electrode befor/after used were also described.

Surface analysis of the electrode

We observed the surface of the deposition electrode of the device ($\phi=3.0$ mm) before and after the stripping process using SEM-EDX. SEM-EDX analysis was performed at the Technical Division, School of Engineering, Tohoku University. The SEM-EDX images were obtained using a JSM-6500F field emission scanning electron microscope (JEOL, Ltd., Japan) operating at 15.0 kV. Deposition process and stripping process were performed with the same way described in the main manuscript. Figure S1A shows an SEM image of the deposition electrode surface after the 60 min deposition process with 1000 EU L^{-1} and reagents in the reaction well. The image shows a large number of crystals of deposited silver, which is similar to that reported by Yasukawa *et al.* (reference 11 in the main manuscript). Figure S1B and S1C show a zoomed image of a crystal and an EDX mapping image of elemental silver on the crystal, respectively. These results confirm that silver certainly deposited onto the electrode as crystal forms through the deposition step. Almost of all of those crystals were removed in the stripping process (Fig. S1D). Figure S1E and S1F show the EDX spectrum corresponding to Fig. S1A and S1D, respectively. Peaks of elemental silver shown before stripping (indicated by the arrow in Fig. S1E) disappeared after the stripping process (Fig. S1F). These data also show that ASV could completely strip the deposited silver with a single sweep.

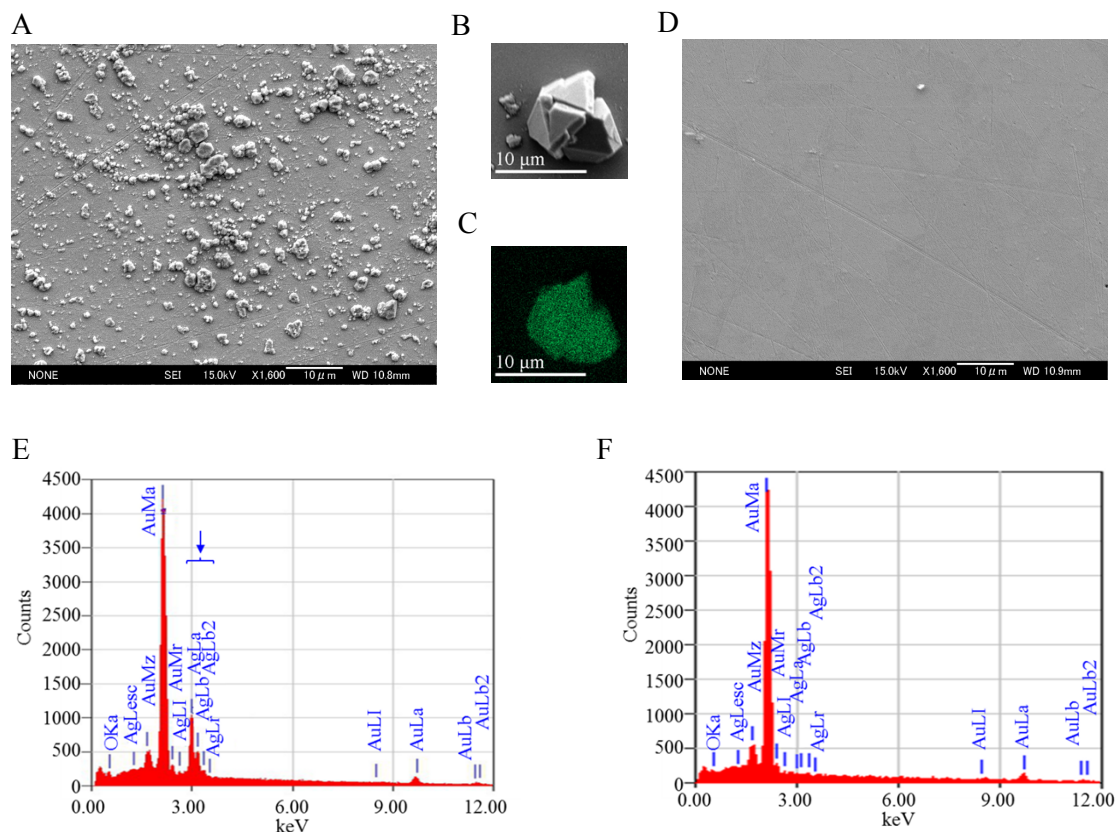


Fig. S 1 Results of SEM-EDX analysis of the deposition electrode. (A) SEM image of the silver-deposited electrode. Deposition time and concentration of endotoxin were set at 60 min and 1000 EU L⁻¹, respectively. (B) Zoomed SEM image of a crystal on the deposition electrode. (C) EDX image of the same area shown in Fig. S1B. Green dots indicate the presence of silver. (D) SEM image of the deposition electrode after ASV. (E) EDX spectrum of the area shown in Fig. S1A. (F) EDX spectrum of the area shown in Fig. S1D.

The dependency of the electrical charge calculated from ASV to pAP concentration.

We evaluated the dependency of the electrical charge calculated from stripping peaks of the deposited Ag to pAP concentration with the following method.

The reaction cell containing 0-0.5 mM pAP / HEPES buffer solution (pH 7.0) was connected with the deposition cell containing 10 mM AgNO₃ solution for 10 min at 37°C to deposit Ag on the electrode. After disconnecting from the reaction cell, the deposition cell was filled with a new 0.1 M KNO₃ solution for ASV. ASV was performed with the same condition described in article. Figure S2 shows the dependency of the electrical charge of deposited Ag to pAP concentration. Electrical charges increased linearly with pAP concentration.

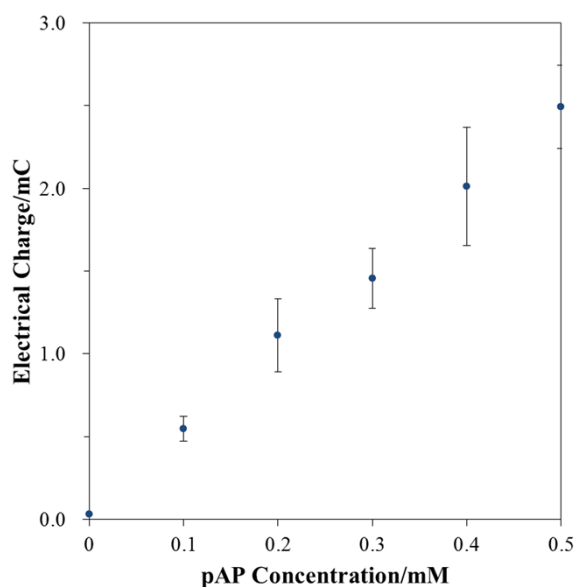


Fig. S2 The dependency of the electrical charge to pAP concentration. Electrical charge was calculated from the voltammogram of ASV. Each plots indicate mean value, and error bars indicate \pm standard deviations (n=3-5).

From the electrical charges obtained above, the amount of silver deposited on the depiisiton electrode (n_{Ag} mol) was estimated. Theoretical amount of pAP oxidized at the reaction electrode (n_{pAP}) was estimated from n_{Ag} . Here, we assume that the oxidation of pAP was two-electron reaction, and electron released from pAP was fully used silver ion reduction. Total amount of pAP ($n_{\text{total,pAP}}$) contained in the standard solution was calculated with the solution volume (125 μL) and the solution concentration (0.1-0.5

mM). Table R1 indicates each value for each concentration. The accumulate efficiency (=the ratio of the number of pAP molecules used for the Ag reduction to the all molecules initially put in the reaction cell) was calculated as 0.20-0.22 for 10 min accumulations.

Table S1 Theoretical value of silver, pAP and accumulate efficiency estimated from electrical charge of silver.

C_{pAP}/M	Electrical Charge/C	n_{Ag}/mol	n_{pAP}/mol	$n_{total.pAP}/mol$	Accumulate efficiency/-
0.1	5.20E-04	5.39E-09	2.69E-09	1.25E-08	0.22
0.2	1.08E-03	1.12E-08	5.61E-09	2.50E-08	0.22
0.3	1.43E-03	1.48E-08	7.39E-09	3.75E-08	0.20
0.4	1.98E-03	2.06E-08	1.03E-08	5.00E-08	0.21
0.5	2.46E-03	2.55E-08	1.28E-08	6.25E-08	0.20

The dependency of the electrical charge calculated from ASV to the connecting time.

To evaluate the effect of the connecting time on the stripping peak, the experiment was done with following method.

The reaction cell containing 125 μL of 0.5 mM pAP / HEPES buffer solution (pH 7.0) was connected with the deposition cell containing 10 mM AgNO_3 solution for 5-60 min at 37°C. After disconnecting from the reaction cell, the deposition cell was filled with a new 0.1 M KNO_3 solution to perform ASV. ASV was conducted with the same condition described in the main manuscript.

Figure R3 shows the plots of electrical charges calculated from the result of ASV versus the connecting time. As shown in this graph, electrical charges increased with the connecting time but this increase slowed down with passage of time. This slow-down is due to the decrease of pAP concentration caused by the consumption of pAP at the electrode.

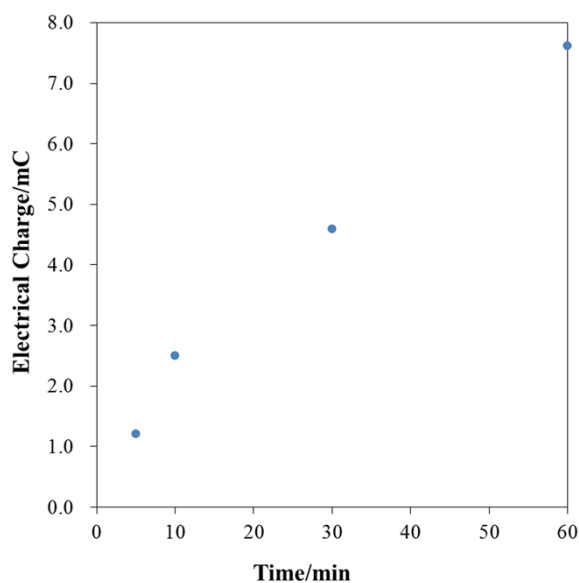


Fig. R3 The dependency of the electrical charge to the connecting time. Electrical charge was calculated from the voltammogram of ASV. The concentration of pAP solution was 0.5 mM.