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Nakano, Michihiko
Faculty of Information Science and Electrical Engineering, Kyushu University

Murayama, Fumitoshi
Graduate School of Information Science and Electrical Engineering, Kyushu University

Inaba, Masafumi
Faculty of Information Science and Electrical Engineering, Kyushu University

Techaumnat, Boonchai
Department of Electrical Engineering, Chulalongkorn University

他

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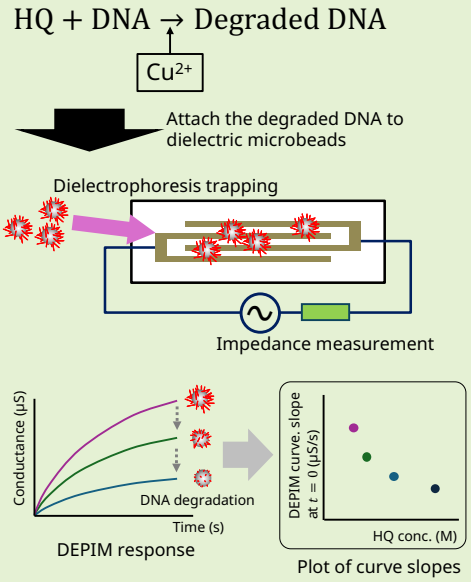
Simple Electrical Detection of Hydroquinone Based on DNA-Degradation-Induced Dielectrophoretic Change

Michihiko Nakano, *Member*, *IEEE*, Fumitoshi Murayama, Masafumi Inaba, Boonchai Techaumnat, *Member*, *IEEE*, and Junya Suehiro

***Abstract*—Hydroquinone (HQ), a phenolic compound present in industrial processes, cosmetics, and tobacco smoke residues, poses health risks due to its reducing properties and potential to damage DNA and cause cancer. Conventional detection methods, such as high-performance liquid chromatography and electrochemical sensors, offer high sensitivity but require complex instrumentation and complex sensor preparation. In this study, we proposed a simple and rapid electrical detection method for HQ based on DNA degradation and dielectrophoretic impedance measurement (DEPIM). Prepared DNA molecules were incubated with HQ to induce degradation and subsequently immobilized on microbeads for DEPIM analysis. The addition of Cu^{2+} ions accelerated the degradation reaction, enabling rapid and sensitive detection. The method demonstrated a clear correlation between the HQ concentration and DEPIM response, with a detection limit of 50 μM and detection time of 60 min. Specificity was confirmed by testing positional isomers, and applicability was validated using artificial urine. The proposed method converts the chemical reaction into an electrical signal through the DEPIM procedure and provides a low-cost, rapid, and straightforward sensing platform for HQ detection, with considerable potential for environmental monitoring and third-hand smoke exposure assessment.**



***Index Terms*—DNA degradation, Hydroquinone, Microbead dielectrophoresis, Third-hand smoke**

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I. Introduction

Hydroquinone (HQ) is an aromatic compound widely used in industrial processes and cosmetics and is also a major metabolite of benzene and phenol. Owing to its potential carcinogenicity and environmental persistence, monitoring HQ in environmental and biological samples is essential for risk assessment and occupational exposure evaluation [1], [2], [3]. In addition to its occupational and environmental sources, HQ is a major component of tobacco smoke and its residues. Third-hand smoke (THS), defined as residual tobacco smoke pollutants that persist on indoor surfaces, dust, and clothing, is a growing health concern. It contains reactive species, including phenolic compounds such as HQ, which can induce oxidative/reductive stress and DNA damage [4], [5]. Consequently, detecting HQ as a marker of THS exposure is critical for evaluating the long-term health risks associated with indoor tobacco contamination.

Conventional analytical methods for HQ detection, such as high-performance liquid chromatography (HPLC) coupled with mass spectrometry, provide excellent sensitivity, but require expensive instrumentation, skilled operators, and time-consuming sample preparation [2]. Electrochemical sensors offer a simpler alternative, but often involve complex electrode modifications, suffer from surface fouling, and exhibit limited selectivity in complex matrices [1]. These limitations highlight the need for a rapid, low-cost, and selective detection strategy that can be implemented without

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M. Nakano, M. Inaba, and J. Suehiro are with Faculty of Information Science and Electrical Engineering, Kyushu University, Fukuoka 819-0395, Japan (e-mail: nakano@ees.kyushu-u.ac.jp, inaba@ees.kyushu-u.ac.jp, and suehiro@ees.kyushu-u.ac.jp).

F. Murayama is with Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka 819-0395, Japan (e-mail: murayama@hv.ees.kyushu-u.ac.jp).

B. Techaumnat is with Department of Electrical Engineering, Chulalongkorn University, Bangkok 10330, Thailand.

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the need for sophisticated equipment.

To address these problems, we proposed a novel detection method based on dielectrophoretic impedance measurement (DEPIM) combined with DNA-labeled microbeads. DEPIM was originally developed to detect bacteria suspended in a low-conductivity liquid. It involves trapping the target bacteria on a microelectrode by dielectrophoresis (DEP) and measuring the microelectrode impedance variation over time [6]. The method was also recently used to detect DNA amplified by polymerase chain reaction (PCR) and other DNA amplification methods [7]. The amplified DNA is attached to dielectric microbeads, which are subsequently trapped by DEP, thereby inducing changes in their dielectrophoretic properties. This enabled the simple and rapid detection of infectious pathogen genes within 30 min with a detection limit of as low as two copies/reaction [8], [9].

In this method, DNA molecules are exposed to HQ and immobilized on microbeads for a measurable change in their dielectrophoretic response. This physical signal transduction mechanism eliminates the need for electrode surface modification and avoids reliance on redox reactions. Moreover, the addition of Cu^{2+} ions accelerates the degradation reaction, shortening the reaction time from 24 h to 30 min. This DNA-degradation-based detection method has been previously demonstrated for DNase I [10].

In this study, we demonstrated the feasibility of HQ detection using DNA-degradation-induced dielectrophoretic impedance changes and evaluated the effect of Cu^{2+} ions on the reaction kinetics. The proposed strategy offers a promising platform for rapid HQ monitoring and considerable potential in environmental analysis, occupational health, and THS exposure assessment applications. It is applicable to liquid samples, such as cigarette smoke extract and urine, which are relevant for monitoring occupational exposure to benzene metabolites. To the best of our knowledge, this is the first study to use DEPIM for the detection of HQ via DNA degradation.

The proposed method offers a unique combination of physical signal transduction, simple and rapid operation, and compatibility with complex matrices, such as artificial urine, making it a promising alternative to conventional chemical sensors.

II. Principle: HQ Detection Using Microbead DEP-based DNA Detection

DEP is the electrokinetic motion of a dielectric particle subjected to a non-uniform electric field [11], [12]. The DEP force acting on a spherical dielectric particle can be expressed as follows:

$$F_{DEP} = 2\pi r^3 \varepsilon_m \mathrm{Re}[K(\omega)] \nabla E^2, \quad (1)$$

where r, ε_m, $K(\omega)$, and E denote the radius, permittivity of the surrounding medium, Clausius–Mossotti factor, and applied electric field, respectively.

The Clausius–Mossotti factor, which represents the particle's polarization, can be expressed as follows:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \quad (2)$$

where $\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega}$ denotes the complex permittivity, where ε, σ, and ω denote the permittivity at infinite frequency, conductivity, and angular frequency ($\omega = 2\pi f$, f: applied voltage frequency), respectively. The subscripts p and m denote the particle and surrounding medium, respectively.

When $\mathrm{Re}[K(\omega)] > 0$, the particle moves toward regions of high electric field (positive DEP, or p-DEP). Conversely, when $\mathrm{Re}[K(\omega)] < 0$, the particle is repelled from the high-electric-field regions (negative DEP, or n-DEP). For small spherical polymer particles, such as polystyrene or latex, the bulk conductivity (σ_b) is negligible, and the particle conductivity (σ_p) can be approximated as follows:

$$\sigma_p = \sigma_b + \frac{2K_s}{r} \approx \frac{2K_s}{r}, \quad (3)$$

where K_s denotes the surface conductance.

The proposed microbead-DEP-based DNA detection method operates under these conditions. DNA molecules carry negative charges on their phosphate backbones. When DNA binds to a small polymer particle, the surface charge density increases, leading to an increase in K_s, and consequently σ_p. This enhances $\mathrm{Re}[K(\omega)]$, shifting the particle's DEP behavior from n-DEP to p-DEP under the appropriate conditions (*i.e.*, suitable frequency and surrounding medium properties). This principle enables simple, rapid, and quantitative DNA detection. The DNA-labeled microbeads are collected at the microelectrodes via p-DEP, and the impedance change in the microelectrodes caused by the DEP collection can be measured simultaneously.

The impedance measurement combined with DEP is called DEPIM. Because the DEP force depends on the number of DNA molecules on the microbeads, the DEPIM response of the DNA-labeled microbeads depends on the presence of DNA. Consequently, the amount of DNA can be estimated using the DEPIM response. This method has been used not only for diagnosing infectious diseases [8], [9], but also for detecting DNase I, a DNA digestion enzyme that is considered a biomarker of acute myocardial infarction. For DNase I detection, the DNA prepared in advance is incubated with DNase I, and the amount of digested DNA is measured using this method [10].

In the present study, we extended this method to detect HQ, which degrades DNA through oxidation and reduction reactions. Upon oxidation (*e.g.*, by dissolved oxygen or ultraviolet light), HQ forms benzoquinone (BQ), which reacts with guanine bases to form adducts. These BQ–

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guanine complexes promote DNA strand cleavage via reduction. Degradation can be accelerated considerably via the addition of Cu^{2+} ions, which facilitates redox cycling [13], [14].

In this study, we proposed a rapid, simple, and quantitative HQ detection method using a microbead-DEP-based DNA detection method with Cu^{2+} acceleration. Although DEPIM has previously been used for enzymatic DNA degradation (*e.g.*, DNase I detection), its extension to chemical degradation by HQ represents a novel application. This study established a new paradigm for detecting genotoxic compounds using physical biosensing principles. Fig. 1 illustrates the concept of the proposed detection strategy.

III. Materials and Methods

A. Materials

HQ, copper(II) sulfate pentahydrate, pyrocatechol, and resorcinol were purchased from Fujifilm Wako Pure Chemical Co. Sigmatrix Urine Diluent was purchased from Merck. DNA molecules (796 bp in length) were prepared by PCR using pUC19 DNA as the template. The amplified DNA was modified with biotin at one 5' terminus. The sequences of the forward and reverse primers were 5'-biotin-TCGGTGATGACGGTGAAAAC and 5'-TCTTTCCTGCGTTATCCCCT, respectively. DNA molecules of 796 bp were selected considering their stability and sufficient length to ensure reliable binding to the microbeads and a measurable dielectrophoretic response [10]. The amplified DNA was purified using a QIAquick PCR purification kit (Qiagen) and quantified using a Quantus fluorometer (Promega) and Qubit dsDNA BR quantification assay kit (ThermoFisher Scientific) before the experiment. DNA was stored at 4°C before use. Streptavidin-coated magnetic microbeads (2.8 μm diameter, Dynabeads M-280 Streptavidin) were purchased from ThermoFisher Scientific.

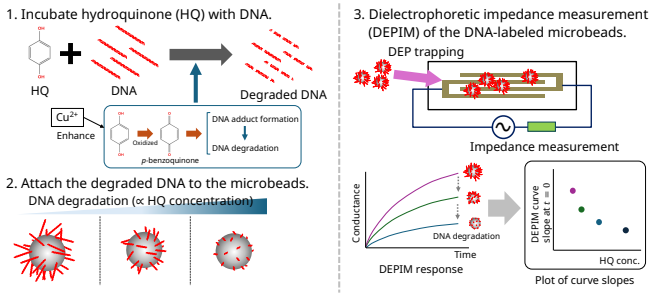

Fig. 1. Schematic diagram of HQ detection using microbead-DEP-based DNA detection. (1) HQ is incubated with DNA, leading to oxidative/reductive degradation via *p*-benzoquinone formation, accelerated by Cu^{2+} ions. (2) Degraded DNA is immobilized on streptavidin-coated microbeads via modification with biotin at one 5' terminus. (3) DNA-labeled microbeads are subjected to DEPIM, where the DEPIM response correlates with the amount of DNA, enabling HQ quantification.

B. HQ Detection by Microbead-DEP-Based DNA Detection

Reactions between DNA and HQ were performed as follows: DNA and HQ were mixed in deionized water (DW) and incubated at 37 °C. For accelerated degradation, copper(II) sulfate was added to the DNA–HQ reaction mixture. After incubation, DNA and the microbeads were suspended in a bind & wash buffer (10 mM Tris-HCl, 1 mM EDTA, and 1 M NaCl) and gently rotated for 30 min at room temperature (~25 °C) to allow the DNA to bind to the microbeads.

The DNA-labeled microbeads were analyzed using a DEP-based DNA detection method, the details of which are described elsewhere [8], [9], [10]. Fig. 2 depicts the experimental setup. Briefly, the DNA-labeled microbead suspension was introduced into a microchamber composed of a polydimethylsiloxane (PDMS) lid, spacer, and glass substrate with microelectrodes. The PDMS lid contained inlet and outlet ports connected to silicone tubing. The spacer (0.1 mm thick) defined the chamber height and shape. The microelectrodes, fabricated from chromium (0.1 μm thick), featured a castle-walled design with the shortest gap being 5 μm.

After introducing the DNA-labeled microbeads into the microchamber, the buffer was replaced with DW (conductivity: 2 μS/cm). During the buffer exchange, the microbeads were retained in the microchamber using a permanent magnet. Fluid flow was controlled using a syringe pump (Fusion 200, ISIS). A sinusoidal wave (2 V_{pp}, 100 kHz) was applied, and the current was measured across a shunt resistance (1 kΩ) using a lock-in amplifier (Signal Recovery 7270, Ametek). The impedance was calculated in real-time using a homemade LabVIEW program. The temporal change in conductance, referred to as the DEPIM curve, was fitted to an exponential function. The slope of the curve reflected the magnitude of the DEP force acting on the microbeads, which correlated with the amount of DNA bound to them

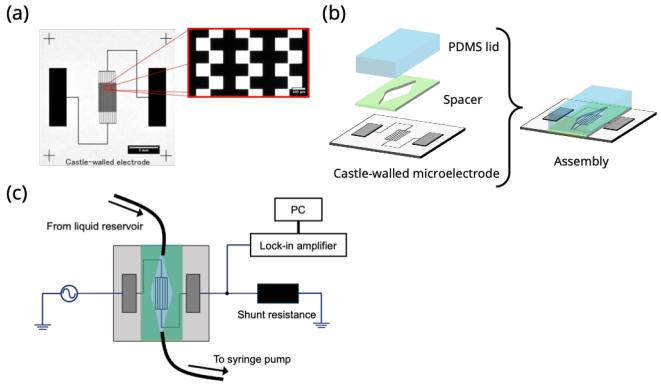

Fig. 2. Schematic illustration of the experimental setup. (a) Castle-walled microelectrode. The inset shows a close-up of the electrode. (b) Assembled chamber consisting of a PDMS lid, double-sided tape spacer, and microelectrode. (c) Instrument used for DEPIM.

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[7]. DEPIM was carried out at room temperature without any temperature control.

IV. Results and Discussion

A. DNA Degradation by HQ and Its Acceleration by Cu^{2+}

HQ is a known carcinogenic chemical used in industrial processes and cosmetics. It is also a major metabolite of benzene and phenol and is found in tobacco smoke. Consequently, the detection of HQ in environmental and biological samples such as urine is important. In this study, HQ was detected using a microbead-DEP-based DNA detection method, which monitored DNA degradation induced by HQ. The degradation process was accelerated *via* the addition of Cu^{2+} ions, enabling rapid and sensitive detection.

HQ-induced DNA degradation was examined by agarose gel electrophoresis. Fig. 2 shows DNA degradation (150 ng/20 µL) by HQ (1 mM) over time. The electropherogram revealed a DNA degradation mechanism consistent with prior studies of HQ–DNA interactions [5], [15]. HQ undergoes oxidation in aqueous environments to form BQ, a reactive electrophile that preferentially forms covalent adducts with guanine bases in DNA [13], [14], [15], [16]. These BQ–guanine adducts destabilize the DNA structure and promote strand cleavage via reductive processes. Notably, the presence of Cu^{2+} ions enhances this degradation pathway by facilitating redox cycling between Cu(I)/Cu(II) and HQ/BQ, thereby generating reactive oxygen species and accelerating DNA cleavage.

As shown in Fig. 3(a), progressive DNA degradation was evidenced by reduced migration and band fading, which indicate DNA adduct formation and DNA degradation, respectively. The adduct formation occurred before the strand break, then both reactions progressed at the concurrently afterward. Fig. 3(b) shows that the addition of Cu^{2+} ions led to complete DNA degradation within 1 h, confirming its catalytic role in HQ-induced DNA damage.

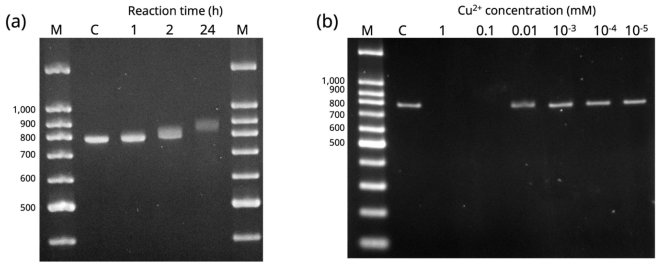

Fig. 3. HQ-induced DNA degradation analyzed by agarose gel electrophoresis. (a) DNA (150 ng/20 μL) was incubated with 1 mM HQ at 37 °C for various durations. The gel shows progressive DNA degradation: reduced migration, band broadening, and eventual disappearance. (b) The addition of Cu^{2+} ions (1 mM) accelerated HQ-induced DNA degradation considerably, with complete disappearance of the DNA band within 1 h. Lanes C and M represent the control (no HQ) and molecular weight marker, respectively. The gels were stained using SYBR Safe DNA Gel Stain (ThermoFisher Scientific).

B. Electrical detection of HQ

For electrical detection, DNA (4,000 ng, approximately 10^{11} molecules) was incubated with HQ at 37 °C. $CuSO_4$ (1 mM) was then added to accelerate the reaction. The incubated DNA was then attached to magnetic microbeads (10^6 beads) and analyzed using the microbead-DEP-based DNA detection method. The ratio of DNA molecules to microbeads, in which the microbeads are fully covered by DNA, was based on previously optimized conditions to ensure efficient binding and a reproducible DEPIM response.

Fig. 4 shows the DEPIM curves for the detection of 1 mM HQ with and without Cu^{2+} ions. DNA degradation resulted in a decrease in the DEPIM response compared with the control. The decrease in DEPIM response is caused by DNA shortening and/or reduced DNA coverage of the microbead surface. Without Cu^{2+} ions, the DEPIM response changed gradually over several hours, and even after 24 h, the curve remained distinguishable, indicating incomplete degradation. By contrast, with Cu^{2+}, the DEPIM response decreased rapidly after just 1 min of reaction, with the degradation appearing saturated after 15 min.

Fig. 5 shows a comparison of the slopes of the DEPIM curves with and without Cu^{2+} ions. The slope, defined as

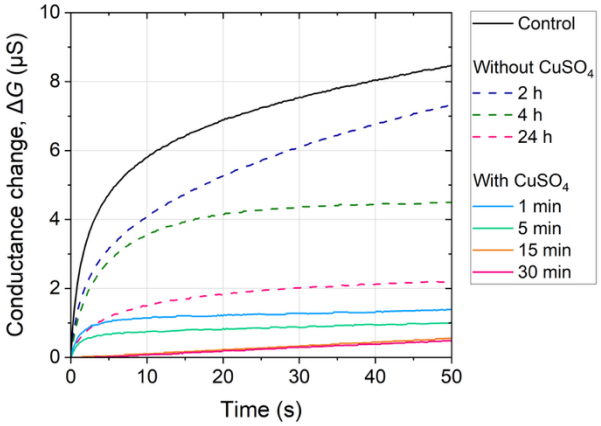

Fig. 4. DEPIM curves of DNA-labeled microbeads after various incubation times with 1 mM of HQ with and without Cu^{2+} ions. Control indicates the sample without HQ incubation, meaning the microbeads were labeled with intact DNA molecules.

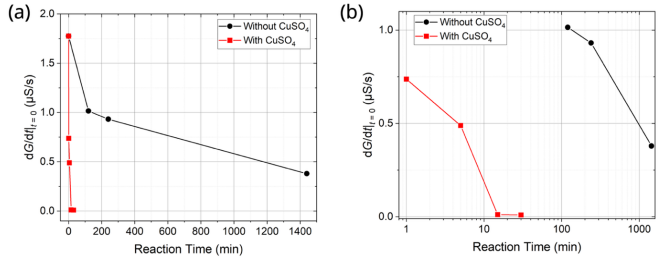

Fig. 5. Comparison of DEPIM curve slopes (dG/dt at $t = 0$) of DNA-labeled microbeads incubated with HQ with and without Cu^{2+}. (a) Linear scale plot showing the decrease in DEPIM slope over time with and without Cu^{2+}, indicating progressive DNA degradation. (b) Semi-logarithmic scale plot emphasizing early reaction kinetics.

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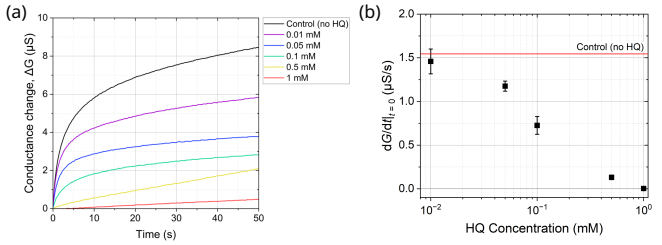

Fig. 6. DEPIM-based detection of HQ at varying concentrations. (a) DEPIM response curves showing the conductance change (ΔG) over time for DNA-labeled microbeads incubated with HQ concentrations ranging from 0.01 to 1 mM for 30 min. (b) Initial slope of DEPIM curves ($dG/dt|_{t=0}$) plotted against HQ concentration. Error bars indicate standard errors. The number of replicates for each data point ranges from 3 to 5.

$dG/dt|_{t=0}$, was calculated by fitting the DEPIM curves to an exponential function. It reflects the DEP force acting on the DNA-labeled microbeads, which depended on the amount of attached DNA. The results clearly show that Cu^{2+} accelerated the HQ-induced DNA degradation. The slope after 30 min of incubation with Cu^{2+} was almost identical to that after 24 h without Cu^{2+}, suggesting a 48-fold acceleration (30 min/24 h = 1/48).

To evaluate detection sensitivity, serial dilutions of HQ were incubated with DNA in Cu^{2+} solution for 30 min, which was sufficient to saturate the reaction. Fig. 6 shows that HQ concentrations as low as 50 μM could be detected. The entire detection process, including the DNA–HQ reaction (30 min), labeling (30 min), and DEPIM (5 min), was completed within approximately 1 h. These results demonstrate that DEPIM could detect HQ-induced DNA degradation with high sensitivity within a short timeframe. Unlike conventional chemical sensors, this method relies on physical signal transduction and offers a novel approach for the detection of genotoxic compounds.

HPLC can detect HQ at concentrations in the range of 0.1–10 μM [17], [18], [19]. Electrochemical sensors have also been developed for HQ detection, achieving high sensitivity (several tens of nanomolar or lower), but requiring complex electrode modifications [20], [21], [22]. Moreover, electrochemical sensors typically involve complex electrode modifications and can be prone to surface fouling, thus requiring careful calibration and handling. These factors limit their suitability for rapid field-based HQ detection. By contrast, the proposed DEPIM-based method offers a simpler and faster alternative without the need for electrode modification or redox reactions. Table 1 summarizes the comparison of the features and performance of these HQ detection methods.

To assess structural specificity, the positional isomers of HQ, pyrocatechol (*ortho*-) and resorcinol (*meta*-), were tested. Fig. 7 shows that DNA degradation induced by these isomers was considerably lower than that induced by HQ. The DEPIM slope at $t = 0$ for 0.1 mM of each isomer (approximately to 1.2 μS/s) was comparable to that for 50 μM HQ, indicating effective selectivity.

The detection of HQ (0.1 mM) in artificial urine (Sigmatrix Urine Diluent) was also examined. Fig. 8 shows that the urine components had a minimal impact on detection performance. Reports indicate that the HQ concentration in urine from occupational exposure can reach several tens of micromolar [17], [23], [24]. Because HQ is a major metabolite of benzene, its presence in urine serves as a biomarker for benzene exposure. Consequently, the ability to detect HQ in artificial urine using DEPIM highlights the potential of the proposed method for non-invasive health monitoring of workers exposed to benzene and related compounds. This application is consistent with occupational hygiene practices, where rapid and cost-effective screening tools are required for routine exposure assessment. The compatibility of the DEPIM method with complex biological matrices such as urine further supports its utility in field-based monitoring.

HQ, a key component of THS, is known for its oxidative DNA-damaging properties, making it a valuable marker for assessing indoor tobacco pollution [4], [5], [23], [25], [26]. THS, the residual contamination from tobacco smoke that persists on indoor surfaces, clothing, and dust, has emerged as a significant public health concern due to its long-term exposure risks. The proposed DEPIM-based detection method enables rapid and cost-effective HQ monitoring without complex instrumentation, thereby offering a practical solution for evaluating THS exposure in homes,

TABLE I
COMPARISON OF HQ DETECTION METHODS

Method	Limit of Quantification	Quantitative Range	Assay Time	On-Site Deployment	Note	Ref.
HPLC	1.82 μM	1.82–90.8 μM	15 min[a]	Difficult	Requires column pretreatment	[17]
	13.6 μM	13.6–890 μM	4 min[b]	Difficult	Requires column pretreatment	[18]
	454 nM	0.454–45.4 μM	15 min[c]	Difficult	Requires column pretreatment	[19]
Electrochemical	5 nM	5 nM–258 μM	4 s[d]	Enabled	Requires complex electrode fabrication	[20]
	500 nM	0.5–2.06 μM	Fast[e]	Enabled	Requires complex electrode fabrication	[21]
	48 nM	0.2–75 μM	Fast[e]	Enabled	Requires complex electrode fabrication	[22]
DEPIM	50 μM	50 μM–1 mM	60 min[f]	Enabled	Employs a simple electrode	This study

[a]Sample run time (additional sample preparation time of >18 h). [b]Sample retention time (additional sample preparation time of >1 h). [c]Sample run time (additional sample preparation time of >14 h). [d]Response time. [e]Actual time not reported. [f]Total assay time.

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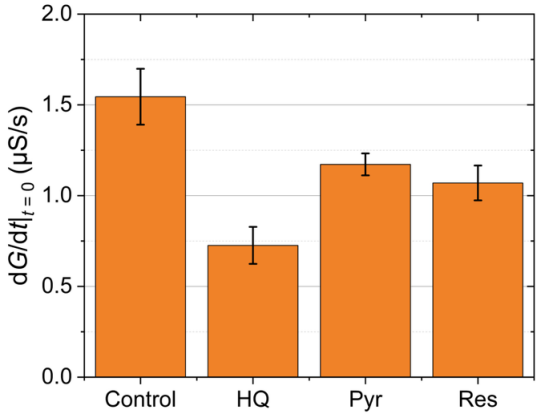

Fig. 7. Cross-reactivity of positional isomers. HQ (*para*-), pyrocatechol (Pyr) (*ortho*-), and resorcinol (Res) (*meta*-) were incubated with DNA for 30 min. Each isomer's concentration was 0.1 mM. Error bars indicate standard errors. The number of replicates for each data point ranges from 3 to 5.

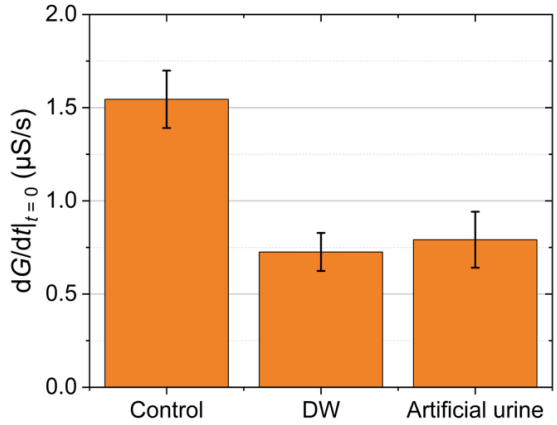

Fig. 8. Detection of HQ in artificial urine. DNA was incubated with 1 mM HQ in deionized water (DW) and artificial urine (Sigmatrix Urine Diluent) for 30 min. Error bars indicate standard errors. The number of replicates for each data point ranges from 3 to 5.

public spaces, and occupational environments. The cost efficiency of this method arises from the absence of expensive instrumentation, such as that in HPLC, and elimination of the complex electrode modifications required for electrochemical sensors. The DEPIM setup uses simple microelectrodes and standard laboratory equipment, making it suitable for a low-cost implementation [27]. By facilitating the on-site screening of HQ in biological and environmental samples, this approach contributes to improved risk assessment and preventive strategies against tobacco-related health hazards.

V. Conclusion

This study demonstrated a simple and rapid method for detecting HQ using DNA-degradation-based dielectrophoretic impedance measurement (DEPIM). The detection process involved three steps: (1) incubation of the DNA with HQ to induce degradation, (2) binding of the reacted DNA to microbeads, and (3) measurement of the dielectrophoretic response of the DNA-labeled microbeads using DEPIM. The addition of Cu^{2+} ions accelerated the DNA degradation reaction considerably, reducing the reaction time to 30 min. The method achieved a detection limit of 50 μM, demonstrated high selectivity against structural isomers, and was successfully applied to an artificial urine sample. Although the detection limit was higher than that of conventional chemical sensors, it was consistent with practical applications, such as THS exposure assessment and occupational monitoring, where HQ concentrations in biological samples typically exceed this threshold. Unlike conventional methods, such as HPLC or electrochemical sensors, the proposed method does not require complex instrumentation, electrode modification, or redox reactions. It offers a low-cost, label-free, and lightweight-equipment-based platform suitable for rapid HQ screening. These findings highlight the potential of DEPIM-based biosensing for environmental monitoring and THS exposure assessments in both public and occupational settings.

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Michihiko Nakano (Member) received the Dr.Eng. degree in environmental and life science engineering from Toyohashi University of Technology, Aichi, Japan, in 2006. He was a research fellow of the Japan Society for the Promotion of Science from 2003 to 2006. After postdoctoral work at Toyohashi University of Technology and the Industrial Research Institute of Shizuoka Prefecture, he joined Kyushu University, where he is currently an Associate Professor. His research focuses on the application of electrostatics and dielectrophoresis to manipulate micro/nanomaterials for biosensing, microfluidic devices, and functional composite fabrication.

Fumitoshi Murayama is a graduate student under the supervision of Associate Professor Michihiko Nakano in Kyushu University, Fukuoka, Japan. His research interests include the application of electrostatics to manipulate micro/nanomaterials for biosensing.

Masafumi Inaba received the Dr.Eng. degree in electronics from Waseda University, Tokyo, Japan, in 2017. He was a research associate in Waseda University from 2014 to 2017 and a postdoctoral research fellow of the Japan Society for the Promotion of Science in Nagoya University from 2017 to 2019. Currently, he is an Assistant Professor in Kyushu University. His research interests include electronic and quantum applications of carbon nanotubes and diamond using electrostatic manipulation.

Boonchai Techaumnat (Member) was born in Bangkok, Thailand, in 1970. He received the B.Eng. and M.Eng. degrees in electrical engineering from Chulalongkorn University, Bangkok, Thailand, in 1990 and 1995, respectively, and the Ph.D. degree in electrical engineering from Kyoto University, Kyoto, Japan, in 2001. He is currently a Professor with the Department of Electrical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand. His research interests include numerical field analysis, electrical insulation, bioengineering, bioelectromagnetics, and particle electrokinetics.

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Junya Suehiro received the B.Eng., M.Eng. and Dr. degrees in electrical engineering from Kyushu University, Fukuoka, Japan, in 1983, 1985 and 1991, respectively. He was with the Nippon Steel Co. from 1985 to 1988. Since April 1988, he has been with Kyushu University. He is now a Professor and involved in research on applied electrostatics and high voltage engineering. He was a Visiting Professor at the University of Wales, Bangor, U.K., from 1997 to 1998.

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