

Nanopore Identification of Nicotine in Cigarette Tobacco and Its Smoke

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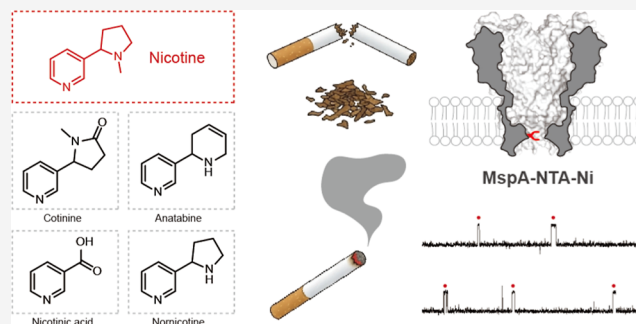
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ABSTRACT: Smoking poses a severe threat to global public health. Nicotine, as the predominant addictive constituent existing in both tobacco matrices and inhaled smoke, requires direct and reliable detection for health risk evaluation and tobacco quality control. In this work, we developed a single-molecule nicotine detection system using a nickel-functionalized *Mycobacterium smegmatis* porin A (MspA) nanopore. Structural analogues of nicotine, including cotinine, nicotinic acid, anatabine, and nornicotine, are also detectable with the same sensing system and can be readily distinguished from nicotine. With the assistance of machine learning, a cross-validation accuracy of 90.85% was achieved for the five analytes. Leveraging the metal coordination chemistry and machine learning algorithm, this system achieves specific identification of nicotine within complex matrices. With a calibrated standard curve, this method enables quantitative analysis of nicotine in tobacco samples. When combined with a smoke collection device, the system successfully detected nicotine in real cigarette smoke, demonstrating great potential for environmental smoke exposure evaluation and on-site field screening.

KEYWORDS: nanopore, nicotine, single-molecule sensing, cigarette, smoke



INTRODUCTION

Since Columbus obtained dried tobacco leaves from the indigenous people of the New World in 1492,¹ global demand for tobacco has increased substantially. Today, tobacco has ranked as the world's second most widely used psychoactive substance, with over one billion active smokers worldwide.² Accordingly, the public health risks associated with tobacco use have attracted widespread concern. For active smokers, long-term inhalation of tobacco smoke is closely linked to numerous severe health disorders, including central nervous system injuries,³ cardiovascular diseases,⁴ chronic obstructive pulmonary disease,^{5–7} and multiple forms of cancer.⁸ For non-smokers, particularly children, exposure to secondhand smoke can trigger respiratory illnesses and exert adverse effects on long-term growth and development.^{9,10} Tobacco and its combustion smoke contain intricate chemical constituents, with nicotine as the primary alkaloid and major addictive agent, whose content directly determines product addictiveness, quality, and population exposure risk.¹¹ Therefore, the development of sensitive, efficient, and accurate methods for detection of nicotine in both tobacco raw materials and cigarette smoke holds great theoretical significance and practical value for tobacco quality control, smoke pollution monitoring, and public health protection.^{12,13}

Conventional methods for nicotine detection, including high-performance liquid chromatography (HPLC),¹⁴ liquid chromatography–mass spectrometry (LC–MS),^{15,16} gas

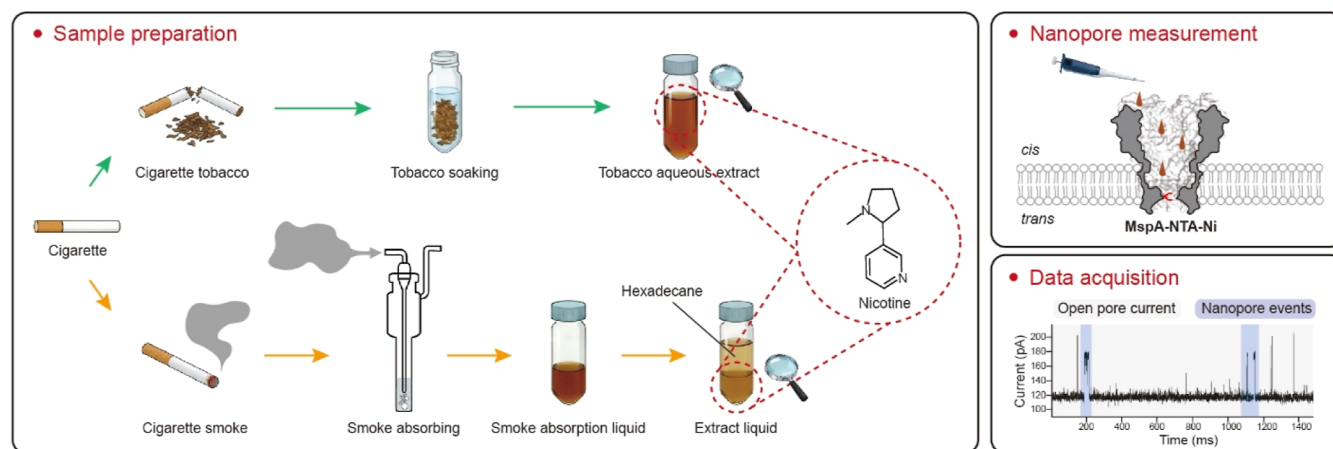
chromatography (GC),^{17–19} capillary electrophoresis (CE),^{20,21} and spectrophotometry,²² have been widely employed in laboratory settings. Nevertheless, these methods typically suffer from certain practical limitations,²³ such as long analysis times, tedious sample preparation, reliance on bulky and expensive instruments, and the requirement for skilled personnel. These factors collectively restrict their applicability for time-efficient, on-site, and real-time monitoring. Electrochemical sensors provide a simpler and more cost-effective alternative.^{24–26} However, they often exhibit insufficient selectivity and are susceptible to interference from the complex cigarette matrix. Therefore, there remains a clear and urgent need for an innovative nicotine sensing platform that is convenient, portable, low-cost, and capable of high-resolution analysis.

Nanopores,^{27,28} an emerging single-molecule analytical platform featured by ultrahigh sensitivity, label-free detection, and considerable portability potential, have achieved remarkable breakthroughs in DNA²⁹ and RNA³⁰ sequencing, protein analysis,^{31,32} small molecule detection,³³ and metal ion sensing.^{34,35} However, since nanopore detection fundamentally relies on an aqueous

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Scheme 1. Schematic of Nanopore Analysis of Nicotine in Cigarettes^a

^aAn NTA-Ni-modified nanopore (MspA-NTA-Ni) was employed for the detection of cigarette samples, covering both cigarette tobacco and cigarette smoke. Both solid tobacco and gaseous smoke can be subjected to nanopore detection after several pretreatment steps. As the core component of cigarettes, nicotine can interact with the NTA-Ni adapter via coordination interaction, generating characteristic nanopore events that enable efficient nicotine detection.

environment, its applications have long been limited to liquid-phase soluble analytes, and the detection of gaseous and volatile substances remains a substantial fundamental challenge.^{36,37} Specifically, gaseous molecules generally exhibit low water solubility, slow diffusion, and large mass-transfer resistance. Furthermore, most of these molecules are small in size and poorly matched with typical nanopore dimensions, making it difficult to produce observable ionic current blockages. Several proof-of-concept strategies have been employed to address these issues. For example, α -hemolysin nanopore integrated with hydrogel enrichment and DNA aptamers have been applied to detect pesticide vapors.³⁸ Solid-state nanopores have been developed to sense HCHO,³⁹ NO,⁴⁰ and CO⁴¹ via ion-current rectification triggered by interactions between gas molecules and functionalized pore lumens. Despite these advances, current approaches still face critical limitations, including insufficient mass-transfer efficiency, slow response, and poor anti-interference ability in complex matrices.

In this work, we present a novel nanopore strategy for nicotine detection in cigarette products (Scheme 1). By functionalizing the pore constriction with a nitrilotriacetic acid-nickel (NTA-Ni) moiety as a “sensing nose”, the system immediately acquires the capability to capture nicotine. This platform is applicable to both cigarette tobacco and smoke, exhibiting favorable practicability and anti-interference ability toward complex real cigarette matrices. Although a previous nanopore study reported nicotine-induced α -synuclein conformational change, neither direct nor quantitative detection of nicotine was achieved.⁴² Another theoretical work proposed a nanopore-based detection scheme for nicotine via density functional theory simulations, but remained purely computational without experimental validation.⁴³ Furthermore, nanopore analysis of tobacco or smoke has not been documented in prior research.

EXPERIMENTAL SECTION

Materials

Nicotine ditartrate was obtained from Macklin (China). L-Tartaric acid and nicotinic acid were obtained from Bidepharm (China). Anatabine was obtained from TargetMol (China). 2-(N-Cyclohexylamino)ethane sulfonic acid (CHES), nickel sulfate (NiSO₄), potassium chloride

(KCl), sodium chloride (NaCl), sodium hydrogen phosphate (Na₂HPO₄), sodium dihydrogen phosphate (NaH₂PO₄), cotinine and Coomassie blue fast staining solution were obtained from Aladdin (China). Nicotine was obtained from Shanghai Yuanye Bio-Technology (China). Tris(hydroxymethyl)aminomethane (Tris), imidazole and kanamycin sulfate were supplied by Solarbio (China). Absolute ethanol was from Guangdong Guanghua Sci-Tech (China). *Escherichia coli* strain BL21(DE3) pLysS and chloramphenicol were obtained from Sangon Biotech (China). Luria Broth (LB) and LB agar were from Hopebio (China). SDS-PAGE sample loading buffer was from Beyotime (China). Chelex 100 Resin, 4–20% Mini-PROTEAN TGX Gel, Precision Plus Protein Dual Xtra Standards, stacking gel buffer (0.5 M Tris–HCl buffer, pH 6.8), resolving gel buffer (1.5 M Tris–HCl, pH 8.8) were purchased from Bio-Rad (USA). Hexadecane, pentane, Genapol X-80, ammonium persulfate, sodium dodecyl sulfate, ethylenediamine tetraacetic acid (EDTA), N,N,N',N'-tetramethylethylenediamine (TEMED), tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and 30% acrylamide/bis-acrylamide solution were obtained from Sigma-Aldrich (USA). 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) was purchased from Avanti Polar Lipids (USA). Maleimido-C3-NTA was purchased from Dojindo Molecular Technologies (Japan). Cigarettes were purchased from local licensed tobacco retailers. KCl buffer (1.5 M KCl, 10 mM CHES, pH 9.0) was prepared with Milli-Q water and filtered through a 0.2 μ m membrane (Whatman, UK) prior to use.

Methods

Nanopore Preparations. (N90C)₁(M2)₇ is a hetero-octameric protein composed of one N90C MspA-H6 monomer and seven M2 MspA-D16H6 monomers.⁴⁴ The presence of a single cysteine residue renders this assembly amenable to site-specific chemical modification. Specifically, the genes encoding N90C MspA-H6 and M2 MspA-D16H6 were synthesized by Genscript (USA) and co-inserted into the pETDuet-1 vector. Following expression in *E. coli* BL21(DE3) pLysS competent cells, the resulting products were purified via nickel affinity chromatography. Different hetero-octameric MspA assemblies were further separated by polyacrylamide gel electrophoresis. The gel band corresponding to (N90C)₁(M2)₇ was excised and recovered. To further incorporate a single nickel ion into (N90C)₁(M2)₇, the purified (N90C)₁(M2)₇ was reacted with maleimido-C3-NTA (20 mM) and nickel sulfate (100 mM) at a volume ratio of 1:8:1. The resulting product, designated as MspA-NTA-Ni,⁴⁵ was used immediately or stored at –80 °C.

M2 MspA is a homo-octamer without chemical modification.⁴⁶ The gene encoding M2 MspA was inserted into the pET-30a (+) vector by Genscript (USA). Following expression in *E. coli* BL21(DE3) pLysS

competent cells, the recombinant protein was purified via nickel affinity chromatography. The resulting product was used immediately or stored at $-80\text{ }^{\circ}\text{C}$.

Nanopore Measurements. Nanopore measurements were conducted inside a Faraday cage mounted on an optical table (Jiangxi Liansheng Technology). The measurement device comprised two chambers separated by a Teflon film, in which an orifice of approximately $100\text{ }\mu\text{m}$ in diameter was created to connect the two chambers. The grounded chamber was designated as the *cis* side, and the opposing chamber as the *trans* side. Prior to each experiment, the orifice was pretreated with 0.5% (v/v) hexadecane in pentane. Both chambers were then filled with $500\text{ }\mu\text{L}$ of KCl buffer (1.5 M KCl, 10 mM CHES, pH 9.0). A pair of Ag/AgCl electrodes, connected to a patch-clamp amplifier, was immersed in the two chambers to complete the electrical circuit. To form a lipid bilayer, a droplet of 5 mg/mL DPhPC in pentane was added to each chamber, allowing spontaneous bilayer formation over the orifice. Nanopores were subsequently introduced into the *cis* chamber to initiate pore insertion. After a single nanopore inserted into the lipid bilayer, the buffer in the *cis* chamber was immediately exchanged with fresh KCl buffer to prevent additional insertions.

All electrophysiological measurements were performed using an Axopatch 200B patch-clamp amplifier coupled with a Digidata 1550B digitizer. Recordings were sampled at 25 kHz and digitally low-pass filtered with a cutoff frequency of 1 kHz . Unless otherwise specified, all electrophysiological measurements were carried out at room temperature under a constant applied potential of $+100\text{ mV}$. All analytes were added to the *cis* chamber to the desired final concentrations.

Data Analysis. All nanopore events were detected from raw current traces using the single-channel search function in Clampfit 11.2 (Molecular Devices, UK). Events with a dwell time shorter than 3 ms were ignored. Event features were extracted for all events using a custom MATLAB program. Subsequent data analysis, including scatter plots, frequency distribution histograms and curve fitting, was performed using Origin 2023b (Origin Lab).

Machine learning was performed using the Classification Learner app in MATLAB R2024b (MathWorks). A labelled dataset was constructed from nicotine, cotinine, nicotinic acid, anatabine, and nornicotine, with each analyte contributing 500 events collected across three independent measurements. Sixteen features were extracted from each event to form a feature matrix, including blockage ratio ($\Delta I/I_0$), standard deviation (SD), skewness (Skew), kurtosis (Kurt), dwell time (t_{off}), median (Med), maximum (Max), minimum (Min), mean of the largest 50% of the data points ($\text{Mean}_{\text{max}50}$), mean of the smallest 50% of the data points ($\text{Mean}_{\text{min}50}$), standard deviation of the largest 50% of the data points ($\text{SD}_{\text{max}50}$), standard deviation of the smallest 50% of the data points ($\text{SD}_{\text{min}50}$), Spectral centroid, Main frequency, Spectral energy, and Spectral entropy. The feature matrix was then randomly divided into a training set (80%) and a testing set (20%) for model training and testing, respectively. Model performance was evaluated by ten-fold cross-validation accuracy.

One-Class SVM, a supervised machine learning algorithm for outlier detection, was used to identify nicotine events from mixtures. A total of 171 sensing events acquired with nicotine ditartrate were used, from which the $\Delta I/I_0$ and SD features were extracted to build the feature matrix for model training. The trained One-Class SVM model was subsequently used to classify events as either inliers or outliers.

Cluster analysis was performed using density-based spatial clustering of applications with noise (DBSCAN). The epsilon and min points parameters were optimized to capture the major clustered event populations in the event scatter plot.

Preparation of Cigarette Tobacco Aqueous Extract. For each cigarette brand, a single cigarette was used. After discarding the filter and wrapping paper, the tobacco was crushed and immersed in 30 mL of Milli-Q water at $4\text{ }^{\circ}\text{C}$ for 24 h . The mixture was then centrifuged at $4\text{ }^{\circ}\text{C}$ and 4500 rpm for 10 min . Subsequently, the supernatant was loaded into an ultracentrifuge tube with a 10 kDa molecular weight cut-off. The filtration was then performed at 4500 rpm for 30 min at $4\text{ }^{\circ}\text{C}$, and the filtrate was collected for subsequent use.

Preparation of Smoke Absorption Solution. A Chunghwa cigarette was lit, and its smoke was collected using a custom-built absorption device. The absorption device consisted of a pipette tip, a bubble absorption tube, a pump, a flow regulator and several rubber tubes. Specifically, the pipette tip served as the cigarette holder, while the rubber tubes connected the pipette tip, bubble absorption tube, and pump to establish a smoke transmission pathway. The bubble absorption tube was preloaded with 1 mL of 95% ethanol, which dispersed the smoke into fine bubbles, ensuring thorough contact between the smoke and the ethanol solution. The pump provided the suction required to draw smoke into the bubble absorption tube. To maintain consistent suction conditions throughout the process, the flow regulator was set to a carrier wave frequency of 8 kHz and an output duty cycle of 27.5%. After the cigarette had burned completely, the resulting absorption solution was extracted with 1 mL of hexadecane. Following phase separation, the lower phase was collected for subsequent use. It should be noted that the actual smoke volume could not be directly measured or reliably estimated with the current setup, as no flow meter or volumetric calibration was incorporated. A standardized smoking machine would be required for accurate volumetric control and measurement.

Nicotine Quantification in Cigarette Tobacco. In nanopore sensing, the event appearance rate is proportional to the analyte concentration, providing the basis for quantification.^{47,48} To enable quantification of nicotine, a calibration curve was first established using nicotine ditartrate. Experiments were performed at varying concentrations, with three independent replicates conducted per condition. For each replicate, inter-event intervals were extracted from a 10 min continuous recording. The calibration curve was then obtained by plotting the reciprocal of the mean inter-event interval ($1/\tau_{\text{on}}$) against the nicotine concentration (Figure 3h).

To quantify the nicotine content in cigarette tobacco, tobacco aqueous extracts of different cigarette brands were analyzed by MspA-Ni. To assess the reliability of the results, three independent trials were carried out. For each condition, a 30 min single-channel current recording was performed. A pre-trained One-Class SVM model was employed to identify nicotine events (Supporting Figures S16–S18), from which the $1/\tau_{\text{on}}$ was derived. The obtained value was then interpolated against the calibration curve to determine the concentration of nicotine in the *cis* chamber of the measurement device. The original nicotine concentration in the tobacco aqueous extract was subsequently calculated using the following equation

$$C_0 = \frac{CV}{V_0} \quad (1)$$

Here, C_0 represents the original nicotine concentration (μM) in the aqueous extract. C is the final nicotine concentration (μM) in the *cis* chamber, as determined from the calibration curve. V_0 is the volume of the tobacco aqueous extract added to the *cis* chamber. V is the total volume of the *cis* chamber solution.

Finally, the total mass of nicotine in the tobacco is calculated according to the following equation

$$m = 10^{-6} C_0 V_{\text{total}} M \quad (2)$$

Here, m represents the calculated mass of nicotine (mg) in the tested cigarette tobacco sample. V_{total} is the volume (mL) of the aqueous extract. M is the molecular weight of nicotine, which is 162.236 g/mol .

HPLC Analysis. HPLC analysis was performed by eceshi (www.eceshi.com) using an Agilent 1290 II HPLC system equipped with an Agilent XDB-C8 column ($150 \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$). Mobile phase A consisted of water containing 0.1% formic acid and 5 mM ammonium acetate, and mobile phase B was methanol. The gradient elution program was set as follows: 0–1 min, 8% B; 1–4 min, 8%–15% B; 4–10 min, 15% B. The flow rate was 1 mL/min , and the column temperature was maintained at $30\text{ }^{\circ}\text{C}$. Detection was performed using a diode array detector (DAD) at a wavelength of 260 nm .

Spike-Recovery Experiment. A Chunghwa tobacco aqueous extract was divided into two portions. One portion was measured directly as the unspiked sample, while the other was used to prepare the

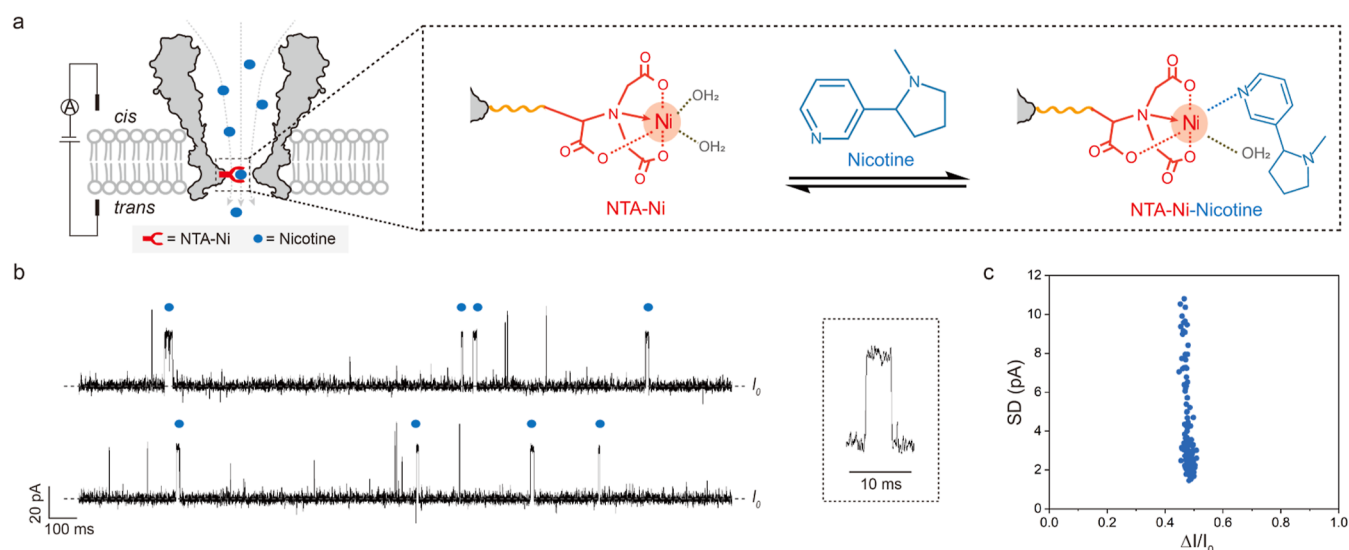


Figure 1. Nanopore sensing of nicotine. (a) Schematic diagram of nicotine detection using MspA-NTA-Ni. MspA-NTA-Ni is a hetero-octameric MspA nanopore equipped with a single NTA-Ni adapter at its constriction. Nicotine reversibly displaces a coordinated water molecule to bind with the Ni²⁺, generating a series of nicotine events. (b) Representative current traces acquired during nicotine sensing. I_0 represents the open pore current of MspA-NTA-Ni. Nicotine events were indicated by blue dots above the traces, with an expanded view of a single event shown in the dashed box on the right. The measurement was conducted in a 1.5 M KCl buffer (1.5 M KCl, 10 mM CHES, pH 9.0) (Methods). A voltage of +100 mV was continually applied. Nicotine ditartrate was added to *cis* with a final concentration of 0.5 mM. (c) The event scatter plot of $\Delta I/I_0$ versus SD for nicotine events. The plot was generated from 171 events ($n = 171$) collected in a single experiment ($N = 1$).

spiked sample by mixing 99 μL of the extract with 1 μL of 300 mM nicotine standard solution. The spiked and unspiked samples were measured separately, with 20 μL of each added to the *cis* chamber. The nicotine concentrations obtained from the two measurements were then used to determine whether the increase observed after spiking was consistent with the added amount of nicotine, and the recovery was calculated as

$$\text{recovery (\%)} = \frac{C_{\text{spiked}} V_{\text{spiked}} - C_{\text{unspiked}} V_{\text{unspiked}}}{C_s V_s} \times 100\% \quad (3)$$

Here, C_{spiked} and C_{unspiked} are the measured nicotine concentrations for the spiked and the unspiked samples, respectively. C_s and V_s denote the concentration and the volume of the added nicotine standard solution. V_{unspiked} is the sample volume before spiking, and V_{spiked} is the total sample volume after spiking ($V_{\text{spiked}} = V_{\text{unspiked}} + V_s$).

RESULTS AND DISCUSSION

Single-Molecule Sensing of Nicotine Using MspA-NTA-Ni

Nicotine, an alkaloid containing a pyridine ring in its structure,⁴⁹ is expected to coordinate with the fixed metal ion at the pore constriction, thereby enabling nanopore detection (Figure 1a). To test this hypothesis, nanopore measurements were performed in a buffer of 1.5 M KCl and 10 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) at pH 9.0, with a constant applied voltage of +100 mV using MspA-NTA-Ni (Methods). MspA-NTA-Ni is a hetero-octameric nanopore functionalized with an NTA-Ni adapter at its constriction (Supporting Figure S1). This design has enabled the detection of various small molecules with coordination ability, such as amino acids,⁴⁵ thyroid hormones,⁵⁰ and biogenic amines.⁵¹ Nicotine was used in the form of nicotine ditartrate, as free-base nicotine is a regulated hazardous chemical and is commercially unavailable in China. Importantly, tartaric acid was confirmed to exert no interference with the measurement (Supporting Figure S2). Experimentally, following the insertion of a single MspA-NTA-

Ni nanopore into the lipid membrane, nicotine ditartrate was added to the *cis* chamber of the measurement device with a final concentration of 0.5 mM. Immediately thereafter, successive nanopore events with consistent signal patterns were observed (Figure 1b). Moreover, the event occurrence rate increased with higher concentrations, confirming that the events were indeed generated by the added analyte (Supporting Figure S3). In contrast, no nanopore events were observed when using M2 MspA nanopore (Supporting Figure S4), which lacks any chemical modifications, highlighting the critical role of the NTA-Ni adapter in event generation.

To quantitatively characterize the nicotine events, the following event features were defined in Supporting Figure S5: the open-pore current (I_0), the event current (I_b), the blockage ratio ($\Delta I/I_0 = (I_b - I_0)/I_0$), the noise amplitude (SD), the event skewness (skew), the event kurtosis (kurt), the event dwell time (t_{off}), and the inter-event interval (t_{on}). An event scatter plot of $\Delta I/I_0$ and SD revealed a single and narrowly distributed population (Figure 1c), indicating high data consistency. The measurements were replicated three times, generating highly reproducible data (Supporting Figure S6). The mean values of the above event parameters were respectively derived and summarized in Supporting Table S1. The limit of detection (LOD) is defined as the minimum concentration yielding at least five detectable events over a 10 min recording period, and the experimental LOD for nicotine is determined to be 5 μM (8 events in 10 min).

Single-Molecule Identification of Nicotine Structural Analogues

To further expand the detection scope and evaluate the selectivity of this strategy, four structural analogues of nicotine: cotinine, nicotinic acid, anatabine, and nornicotine (Figure 2a–d), were analyzed using MspA-NTA-Ni under conditions identical to those employed for nicotine. Each compound successfully reported nanopore binding events (Figure 2e–h), and their features were

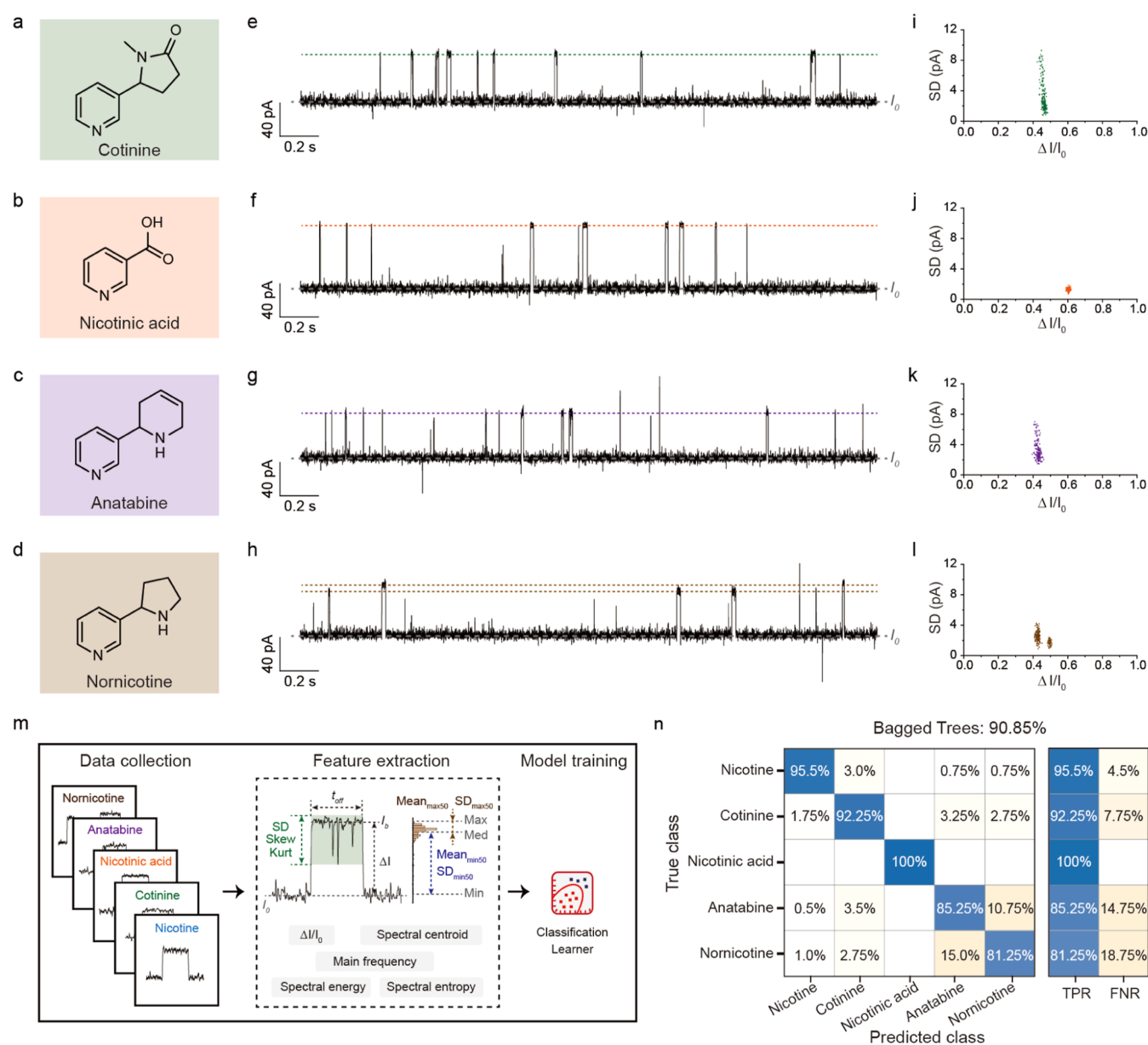


Figure 2. Nanopore analysis of nicotine structural analogues. (a–d) Chemical structures of cotinine, nicotinic acid, anatabine, and nornicotine. (e–h) Representative current traces showing events induced by cotinine (e), nicotinic acid (f), anatabine (g), and nornicotine (h). I_0 denotes the open pore current of MspA-NTA-Ni. Nanopore events are indicated by colored dashed lines. The measurement was conducted in a 1.5 M KCl buffer (1.5 M KCl, 10 mM CHES, pH 9.0) (Methods). A voltage of +100 mV was continually applied. Each analyte was separately added to *cis* with a final concentration of 0.25 mM (anatabine and nornicotine) or 0.5 mM (cotinine and nicotinic acid). (i–l) The event scatter plots of $\Delta I/I_0$ versus SD respectively derived from sensing results of cotinine (i), nicotinic acid (j), anatabine (k), and nornicotine (l). Each plot was generated from 200 events ($n = 200$) collected in a single experiment ($N = 1$). (m) The workflow of machine learning. Briefly, nanopore events acquired separately with nicotine, cotinine, nicotinic acid, anatabine, and nornicotine were combined into a labeled dataset. Sixteen features were extracted from each event to construct a feature matrix, which was then imported into the Classification Learner app in MATLAB for model training and validation. (n) The confusion matrix. The true positive rate (TPR) and false negative rate (FNR) are shown on the right.

clearly distinct from those of nicotine (Supporting Table S1). Cotinine, nicotinic acid, and anatabine each gave rise to a single event type, whereas nornicotine produced two distinct event populations (Figure 2i–l). This divergence is likely attributable to differences in coordination behavior (Supporting Figure S7): nicotine, cotinine, nicotinic acid, and anatabine coordinate to the metal center predominantly through the pyridine nitrogen, whereas nornicotine can engage in monodentate coordination through both its pyridine nitrogen and pyrrolidine nitrogen, yielding multiple coordination modes and consequently more than

one event type. Three independent measurements were performed for each analyte, and the event features were highly reproducible (Supporting Figure S8).

To quantitatively assess the differentiability of these analytes, a machine learning classification model was constructed (Methods). A labeled dataset was assembled from 2500 events across nicotine, cotinine, nicotinic acid, anatabine, and nornicotine, with sixteen features extracted per event to form a feature matrix (Figure 2m). The matrix was then randomly partitioned into a training set (80%) and a testing set (20%) for model

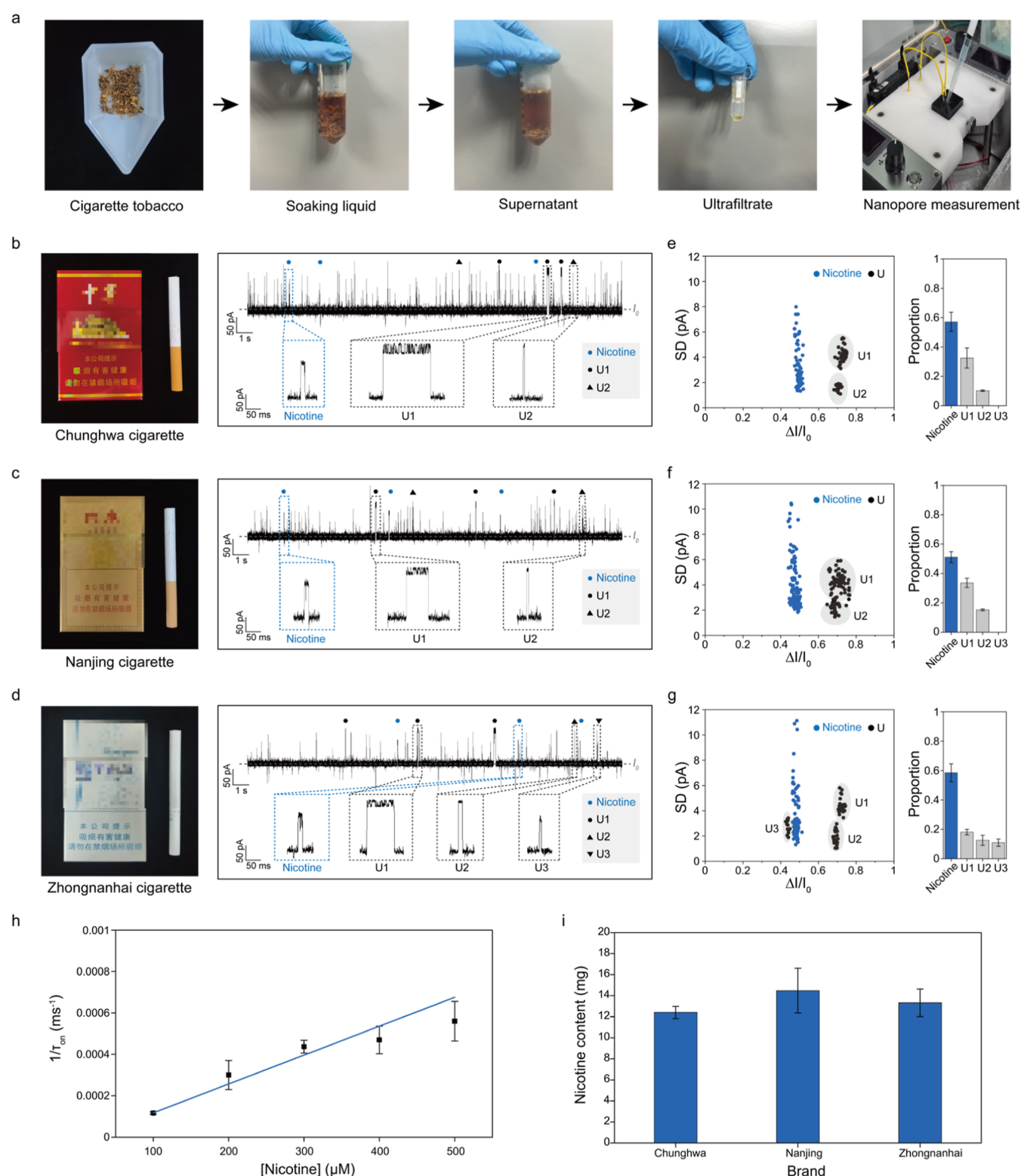


Figure 3. Nanopore analysis of cigarette tobacco from different brands. (a) Workflow of nanopore analysis of tobacco directly from commercially available cigarettes. Tobacco shreds were first collected, ground, and soaked in Milli-Q water. The soaking liquid was then centrifuged to remove insoluble debris, and the resulting supernatant was subjected to ultrafiltration. The final filtrate was directly added to the *cis* chamber to initiate nanopore measurement. (b–d) Left: images of Chunghwa (b), Nanjing (c), and Zhongnanhai (d) cigarettes. Right: representative current traces acquired with tobacco extracts of Chunghwa (b), Nanjing (c), and Zhongnanhai (d) cigarettes. Shaded regions were enlarged below the corresponding traces to show event details. The measurements were conducted in a 1.5 M KCl buffer (1.5 M KCl, 10 mM CHES, pH 9.0) (Methods). A voltage of +100 mV was continually applied. For each sample, 20 μ L of filtrate was added to the *cis* chamber to initiate the measurement. I_0 represents the open pore current of MspA-NTA-Ni. Nicotine events were observed in all samples. Two additional event types (U1, U2) were observed in Chunghwa cigarette (b) and Nanjing cigarette (c), while three additional event types (U1, U2, U3) were observed in Zhongnanhai cigarette (d). (e–g) Left: the event scatter plots of $\Delta I/I_0$ versus SD for events acquired with tobacco extracts of Chunghwa (e), Nanjing (f), and Zhongnanhai (g)

Figure 3. continued

cigarettes. A total of 151, 141, and 171 events were respectively used for plot generation, each collected from a single experiment per brand. Nicotine events were identified by a pre-trained One-Class SVM model. Unknown but clustered event clusters (U1, U2, U3) were detected by the unsupervised DBSCAN algorithm, consistent with the observations shown in (b–d). Right: event proportions for Chunghwa (e), Nanjing (f), and Zhongnanhai (g) cigarettes. (h) Calibration curve for nicotine. The curve was generated using nicotine ditartrate at a series of known concentrations (Supporting Figure S3 and Supporting Table S3). (i) Quantitative analysis of nicotine in different cigarette brands.

training and testing, respectively. Machine learning was conducted using the Classification Learner application in MATLAB, and ten-fold cross-validation was applied to evaluate model performance and mitigate potential bias. The Bagged Trees model achieved a validation accuracy of 90.85% and a testing accuracy of 90.6%. The corresponding confusion matrix was presented in Figure 2n, where the sensitivity (true positive rate, TPR) for all classes exceeded 80%. Nicotinic acid exhibited the highest TPR of 100%, and nicotine and cotinine each surpassed 90%. The specificity (true negative rate, TNR), exceeded 95% for all classes and reached 99.19% for nicotine (Supporting Table S2), indicating that non-nicotine events were rarely misclassified into the nicotine class.

Collectively, these results demonstrate that the sensing platform responds broadly to pyridine-containing analytes, and the high-resolution MspA nanopore enables specific discrimination among structurally similar compounds, reinforcing the selectivity of this strategy for nicotine identification in complex matrices.

Nanopore Analysis of Cigarette Tobacco

To further investigate the capability of this sensing platform for real-world cigarettes, tobacco from different cigarette brands (Chunghwa, Nanjing, Zhongnanhai) was analyzed (Figure 3a and Methods). All cigarette samples were purchased from local tobacco monopoly stores.

Prior to the measurement, the tobacco was separated from the cigarettes by removing the filter and wrapping paper. The tobacco was then crushed and soaked in Milli-Q water overnight. Following this, the solution was centrifuged at 4 °C and 4500 rpm for 10 min. The resulting supernatant was subsequently ultrafiltered through a 10 kDa molecular weight cutoff (MWCO) filter to obtain the filtrate. 20 μ L of the filtrate from each of the three tobacco brand samples was separately added to the *cis* chamber to initiate the nanopore measurements. Nicotine events were successfully detected across all three samples (Figure 3b–d and Supporting Figures S9–S11). Tobacco samples from three individual Chunghwa cigarettes obtained from three different packs were also prepared and measured, and consistent results were observed across these samples (Supporting Figure S12). Besides nicotine events, other events with distinct features were also observed, which may originate from other components in the tobacco extract capable of coordinating with the NTA-Ni adapter. This observation suggests that MspA-NTA-Ni nanopore holds potential for detecting a broader range of analytes.

To accurately identify nicotine events from interference, One-Class SVM, an outlier analysis algorithm, was employed (Methods). Using training events acquired from nicotine ditartrate (Figure 1), the algorithm automatically classified each unknown event as either an inlier or an outlier, and treated the two categories separately (Supporting Figures S13–S15). Inlier events were directly identified as nicotine events. Outlier events, which also contain rich sensing information, were subjected to cluster

analysis, where event clusters with consistent features were identified (Methods). Among these, two event clusters were consistently observed across all three cigarette brands, and we denoted these unresolved event clusters as U1 and U2. In addition, an extra event cluster, denoted as U3, appeared only in the Zhongnanhai cigarette (Figure 3e–g). Based on the event features and the abundance of these unresolved signals, we tentatively infer that U1–U3 are unlikely to originate from the non-nicotine analytes tested in this study. They may derive from other coordinating species present in tobacco, such as amino acids, organic acids, or phenolic acids, while their exact identities remain to be determined by further experiments and orthogonal analysis. For clarity of demonstration, the inlier events and the clustered outlier events were combined and summarized (Figure 3e–g). The proportional distribution of nicotine and U events for each brand was also shown (Figure 3e–g).

Nicotine content is the total amount of nicotine naturally present in cigarette tobacco, serving as a core determinant of both tobacco quality and its addictive potential. Therefore, accurate quantification of nicotine in tobacco is essential for quality control in cigarette manufacturing and for the assessment of associated health risks.^{52,53} To quantify nicotine content in the cigarette tobacco, a calibration curve was constructed using nicotine ditartrate at a series of known concentrations (Figure 3h). The reciprocal of the mean inter-event interval ($1/\tau_{\text{on}}$) was calculated for each concentration and plotted against the concentration to generate the calibration curve (Supporting Figure S3 and Supporting Table S3). A clear linear relationship was observed, with $1/\tau_{\text{on}}$ increasing as a function of concentration, enabling quantification of nicotine content based on experimentally measured $1/\tau_{\text{on}}$ values. Applying this to real samples, the $1/\tau_{\text{on}}$ values derived from the nicotine events of different cigarette brands were interpolated into the calibration curve (Supporting Figures S16–S18), and the resulting nicotine content per cigarette was determined as follows: 12.3 ± 0.7 mg for Chunghwa, 14 ± 2 mg for Nanjing, and 13.3 ± 1.3 mg for Zhongnanhai (Figure 3i and Supporting Table S4). These values are in general agreement with those reported for commercial cigarettes in the previous literature,^{54,55} supporting the reliability of the nanopore-based detection and quantification of nicotine in the cigarette tobacco. High-performance liquid chromatography (HPLC) was further employed as an orthogonal method to independently quantify the nicotine content in the Chunghwa tobacco extract as a representative example (Methods and Supporting Figure S19). The determined value, 8.08 mg per cigarette, is of the same order of magnitude as the nanopore result, further supporting the reliability of the nanopore measurement.

To assess potential interference from the tobacco matrix, a spike-recovery experiment was conducted (Methods). The spiked sample was prepared by adding 1 μ L of 300 mM nicotine tartrate solution to 99 μ L of tobacco aqueous extract. Aliquots of 20 μ L from both the unspiked and spiked samples were separately added to the *cis* chamber to initiate the nanopore measurement. A marked increase in the frequency of nicotine-

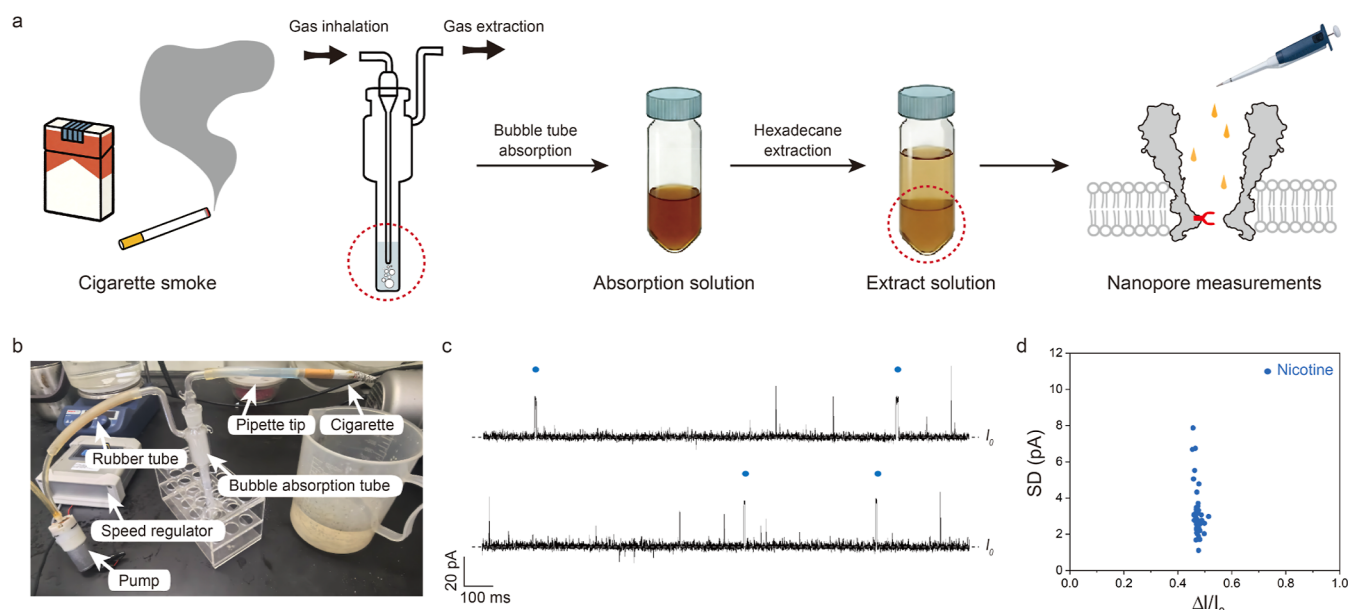


Figure 4. Nanopore analysis of cigarette smoke. (a) Workflow for collection and measurement of cigarette smoke. Cigarette smoke from a lit Chunghwa cigarette was introduced and washed with 95% ethanol via gas–liquid absorption. The resulting solution was extracted with hexadecane, and the lower phase was subsequently subjected to nanopore measurements. (b) Photograph of the smoke collection setup. A pipette tip serves to secure the cigarette and guide smoke into the rubber tubing. The tubing connects the bubble absorption tube to the pipette tip and vacuum pump, enabling stable smoke delivery. The bubble absorption tube is loaded with 1 mL of 95% ethanol, which disperses smoke into fine bubbles. The vacuum pump provides continuous suction, while the flow regulator is adjusted to a carrier wave frequency of 8 kHz and an output duty cycle of 27.5%. (c) Representative current traces acquired with smoke sample. The measurements were conducted in a 1.5 M KCl buffer (1.5 M KCl, 10 mM CHES, pH 9.0) (Methods). A voltage of +100 mV was continually applied. 10 μ L of smoke absorption solution was added to the *cis* chamber. I_0 represents the open pore current of MspA-NTA-Ni. Nicotine events were marked with blue dots. (d) The event scatter plots of $\Delta I/I_0$ versus SD for events acquired with the smoke sample. The plot was generated from 45 events ($n = 45$) collected in a single experiment ($N = 1$). Nicotine events were identified by the trained One-Class SVM model.

corresponding events was observed after spiking (Supporting Figure S20a,b), confirming that this event population indeed originates from nicotine. The $1/\tau_{\text{on}}$ values were subsequently derived and interpolated to the calibration curve to determine nicotine concentrations (Supporting Figure S20c–f), which were 2.96 mM and 5.56 mM for the unspiked and spiked samples, respectively. The calculated recovery was 87.65%, falling within the commonly accepted range. Since aspects such as moisture correction, extraction efficiency, and stepwise recovery have not yet been fully characterized in the current workflow, slight variations in quantitative results may occur. Further standardization of these procedures would help refine the quantitative accuracy and support broader applications, including tobacco quality inspection.

Nanopore Analysis of Cigarette Smoke

Cigarette smoke is a complex gaseous mixture containing various harmful substances,⁵⁶ among which nicotine is a major addictive and toxic component closely associated with human health risks.^{57–59} Accurate assessment of nicotine exposure levels and efficient detection of nicotine in cigarette smoke are of great practical significance. At present, nicotine exposure assessment mainly adopts methods such as questionnaires, airborne PM (particulate matter, mixture of solid particles and liquid droplets in air) monitoring, and cotinine levels in biological samples.⁶⁰ However, these approaches fall short in either quantification accuracy, objectivity, or specificity. Although chromatography-mass spectrometry analysis of airborne nicotine offers high accuracy and sensitivity, its application is hindered by sophisticated equipment and complex sample

preparation.⁶¹ Consequently, the development of an efficient, straightforward, and low-cost strategy for nicotine detection in cigarette smoke is of great significance.

To analyze the cigarette smoke, a custom-built smoking generation and sampling device was constructed (Figure 4a,b). The entire device was configured as a continuous gas circuit, where a pipette tip at the inlet served as the cigarette holder, followed in series by a bubble absorption tube. A vacuum pump was installed at the end of the circuit to generate negative pressure, thereby drawing the smoke through the entire system. Meanwhile, a flow regulator was employed to maintain a stable and controllable smoking flow rate. All components were connected via flexible tubing to ensure a sealed, unobstructed gas path throughout the setup. During the measurement, a Chunghwa cigarette was fixed onto the pipette tip holder and then lit. The mainstream smoke generated during combustion was continuously drawn into the bubble absorption tube and absorbed by 1 mL of 95% ethanol solution. After the cigarette had burned completely, the absorption solution underwent liquid–liquid extraction with 1 mL of hexadecane, and the extraction was repeated three to five times. Finally, the lower phase was collected and subsequently subjected to nanopore measurements (Supporting Figure S21).

To initiate the nanopore measurement, 10 μ L of the obtained smoke sample was loaded into the *cis* chamber of the measurement device, from which nicotine events were clearly observable (Figure 4c). Notably, the extraction step efficiently removed lipophilic organic compounds that could potentially destabilize the lipid bilayer, enabling prolonged measurements of the smoke sample (Supporting Figure S22). To avoid interference from noise events, outlier analysis was applied to specifically

identify the nicotine events (Figure 4d). The robustness of these observations was confirmed by three independent measurements, in which nicotine events were consistently observed (Supporting Figure S23). HPLC analysis was also performed with the smoke sample (Methods). A peak matching the nicotine standard was observed, confirming the presence of nicotine in cigarette smoke (Supporting Figure S24). These results collectively indicate that the sensing platform enables reliable detection of nicotine in mainstream cigarette smoke, a gas-phase matrix characterized by high complexity and substantial interfering substances. Unlike conventional application of nanopore sensing, which is typically limited to liquid-phase samples, this study extends the application to gas-phase detection, successfully achieving effective identification of the target analyte within a highly complex gas matrix and thereby offering new insights and a feasible approach for the development of nanopore-based gas sensing.

CONCLUSION

In summary, this work established a robust nicotine sensing platform based on an NTA-Ni modified MspA nanopore (MspA-NTA-Ni), and further validated its practical applicability for cigarette analysis. The metal ion immobilized within the pore lumen functions as a biomimetic sensing nose, capable of specifically recognizing nicotine through metal coordination interactions. Nicotine-related compounds, including cotinine, nicotinic acid, anatabine, and normicotine, can also be detected by MspA-NTA-Ni, yielding distinct nanopore event types and coordination behaviors. An overall validation accuracy of 90.85% was achieved by the Bagged Trees model for the classification of the five compounds. The intrinsic high resolution of MspA, coupled with machine learning algorithm, ensures credible identification of nicotine in intricate cigarette-derived matrices, covering both solid tobacco materials and gaseous cigarette smoke. Benefiting from this platform, nicotine content in tobacco products of different commercial brands were accurately quantified and compared. Meanwhile, a set of characteristic current events distinct from nicotine signals were consistently observed in tobacco samples. Although their chemical identities were not determined, these statistically distinct event populations may provide auxiliary information for the analysis of cigarette tobacco. Further efforts can focus on constructing a molecular fingerprint database by incorporating additional standard substances, thereby enabling more comprehensive profiling of cigarette matrices. With a custom-designed smoke sampling system, effective nicotine detection in mainstream cigarette smoke was achieved, extending the scope of nanopore sensing from liquid-phase analytes to gas-phase samples. Although this simple setup was sufficient for capturing and detecting nicotine in smoke at the proof-of-concept stage, extending this method toward accurate quantitative analysis of smoke constituents will require the use of a standardized smoking machine operated according to protocols recommended by the International Organization for Standardization (ISO) or the World Health Organization (WHO), thereby providing a standardized and reproducible sampling basis for smoke analysis. This sensing platform may be further integrated into miniaturized sampling modules, holding great potential for on-site monitoring and risk assessment of secondhand smoke exposure in complex environmental conditions. Although in situ detection of gaseous smoke remains challenging for the current platform, further technical improvements can be implemented in follow-up research by optimizing

gas–liquid interfacial contact patterns and enhancing mass transfer efficiency, which may inspire broader applications of nanopore sensing in gas-phase analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.6c04163>.

Materials, detailed methods for nanopore preparations, nanopore measurement, and data analysis, statistics of event core parameters, continuous current traces during single-channel recordings, histograms and scatter plots (DOCX)

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Author Contributions

S.H., Y.F.W. and K.F.W. conceived the project. K.F.W. performed the nanopore engineering. Y.F.W., X.Y.D., L.L.Z. and

L.Q. performed the measurements. P.K.Z. set up the instruments. S.H., Y.F.W. and K.F.W. wrote the paper. S.H. supervised the project.

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Notes

The authors declare the following competing financial interest(s): S.H. and K.F.W. have filed patents describing the preparation of heterogeneous MspA and its applications thereof. The remaining authors declare no other competing interests.

ABBREVIATIONS

MspA, *Mycobacterium smegmatis* porin A; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; GC, gas chromatography; CE, capillary electrophoresis; CHES, *N*-cyclohexyl-2-aminoethanesulfonic acid; NTA-Ni, nitrilotriacetic acid-nickel.

REFERENCES

- (1) Stolberg, V. B. A Cross-Cultural and Historical Survey of Tobacco Use among Various Ethnic Groups. *J. Ethn. Subst. Abuse* **2008**, *6* (3–4), 9–80.
- (2) Reitsma, M. B.; Kendrick, P. J.; Ababneh, E.; Abbafati, C.; Abbasi-Kangevari, M.; Abdoli, A.; Abedi, A.; Abhilash, E. S.; Abila, D. B.; Aboyans, V.; et al. Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and attributable disease burden in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet* **2021**, *397* (10292), 2337–2360.
- (3) Domino, E. F. Tobacco Smoking and Nicotine Neuropsychopharmacology: Some Future Research Directions. *Neuropsychopharmacology* **1998**, *18* (6), 456–468.
- (4) Benowitz, N. L.; Liakoni, E. Tobacco use disorder and cardiovascular health. *Addiction* **2022**, *117* (4), 1128–1138.
- (5) Lugg, S. T.; Scott, A.; Parekh, D.; Naidu, B.; Thickett, D. R. Cigarette smoke exposure and alveolar macrophages: mechanisms for lung disease. *Thorax* **2022**, *77* (1), 94–101.
- (6) Wang, L.; Yu, Q.; Xiao, J.; Chen, Q.; Fang, M.; Zhao, H. Cigarette Smoke Extract-Treated Mouse Airway Epithelial Cells-Derived Exosomal LncRNA MEG3 Promotes M1 Macrophage Polarization and Pyroptosis in Chronic Obstructive Pulmonary Disease by Upregulating TREM-1 via m(6)A Methylation. *Immune Netw.* **2024**, *24* (2), e3.
- (7) Shen, Q.; Chen, J.; Yang, S.; Zhang, H.; Yu, H.; Wang, S.; Li, J. Protection against cigarette smoke-induced chronic obstructive pulmonary disease via activation of the SIRT1/FoxO1 axis by targeting microRNA-132. *Am. J. Transl. Res.* **2024**, *16* (10), 5516–5524.
- (8) Jha, P. Avoidable global cancer deaths and total deaths from smoking. *Nat. Rev. Cancer* **2009**, *9* (9), 655–664.
- (9) Musso, F.; Bettermann, F.; Vucurevic, G.; Stoeter, P.; Konrad, A.; Winterer, G. Smoking impacts on prefrontal attentional network function in young adult brains. *Psychopharmacology* **2007**, *191* (1), 159–169.
- (10) Goriounova, N. A.; Mansvelter, H. D. Short- and Long-Term Consequences of Nicotine Exposure during Adolescence for Prefrontal Cortex Neuronal Network Function. *Cold Spring Harbor Perspect. Med.* **2012**, *2* (12), a012120.
- (11) Sansone, L.; Milani, F.; Fabrizi, R.; Belli, M.; Cristina, M.; Zaga, V.; de Iure, A.; Cicconi, L.; Bonassi, S.; Russo, P. Nicotine: From Discovery to Biological Effects. *Int. J. Mol. Sci.* **2023**, *24* (19), 14570.
- (12) Peto, R.; Lopez, A. D. The future worldwide health effects of current smoking patterns. In *Tobacco and Public Health: Science and Policy*; Oxford University Press, 2004, pp 281–286.
- (13) Jha, P.; Ramasundarahettige, C.; Landsman, V.; Rostron, B.; Thun, M.; Anderson, R. N.; McAfee, T.; Peto, R. 21st-Century Hazards of Smoking and Benefits of Cessation in the United States. *N. Engl. J. Med.* **2013**, *368* (4), 341–350.
- (14) Santos e Silva, S. I.; Bowdler, P.; Giltrow, D.; Riddell, S.; Honeychurch, K. A simple and rapid method for the determination of nicotine in third-hand smoke by liquid chromatography and its application for the assessment of contaminated outdoor communal areas. *Drug Test. Anal.* **2016**, *8* (7), 676–681.
- (15) Abdallah, I. A.; Hammell, D. C.; Stinchcomb, A. L.; Hassan, H. E. A fully validated LC–MS/MS method for simultaneous determination of nicotine and its metabolite cotinine in human serum and its application to a pharmacokinetic study after using nicotine transdermal delivery systems with standard heat application in adult smokers. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2016**, *1020*, 67–77.
- (16) Attalah, E. R.; El-Shabrawy, M. S.; Abo-Aly, M. M. Development and validation of a new simple analytical method for the analysis of nicotine in mushroom using LC–MS/MS. *Int. J. Environ.* **2017**, *6* (1), 16–25.
- (17) Goniewicz, M. L.; Gupta, R.; Lee, Y. H.; Reinhardt, S.; Kim, S.; Kim, B.; Kosmider, L.; Sobczak, A. Nicotine levels in electronic cigarette refill solutions: A comparative analysis of products from the US, Korea, and Poland. *Int. J. Drug Policy* **2015**, *26* (6), 583–588.
- (18) Pagano, T.; DiFrancesco, A. G.; Smith, S. B.; George, J.; Wink, G.; Rahman, I.; Robinson, R. J. Determination of nicotine content and delivery in disposable electronic cigarettes available in the United States by gas chromatography-mass spectrometry. *Nicotine Tob. Res.* **2016**, *18* (5), 700–707.
- (19) Ghiasvand, A.; Koonani, S.; Yazdankhah, F.; Farhadi, S. A comparison study on a sulfonated graphene-polyaniline nanocomposite coated fiber for analysis of nicotine in solid samples through the traditional and vacuum-assisted HS-SPME. *J. Pharm. Biomed. Anal.* **2018**, *149*, 271–277.
- (20) Copper, C. L.; Newman, C. I.; Collins, G. E. Simple and rapid extraction, separation, and detection of alkaloids in beverages. *J. Sep. Sci.* **2008**, *31* (21), 3727–3731.
- (21) Carolina Soto, V.; Jofré, V. P.; Galmarini, C. R.; Silva, M. F. Determination of alkaloids in onion nectar by micellar electrokinetic chromatography. *Electrophoresis* **2016**, *37* (13), 1909–1915.
- (22) Al-Tamrah, S. Spectrophotometric determination of nicotine. *Anal. Chim. Acta* **1999**, *379* (1–2), 75–80.
- (23) Ray, M.; Ray, A.; Dash, S.; Mishra, A.; Achary, K. G.; Nayak, S.; Singh, S. Fungal disease detection in plants: Traditional assays, novel diagnostic techniques and biosensors. *Biosens. Bioelectron.* **2017**, *87*, 708–723.
- (24) Jing, Y.; Yuan, X.; Yuan, Q.; He, K.; Liu, Y.; Lu, P.; Li, H.; Li, B.; Zhan, H.; Li, G. Determination of nicotine in tobacco products based on mussel-inspired reduced graphene oxide-supported gold nanoparticles. *Sci. Rep.* **2016**, *6* (1), 29230.
- (25) Mehmeti, E.; Kilic, T.; Laur, C.; Carrara, S. Electrochemical determination of nicotine in smokers' sweat. *Microchem. J.* **2020**, *158*, 105155.
- (26) Jerome, R.; Sundramoorthy, A. K. Preparation of hexagonal boron nitride doped graphene film modified sensor for selective

electrochemical detection of nicotine in tobacco sample. *Anal. Chim. Acta* **2020**, *1132*, 110–120.

(27) Fu, K.; Kwon, S.-R.; Han, D.; Bohn, P. W. Single Entity Electrochemistry in Nanopore Electrode Arrays: Ion Transport Meets Electron Transfer in Confined Geometries. *Acc. Chem. Res.* **2020**, *53* (4), 719–728.

(28) Reitemeier, J.; Metro, J.; Fu, K. X. Nanopore sensing and beyond: Electrochemical systems for optically-coupled single-entity studies, stimulus-responsive gating applications, and point-of-care sensors. *Sens. Actuators Rep.* **2024**, *8*, 100225.

(29) Manrao, E. A.; Derrington, I. M.; Laszlo, A. H.; Langford, K. W.; Hopper, M. K.; Gillgren, N.; Pavlenok, M.; Niederweis, M.; Gundlach, J. H. Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. *Nat. Biotechnol.* **2012**, *30* (4), 349–353.

(30) Jain, M.; Abu-Shumays, R.; Olsen, H. E.; Akeson, M. Advances in nanopore direct RNA sequencing. *Nat. Methods* **2022**, *19* (10), 1160–1164.

(31) Nivala, J.; Marks, D. B.; Akeson, M. Unfoldase-mediated protein translocation through an α -hemolysin nanopore. *Nat. Biotechnol.* **2013**, *31* (3), 247–250.

(32) Cheng, L.; Cai, C.; Yuan, Z.; Ma, L.; Hou, M.; Liu, H.; Xiao, Y.; Zheng, M.; Chen, Y.; Chen, X.; Wong, C. Sub-10 nm Silicon-Based Pyramidal Nanopore Enabling High Performance Single Protein Molecule Detection. *Adv. Funct. Mater.* **2026**, *36* (52), e76232.

(33) Xie, L.; Liu, L.; Xu, S.; Wang, T.; Yue, X.; Li, G. An efficient voltammetric sensing platform for trace determination of Norfloxacin based on nanoplate-like α -zirconium phosphate/carboxylated multi-walled carbon nanotube nanocomposites. *Microchem. J.* **2024**, *206*, 111451.

(34) Sun, W.; Xiao, Y.; Wang, K.; Zhang, S.; Yao, L.; Li, T.; Cheng, B.; Zhang, P.; Huang, S. Nanopore discrimination of rare earth elements. *Nat. Nanotechnol.* **2025**, *20* (4), 523–531.

(35) Braha, O.; Gu, L.-Q.; Zhou, L.; Lu, X.; Cheley, S.; Bayley, H. Simultaneous stochastic sensing of divalent metal ions. *Nat. Biotechnol.* **2000**, *18* (9), 1005–1007.

(36) Yuan, J.; Ma, Y.; Wang, M.; Zhao, Y.; Liu, M.; Du, L.; Wu, C. Advances in Nanopore Sensors for Olfactory Sensing. *Adv. Mater. Technol.* **2026**, *11* (6), e01598.

(37) Lu, X.; Du, X.; Zhong, D.; Li, R.; Cao, J.; Huang, S.; Wang, Y. Nanopore Environmental Analysis. *JACS Au* **2025**, *5* (4), 1570–1590.

(38) Fujii, S.; Nobukawa, A.; Osaki, T.; Morimoto, Y.; Kamiya, K.; Misawa, N.; Takeuchi, S. Pesticide vapor sensing using an aptamer, nanopore, and agarose gel on a chip. *Lab Chip* **2017**, *17* (14), 2421–2425.

(39) Wu, K.; Kong, X.-Y.; Xiao, K.; Wei, Y.; Zhu, C.; Zhou, R.; Si, M.; Wang, J.; Zhang, Y.; Wen, L. Engineered Smart Gating Nanochannels for High Performance in Formaldehyde Detection and Removal. *Adv. Funct. Mater.* **2019**, *29* (14), 1807953.

(40) Li, R.; Sui, X.; Li, C.; Jiang, J.; Zhai, J.; Gao, L. Artificial NO and Light Cooperative Nanofluidic Diode Inspired by Stomatal Closure of Guard Cells. *ACS Appl. Mater. Interfaces* **2018**, *10* (4), 3241–3247.

(41) Ouyang, Q.; Tu, L.; Zhang, Y.; Chen, H.; Fan, Y.; Tu, Y.; Li, Y.; Sun, Y. Construction of a Smart Nanofluidic Sensor through a Redox Reaction Strategy for High-Performance Carbon Monoxide Sensing. *Anal. Chem.* **2020**, *92* (22), 14947–14952.

(42) Tavassoly, O.; Kakish, J.; Nokhrin, S.; Dmitriev, O.; Lee, J. S. The use of nanopore analysis for discovering drugs which bind to α -synuclein for treatment of Parkinson's disease. *Eur. J. Med. Chem.* **2014**, *88*, 42–54.

(43) Ray, S. J. Single molecule transistor based nanopore for the detection of nicotine. *J. Appl. Phys.* **2014**, *116* (24), 244307.

(44) Zhang, S.; Cao, Z.; Fan, P.; Wang, Y.; Jia, W.; Wang, L.; Wang, K.; Liu, Y.; Du, X.; Hu, C.; Zhang, P.; Chen, H.; et al. A Nanopore-Based Saccharide Sensor. *Angew. Chem., Int. Ed.* **2022**, *61* (33), e202203769.

(45) Wang, K.; Zhang, S.; Zhou, X.; Yang, X.; Li, X.; Wang, Y.; Fan, P.; Xiao, Y.; Sun, W.; Zhang, P.; Li, W.; Huang, S. Unambiguous

discrimination of all 20 proteinogenic amino acids and their modifications by nanopore. *Nat. Methods* **2024**, *21* (1), 92–101.

(46) Wang, Y.; Yan, S.; Zhang, P.; Zeng, Z.; Zhao, D.; Wang, J.; Chen, H.; Huang, S. Osmosis-Driven Motion-Type Modulation of Biological Nanopores for Parallel Optical Nucleic Acid Sensing. *ACS Appl. Mater. Interfaces* **2018**, *10* (9), 7788–7797.

(47) Cheley, S.; Gu, L. Q.; Bayley, H. Stochastic sensing of nanomolar inositol 1,4,5-trisphosphate with an engineered pore. *Chem. Biol.* **2002**, *9* (7), 829–838.

(48) Ouyang, Y.; Wang, K.; Jia, W.; Zhang, P.; Huang, S. Simultaneous Identification of Vitamins B1, B3, B5, and B6 by an Engineered Nanopore. *Nano Lett.* **2024**, *24* (38), 11944–11953.

(49) Bundjaja, V.; Santoso, S. P.; Go, A. W.; Wijaya, R.; Truong, C. T.; Yuliana, M.; Soetaredjo, F. E.; Ju, Y.-H.; Angkawijaya, A. E. Protocatechuic acid-metal-nicotine complexation study for chelation of smoking-related poisoning. *J. Mol. Liq.* **2020**, *312*, 113428.

(50) Wang, K.; Yang, X.; Xiao, Y.; Cao, Z.; Zhang, S.; Zhang, P.; Huang, S. Simultaneous Identification of Major Thyroid Hormones by a Nickel Immobilized Biological Nanopore. *Nano Lett.* **2024**, *24* (1), 305–311.

(51) Xiao, Y.; Du, X.; Zhao, L.; Dai, X.; Qian, L.; Zhang, S.; Zhang, P.; Huang, S.; Wang, K. Nanopore Identification of Biogenic Amines and Its Applications in Food Freshness Evaluation. *ACS Nano* **2026**, *20* (3), 2740–2751.

(52) Rose, J. E.; Behm, F. M. Effects of low nicotine content cigarettes on smoke intake. *Nicotine Tob. Res.* **2004**, *6* (2), 309–319.

(53) Benowitz, N. L.; Hall, S. M.; Stewart, S.; Wilson, M.; Dempsey, D.; Jacob, P. III Nicotine and Carcinogen Exposure with Smoking of Progressively Reduced Nicotine Content Cigarette. *Cancer Epidemiol., Biomarkers Prev.* **2007**, *16* (11), 2479–2485.

(54) Taghavi, S.; Khashyarmansh, Z.; Moalemzadeh-Haghighi, H.; Nassirli, H.; Eshraghi, P.; Jalali, N.; Hassanzadeh-Khayyat, M. Nicotine content of domestic cigarettes, imported cigarettes and pipe tobacco in Iran. *Addict. Health* **2012**, *4* (1–2), 28–35.

(55) Ershad, M.; Beld, J.; Mostafa, A.; Dela Cruz, M.; Vearrier, D.; Greenberg, M. I. Nicotine Content from Cigarettes Submerged in Soda. *J. Med. Toxicol.* **2020**, *16* (4), 452–457.

(56) Tsai, J.; Homa, D. M.; Gentzke, A. S.; Mahoney, M.; Sharapova, S. R.; Sosnoff, C. S.; Caron, K. T.; Wang, L.; Melstrom, P. C.; Trivers, K. F. Exposure to secondhand smoke among nonsmokers—United States, 1988–2014. *MMWR Morb. Mortal. Wkly. Rep.* **2018**, *67* (48), 1342–1346.

(57) Loomis, D.; Grosse, Y.; Lauby-Secretan, B.; Ghissassi, F. E.; Bouvard, V.; Benbrahim-Tallaa, L.; Guha, N.; Baan, R.; Mattock, H.; Straif, K. The carcinogenicity of outdoor air pollution. *Lancet Oncol.* **2013**, *14* (13), 1262–1263.

(58) Kostikas, K.; Minas, M.; Nikolaou, E.; Papaioannou, A. I.; Liakos, P.; Gougoura, S.; Gourgoulanis, K. I.; Dinas, P. C.; Metsios, G. S.; Jamurtas, A. Z.; et al. Secondhand smoke exposure induces acutely airway acidification and oxidative stress. *Respir. Med.* **2013**, *107* (2), 172–179.

(59) Franklin, B. A.; Brook, R.; Arden Pope, C. III Air pollution and cardiovascular disease. *Curr. Probl. Cardiol.* **2015**, *40* (5), 207–238.

(60) Zhang, T.; Chillrud, S. N.; Yang, Q.; Pitiranggon, M.; Ross, J.; Perera, F.; Ji, J.; Spira, A.; Breyse, P. N.; Rodes, C. E.; Miller, R.; Yan, B. Characterizing peak exposure of secondhand smoke using a real-time PM_{2.5} monitor. *Indoor Air* **2020**, *30* (1), 98–107.

(61) Li, L.; Wen, J.; Deng, Y.; Yang, J.; Yuan, Y.; Shen, Y.; Liu, G.; Tian, Y.; Lei, D. Direct Extraction and Determination of Free Nicotine in Cigarette Smoke. *J. Anal. Methods Chem.* **2024**, *2024*, 1–13.