

Forensic Analysis of Saliva: Development of Crown-Ether-Based Potassium Biosensors from Cigarette Butts for Crime Scene Investigation

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Abstract

This work outlines the development and forensic use of a new electrochemical biosensor for the detection and profiling of potassium ions in saliva residue recovered from cigarette butts. The sensor was specifically constructed to work on the Nernstian principle using a dibenzo-18-crown-6 ether film coating over a carbon counter electrode to selectively chelate potassium. Standard calibration using potassium chloride (KCl) solutions provided a sensitivity of 57.4 mV/decade in the range of 8 to 70 mmol/L. Analysis of saliva residues collected from 120 cigarette butts belonging to 20 individuals revealed high potassium sensitivity compared to sodium (35 mV/decade), with negligible interference from sodium ions. Atomic absorption spectroscopy validated the biosensor readings. The findings confirm that potassium profiling from saliva traces on cigarette butts offers an accurate, non-destructive forensic method for human identification and evidence evaluation.

Keywords: Forensic Investigation, Potassium, Dibenzo-18-Crown-6, Potentiometric Sensor, Cigarette Butts, Forensic Biosensor

1. Introduction:

Because of its complex biochemical makeup, saliva—a hypotonic secretion mainly produced by the parotid, submandibular, and sublingual glands¹—is an essential biological fluid for forensic and diagnostic investigations. The physiological and pathological conditions of an individual are reflected in its composition. Saliva contains electrolytes like sodium, potassium, chloride, calcium, magnesium, and phosphate, as well as proteins, enzymes, and more than 700 microbial species^{2&3}. It is composed of 99% water and 1% dissolved organic and inorganic substances. Saliva traces recovered from personal objects such as drinking glasses, envelopes, and cigarette butts are extremely valuable from a forensic stand point⁴. These types of evidence offer biochemical and DNA markers that can help with health evaluation and individual identification.

1.1. Crown ethers:

Crown ethers are cyclic polyethers with internal cavities that bind cations selectively. Pedersen first introduced them in the 1960s. Because the ion's radius and the ether cavity have the ideal size match,

dibenzo-18-crown-6 is very selective for potassium ions. This molecular recognition property is particularly useful for designing ion-selective devices. Dibenzo-18-crown-6, the first crown ether discovered, was a small amount of an unexpected by-product in a 0.4% yield rather than the intended synthesis target. The basis for crown ethers' current position in chemistry was laid by the significance of the discovery and the fervent research that followed. Crown ether's discovery and its unique ability to complex alkali and alkaline earth metal salts were first announced orally on September 15th at the Xth International Conference on Coordination Chemistry in Nikko, Japan ^[5,6]. Because of their chemical makeup and the fact that the complexes' shape resembled a crown on a metal ion, Pedersen called these macrocyclic polyethers with particular properties "Crown ethers." These unique properties includes.

1.2. Intricacy Features of crown ethers include:

Crown ethers' capacity for selective complexity is one of their most remarkable features. They bind the alkali's cationic component. The size and charge of the potassium ion complement the crown ether's cavities and clefts (Figure 1). Very selective interactions result from the excellent match between the ionic diameter of K^+ and the size of the cavity of crown ether. The enhanced potassium ion sensitivity of the ISFET with crown ether is caused by these interactions.

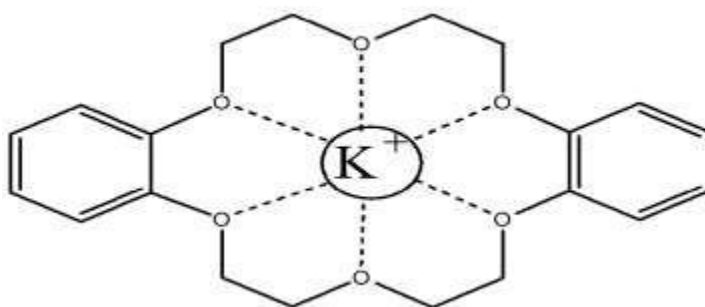


Figure 1. Binding of K^+ ion within the crown ether molecular structure (Host-guest Interaction)

Mechanism through which the crown ether layer enhances the ISFET's sensitivity to potassium. Complexation of oxygen atoms with different ionic species is the primary feature of crown ethers. Stable complexes are created when metallic elements adhere to oxygen atoms after passing through the hole's center. The crown ether serves as the "host" and ionic species as the "guest" in

what is then referred to as "host-guest" chemistry. Crown ether's clefts and cavities complement the potassium ion's size and charge. Very selective interactions result from the perfect match between the ionic diameter of potassium ion (K^+) and the size of the crown ether cavity. The higher potassium ion sensitivity of ISFET is caused by these interactions.

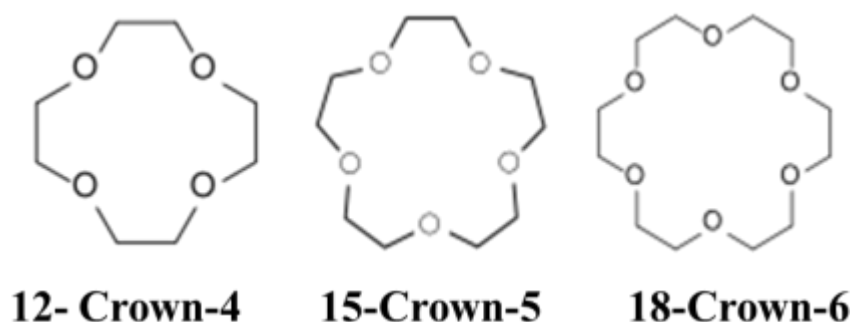


Figure 2. Structures of Crown Ethers used in this study

Food analysis, environmental pollutant monitoring, clinical diagnostics, pharmaceuticals, crime investigation, and biological warfare agent detection all make use of electrochemical sensors. A biochemical sensor combines a physical or chemical transducer with a biochemical recognition element. The recognition element needs to be stable in a wide range of conditions and specific to the target analyte. Because of their simplicity, ease of preparation, speed, accuracy, rapidity, non-destructive nature, simplicity of the device, and low cost, ion selective electrode development and application remain an intriguing area of analytical research⁸. In addition, these devices allow for online monitoring; however, the development of effective zinc-selective electrodes has not been particularly successful thus far. The majority of them exhibit low stability, sensitivity, and selectivity.

Table 1. Complexation in Crown Ethers according to Cavity and Ion Size

Cation	Ion Radius	Crown Ether	Cavity Diameter	Strongest Binding
Li ⁺	1.39 Å ⁰	12-Crown-4	1.2-1.4 Å ⁰	Li ⁺
Na ⁺	1.94 Å ⁰	15-Crown-5	1.7-2.2 Å ⁰	Na ⁺
K ⁺	2.66 Å ⁰	18-Crown-6	2.6-3.2 Å ⁰	K ⁺

2. MATERIALS AND TECHNIQUES:

2.1. Substances:

All of the analytical-grade reagents used in this study were acquired from Merck in India and included potassium chloride (KCl), dibenzo-18-Crown-6, sodium chloride (NaCl), serum, and chloroform. All aqueous solutions were prepared using deionized water.

2.2. Standard Potassium Solution Preparation

The stock (100 millimole/l) is made by adding 0.1M HCl to 100ml of a standard flask containing 7.456g of KCl and stirring. Using distilled water, various dilutions of 10 ml, such as 5, 10, 20, 30, 40, 50, 60, and 70 (millimole/lit), were made from the stock.

2.3. Crown ether membrane deposition on the carbon counter electrode

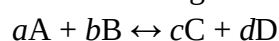
A layer of Dibenzo 18-Crown-6 (DB18C6) ether has been applied to the Carbon Counter Electrode's

surface. A paste was created by dissolving a small amount of Crown ether (200–400 mg) in chloroform solvent. The paste was applied to the carbon electrode's surface in a single layer. Chloroform instantly evaporated when exposed to room temperature air, leaving the ionophore behind.

2.4. Standard Procedure for Measuring Potassium Concentration measurements of the potassium ion's concentration and distribution in human saliva. Saliva samples from cigarette butts are gathered on filter paper and then dipped in the buffer saline solution. Crown ether (DB18C6) was used to estimate the samples, which were stored in a freezing box at -200 C in a refrigerator. KCl standard solutions were used to measure sensitivity, and an atomic absorption spectrophotometer was used to measure the concentration of potassium ion samples

2.5. Principle based on Nernst Equation

The general Nernst equation⁹ correlates the Gibb's Free Energy ΔG and the EMF of a chemical system known as the galvanic cell. For the reaction,



and

$$Q = \frac{C_c D_d}{A_a B_b}$$

and Gibbs Free Energy, $G = G^\circ + RT \ln Q$ and $G = -nFE$

So, $-nFE = -nFE^\circ + RT \ln Q$

where R , T , Q and F are the gas constant ($= 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), temperature (in K), reaction quotient, and Faraday constant ($= 96485 \text{ }^\circ\text{C}$) respectively. Thus, it results the following Nernst equation which allows calculation of cell potential for any galvanic cell at any given concentrations:

3. RESULTS AND DISCUSSION

$$E = E^\circ - \frac{RT}{nF} \ln \frac{C_c d}{A_a B_b}$$

Crown ether was dissolved in a few drops of chloroform to fabricate and characterize an electrode using crown monolayer coating in order to detect potassium in cigarette butt saliva using Dibenzo-18-crown (as ionophore). The ionophore was left behind when the chloroform instantly evaporated when exposed to room temperature air. An Ag/AgCl reference electrode was used (Figure 3) and an in-house assembled carbon counter electrode (as shown in Table 2) for potassium measurements in order to maintain uniformity.

Table 2: Comparison of in-house assembled electrodes with Dibenzo-18-crown-6 sensitivities towards potassium and sodium ions

S. No.	Ion studied	Concentration range examined	without crown ether	with crown ether
1.	Potassium	5-100 mmol/lit	35.27	58.9
2.	Sodium	42-72 mg/dl	45.4	70.4

The electrodes were dipped in 10 milliliters of standard solutions and cigarette butt saliva to measure potential, and the potential was recorded for each sample (Table 3). In order to match the read potential with standard values, a standard plot of the potential difference or concentration was created for standard samples.

Table 3. Item, source and collection and packing for forensic investigation

Item containing Evidence	Possible location of evidence	Possible source of Evidence	Collection and Packaging
Used Cigarette	Cigarette butts	Saliva	Place it in watch glass Allow it to air dry place in a clean watsman filter paper paper

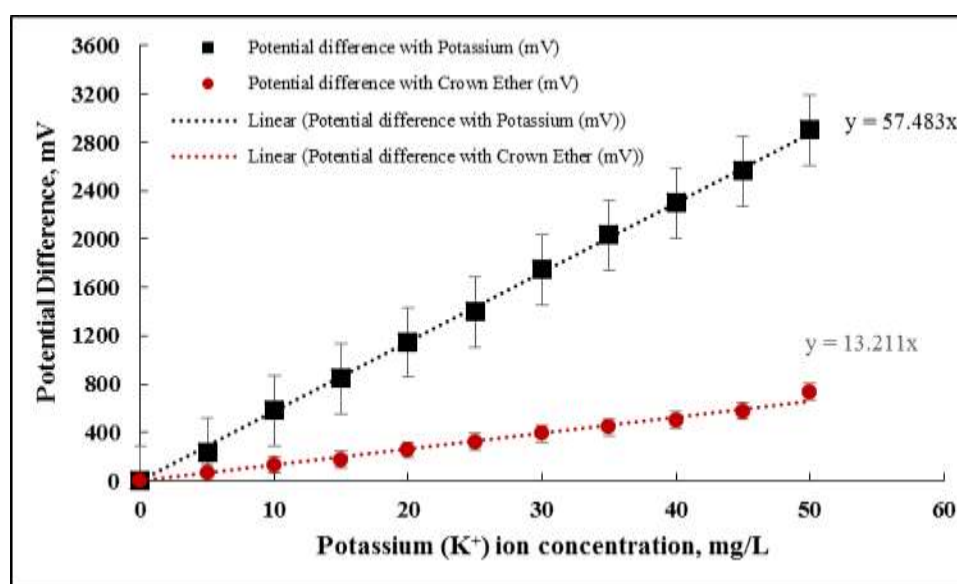


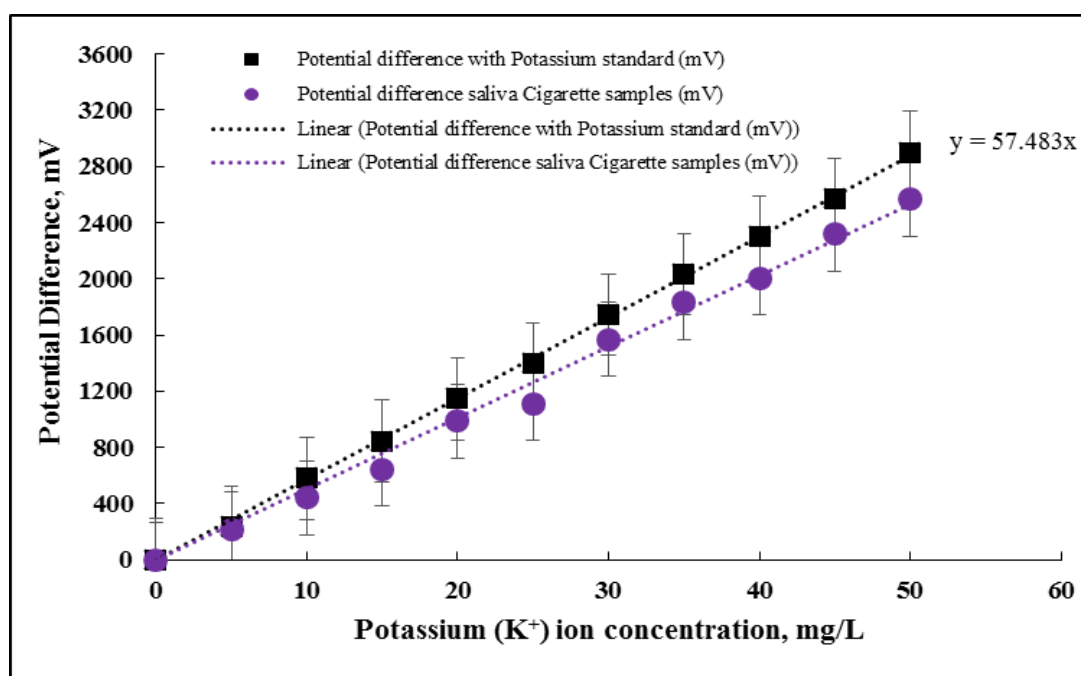
Figure 3. Calibration curve of Standard Potassium concentration vs Potential with Crown ether and without Crown ether

Table 4. Comparison of Biomarker and analyte transducer, sample vol and working range

Biomarker	Biorecognition	Transducer	Sample vol (ml)	Working range
Blood serum	K ⁺	Electrochemical	1ml	1-10 mmol/dl
Blood Serum	Na ⁺	Electrochemical	1ml	5-100 mmol/dl
Saliva	K ⁺	Electrochemical	1ml	1-10 mmol/dl
Saliva	Na ⁺	Electrochemical	1ml	10- mmol/dl

A standard graph was used to determine the potassium concentration in the cigarette butt saliva sample. Additionally, the electrode was standardized using crown ether and KCl solutions (Figure 4). The atomic absorption spectrophotometer was used to calibrate the electrode by comparing it to the KCl standards. Using an Atomic Absorption Spectrophotometer, the potassium concentrations in sample saliva from cigarette butts were measured while the interfering ion sodium was present (Figure 5)¹⁰. On-field, non-destructive, confirmatory techniques for body fluid identification at a crime scene are made possible by the use of this innovative potassium biosensor in cigarette butt saliva for forensic purposes. y the use of this innovative potassium biosensor in cigarette butt saliva for forensic purposes.

Figure 5. Plot of potentials relative to potassium ion concentration obtained from the in- house assembled carbon and Ag/AgCl of saliva-Cigarette butts' samples



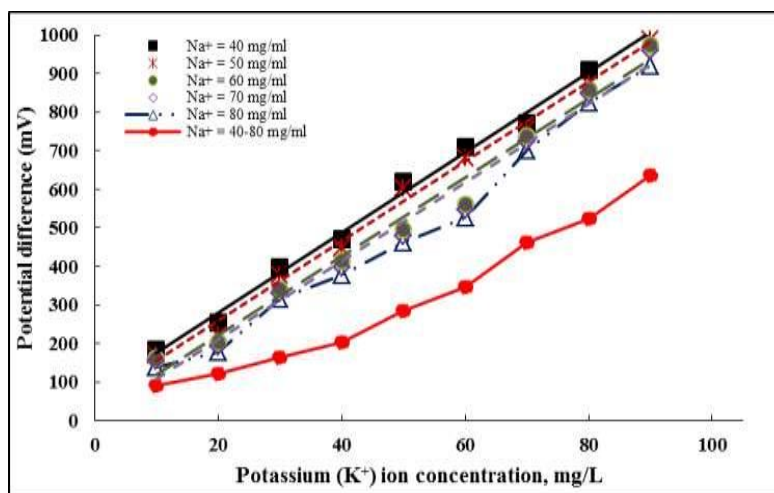


Figure 5. Calibration curve of potential vs Standard Potassium ion concentration with Dibenzo-18-crown-6 in presence of interfering sodium ion.

4. Comparison

Saliva is a biological fluid that is easily obtained through non-invasive techniques¹¹, is becoming a popular source of DNA samples, and is simple to collect and handle in cases of drug and alcohol abuse, poisoning, and animal bites¹². Among the factors contributing to this recognition are the advantages of saliva-based biosensors over blood sampling as a non-invasive monitoring tool for clinical diagnostics¹³. Uric acid levels in human saliva can be measured non-invasively to diagnose severe illnesses¹⁴. Using two anti-salivary amylase monoclonal antibodies, the Rapid Stain Identification (RSID™-Saliva) lateral flow immunochromatographic strip test detects the presence of salivary amylase rather than the enzyme's activity¹⁵, which reveals more saliva by detecting oral streptococci using PCR¹⁶. Additionally, methylation-sensitive high resolution melting was used to predict the forensic age of saliva samples¹⁷. Saliva and cigarette butts are frequently encountered forensic trace materials that are analyzed for one MZ twin pair epigenetic discrimination of identical twins¹⁸, and the identification of saliva in forensic samples by RT-LAMP¹⁹ was also investigated. Buccal swabs are a common forensic reference material. Other studies have reported using the MVR-PCR technique to identify a person using DNA extracted from cigarette butts, fluorescent spectroscopy, and single and multiple discs (3 mm) punched from a licked stamp attached to a postal stamp^{20&21}. DNA analysis, blood group analysis from cigarette butts using the absorption inhibition method²², and forensic DNA identification using Polymerase Chain Reaction (PCR) in Short Tandem Repeat²³, DNA analysis²⁴, DNA identification²⁵, blood group analysis from cigarette butts using the absorption inhibition method²⁶, and the effects of heat exposure duration on saliva traces on cigarette butts to examine the effects of heat exposure duration on the protein content of the saliva traces on cigarette butts to aid in the forensic blood group identification process²⁷. A self-assembled monolayer of 4-aminobenzo-18-Crown-6 ether has been used as a selective ionophore to create a potassium ion (K⁺) selective chemical sensor. Conductance studies, UV-Vis, and FTIR spectroscopic measurements were used to confirm the host-guest complexation of potassium ions and ionophore²⁸. Rapid Point-of-Care Measurement of Salivary Potassium was also investigated using hybrid integrated colorimetric sensing made possible by acoustic-fluidic micro-

mixing²⁹.

5. Conclusions

In conclusion Finally, we have shown that these new materials are effective at sensing potassium. When Di-benzo-18-Crown-6 is applied to the counter electrode, the K⁺ ion sensitivity of the electrode is greatly enhanced. With a wide concentration range (mg/dl) of saliva and a slope of 57.48 mv/decade, the resulting sensor demonstrated the best performance K⁺. The test is useful for determining the potassium content of saliva from cigarette butts. Additionally, it demonstrated accuracy, and the electrode's selectivity toward K was superior to that of other Na⁺ cations, and the assembly's lifespan exceeded three months.

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Conflict of Interest:

The authors have no conflicts of interest regarding this investigation.

References

1. Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X, Zhou XD. Saliva in the diagnosis of diseases. International journal of oral science. 2016 Sep; 8 (3):133-7.
2. Hildes JA, Ferguson MH. The concentration of electrolytes in normal human saliva. Canadian journal of biochemistry and physiology. 1955 Mar 1; 33 (2):217-25.
3. Virkler K, Lednev IK. Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic science international. 2009 Jul 1; 188 (1-3):1-7.
4. Pedersen CJ. Cyclic polyethers and their complexes with metal salts. Journal of the American Chemical Society. 1967 Dec; 89 (26):7017-36.
5. Lee DK, Jeong GS, Kim KC. Unexpected Electrochemical Behavior of Crown-Based Organic Compounds for Lithium-Ion Battery Cathodes. Industrial & Engineering Chemistry Research. 2021 May 20.
6. Hiraoka M, editor. Crown ethers and analogous compounds. Elsevier; 2016 May 4.
7. Sze SM. „Semiconductor Sensors —John Wiley & Sons. New York. 1994.
8. Lim HR, Lee SM, Mahmood M, Kwon S, Kim YS, Lee Y, Yeo WH. Development of Flexible Ion-Selective Electrodes for Saliva Sodium Detection. Sensors. 2021 Jan; 21(5):1642.
9. Chatterjee S, Nagi R, Aravinda K, Rakesh N, Jain S, Kaur N, Mann AK, Jayachandran S, Aruna P,

Preethi M, Yuvaraj M. Forensic Dental Sciences.

10. John SJ, Rajaji D, Jaleel D, Mohan A, Kadar N, Venugopal V. Application of Saliva in Forensics. *Oral & Maxillofacial Pathology Journal*. 2018 Jul 1; 9(2).
11. Malon RS, Sadir S, Balakrishnan M, Córcoles EP. Saliva-based biosensors: noninvasive monitoring tool for clinical diagnostics. *BioMed research international*. 2014 Sep 8; 2014.
12. Vernerová A, Krčmová LK, Melichar B, Švec F. Non-invasive determination of uric acid in human saliva in the diagnosis of serious disorders. *Clinical Chemistry and Laboratory Medicine (CCLM)*. 2021 Apr 1; 59(5):797-812.
13. Old JB, Schweers BA, Boonlayangoor PW, Reich KA. Developmental validation of RSID™-saliva: A lateral flow immunochromatographic strip test for the forensic detection of saliva. *Journal of forensic sciences*. 2009 Jul; 54 (4):866-73.
14. Nakanishi H, Kido A, Ohmori T, Takada A, Hara M, Adachi N, Saito K. A novel method for the identification of saliva by detecting oral streptococci using PCR. *Forensic science international*. 2009 Jan 10; 183(1-3):20-3.
15. Hamano Y, Manabe S, Morimoto C, Fujimoto S, Tamaki K. Forensic age prediction for saliva samples using methylation-sensitive highresolution melting: exploratory application for cigarette butts. *Scientific reports*. 2017 Sep 5; 7 (1):1-8.
16. Vidaki A, Kalamara V, Carnero-Montoro E, Spector TD, Bell JT, Kayser M. Investigating the epigenetic discrimination of identical twins using buccal swabs, saliva, and cigarette butts in the forensic setting. *Genes*. 2018 May; 9 (5):252.
17. Tsai LC, Su CW, Lee JC, Lu YS, Chen HC, Lin YC, Linacre A, Hsieh HM. The detection and identification of saliva in forensic samples by RT-LAMP. *Forensic Science, Medicine and Pathology*. 2018 Dec; 14 (4):469-77.
18. Hopkins B, Williams NJ, Webb MB, Debenham PG, Jeffreys AJ. The Use of Minisatellite Variant Repeat—Polymerase Chain Reaction (MVR-PCR) to Determine the Source of Saliva on a Used Postage Stamp. *Journal of Forensic Science*. 1994 Mar 1; 39(2):526-31.
19. Raj CK, Garlapati K, Karunakar P, Badam R, Soni P, Lavanya R. Saliva as Forensic Evidence using Fluorescent Spectroscopy: A Pilot Study. *Journal of Clinical & Diagnostic Research*. 2018 Sep 1; 12 (9).
20. Yudianto A. Effectiveness of Cigarette Butts as an Alternative Material for Forensic DNA Identification with Polymerase Chain Reaction (PCR) in Short Tandem Repeat (STR) Loci. *Folia Medica Indonesiana*. 2009 Apr 1; 45 (2):112.
21. Ruth MS, Purnadianti M, Marini MI. Blood group analysis from cigarette butts by absorption inhibition method: An experimental study. *Journal of International Oral Health*. 2020 May 1;12(3):275.
22. Thirunavakarasu T, Ahmad UK, Khan HO, Rahmat Z. DNA analysis of saliva traces on cigarette butts exposed to indoor and outdoor environmental conditions. *Malaysian J Forensic Sci*. 2016;7:10-6.
23. Apostolov A. DNA identification of biological traces on cigarettes: Vices reveal. *Biotechnology & Biotechnological Equipment*. 2012 Jan 1; 26 (3):2994-8.
24. Purnadianti, M., Aliviameita, A., Rochmawati, D.A.N. and Amanovitasari, D., 2017, April. Effects of Heat Exposure Duration on Saliva Traces on Cigarette butts as Forensic Identification Tools. In 1st International Conference Postgraduate School Universitas Airlangga: "Implementation of



Climate Change Agreement to Meet Sustainable Development Goals"(ICPSUAS 2017) (pp. 64-66). Atlantis Press.

25. Kumbhat S, Singh U. A potassium-selective electrochemical sensor based on crown-ether functionalized self assembled monolayer. *Journal of Electroanalytical Chemistry*. 2018 Jan 15; 809: 31-5.
26. Surendran V, Chiulli T, Manoharan S, Knisley S, Packirisamy M, Chandrasekaran A. Acoustofluidic Micromixing Enabled Hybrid Integrated Colorimetric Sensing, for Rapid Point- of- Care Measurement of Salivary Potassium. *Biosensors*. 2019 Jun; 9(2):73.