Chapter 15

Vanillin and Its Detection in Air

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Additional information is available at the end of the chapter

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Abstract

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an important flavor and aroma molecule, which has been widely used in not only foods and beverages such as chocolate and dairy products, but also masking unpleasant tastes in medicines or livestock fodder. Its chemical properties, manufacturing methods, novel applications, and developments in fast detections in air are discussed in detail.

Keywords: vanillin, properties, detection, capillary electrophoresis

1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an important flavor and aroma molecule but is also of interest because of its biogenetic relationship to the phenylpropanoid pathway and to other molecules of physiological significance, notably salicylate [1]. Vanillin is the most important ingredient of the well-known vanilla, which is a complex blend of flavor and fragrance ingredients extracted from the seed pods of the vanilla orchid. As a flavorings agent, vanillin is used in not only foods and beverages such as chocolate and dairy products, but also masking unpleasant tastes in medicines or livestock fodder [2].

Here, its properties, manufacturing methods, and novel applications are discussed. Furthermore, its detection in air is introduced.

2. Molecular structure and properties of vanillin

Vanillin is the common name for 3-methoxy-4-hydroxybenzaldehyde, and its molecular structure is shown in **Figure 1**.

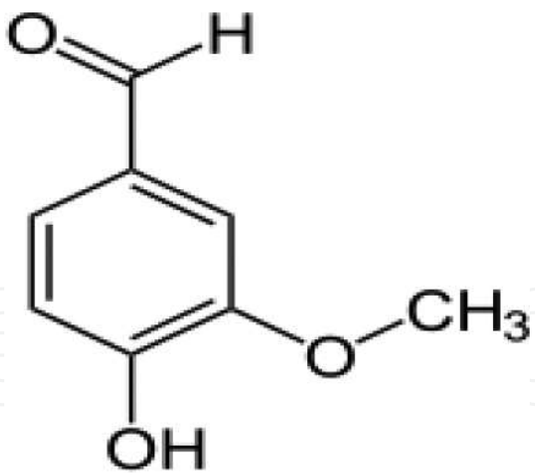

Figure 1. Molecular structure of vanillin.

2.1. Toxicity about vanillin

Toxicity about vanillin was studied as early as 1940 [3]. Generally, vanillin does not give human skin irritation and produce no sensitization reactions. Vanillin is considered to be a secondary allergen because sensitivity was found only in patients sensitive to vanilla, isoeugenol, and coniferyl benzoate. Animal studies showed that vanillin was not carcinogenic.

2.2. Antioxidant, antifungal or antimicrobial, and antimutagenic properties of vanillin and vanillin derivatives

Vanillin and vanillin derivatives have antioxidant and antimutagenic properties. Antifungal activities of vanillin and 33 vanillin derivatives against the human fungal pathogen Cryptococcus neoformans, which was the main pathogen of cryptococcal meningitis in immunocompromised patients, have been studied [4]. Functional groups in the vanillin derivatives seemed to affect antifungal activity. The hydroxyl or alkoxy group seemed to be more effective than the halogenated or nitrated group in benzaldehyde in antifungal ability. O-vanillin and o-ethyl vanillin were with the highest antifungal activity against *C. neoformans* in the vanillin derivatives. O-Vanillin was further found to be able to cause mitochondrial dysfunction and trigger oxidative stress. These antifungal mechanisms of o-vanillin were experimentally confirmed by the significantly reduced growth of the mutants lacking the genes involved in mitochondrial functions and oxidative stress response.

Evaluation in which structural elements of the vanillin molecule are responsible for its antifungal activity was also investigated [5]. Minimum inhibitory concentrations (MICs) of vanillin, its six direct structural analogs, and several other related compounds were determined in yeast extract peptone dextrose broth against a total of 18 different food spoilage molds and yeasts. Experimental results showed that the antifungal order of isomers of hydroxybenzaldehyde and anisaldehyde was 2- > 3- > 4- and 3- > 2- > 4-, respectively. The aldehyde moiety of vanillin seems to play a key role in its antifungal activity, but side-group position on the benzene ring also influences this activity.

Antimicrobial activities and the MICs of solutions containing vanillin and vanillic acid against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Enterobacter aerogenes, Escherichia coli*, and *Yersinia enterocolitica* were experimental studied by the agar well-diffusion method [6]. The experimental results showed that the vanillin and vanillic acid was with inhibitory activity against all of the bacteria. Moreover, the MIC of the vanillin and vanillic acid decreased with the vanillic acid concentration. It suggested that thermal treatment of vanillin-containing food may lead to products with higher antioxidant and antimicrobial properties.

2.3. Protection human keratinocyte stem cells against ultraviolet-B irradiation

Ultraviolet-B (UVB) irradiation is one of major factors, which induce cellular damages in the epidermis. Protective effects and mechanisms of vanillin against UVB-induced cellular damages in keratinocyte stem cells (KSC) have been investigated recently [7]. Experimental results indicated that vanillin significantly decreased the UVB irradiation-induced cytotoxicity. Also, vanillin seemed to be able to induce production of pro-inflammatory cytokines. It was explained that vanillin could significantly reduce phosphorylation of ataxia telangiectasia mutated (ATM), tumor suppressor protein 53 (p53), serine threonine kinase checkpoint kinase 2 (Chk2), c-Jun N-terminal kinase/stress-activated protein kinase (JNK), S6 ribosomal protein (S6RP), p38/mitogen-activated protein kinase (p38), and histone 2A family member X (H2A.X) generated by the UVB. Vanillin also could inhibit UVB-induced activation of p53 luciferase reporter. The results suggested that vanillin protects KSC from UVB irradiation. Vanillin may play its role through the suppression of downstream step of MDM2 in UVB irradiation-induced p53 activation.

3. Manufacturing methods of vanillin

A simple laboratory synthesis is illustrated in **Figure 2** to make a small amount of vanillin. This synthesis scheme involves electrophilic bromination of 4-hydroxybenzaldehyde, followed by copper-catalyzed methoxylation.

For large-scale industrial syntheses, a classic early method starts from eugenol, which occurs naturally in cloves, nutmeg, and cinnamon. This isomerizes to isoeugenol in alkaline solution, and this in turn can be oxidized (by nitrobenzene) to vanillin (**Figure 3**).

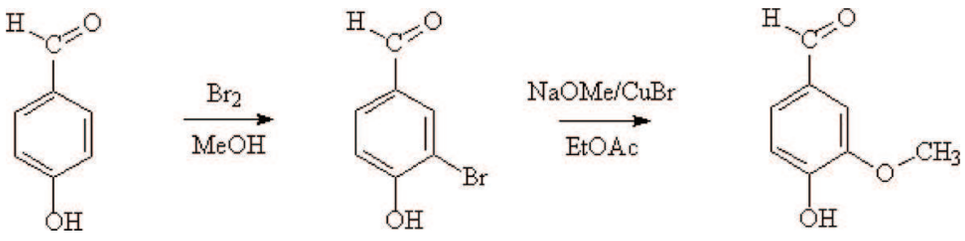

Figure 2. Laboratory synthesis scheme of vanillin.

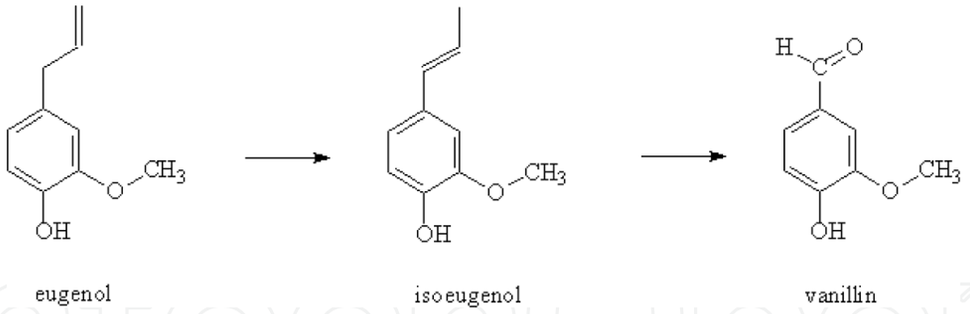

Figure 3. Industrial synthesis scheme of vanillin.

Lignin was known to be a source of vanillin as early as at the beginning of the twentieth century. Lignin is a well-known polymer, which plays strengthening role in woods and in the cell walls of plants. Since the 1920s, much of the world's vanillin was extracted from lignin waste from the cellulose industry [8].

Recently, potential of industrial Eucalyptus globulus sulfite liquor and kraft liquors was evaluated for the production of syringaldehyde and vanillin by oxidation with O_2 in alkaline medium [9]. The Eucalyptus globulus sulfite liquor and kraft liquors were collected at different stages of processing before the recovery boiler. Under controlled temperature and pressure, the oxidations were performed in a jacketed reactor by two methods. One was the direct oxidation of pulping liquors, and the other was the one of kraft lignins isolated from liquors. Products profiles were established, as well as the yields, temperature and O_2 uptake during the reaction. Results showed that sulfite liquor was the best raw material leading to the highest yield by direct oxidation. Thin kraft liquor (KL) was with the second high yield. Proportion of by-products such as syringic and vanillic acids was low.

Natural vanillin was obtained by plant tissue culture early. Molecular biology and microbial biotransformation techniques can also be used to produce natural vanillin [10]. These techniques rely on natural vanillin precursor molecules (eugenol, isoeugenol, curcumin, or ferulic acid), and their enzymatic reaction pathways are very different. Among them, microbial biotransformation method seems to be the most promising for large amount of natural vanillin production with high efficiency and high quality.

Screening of bacteria to produce vanillin and/or vanillic acid from isoeugenol was carried out [11]. *Achromobacter*, *Aeromonas*, *Agrobacerium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Rhodobacter*, and *Rhodococcus* were found to be able to produce vanillin and/or vanillic acid, in addition of isoeugenol to the culture medium [11]. In particular, a soil isolate strain IE27 showed the highest vanillin-producing activity, and it was identified as *Pseudomonas putida*. Under the optimized culture conditions, *P. putida* IE27 cells produced 16.1 g/l vanillin from 150 mM isoeugenol. The molar conversion yield from isoeugenol to vanillin was as high as 71% at 20°C after a 24-h incubation. Therefore, it is expectable to produce natural vanillin with high efficiency.

Production of vanillin from vanillic acid and *O*-benzylvanillic acid was investigated by using whole cells and enzyme preparations of *Nocardia* sp. strain NRRL 5646 [12]. With growing

cultures of the whole cells, 69 and 11% of vanillic acid were found to be decarboxylated to guaiacol and reduced to vanillyl alcohol, respectively. On the other hand, no decarboxylation of 4-*O*-benzylvanillic acid was found in conversion to the corresponding alcohol product. Purified *Nocardia* carboxylic acid reductase, an ATP and NADPH-dependent enzyme, was found to be able to reduce vanillic acid to vanillin quantitatively.

In addition to make use of the microbial biotransformation, enzymatic synthesis of natural vanillin was studied [13]. Flavoprotein vanillyl alcohol oxidase (VAO) could convert both creosol and vanillylamine to vanillin with high production yield. This conversion of creosol was realized via a two-step process. The first step was to convert creosol to vanillyl alcohol, and then, the second step was the oxidation of the vanillyl alcohol to vanillin. In the second step, the conversion of vanillyl alcohol to vanillin was inhibited by the competitive binding of creosol. The VAO-catalyzed conversion yield of vanillylamine to vanillin was high at alkaline solutions. Furthermore, mechanism study showed that vanillylamine was firstly converted to a vanillylimine intermediate product. The intermediate product was then hydrolyzed to vanillin nonenzymatically.

4. Novel applications of vanillin

4.1. Preparation of benzoxazine resin and reactive monomeric surfactant containing oxazine ring

Vanillin is used to synthesize polybenzoxazine with the expected desirable benzoxazine properties as well as a high char yield of 55.3% [14]. The synthesized monomer provides an unused aldehyde group from vanillin. The aldehyde can be further reacted with other materials to enhance properties. As a model, the unused aldehyde is reacted with amine terminated poly (ethylene oxide) to form a surfactant, which retains 1,3-benzoxazine's reactivity. The chemical structure of the synthesized monomers, surfactant, and polymers is characterized by Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). Thermal properties are also characterized by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Miniemulsions with stability up to 2 weeks are created with the newly synthesized surfactant and polystyrene. Dynamic light scattering (DLS) indicates 627 nm as the average diameter of the emulsion droplets.

4.2. Renewable polymers prepared from vanillin and its derivatives

Methacrylated derivatives of vanillin and vanillyl alcohol are synthesized and used as two monomers, respectively. The two monomers were further polymerized by a free-radical process [15]. Rheokinetics of their polymerization were studied to determine the cure behaviors. Thermomechanical properties of the resulting polymers affected by the structure and functionalities of the monomers were discussed. In comparison with methacrylated vanillin monomer, the methacrylated vanillyl alcohol gave a higher cross-linking density of the polymers, which in turn resulted a higher storage modulus and glass transition temperature, a better thermal resistance. Methacrylated vanillyl alcohol monomer was also with low viscosity at

room temperature. Therefore, methacrylated vanillyl alcohol monomer is an expectable bio-based reactive diluent for unsaturated polyester resins and vinyl esters.

5. Detection and analysis of vanillin and vanillin-type aromatic compounds

Vanillin and other purely olfactory odorants such as coumarin, octanoic acid, and phenylethyl alcohol cannot be identified when presented oral cavity only (OCO), because the oral cavity trigeminal system is fully unresponsive to these odorants in vapor phase [16]. Therefore, modern analytical methods are usually required for detection and analysis of vanillin and its derivatives.

5.1. Detection of real natural vanilla from synthetic one

Because of the extremely different in price of natural real vanilla from synthetic one, detection of fake vanilla is required. Basically, gas or liquid chromatography can tell the real natural from synthetic vanilla because some impurities such as 4-hydroxybenzaldehyde present in natural vanilla essence could be detected. Another way is to investigate amount of radioactive carbon-14. Natural plant-sourced vanillin contains a certain level of carbon-14, whose half-life is 5730 years. On the other hand, vanillin derived from crude oil has no radiocarbon since it has decayed away over the millions of years; the oil was trapped underground. Ratio of the natural isotopes, carbon-13 to carbon-12, also can be used for identify the real natural vanilla from the synthetic ones, because the vanilla orchid uses a different biosynthetic pathway to other plants. Orchid-derived vanillin has a greater ratio of carbon-13 to carbon-12 than synthetic vanillin [17].

A solid phase micro-extraction (SPME)-GC-MS method seems to be able to distinguish the natural real vanilla extracts from the synthetic one too [18]. The fiber material in SPME, sampling time, desorption time, and other experimental conditions were optimized. Under the optimized conditions, a relative standard deviation (RSD) of 2.5–6.4% indicated good reproducibility of the method. Because GC profile of the natural extracts was different from synthetic ones, it is easily to determine whether the sample is natural or synthetic. The method is also applicable to identify the type of vanilla extract/flavoring used in flavor foods.

5.2. Analysis of vanillin

The presence of vanillin in orange, tangerine, lemon, lime, and grapefruit juices could be easily identified and confirmed using GC-MS [19]. Vanillin concentrations in the orange, tangerine, lemon, lime, and grapefruit juices were determined to be 0.20, 0.35, 0.41, 0.35, and 0.60 ppm, respectively.

A headspace-solid phase micro-extraction (HS-SPME) GC-MS method was also proposed to determine of vanillin in vanilla products [20]. Detection limits were reported to be 1.33–13.2 ppb. Furthermore, LC-ESI-MS determinations of the vanillin were carried out at the same time, and the results were compared. Totally, 24 commercially available vanilla products were analyzed

with the two methods. Vanillin was detected in all of the 24 products. Also, 18 other flavor related compounds were detected in the samples.

As illustrated in **Figure 4**, the sampling system allowed real-time and continuously sampling of the aroma volatiles from model liquid foods [21]. The sample samples could be kept at a certain temperature (e.g., at 37°C), and aroma volatiles released into the headspace. A carrier gas was flowed into the headspace and further into a quadrupole MS via a jet separator. The MS analysis could monitor and identify the volatile molecular weight. Also, this sampling system can examine the dynamic flavor releasing process of liquid samples.

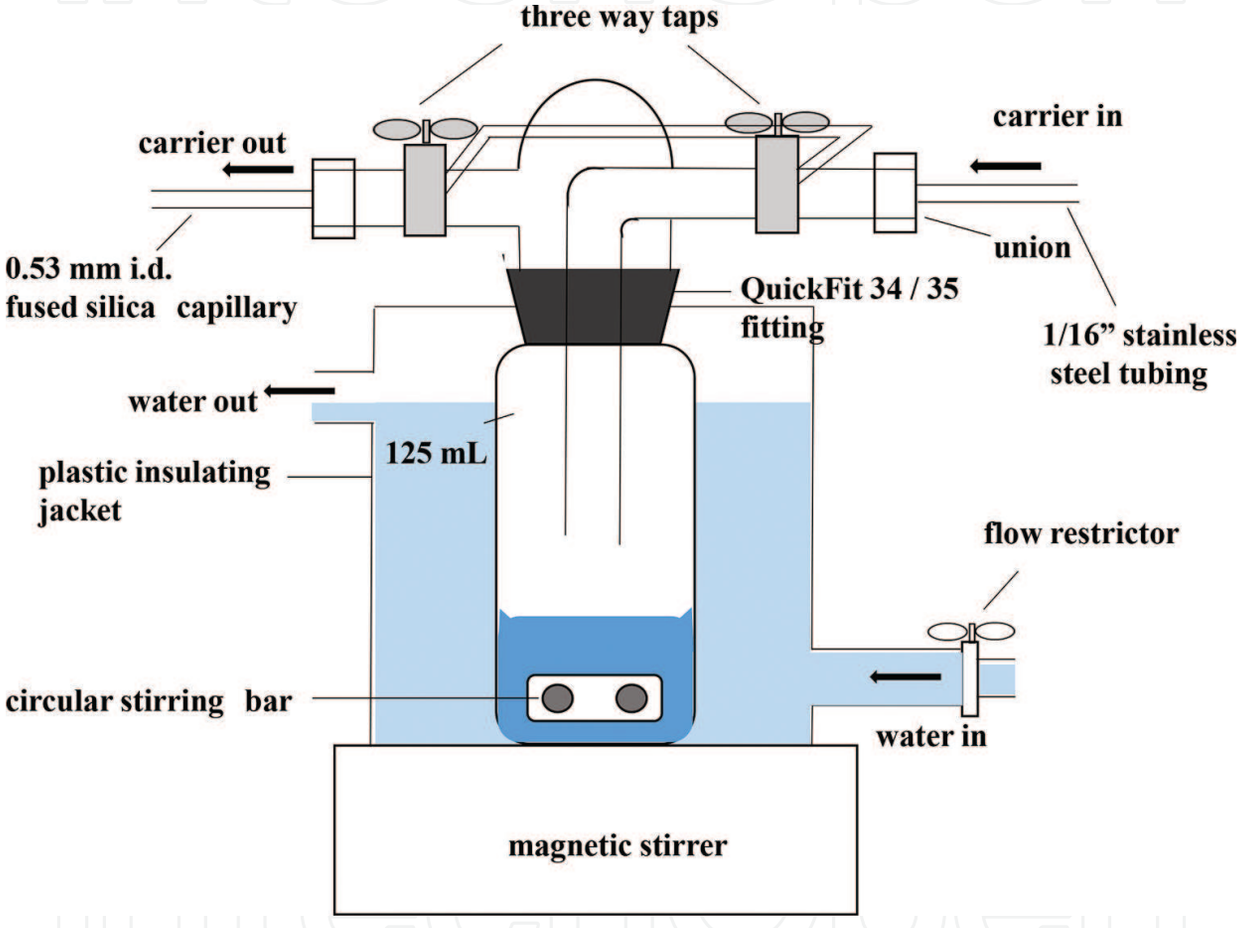

Figure 4. Illustration of a sampling system for the measurement of dynamic flavor release.

Recently, odor imaging sensing with multi-probe film is reported [22]. Odor substances are determined by fluorescent quenching with imaging film and a cooled CCD camera. The system could detect gaseous odor flow and visualize shape, spread, and concentration distribution of odor. A multi-film and FRET probes consisted of a certain combination of fluorescence dyes such as tryptophan and vanillin have been used for high sensitive and selective detection of odor. This approach is expectable in near future for more sensitivity and selectivity.

There are a lots of other analytical methods for the analysis of vanillin, based on spectrophotometric [23, 24], FIA [25], ion selective electrodes [26], flourimetric [27], thin layer chromatog-

raphy [28], GC-MS [20, 29], HPLC [30, 31], and capillary electrophoresis (CE) [32–35]. Because CE is not only fast (usually shorter than 10 min), but also with nano-liter amount of injection volume for samples, it is particularly noticeable. Recently, direct sampling method for CE determination of vanillin in indoor air has been developed [36]. Here, the CE determination of vanillin is discussed in detail.

5.3. CE detection of vanillin in indoor air

Generally, a fused silica capillary is used in CE. When running buffer solution is filled into the capillary, silanol groups (**Figure 5A**) in the surface of capillary dissociate, and inner surface of the capillary is charged negatively (**Figure 5B**). Cations in the running buffer solution are pulled toward the inner surface. As a result, an electric double layer is formed (**Figure 5C**). When an electric voltage is applied across the two ends of the capillary, an electroosmotic flow (EOF) toward cathode arises.

$$EOF = (\varepsilon Z/(4\pi\rho))E \quad (1)$$

where ε, Z, ρ, and E are the dielectric constant, zeta potential, solution viscosity, and electric field, respectively.

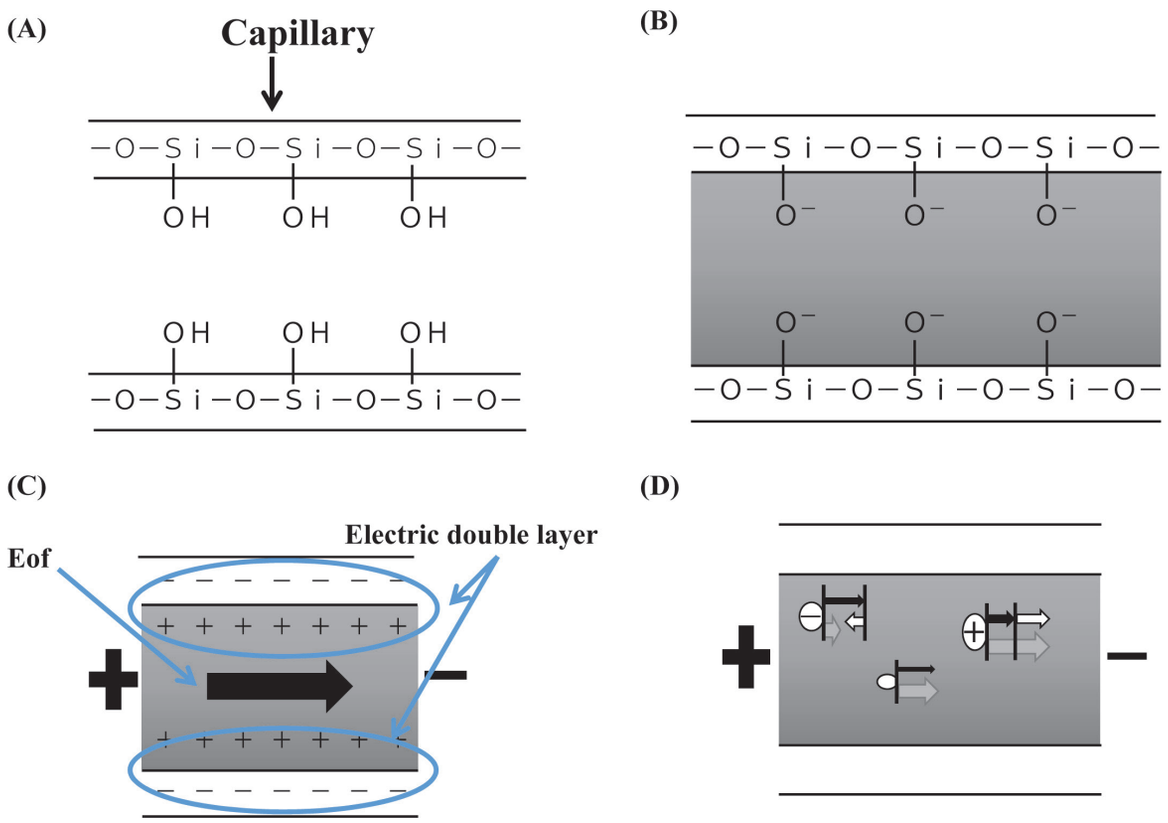

Figure 5. Illustration of molecular structure of the capillary inner surface and principle of CE separation. (A) molecular structure of inner surface of capillary; (B) dissociation of silanol group; (C) formation of electric double layer; (D) electromigration of analytes in CE.

On the other hand, electrophoretic velocity (Vep) of an ion toward the electrode of opposite charge is:

$$Vep = (q/(6\pi\rho r))E \quad (2)$$

where q and r are the electric charge and radius of the ion, respectively. Usually, EOF is larger than Vep for most of ions, and detector is usually close to the cathode end of the capillary. Electromigration velocity of a cation will be (EOF + Vep) while that of an anion (EOF−Vep). Neutral molecule will move with the electroosmotic flow (EOF). Then, cations, anions, and neutral molecules are separated (**Figure 5D**). Because different ions are with different q/r, their Vep is different. Thus, ions can be separated from each other by CE.

CE instrument is relatively simple. Basically, it consists of an electric power supply, a capillary, and a detector. **Figure 6** illustrates a typical laboratory-built CE apparatus [35]. It was consisted of a 30 kV high-voltage power supply and an UV-absorbance detector. Wavelength of the UV-absorbance detector could be set at 254 nm for detection of aromatic compounds. A capillary (inner diameter of 50–100 μm, out diameter of 364 μm) could be used. Its total length and the effective length (length from the anode end to the detector) could be about 30–70 and 20–60 cm, respectively. The capillary was usually cleaned thoroughly by subsequently flushing 1 mol/L NaOH, distilled-deionized water, and finally running buffer. Sample injection could be performed with either an electrophoretic method (e.g., injection voltage 1 kV, injecting time 30 s) or a hydrodynamic flow method with a height difference (e.g., 1 cm) between the two ends of the capillary.

Buffer solutions such as phosphate buffers or boric buffers with certain pH and concentration can be used in CE. Samples are usually dissolved or diluted with the buffers. For example, vanillin stock solution was prepared by dissolving a certain amount of vanillin into distilled-deionized water directly [35]. Its concentration was 10^{-3} mol/L. This stock solution was diluted to required concentrations with the running buffer when used. Vanilla perfume was also diluted with the buffer solutions to concentrations of 1 and 10%. For vanillin spiked vanilla perfume sample, a certain amount of the vanillin standard solution was added into the diluted vanilla perfume sample.

5.3.1. CE of vanillin standard solution [35]

Figure 7 showed the CE results of vanillin at running buffers with different pH. It is well known that the higher the pH of the running buffer, the faster the EOF. Therefore, EOF was the fastest in running buffer of pH 11.5, while slowest in running buffer of pH 7.2. On the other hand, vanillin is a weak acid, and its acid dissociation constant Ka is about $10^{-9.25}$. At pH 11.5, almost all of vanillin molecules behavior as anions, while at pH 7.2 most of them as neutral molecules. At pH 9.3, about half vanillin molecules dissociated to anions.

Therefore, Vep of vanillin was the largest at pH 11.5, slowest at pH 7.2. As a result of V = EOF-Vep, the vanillin was detected at about 500, 450, and 600 s at pH of 11.5, 9.3, and 7.2, respectively.

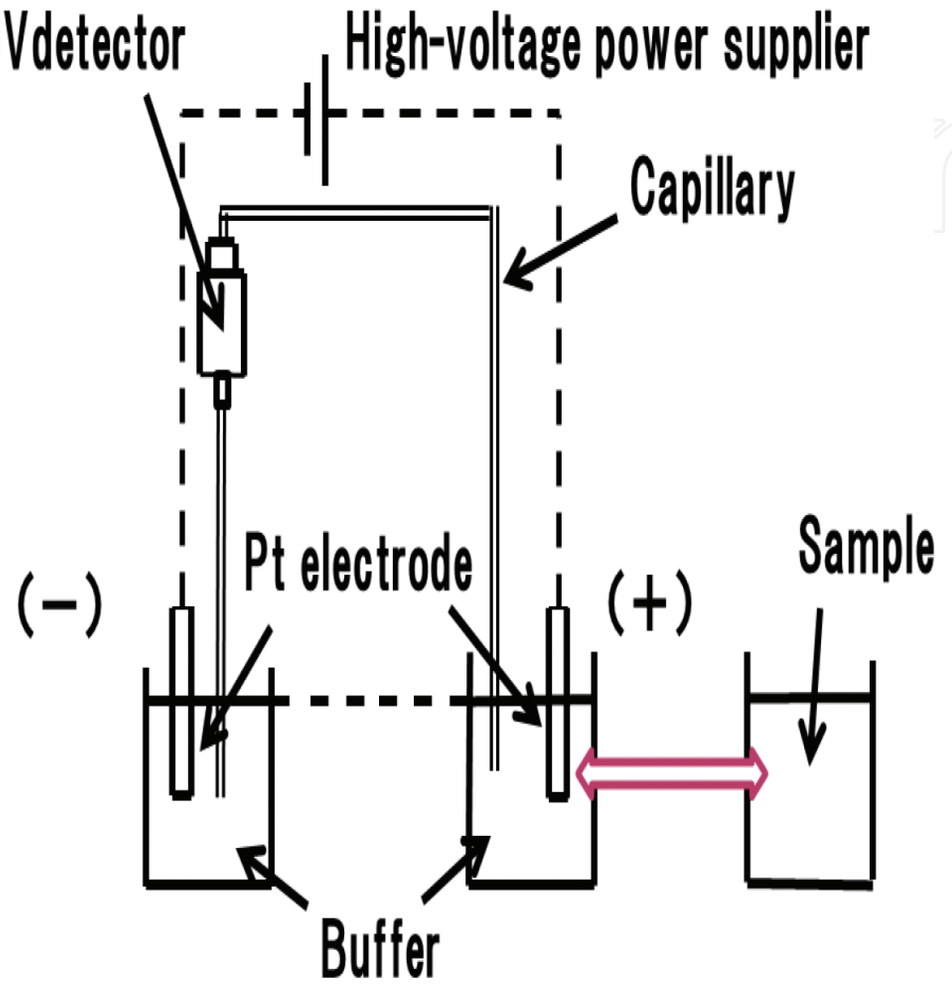

Figure 6. Illustration of a laboratory-built CE instrument.

Figure 8 showed the calibration curve of vanillin at the running buffer of pH 9.3. It can be seen that the peak area is proportional to the vanillin concentration in the range of 10^{-6}–10^{-2} mol/L. The detection limit was about 10^{-6} mol/L.

5.3.2. CE of vanilla perfume [35]

Figure 9 showed electropherogram of 10% vanilla perfume sample solution (A) and 10% vanilla perfume sample solution spiked with 10^{-3} mol/L vanillin standard solution (B). In **Figure 9A**, three peaks were detected. In order to confirm which peak was vanillin, the vanilla perfume sample was spiked with vanillin standard solution. **Figure 9B** showed that only the third peak became large in the spiked sample. Therefore, the third peak was confirmed to be vanillin in the vanilla perfume. Vanillin concentration in the 10% vanilla perfume sample was determined to be about 3×10^{-3} mol/L by a standard addition method. The first and second peaks have not been identified.

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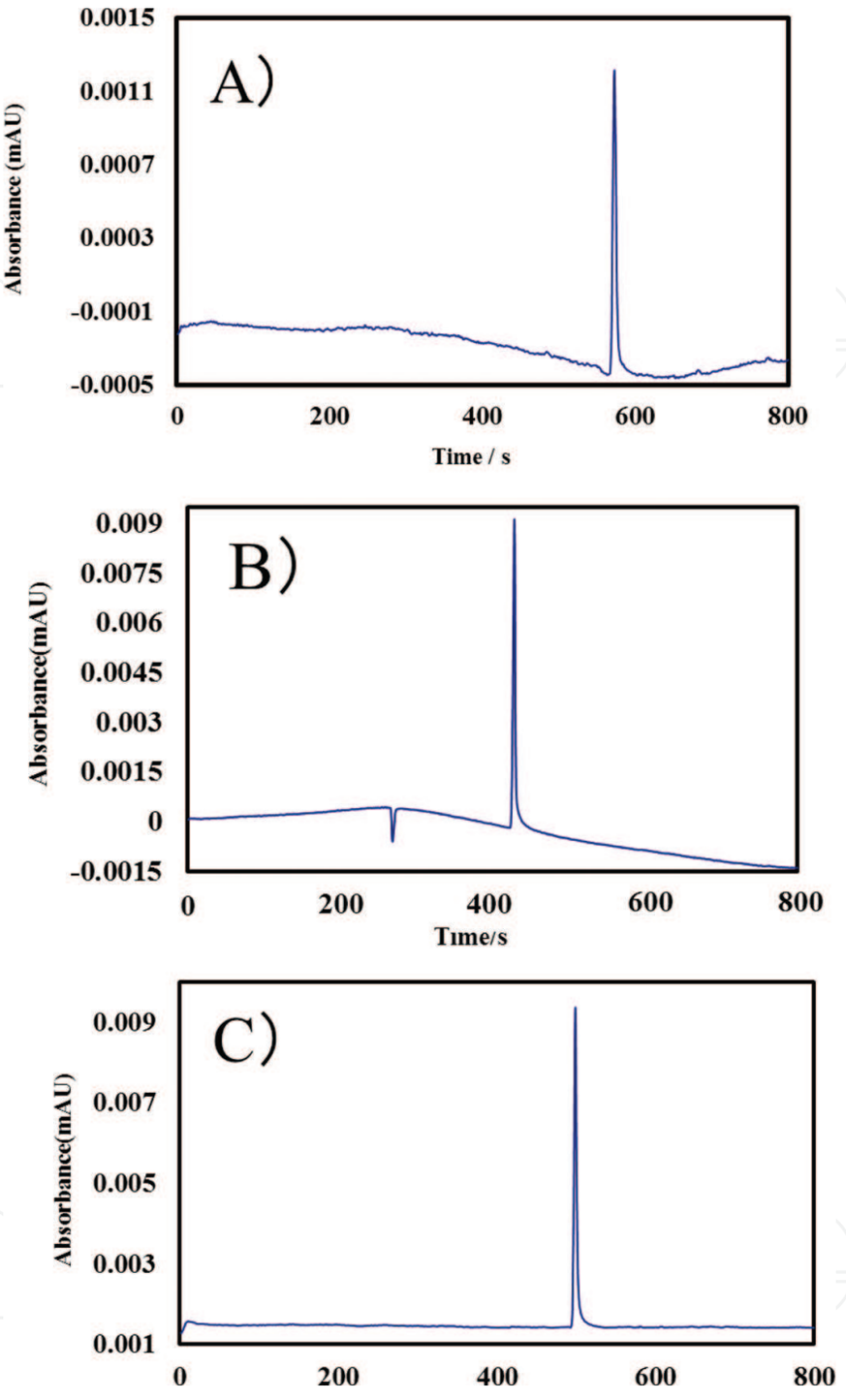

Figure 7. Electropherograms of vanillin standard solution (10^{-4} mol/L) in running buffers of pH 7.2 (A), 9.3 (B), and 11.5 (C).

5.3.3. Detection of vanillin in indoor air by offline combination of CE with absorption and desorption of vanillin with active carbon [35]

In order to detect vanillin in indoor air with CE, about 0.02 g active carbon was spread on a glass slide for adsorption of vanillin in air. The glass slide was placed in a room of about 80 m^2,

where a vanilla perfume of 5 ml was placed too for a certain period of time. Then, the active carbon on the glass slide was collected into a vial, and 0.5 ml ethanol or mixture of ethanol/pH 11.5 running buffer with a mixing ratio of 1:1 was added for desorption of vanillin adsorpted on the active carbon. The vial was centrifuged for 5 min at a centrifugation speed of 3000 rpm. The supernatant was directly injected into the capillary, and CE was carried out. **Figure 11** showed CE results.

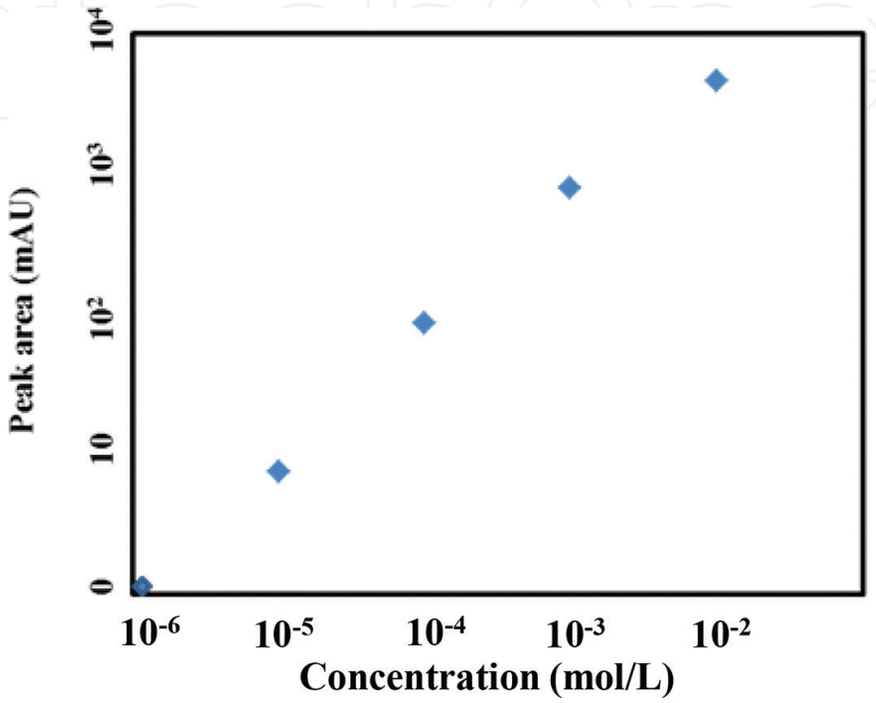

Figure 8. Vanillin calibration curves in running buffers of pH 9.3.

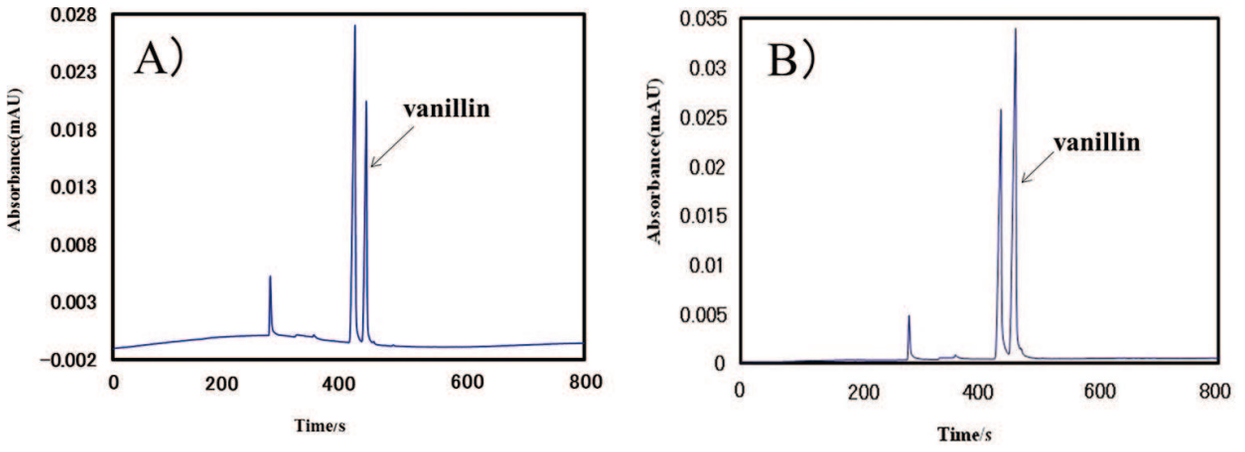

Figure 9. Electropherograms of 10% vanilla perfume sample (A) and the 10% vanilla perfume spiked with 10^{-3} (mol/L) vanillin (B) in the pH 9.3 phosphate buffer.

When the active carbon placed in the room for 2 days, the peak of vanillin was not detectable (top figure in **Figure 10**). When the active carbon placed in the room for 4 days, the vanillin

peak was clearly detected (bottom figure in **Figure 10**). This meant that the CE method could be used for detection of vanillin in air by combination with the active carbon adsorption.

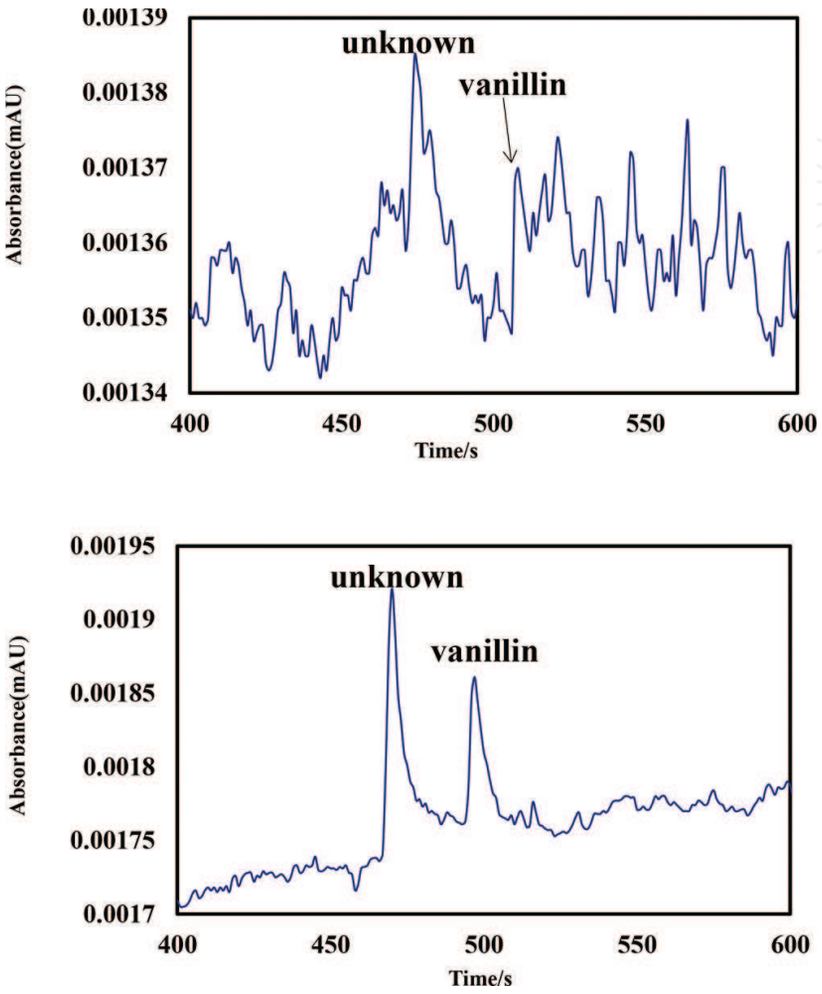

Figure 10. Electropherograms of supernatant of the ethanol/pH 11.5 running buffer after desorption from the active carbons. The active carbons adsorption time was 2 (top) and 4 (bottom) days, respectively.

5.3.4. Fast detection for trace vanillin and vanillin-type aromatic compounds in air by direct sampling [36]

As stated above, CE could detect vanillin in air by offline combining with the adsorption/desorption method. However, it is usually time- and labor-consuming. Recently, a direct sampling of vanillin in the air of CE was demonstrated [36].

As shown in **Figure 11**, the inlet end of a fused silica capillary filled with a pH 7.2 phosphate buffer was directly placed in the air, while the outlet end was immersed into a buffer vial at the low electric potential side. Then, gaseous or volatile components such as vanillin and its derivatives would absorb at the air/buffer interface of the capillary inlet end. That meant a direct sampling of the vanillin in air at capillary inlet end for CE. After a certain period of sampling time, the inlet end was immersed into another buffer vial at the high electric potential

side; CE was carried out by applying a high electric voltage of 20 kV. Evaporated vanillin in indoor air was detected fast.

It was found that the CE peak area increased with the direct sampling time. This was easily understood because the longer the sampling time, the more the vanillin absorbed at the running /air/buffer interface, the larger the peak area. Also, the peak area in the direct sampling-CE was considered to be proportional to the vanillin concentrations in air.

Figure 12 showed results of a conventional CE of 10% vanilla perfume sample (A) and direct sampling-CE in indoor air with a sampling time of 5 min (B), respectively. A conventional CE usually gave two peaks for vanilla perfume sample. The detection time of the two peaks was about 420–600 s. In particular, the second peak was identified to be vanillin [35].

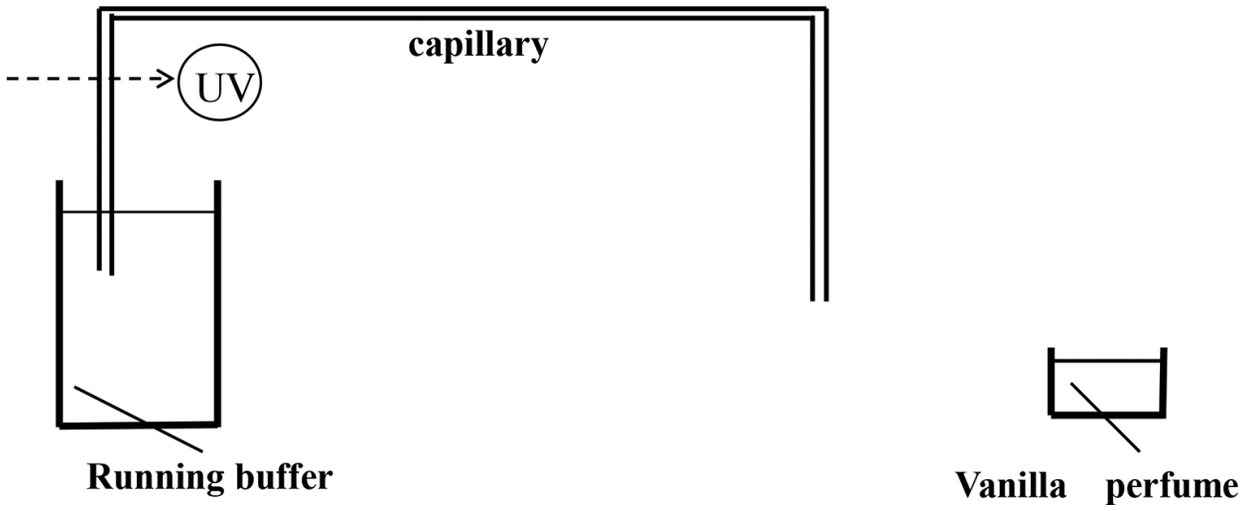

Figure 11. Illustration of the direct sampling of CE in air for vanillin in indoor air.

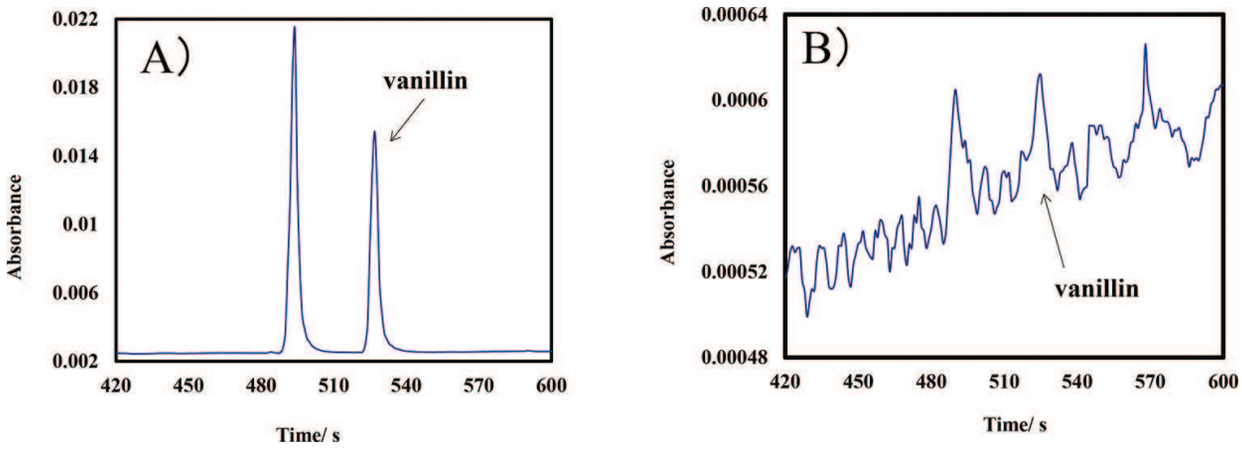

Figure 12. Results of a conventional CE of 10% vanilla perfume solution (A) and direct sampling-CE in indoor air with a sampling time of 5 min (B), respectively.

As shown in **Figure 12B**, there were also two peaks detected in the direct sampling-CE in air, and they were from vanilla perfume. Moreover, they were detected even with a sampling

time as short as 5 min. This sampling time was extremely short in comparison with the offline active carbon adsorption/desorption-CE determinations [35]. In the offline active carbon adsorption/desorption-CE, it took more than 2 days to detect vanillin in indoor air [35]. Therefore, the direct sampling-CE is much faster and simpler than the offline active carbon adsorption/desorption-CE. The direct sampling-CE is promising and expectable in fast gas analysis.

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References

[1] Walton N.J., Mayer M.J., Narbad A.: Phytochemistry. 2003; **63**: 505–515. doi: 10.1002/jps.3030291001

[2] Vanillin: PubChem Open Chemistry Database [Internet] 2004. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/vanillin

[3] Deichmann W., Kitzmiller K.V.: J Am Pharm Assoc (Scientific ed.). 1940; **29**(10): 425–428. doi: 10.1002/jps.3030291001

[4] Kim J.H., et al.: PLoS One. 2014; **9**(2): e89122. doi: 10.1371/journal.pone.0089122

[5] Fitzgerald D.J., Gasson M.J., Narbad A.: J Agric Food Chem. 2005; **53**: 1769–1775. doi: 10.1021/jf048575t

[6] Mourtzinos I., Kalogeropoulos N., Karathanos V.T., Konteles S.: Food Chem. 2009; **114**: 791–797. doi: 10.1016/f.foodchem.2008.10.014

[7] Lee J., Lee K.-W., Song J.-Y., Cho J.Y., et al.: Food Chem Toxicol. 2014; **63** :30–37. doi: 10.1016/j.fct.2013.10.031

[8] Fahlbusch K.-G., et al: Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry, 7th ed. (1999–2015) New York, NY: John Wiley & Sons. Online Posting Date: January 15, 2003.

[9] Pinto P.C.R., Costa C.E., Rodrigues A.E.: Ind Eng Chem Res. 2013; **52**: 4421–4428. doi: 10.1021/ie303349j

[10] Burdock G.A. (ed.): Fenaroli's Handbook of Flavor Ingredients. 6th ed. CRC Press, Taylor & Francis Group, Boca Raton, FL 2010. 2001 p.

[11] Yamada M., Okada Y., Yoshida T., Nagasawa T.: Appl Microbiol Biotechnol. 2007; **73**: 1025–1030. doi: 10.1007/s00253-006-0569-1

[12] Li T., Rosazza J.P.N.: Appl Environ Microbiol. 2000; **66**: 684–687. doi: 10.1128/AEM.66.2.684-687.2000

[13] Robert H. H. van den Heuvel, Marco W. Fraaije, Colja Laane, and Willem J. H. van Berkel: J Agric Food Chem. 2001;**49**: 2954–2958. doi:10.1021/jf010093j

[14] Van A., Chiou K., Ishida H.: Polymer. 2014; **55**: 1443–1451. doi: 10.1016/j.polymer.2014.01.041

[15] Zhang C., Kessler M.R., Madbouly S.A.: Macromol Chem Phys. 2015; **216**: 1816–1822. doi: 10.1002/macp.201500194

[16] Stephensin D., Halpern B.P.: Chem Senses. 2009; **34**: 121–126. doi: 10.1093/chemse/bjn063

[17] Vanillin. Toxicology Data Nerwork [Internet] 1983. Available from http://www.rsc.org/chemistryworld/podcast/CIIEcompounds/transcripts/vanillin.asp

[18] Sostaric T., Boyce M.C., Spickett E.E.: J Agric Food Chem. 2000; **12**: 5802–5807. doi: 10.1021/jf000515+

[19] Goodner K.L., Jella P, Rouseff R.L.: J Agric Food Chem. 2000; **48**: 2882–2886. doi: 10.1021/jf990561d

[20] de Jager L.S., Perfetti G.A., Diachenko G.W.: Food Chem. 2008; **107**: 1701–1709. doi: 10.1016/j.foodchem.2007.09.070

[21] Elmore J.S., Langley K.R.: J Agric Food Chem. 1996; **44**: 3560–3563. doi: 10.1021/jf950687k

[22] Frusawa Y., Yokoyama R., Liu C., Hayashi K.: Inst Electr Eng Japan. 2013; **133**: 199–205. doi: 10.1541/ieejsmas.133.199

[23] Rind F.M.A., Mughal U.R., Memon A.H. et al.: Asian J Chem. 2009; **21**: 2849–2856.

[24] Longares-Patron A., Canizares-Macias M.P.: Talanta. 2006; **69**: 882–887. doi: 10.1016/j.talanta.2005.11.030

[25] Linares P., Luque de Castro M.D., Valcarcel M.: Microchem. J. 1987; **35**: 120–124. doi: 10.1016/0026-265x(87)90206-2

[26] Chan W.H., Lee W.M., Foo C.L., Tang W.K.: Analyst. 1987; **112**: 845–848. doi: 10.1039/AN9871200845

[27] Katayama M., Mukai Y., Taniguchi H.: Anal Sci. 1987; **3**: 369–372. doi: 10.2116/analsci.3.369

[28] Belay M.T., Poole C.F.: Chromatographia. 1993; **37**: 365–373. doi: 10.1007/BF02272250

[29] Gassenmeier K.: Lebensm Wiss Technol. 2003; **36**: 99–103. doi: 10.1016/S0023-6438(02)00183-4

[30] Sinha A.N., Verma S.C., Sharma U.K.: J Separation Sci. 2007; **30**: 15–20. doi: 10.1002/jssc.200600193

[31] Guenther L.: Food Chem. 2009; **114**: 1130–1134. doi: 10.1016/j.foodchem.2008.10.071

[32] Masataka O., Hisako O., Misako H., et al.: J Chromatogr A. 2007; **1138**: 262–267. doi: 10.1016/j.chroma.2006.10.031

[33] Toshiro W., Akira Y., Shiro N., et al.: J Chromatogr A. 1998; **793**: 409–413. doi: 10.1016/S0021-9673(97)00931-X

[34] Jozef L.B.: J Chem Educ. 2005; **82**: 604–606. doi: 10.1021/ed082p604

[35] Minematsu S., Xuan G.S., Wu X.-Z.: J Environ Sci. 2013; **25**: 8–14. doi: 10.1016/S1001-0742(14)60617-3

[36] Minematsu S., Wu X.-Z.: Anal. Sci. 2013; **29**: 373–375. doi: 10.2116/analsci.29.373

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