Colorimetric paper sensor for visual detection of date-rape drug γ-hydroxybutyric acid (GHB)

Seong Uk Son a,b,1, Soojin Jang a,b,1, Byunhoon Kang a,1, Junseok Kim c, Jaewoo Lim a,b, Seunbeom Seo a,c, Taejoon Kang a, Juyeon Jung a,b, Kyu-Sun Lee a, Hyungjun Kim a,d,*, Eun-Kyung Lim a,b,***

a BioNanotechnology Research Center, KRIBB, 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, South Korea
b Department of Nanobiotechnology, KRIBB School of Biotechnology, 217 Gajeong-ro, Yuseong-gu, UST, Daejeon 34113, South Korea
c Research Institute of basic Sciences, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, South Korea
d Department of Cogno-Mechatronics Engineering, Pusan National University, 2 Busandaehak-ro, Gumjeong-gu, Busan 46241, South Korea

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ABSTRACT

Drug-facilitated crimes using date-rape drugs are on the rise. Gamma-hydroxybutyric acid (GHB), a notorious date-rape drug, is colorless, odorless, and tasteless, and therefore difficult to detect in beverages. The ingestion of GHB can severely incapacitate individuals by inducing dizziness, drowsiness, and unconsciousness, making them vulnerable to crimes. To prevent drug-facilitated crimes, a GHB detection kit is developed in which PCDA-gabazine and PCDA, which strongly interact with GHB, are used as sensing materials, while PEO and PVDF are used as matrix materials that enable rapid GHB detection in a short time by increasing the penetration rate of the liquid samples. Detection kits are fabricated by electrospinning a solution containing the sensing materials and matrix materials mixed in an optimal ratio. The color of the kit distinctly changes from blue to red when a drink spiked with GHB is dropped on it (limit of detection: about 0.0096 wt%). The fabricated GHB detection kit is easy to carry and provides a visually distinguishable detection result without requiring any complicated procedure, equipment, or trained personnel. Importantly, the kit can detect GHB in colored and alcoholic beverages. Furthermore, a mobile application is developed that can aid GHB detection using the kit in diverse environments.

1. Introduction

Drug-facilitated crimes are increasing due to the illegal abuse of gamma-hydroxybutyric acid (GHB), a notorious date-rape drug [1–3]. GHB is colorless and odorless, and therefore almost undetectable when mixed with water and beverages [4,5]. The effects of GHB include disinhibition, dizziness, amnesia, and unconsciousness [6]. The ingestion of GHB can severely incapacitate individuals, making them vulnerable to crimes. Because of its use in drug-facilitated sexual assault (DFSA), GHB has attracted the attention of both the media and the scientific community. Since GHB is an endogenous neurotransmitter that is naturally present in the mammalian brain, its detection is difficult as it is rapidly eliminated from the body (plasma half-life: 20–45 min) after oral intake [7]. Research is being conducted to detect GHB metabolites based on NMR [8,9], however it is difficult to prove that GHB was illegally used and that a crime was committed. Although much effort has been devoted to the development of methodologies for GHB detection in forensic samples, these methods need to be performed in the laboratory and require trained personnel and multiple steps with dedicated analytical devices [5,10–16]. Hence, current research is focused on the pharmacokinetic analysis and detectability of GHB after ingestion. Therefore, to prevent DFSA, the demand for simple, easy-to-use sensors capable of detecting GHB in beverages has increased [4]. In this study, we developed a colorimetric paper sensor that can detect the presence of GHB in beverages with a color change from blue to red (Fig. 1).
2. Experimental section

2.1. Materials

10,12-Pentacosadiynoic acid (PCDA) was purchased from Alfa Aesar (Haverhill, MA, USA). N-hydroxysuccinimide (NHS), ethylenediamine, trimethylamine, dichloromethane (DCM) and dimethylformamide (DMF) were purchased from Sigma Aldrich (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased form Thermo Fisher Scientific (Waltham, MA, USA). Gabazine (purity > 97%) was synthesized at the 4-Chem Laboratory (Suwon, Gyeonggi-do, Korea) according to reference [17]. GHB was legally purchased from Sam Eung Industrial Co., LTD.

2.2. Synthesis of the PDCA-NHS

PCDA (2.67 mmol, 1 g) and EDC (4 mmol, 620 mg) were dissolved in 50 mL of DCM. To this solution, NHS (4 mmol, 460 mg) was added, and the resulting solution was stirred for 8 h at room temperature. After completion of reaction, the organic solvent was removed in vacuo. The crude product was purified by pouring into distilled water and extracting with ethyl acetate three times. The organic solvent was then dried over anhydrous magnesium sulfate and concentrated in vacuo. The resulting solution was stirred overnight at room temperature. After completion of reaction, the organic solvent was separated by filtration, and the crude product was purified by pouring into distilled water and extracting with DCM three times. The organic solvent was then dried over anhydrous magnesium sulfate and concentrated in vacuo. The resulting light-blue powder was analyzed by TLC (methanol/ethyl acetate, 3:7), and its chemical structure was determined by $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) spectroscopy (Fig. S3d and S4d). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 0.88(t,3H), 1.26–1.63(m,32H), 2.18(t,2H), 2.24 (t,4H), 2.83(m,2H), 3.30 (q,2H), 5.87(brs,1H). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 14.13, 19.22, 22.70, 25.76, 28.31, 28.38, 28.77, 28.88, 28.93, 29.12, 29.18, 29.26, 29.36, 29.49, 29.64, 31.93, 36.86, 41.45, 41.91, 66.25, 77.63, 169.15.

2.3. Synthesis of the PCDA-NHS

PCDA-NHS (1 mmol, 442 mg) was then dissolved in 20 mL of DMF followed by the addition of EDC (2.4 mmol, 372 mg) and NHS (2.4 mmol, 276 mg). This solution was stirred for 8 h at room temperature. Then, PCDA-NHS (1 mmol, 419 mg) was added to this reactant solution and stirred overnight at room temperature. The obtained powder was purified by extracting with ethyl acetate and removing DMF using distilled water three times. Then, the organic solvent was dried over anhydrous magnesium sulfate and concentrated in vacuo. The resulting light-blue powder was analyzed by TLC (methanol/ethyl acetate, 3:7), and its chemical structure was determined by $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) spectroscopy (Fig. S3e and S4e). $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 2.07(m,2H), 2.43(t,2H), 3.84(s,3H), 4.34(t,2H), 7.11 (d,2H), 7.61(d,1H), 7.94(d,2H), 8.38(d,1H), 8.98(brs,2H). $^{13}$C NMR (150 MHz, DMSO-d$_6$): $\delta$ 22.18, 30.67, 55.56, 55.95, 115.13, 125.57, 126.18, 128.54, 131.53, 149.77, 152.51, 161.91, 174.18. Gabazine (1.2 mmol, 442 mg) was then dissolved in 20 mL of DCM solution containing ethylenediamine (50 mmol, 3 g), and the crude product was purified by pouring into distilled water and extracting with DCM three times. The organic solvent was then dried over anhydrous magnesium sulfate and concentrated in vacuo. The resulting light-blue powder was analyzed by TLC (methanol/ethyl acetate, 3:7), and its chemical structure was determined by $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) spectroscopy (Fig. S3f and S4f). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 0.88(t,3H), 1.26–1.63(m,32H), 2.18(t,2H), 2.24 (t,4H), 2.83(m,2H), 3.30 (q,2H), 5.87(brs,1H). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 14.18, 19.19, 21.02, 22.67, 25.57, 28.79, 28.90, 29.11, 29.35, 29.49, 29.62, 29.66, 30.94, 31.93, 65.24, 65.33, 77.44, 77.62, 169.15.

2.4. Synthesis of the PCDA-gabazine

First, gabazine was synthesized according to a method reported in literature and its chemical structure was determined by $^1$H NMR and $^{13}$C NMR spectroscopy (Fig. S1, S3d and S4d). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 0.88(t,3H), 1.26–1.63(m,32H), 2.18–2.63(m,32H), 2.64–2.67(t,2H), 2.68–2.71(t,4H). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 14.13, 19.22, 22.70, 25.76, 28.31, 28.38, 28.77, 28.88, 28.93, 29.12, 29.18, 29.26, 29.36, 29.49, 29.64, 31.93, 36.86, 41.45, 41.91, 66.25, 77.63, 169.15.

2.5. Preparation of GHB detection kit

Paper-type GHB detection kits were fabricated by electrospinning (Fig. S5) [20–26]. For the matrix layer, a solution of PVDF and PEO was prepared in NMP. The chemical structure of PVDF and PEO were analyzed by $^1$H NMR and $^{13}$C NMR spectroscopy, respectively (Fig. S5).
For the sensing layer, PCDA and PCDA-Gabazine (or gabazine) were dissolved in acetone.

<table>
<thead>
<tr>
<th>GHB detection kit</th>
<th>Matrix solution</th>
<th>Sensing solution</th>
</tr>
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<tbody>
<tr>
<td>(i)</td>
<td>PVDF 600 mg</td>
<td>PCDA 11.25 mg</td>
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<tr>
<td></td>
<td></td>
<td>30 μmole</td>
</tr>
<tr>
<td></td>
<td>PEO 300 mg</td>
<td>Gabazine 11.25 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 μmole</td>
</tr>
<tr>
<td></td>
<td>NMP 1.28 mL</td>
<td>Acetone 5.7 mL</td>
</tr>
<tr>
<td>(ii)</td>
<td>PVDF 810 mg</td>
<td>PCDA 11.25 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 μmole</td>
</tr>
<tr>
<td></td>
<td>PEO 90 mg</td>
<td>PCDA-Gabazine 11.05 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 μmole</td>
</tr>
<tr>
<td></td>
<td>NMP 1.65 mL</td>
<td>Acetone 6.2 mL</td>
</tr>
</tbody>
</table>

Then, the two solutions were uniformly mixed at 40 °C. The mixture was then loaded into a 15 mL syringe capped with a 22G blunt metal needle and electrosprun for 2 h at 3 mL/h and 20 kV using an electrospinning system (ESR200RD, NanoNC Co. Ltd., Korea). The electrosput nanofibers were collected on an aluminum foil-coated rotating drum to obtain the paper-type GHB sensor. The prepared paper sensor was completely dried for more than 6 h to evaporate the solvents and stored at room temperature in the dark before use. The paper sensor was then cut into specific sizes and irradiated by a UV light at 254 nm for 1 min, which resulted in a change in the color of the paper sensor from white to blue due to the polymerization of PCDA (Fig. S5).

2.6. GHB detection test

To legally obtain GHB, permission for drug handling was first obtained from the Korea Food & Drug Administration (Permission No. 443). For the GHB detection test, GHB was dissolved in distilled water at a high concentration (25 wt% = 250 mg/mL). Since there is no known accurate concentration information for illegal GHB use, we set the screening concentrations based on the references [10–16]. It has reported that 150 pounds (≈ 68. 0 kg) of women consuming more than 2.5 g of GHB can be dangerous to the body. Based on them, we determined the tests concentration range (≈ 5 wt%) and dilute GHB solution with drinking waters to prepare solutions of different GHB concentrations. After that, each GHB solution (10 μL) was dropped on the GHB detection kit. The 0% GHB solution indicates pure water without GHB and was used as a control. The intensity of the red color of the GHB detection kit before and after GHB detection was analyzed by Image J software. Furthermore, to visually check the presence of GHB in beverages, beverages containing 4 wt% (≈ 40 μg/mL) GHB were prepared by mixing GHB with various beverages (water, sports drink, soda, Yakult, coffee, soju, beer, cognac, and wine). All tests were performed three or more times. Furthermore, a mobile app was developed using the MIT app inventor program for a more accurate detection of GHB in beverages using the GHB detection kit [26].

2.7. Quantum chemical simulation

Quantum chemical simulations were performed to understand the interaction between PCDA and GHB. Also, the difference in interaction between GHB and the two detection kits (GHB detection kits), PCDA with free gabazine (i) and PCDA-Gabazine (ii), was computed and analyzed. As it is impossible to use nearly infinite chains of polymer structures in quantum chemical simulations, we truncated PCDA and PCDA-Gabazine to different degree depending on the purpose of simulations. As the color change is widely known to occur due to the twist in the PCDA backbone [21,27,28], the model structures should contain more than three repeating units. Therefore, we used the minimal model consisting of four repeating units. Preliminary geometry optimizations with four repeating units resulted in artificial bending of both ends, which is quite different from known planar structures. This phenomena was not cured by increasing the number of repeating units. We used reasonable tricks to obtain planar four-repeating-unit model: optimize the six-repeating-unit model, and truncate both ends, and fix the position of hydrogen atoms of carboxylic acid groups. This approach was expected to overcome the limitations of truncated models. The long alkyl chains (C11) attached to the PCDA backbone were removed, and octyl groups where the carboxyl acid group is attached are shorten to propyl groups. Meanwhile, the shorter model consisting of two repeating units was selected for the interaction analysis. For this simulation, only the C11 alkyl chains were simplified to propyl groups. All truncated model structures have essential features to mimic real molecules, and such modification would not cause severe loss in accuracy. Also, there could be a number of different local minima for the complex bimolecular systems during interaction analysis, and the results of geometry optimization strongly depend on the initial structures. To overcome this limitation, we systematically varied initial positions of the two molecules (the analyte and the detection kit) and sampled unique final geometries. Density functional theory (DFT) employing the B3LYP functional [29–31] with 3–21G(d) [32–34] basis sets was used to optimize the ground state geometries and estimate the electronic energies and analyze the torsion angle for the σ-conjugated geometry. Dispersion interaction was included via Grimme’s empirical correction [35].

2.8. Results and discussion

3.1. Characterization of PCDA-gabazine

PCDA, a monomer of polydiacetylene (PDA) with intrinsic optical properties, was used as the colorimetric sensing material, and gabazine, which has a high binding affinity for GHB (Kd = 16 μM), was introduced into the PCDA backbone as a chemical receptor (Fig. S3) [18,19]. A paper-type colorimetric sensor (colorimetric paper sensor) that is easy to carry and use anytime and anywhere was produced by electrospinning these materials [20–25]. To prepare PCDA-Gabazine conjugates, PCDA-NHS was synthesized according to our previously reported method [26]. Then, ethylenediamine was bound to the NHS group of PCDA-NHS to synthesize PCDA-NH2, and its chemical structure was confirmed by NMR spectroscopy. Especially, in the 1H NMR spectra, PCDA exhibited carboxylic acid (−COOH) peak at 10.59 ppm (Fig. S3a), whereas PCDA-NHS and PCDA-NH2 did not show this peak (Fig. S3b and c). However, a new peak at 5.87 ppm corresponding to the in secondary amide (−CO-NH−) for PCDA-NH2 (Fig. S3c). PCDA-Gabazine was synthesized by chemically conjugating gabazine to PCDA-NH2 whose chemical structure was also confirmed (Fig. S3d–e). Particularly, the chemical structure indicating the aromatic ring of gabazine in PCDA-Gabazine was clearly confirmed by NMR spectroscopy (Fig. S3 and S4). In detail, peaks corresponding to methylene (−CH3) and benzylidenimin (−CH−) in aromatic ring of gabazine were observed at 3.81 and 7.5 ~ 8.0 ppm, respectively.

3.2. Fabrication of GHB detection kit

GHB detection kit was fabricated by electrospinning PCDA-Gabazine (Fig. S6). The synthesis method not only improves the detection sensitivity by increasing the contact area between GHB and the colorimetric sensing materials (PCDA-Gabazine and PCDA), but also enables mass production. PCDA mixtures containing PCDA and PCDA-Gabazine were prepared as colorimetric sensing materials, while PEO and PVDF
mixtures were prepared as matrix materials. The optimal ratio of hydrophilic PEO to hydrophobic PVDF in the matrix material allowed the rapid detection of GHB (within seconds). At a high proportion of hydrophilic PEO, the kit dissolves like tissue paper in the liquid sample, whereas at a high proportion of hydrophobic PVDF, the sample does not penetrate the kit. The two mixtures were uniformly mixed, and the mixed solution was electrospun to form a PCDA-Gabazine nanofiber-embedded sheet (Fig. S7). With complete solvent evaporation during the fabrication process, uniform nanofibers were formed and stacked to produce a white sheet. PCDA and PCDA-Gabazine were photo-polymerized by UV irradiation, and the white sheet turned blue. Thus, GHB detection kits were prepared. As a control, only gabazine was used instead of PCDA-Gabazine.

3.3. GHB detection using GHB detection kits

First, we tested the detection ability of the GHB detection kits using solution of different GHB concentrations. Additionally, to determine the appropriate composition of the GHB detection kit for beverage testing, we analyzed the interaction between the molecules by quantum chemical simulations. Here, the important thing was whether a distinct color change was shown in drinks containing GHB. As previously mentioned, GHB and free gabazine interact strongly; their interaction energies ranged from 41.2 kcal/mol to 60.5 kcal/mol depending on the orientations, as predicted by quantum chemical simulation (Fig. S8).

Based on this, we fabricated GHB detection kit (i) containing PCDA and free gabazine. In GHB detection kit (i), red spots appeared when solutions with GHB concentrations higher than 1 wt% were dropped (Fig. 2). The intensity of the red color increased with increasing GHB concentration. The color change is presumed to occur due to the twisting of the $\pi$ conjugation in the PCDA backbone by interaction with GHB. Fig. S9 shows the PCDA backbone remains planar and all torsion angles are very close to $180^\circ$. When one GHB molecule is introduced, the planarity of PCDA backbone is severely broken. Torsion angle changes significantly at the edges ($-178^\circ$ to $-143^\circ$, $-179^\circ$ to $-156^\circ$), and this would cause the torsion of $\pi$ conjugated chain. TDDFT calculation predicted the first allowed singlet excited state is blue shifted by 0.14 eV due to the presence of GHB (from 3.13 eV to 3.27 eV, Fig. S9c). From this simulation, the increasing red intensity with GHB concentration could be understood based on the modulation of $\pi$ conjugation by GHB.

In Fig. 2 (a), the little color change even in the absence of GHB suggests that there is slight structural change in PCDA due to gabazine or water in kit (i), but the degree of color change by these molecule is weaker than that of GHB. Having confirmed the color change mechanism of PCDA, we examined the three intermolecular interactions present in the GHB detection kit (i): As shown in Fig. 3, both interaction energy of Gabazine⋯PCDA (max.: 72.3 kcal/mol) and interaction energy of Gabazine⋯GHB (max.: 60.5 kcal/mol) are much stronger than the largest interaction energy of GHB⋯PCDA (52.2 kcal/mol). It can be seen that the interaction of gabazine and GHB, and the interaction of gabazine and PCDA are much more preferred to the interaction of GHB and PCDA. In the case of the kit (i) containing free gabazine, it can be expected that interaction of GHB and PCDA, which affects a large color change, is not preferred due to the strong interaction of gabazine with GHB and PCDA. Quantum chemical simulations confirmed that the color change occurs due to interaction of GHB and PCDA, and could explain low GHB detection performance of the kit (i) with the presence of free gabazine. However, the performance of GHB detection kit (ii) is noteworthy. As shown in Fig. 2(b), when a GHB solution was dropped on GHB detection kit (ii), the color change was instantaneous regardless of the GHB
concentration. Moreover, its red intensity was significantly increased compared to that of the GHB detection kit (i). As revealed by the computation, GHB favors interaction with PCDA-Gabazine than unfunctionalized PCDA (52.2 kcal/mol vs 66.8 kcal/mol) (Fig. 3). In the absence of GHB (0%), the color change is rarely observed, which implies that gabazine covalently bonded to PCDA has no direct interaction with the backbone of PCDA. In other words, unlike the free gabazine of kit (i), gabazine covalently bound to PCDA in kit (ii) does not compete with GHB. In addition, GHB which is more strongly bound to PCDA-Gabazine, is expected to alter $\pi$ conjugation and electronic structure of PCDA backbone more effectively. And, this is affected by the hydrogen bonding between GHB and the gabazine pendant groups, and the dispersion interaction between the GHB carbon chains and the PCDA side chains, resulting in a reduction in the stability of the sensing material (PCDA-Gabazine and PCDA) backbone perturbed by GHB binding [18]. GHB detection kit (i) was unstable in detection of low concentrations of GHB due to the combination of gabazine and PCDA, as mentioned above. However, GHB detection kit (ii) was confirmed to

Fig. 3. (a) Three representative chemical structures of each interaction (Gabazine-PCDA, GHB-PCDA, and GHB-PCDA-Gabazine), and (b) the corresponding interaction energies. Small molecules (Gabazine and GHB) are highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Fig. 4. Detection of GHB in various drinks using GHB detection kit (ii). Color transition images of GHB detection kit (ii) after dropping drinks spiked with GHB on the kit. GHB-free drinks were used as controls.

Fig. 5. GHB detection procedure using the GHB detection kit and the developed mobile app. Each panel shows the mobile phone screen: (a) start-up screen, (b) background setting of GHB detection kit before dropping the drug (or drink), (c) screen after background setting, and (d) analysis process after dropping the drug (or drink). When the actual app is run, the finger image is not visible on the screen.
have a detection limit (limit of detection: LoD) of about 0.0096 wt% (≈ 0.096 μg/L, 9.6 μg/mL) (Fig. S10).

3.4. Colorimetric detection of GHB using GHB detection kits

Based on the results, we used GHB detection kit (ii) for further experiments. As previously mentioned, GHB is colorless, odorless, and tasteless, and therefore difficult to detect. Moreover, when mixed with colored non-alcoholic or alcoholic beverages, the drug is even more difficult to notice. Therefore, we examined the detection ability of GHB detection kit (ii) using various alcoholic and non-alcoholic drinks. Five types of non-alcoholic drinks (water, sports drink, soda, Yakult, and coffee) and four types of alcoholic beverages (soju, beer, cognac, and wine) were used in the test. It should be noted that GHB naturally occurs in red wine by the fermentation of red grapes (4.1–2.14 mg/L), and that unintended color changes could occur due to the presence of various additives in the drinks [37]. As shown in Fig. 4, no color change occurred when pure drinks were dropped on GHB detection kit (ii). However, the color of GHB detection kit (ii) changed from blue to red immediately after GHB-containing drinks were dropped, regardless of the type of drink (Fig. 4). In addition, the color changes were distinct and can be distinguished by the naked eye without the need of sophisticated analytical equipment. With time, the red intensity increased and the color change became more distinguishable. Thus, GHB detection kit (ii) enables the facile and visual detection of GHB in both alcoholic and non-alcoholic drinks. People have different sense of color, and their surrounding environments can be different during the test. Moreover, date-rape drugs, such as GHB, are more likely to be given in dark environments; hence, the perception of the result may differ depending on the situation. To solve this problem, we additionally developed a mobile application (app) that can be used as an auxiliary means to confirm the sensitivity and reliability of the result. The app can provide detection information by analyzing the change in the red intensity of the GHB detection kit before and after dropping a drink. In the first step, after starting this program (app.), the detection kit is photographed before dropping (press "Take a picture" button) (Step1, Fig. 5(a)) and touched a "Detection point" (the center of the detection area) (Step 2, Fig. 5(b)). If you do not touch the blue colored point correctly, the NEXT button will not be activated. After that, the "NEXT" button press to set this kit’s background. After dropping the sample on kit, take a picture of this kit again (Step 3, Fig. 5(c)) and touch the detection point to analyze it (Step 4, Fig. 5(d))[26]. If the drink contains GHB, a “Danger” sign appears in red in the app; otherwise, a “Safety” sign appears (Fig. 5).

4. Conclusions

In conclusion, we developed a kit to detect GHB, a date-rape drug, easily and quickly. The GHB detection kit allows the visual detection of GHB in drinks through a color change from blue to red immediately after GHB-containing beverages are dropped on the kit. The color change occurs because of the strong interaction between GHB and the sensing materials (PCDA-Gabazine). Even alcoholic drinks containing GHB can provide a visual indication through color change. Our kit can help protecting people against drug-facilitated crimes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2021.130598.

References


