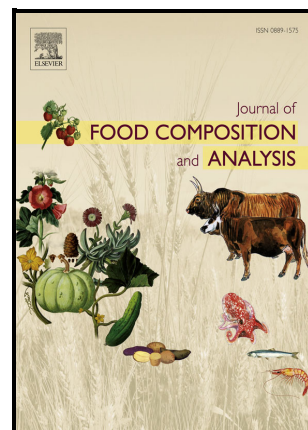


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Carbon nanodots derived from glycine for rapid detection of caffeine in beverages

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Abstracts

Caffeine, a widely consumed stimulant, requires precise monitoring in food and beverages to ensure consumer safety. However, conventional detection methods, such as high-performance liquid chromatography (HPLC), are time-consuming, costly, and equipment-intensive. To overcome these limitations, we present a novel fluorescent sensing platform based on glycine-derived carbon nanodots (Gly-CNDs) for rapid and cost-effective caffeine detection. Unlike existing sensors, our food-grade Gly-CNDs offer enhanced biocompatibility, stability, and selectivity while eliminating the need for complex synthesis or toxic reagents. The synthesized Gly-CNDs, with an average size of 4.2 ± 1.2 nm, exhibited a significant fluorescence enhancement upon caffeine binding, enabling highly sensitive detection within 39–2060 nM range with a low limit of detection (LOD) of 36.5 nM. Tested with beverage samples, this green method offers a promising alternative for monitoring caffeine in foods and could be applied to detect other contaminants and bioactive compounds.

Keywords: carbon dots; green synthesis; glycine-derived carbon dots; caffeine detection; fluorescence sensing; food analysis.

1-Introduction

Caffeine, 1,3,7-Trimethylpurine-2,6-dione, a naturally occurring purine alkaloid, is one of the most widely consumed psychoactive substances globally [1]. Estimates suggest that over 80% of the world's population consumes caffeine daily, primarily through beverages like coffee, tea, and energy drinks [1]. This popularity stems from caffeine's stimulating effects on the central nervous system, leading to increased alertness, improved cognitive function, enhanced physical performance [2], and reduced cancer risk [3]. Despite these health benefits of caffeine, consuming high amounts of caffeine can lead to acute symptoms like hypokalemia, vomiting, nausea, palpitations, seizures, and dysrhythmia [4]. In addition to coffee overdose, coffee adulteration is another problem that needs attention. Coffee adulteration, a widespread issue in the industry, involves mixing low-value materials into roasted and ground coffee to reduce costs [5]. Common adulterants include spent coffee grounds, cereals, legumes, and coffee husks [6]. This highlights the need for rapid caffeine detection, as it indicates adulteration and helps prevent

caffeine overdose. Hence, developing accurate and efficient methods for quantifying caffeine content is essential.

Traditional methods for detecting caffeine typically rely on chromatographic methods such as high-performance liquid chromatography (HPLC) [7], gas chromatography-mass spectrometry (GC-MS), or thin-layer chromatography (TLC) [8]. While HPLC offers high accuracy and sensitivity, it requires expensive equipment, trained personnel, and relatively lengthy analysis times. This can be a significant disadvantage in situations demanding rapid on-site detection or real-time monitoring of caffeine content. Recently, various methods have been developed for detecting caffeine, such as electrochemical sensors [9,10], fluorescent sensors [11] and mass spectrometry [12]. While several sensors, including Cu-MOF/graphene electrochemical sensors [9] and BODIPY-based fluorescent sensors [11], have been developed for caffeine detection in beverages, they frequently necessitate complex synthesis, expensive materials, or extensive sample preparation. Consequently, a rapid, cost-effective, and eco-friendly carbon dot-based detection method is greatly needed.

Carbon nanodots (CNDs) have recently garnered attention as promising candidates for sensing applications due to their unique optical properties, including intense fluorescence, high photostability, and biocompatibility [13]. Due to its strong fluorescence and ability to quench and enhance the fluorescence with a wide range of components, numerous studies were focused on developing CND-based sensors to monitor food components [14,15] and detect food contaminants [16,17]. Various synthesis methods have been employed to produce CNDs, including hydrothermal treatment [18], plasma, electrochemical oxidation, and thermal decomposition [19]. In our previous publication [20], the safety of using CNDs was still controversial and necessitates further research. Prioritizing the selection of CNDs from safe sources is essential to mitigate potential risks. The majority of CNDs detected in foods have been attributed to the formation of Maillard reactions like bread [21], caramelized sugar [21], coffee [22,23], roasted chicken breast [24], and roasted salmon [25]. Therefore, glycine-sugars, which serve as the primary model of the Maillard reaction in foods, were selected as precursors to synthesize safe CNDs for food applications. Furthermore, among these sources, glycine and sugars present an attractive, sustainable, and cost-effective precursor for CND synthesis [26].

In this study, a rapid method to detect and measure caffeine in beverages was developed with focus on the following topics: (1) CNDs were derived from glycine and characterized in size, morphology, and optical properties. (2) The properties of carbon nanodots derived from glycine (Gly-CNDs) were assessed under different environmental conditions, including varying NaCl concentrations, pH levels, and over time. (3) The quantitative methods were developed to detect caffeine with Gly-CNDs. (4) Finally, the accuracy and reliability of the proposed method were validated by detecting caffeine in actual beverage samples and comparing the results with traditional HPLC measurements. The findings from this study highlight the potential of using Gly-CNDs as a rapid, efficient, and cost-effective tool for rapid caffeine detection, which could significantly benefit quality control processes in the food and beverage industry. This work not only provides a novel application for Gly-CNDs but also contributes to the broader field of nanotechnology-based sensing methods for food safety and quality assurance.

2-Material and Methods

2.1. Materials

Coffee beans (*Coffea arabica* L.) originated from Peru and were purchased from ALDI supermarket in Essen, Germany (roasting level 8/10). Three types of energy drinks were bought to test the caffeine in actual samples from different companies, including HELL ENERGY Magyarország Ltd., Hungary; XIXO Company Ltd., Hungary; Hansen Natural Company, USA. Ultrapure water was used for all experiments. VWR International (Pennsylvania, USA) supplied all chemicals, including 0.01 mol/L hydrochloric acid solution, $\geq 99.5\%$ ethanol solution, 0.01 mol/L sodium hydroxide solution, and $\geq 99.5\%$ HPLC-grade acetonitrile. Caffeine standard (analytical grade) was bought from Sigma-Aldrich company in Missouri, USA.

2.2. Experimental design

Figure 1 illustrates the experimental design to develop a method for rapidly detecting caffeine content in beverages using carbon nanodots derived from glycine (Gly-CNDs). (1) First, the Gly-CNDs were synthesized and characterized using a transmittance electron microscopy (TEM), fluorescence spectrophotometer, and UV-Vis spectrophotometer. The stability of Gly-CNDs in different conditions was tested, including NaCl concentrations ranging from 0.1M to 1M, pH conditions from 3.0 to 11.0, and stability over time. (2) Gly-CNDs were used to develop a method for detecting caffeine content using a fluorescence spectrophotometer. The caffeine standard was added into Gly-CNDs from 0 to 7500 μM . (3) the selectivity of the proposed method was assessed along with the stability of Gly-CNDs in the presence of caffeine. (4) Finally, this method was used to measure the caffeine content in energy drinks and then compared with the results of the HPLC method.

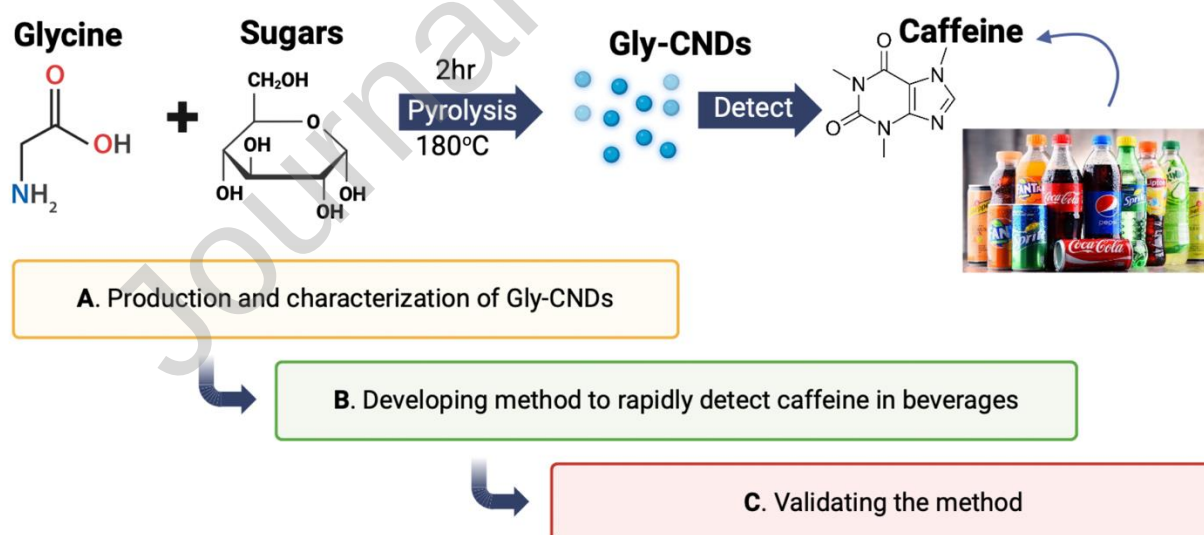


Figure 1. Schematic diagram of the experimental design for developing methods to rapidly detect caffeine in foods using glycine-based carbon nanodots (Gly-CNDs). The design includes three main stages: **A.** Production and characterization of Gly-CNDs, **B.** Development of a method to rapidly detect caffeine in beverages using Gly-CNDs, and **C.** Validation of the proposed methods.

2.3. Production and characterization of carbon nanodots derived from glycine (Gly-CNDs)

Gly-CNDs were synthesized by mixing glycine and dextrose in a 4:1 ratio. The mixture was then subjected to pyrolysis at 180°C for 2 hours in an oven, ensuring the reaction occurred in the absence of oxygen. After the reaction, 2 L of ultrapure water was added and sonicated for 30 min to dissolve the sample. Then, the isolated Gly-CNDs solution was obtained after passing the microcrystalline cellulose column completely and filtering with a 0.22 µm filter (VWR International Co., Ltd., Pennsylvania, USA). The sample was purified using the HPLC system (ECOM ECB2000, Praha, Czech Republic) with a size-exclusion column 60 x 21.20 mm (Phenomenex BioSep-SEC-S 2000 60 x 21.20 mm, California, USA) connected with a RF-20A XS fluorescent detector (Shimadzu Co., Kyoto, Japan). Gly-CNDs were lyophilized and stored for further measurements. The production yield of Gly-CNDs is approximately 30%. A Lambda 35 UV-Vis spectrophotometer (Norwalk, USA), an FP-8500 fluorescence spectrophotometer (Jasco Co., Oklahoma, USA), 4300 Handheld FTIR Spectrometer (Agilent Technologies Co., California, USA) and a transmittance electron microscopy (TEM) (JEM-2000FXII model, JEOL Ltd., Tokyo, Japan) were used to characterize the isolated Gly-CNDs.

2.4. Measurement of caffeine content using the HPLC system

Caffeine content in foods was measured following the method described in ISO 20481 – 2008 [27]. For measurement, The ECS05 Gradient Analytical HPLC System (ECOM Co., Praha, Czech Republic) was operated with a UV-VIS PDA detector (ECDA2800, ECOM Co., Praha, Czech Republic) and Nucleosil C18 100 Å column 5 µm, 150 × 4.6 mm (Phenomenex Corporation, California, USA). Samples were measured at 25°C using a mobile phase that contains acetonitrile (ACN) and 0.01 M phosphate buffer (pH 6.4) in a 90:10 (v/v) ratio with a flow rate of 2 mL/min and a run time of 8 min. The retention time of caffeine was observed to be around 5.3 – 5.6 min. Caffeine standard, purchased from Sigma-Aldrich Company (Missouri, USA), was used to plot the standard curve.

2.5. Method validation

The calibration curve was created using caffeine concentrations ranging from 0 to 2060 nM, prepared in triplicate. The method was validated by establishing the linear range of the analyte and calculating the limit of detection (LOD) and the limit of quantification (LOQ). The precision of the analysis was confirmed through recovery samples, which were prepared by adding varying concentrations of each analyte to real samples.

2.6. Preparation of real sample solutions

Three samples were collected to analyze the caffeine content using Gly-CNDs and HPLC. All samples were opened and sonicated for 1 hour to completely degases. The sample was filtered with a 0.22 µm filter (VWR International Co., Ltd., Pennsylvania, USA). Depending on the measurement method, samples were diluted using ultrapure water,

2.5. Statistical analyses

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA, and differences were considered significant at $p < 0.05$. The statistical analysis used SPSS software with 25.0 version (IBM Ltd., New York, USA). ImageJ software version 1.54D (New York, USA) was used to analyze

and disperse particles from TEM pictures. GraphPad Prism version 9.0 (GraphPad Software Inc., California, USA) was used to construct all charts.

3. Results and discussion

3.1. Characterization of carbon nanodots derived from glycine (Gly-CNDs)

The characterization of purified carbon nanodots derived from glycine (Gly-CNDs) is comprehensively illustrated in **Figure 2**. The TEM image with a 10 nm scale reveals that uniformly dispersed Gly-CNDs are roughly spherical, indicating successful synthesis (**Figure 2A**). The size distribution histogram shows a narrow size distribution centered around 4.2 ± 1.2 nm, highlighting the consistency in particle size (**Figure 2B**). The fluorescence properties of Gly-CNDs were scanned with the excitation wavelength ranging from 280 – 460 nm and the emission wavelength ranging from 288 – 748 nm. As a result (**Figure 2C**), the optimum excitation wavelength was recorded at 360 nm with the highest emission wavelength at 448 nm, similar to CNDs from the Maillard reaction [26,28]. This tunable fluorescence is advantageous for optimizing detection sensitivity and selectivity. **Figure 2D** displays the UV-vis absorption spectra of the Gly-CNDs. It exhibits distinct peaks, with a prominent peak around 280 nm corresponding to the π - π^* transition of aromatic sp^2 domains and a broad absorption tail into the visible range, suggesting surface defects and functional groups, similar to the previous studies about CNDs [26,28,29]. The Raman spectra of Gly-CNDs show two prominent peaks at around 1350 cm^{-1} (D-band) and 1600 cm^{-1} (G-band), confirming the graphitic structure of Gly-CNDs (**Figure 2D**), similarly with Raman spectra of nitrogen-doped CNDs [30]. However, the Raman spectra of Gly-CNDs are relatively weak and broad due to the presence of surface functional groups that mask the underlying carbon core. X-ray diffraction (XRD) was measured to understand the core structure of Gly-CNDs in **Figure 2G**. The XRD patterns of Gly-CNDs exhibit broad, diffuse peaks centered around $2\theta = 25^\circ$, indicative of an amorphous carbon structure with a high degree of disorder. This is attributed to the presence of various functional groups and defects on the surface of CNDs. The elemental composition of Gly-CNDs presents in **Figure 2E** with the percentage of carbon (33.1%) and nitrogen (18.2%). To understand further the structure of Gly-CNDs, the FTIR spectra indicate significant changes compared to glycine, demonstrating structural transformation during pyrolysis (**Figure 2F**). The peak in the $1600\text{--}1650\text{ cm}^{-1}$ and $1500\text{--}1400\text{ cm}^{-1}$ range, corresponding to the COO^- asymmetric stretch in glycine [31], appears more pronounced in CNDs, suggesting the formation of aromatic $\text{C}=\text{C}$ domain ($1600\text{--}1650\text{ cm}^{-1}$ region) [32], and indicating modifications in carboxyl functional groups (broadens in $1500\text{--}1400\text{ cm}^{-1}$ range) [33]. The peak at 1350 cm^{-1} , attributed to CH/NH bending in glycine, is enhanced in CNDs, likely due to the formation of sp^2 carbon domains [34]. An increase in the $1100\text{--}1050\text{ cm}^{-1}$ range suggests the presence of C-N or C-O stretching linked to oxidized surface groups, confirming surface functionalization [35]. At 900 cm^{-1} , glycine exhibits C-H or N-H wagging, which sharpens in CNDs due to surface modifications, likely from the introduction of amine or hydroxyl groups [30,31]. Lastly, the $700\text{--}800\text{ cm}^{-1}$ region, corresponding to C-H bending or skeletal vibrations, remains largely unchanged but shows minor shifts, indicating subtle molecular rearrangements [30]. These spectral changes reflect the transformation of glycine into CNDs through aromaticity development, sp^2 carbon formation, and oxygen/nitrogen surface functionalization. The HPLC-SEC-FD chromatogram of isolated Gly-CNDs is illustrated in **Figure 2H**. By calculating the molecular

mesh of each peak, the Gly-CNDs were identified and collected as the first peak to obtain purified Gly-CNDs [36]. The sample was lyophilized and stored for further use.

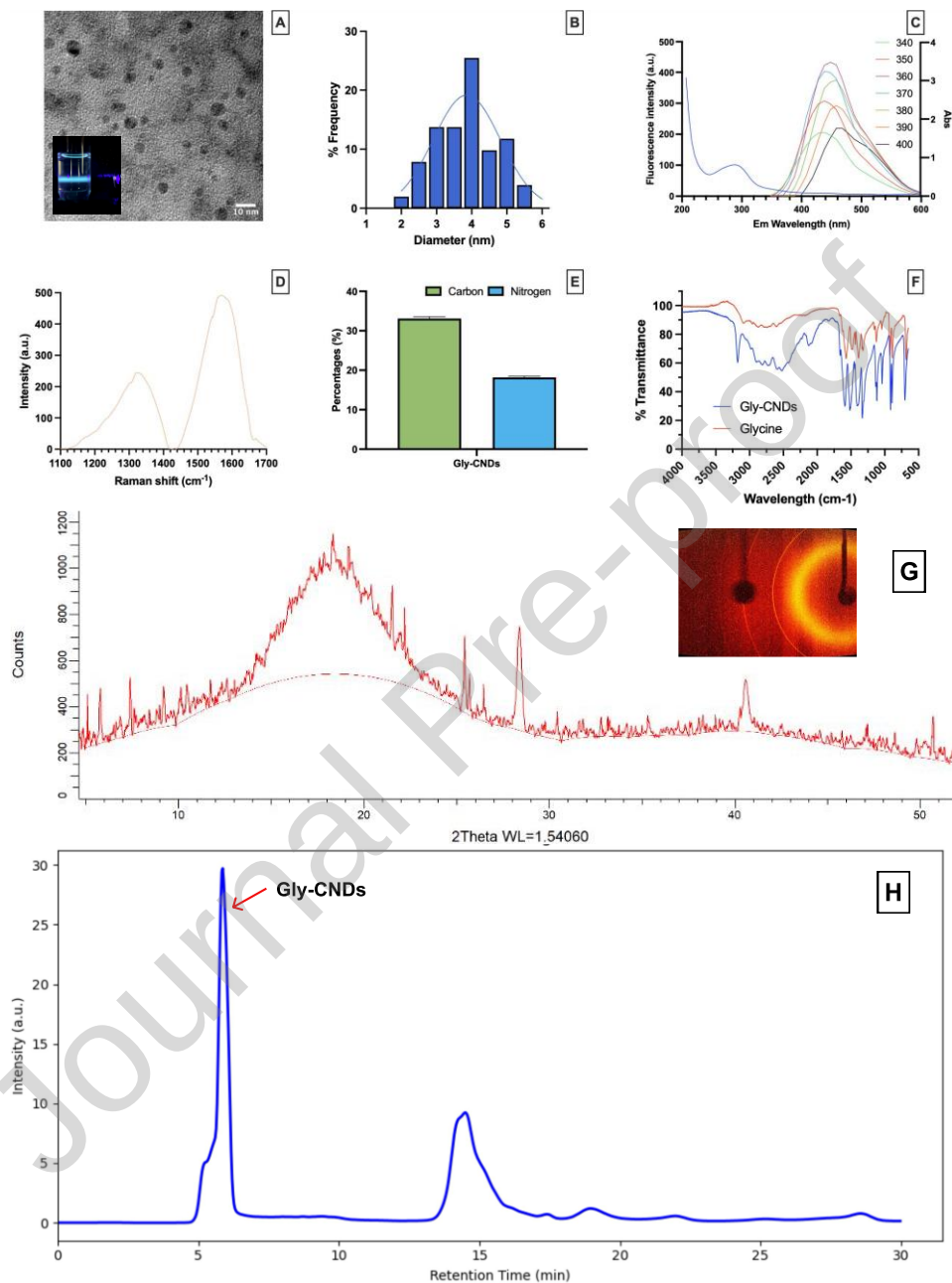


Figure 2. Characterization of carbon nanodots derived from glycine (Gly-CNDs) including **A**, TEM images with 10 nm scale. **B**, size distribution histogram with the average size at 4.2 ± 1.2 nm; **C**, the emission fluorescent spectra at different excitation wavelengths (right) and UV spectra with real sample under UV lamp (left). **D**, Raman spectra. **E**, Carbon and nitrogen percentage. **F**, FTIR spectra of Gly-CNDs (blue line) and glycine (red line). **G**, X-ray diffraction (XRD) spectra. **H**, HPLC-SEC-FD chromatogram of isolated Gly-CNDs before the final purification.

3.2. Stability of carbon nanodots derived from glycine (Gly-CNDs)

Figure 3 investigates the stability of the glycine-derived carbon nanodots (Gly-CNDs) under various conditions relevant to practical applications. The Gly-CNDs exhibit good stability in different NaCl concentrations from 0.1M to 1M, with the fluorescence intensity remaining relatively constant (**Figure 3A**). This suggests that the Gly-CNDs are resistant to aggregation induced by salt, making them suitable for applications in physiological environments. Furthermore, the fluorescence intensity of Gly-CNDs remains largely unchanged over a 60-minute, indicating good stability over time (**Figure 3B**). However, their stability is pH-dependent, with optimal performance at acidic pH 4.0 (**Figure 3C** and **Figure 3D**). The fluorescence intensity of Gly-CNDs was observed to significantly decrease at alkaline conditions, suggesting a potential link to surface charge properties. **Figure 3D** presents the emission fluorescence spectra of the Gly-CNDs at an excitation wavelength of 370 nm but at various pH conditions (4.0 to 11.0). The emission peak remains around 440 nm across the different pH values, further highlighting the stability of the Gly-CNDs optical properties. Overall, Gly-CNDs exhibited excellent stability across various salt concentrations and over time, maintaining consistent performance under acidic pH conditions.

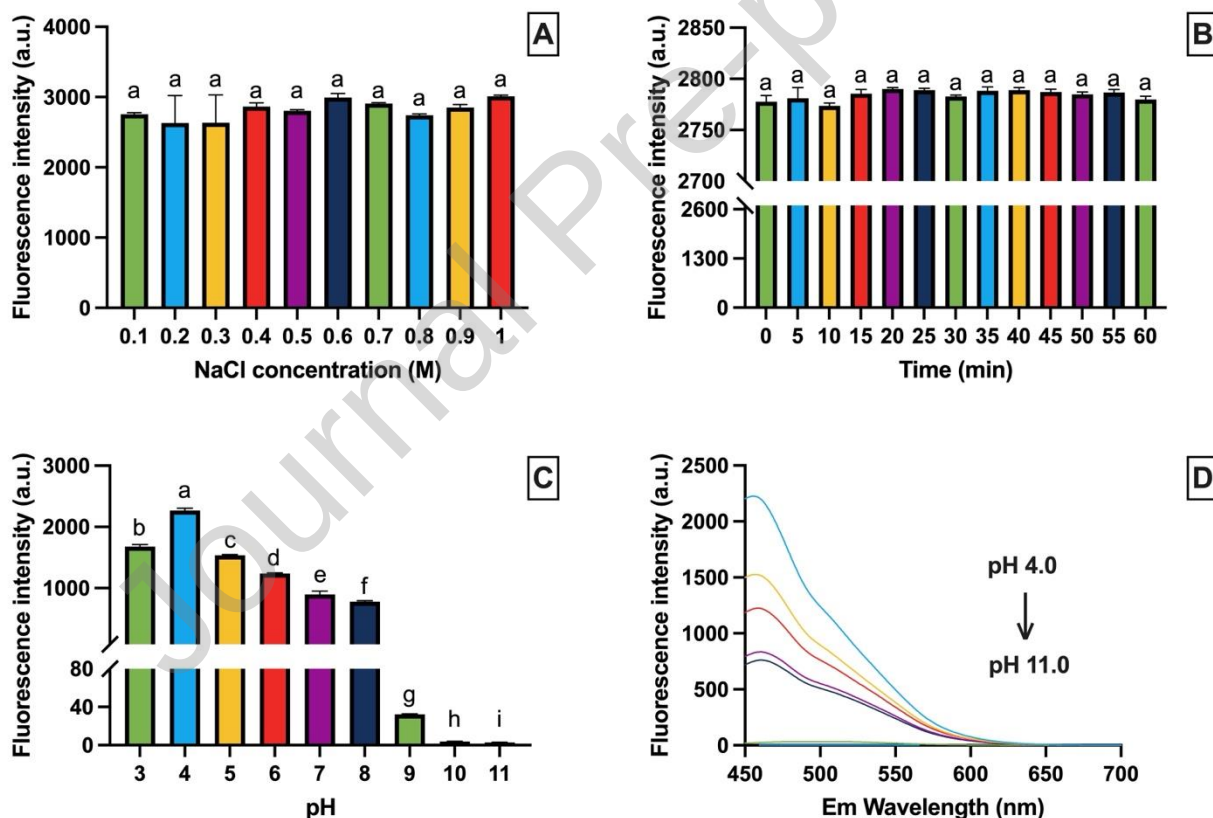


Figure 3. Stability of carbon nanodots derived from glycine (Gly-CNDs). **A**, Gly-CNDs in different NaCl concentrations ranging from 0.1M to 1M. **B**, Stability of Gly-CNDs over time (min). **C**, Stability of Gly-CNDs in different pH conditions. **D**, the emission fluorescence spectra of Gly-CNDs at the excitation wavelength 360 nm with pH ranging from 4.0 to 11.0. All samples were measured in triplicate. Error bars expressed the difference of means. Letter a-i indicates the significant differences at $p < 0.05$.

3.3. Selectivity of this methods

The examination of selectivity holds significant importance for each reaction system. In order to evaluate the selectivity of the proposed method, the effects of common sweeteners (glucose, fructose), beverage additives (ascorbic acid, citric acid, benzoic acid (preservative, E210), some ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) and some toxins compounds (acrylamide, 5-HMF) on the reaction system were investigated. **Figure 4** illustrates that the interfering species did not significantly impact the reaction system due to the strong specificity of Gly-CDs for caffeine. Additionally, it demonstrated that the proposed method can selectively identify caffeine even in the presence of high concentrations (500 μM) of interfering species in beverages.

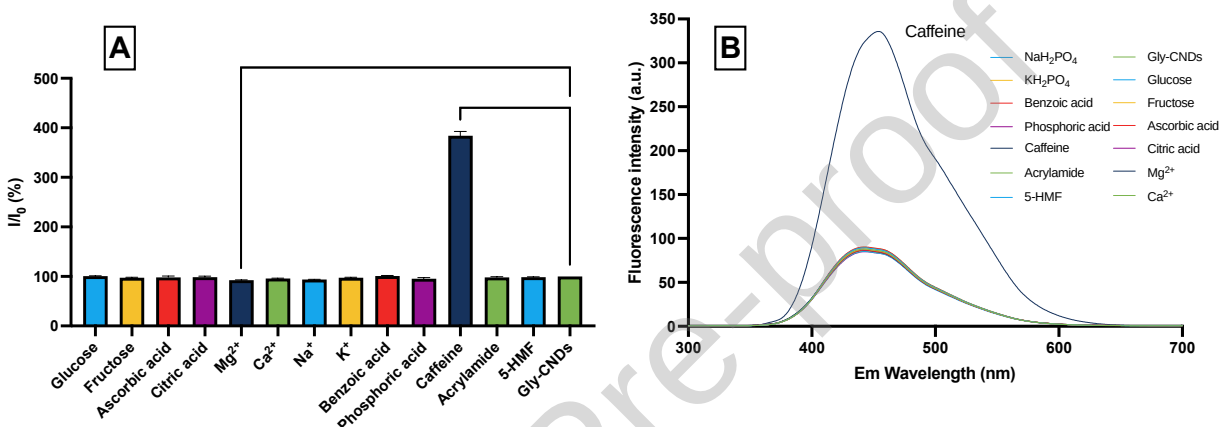


Figure 4. Selectivity of carbon nanodots derived from glycine (Gly-CNDs) in the presence of other interfering compounds at the concentration of 500 μM . **A**, the relative fluorescence intensity changes of the interfering compound's response (I/I_0 (%)) before and after interacting with Gly-CNDs. **B**, the emission fluorescence spectra of other compounds after mixing with Gly-CNDs at the excitation wavelength 360 nm. All samples were measured in triplicate. Error bars expressed the difference between means. The asterisk (*) indicates a significant difference from the control sample (Gly-CNDs) (*, $p < 0.05$ and ****, $p < 0.001$).

3.4. Building the assay for measuring caffeine using carbon nanodots derived from glycine (Gly-CNDs)

Figure 5 presents the emission spectra of carbon nanodots derived from glycine (Gly-CNDs) at an excitation wavelength of 360 nm, demonstrating the fluorescence response under different conditions: (1) Gly-CNDs in the presence of 5000 μM caffeine (red line), (2) only Gly-CNDs without caffeine (black line), and (3) a caffeine solution of 5000 μM without Gly-CNDs (green line). In **Figure 5**, the emission spectrum of Gly-CNDs without caffeine (black line) exhibits a strong fluorescence peak with the maximum fluorescence intensity at 2232.82 (a.u.), indicating the typical fluorescence properties of the CNDs. When 5000 μM of caffeine is added to the Gly-CNDs solution (red line), a significant enhancement of fluorescence intensity is observed over than 50% with the obtained maximum value at 3722.57 (a.u.), confirming the interaction between caffeine molecules and Gly-CNDs. Caffeine has a very low fluorescent intensity (green line) at the excitation wavelength at 360 nm. When the CNDs solution was added to the caffeine solution, the fluorescent intensity increased significantly to 50% compared to the fluorescence of CNDs,

and 4000 times compared to the fluorescence of caffeine. These results underscore the effectiveness of Gly-CNDs as a fluorescent probe for caffeine detection.

Figure 6 illustrates the relationship between caffeine concentration and the fluorescence enhancement efficiency of carbon nanodots derived from glycine (Gly-CNDs). **Figure 6A** presents a plot of quenching efficiency (F/F_0) against caffeine concentration, showing a linear relationship over the range from 0 to 2060 nM. The linear fit equation $y = 0.0008225 \times X + 1.006$ with a high correlation coefficient ($R^2=0.9929$), indicating a strong linear dependency of fluorescence enhancement on caffeine concentration. The low of detection (LOD) is recorded with 36.5 nM caffeine concentration, and the limit of quantification (LOQ) is 122.1 nM. This high correlation suggests that Gly-CNDs are highly sensitive to caffeine, enabling accurate quantification over a wide concentration range. The linearity and sensitivity are crucial for developing a reliable sensing method for caffeine detection in various food matrices. The increase of emission spectra of Gly-CNDs in the presence of caffeine at concentrations ranging from 0 to 2060 nM illustrated in **Figure 6B** with an excitation wavelength of 360 nm, and in **Figure 6C** for concentration ranging from 0 to 309 nM. The fluorescence intensity Gly-CNDs demonstrates a positive correlation with increasing concentrations of caffeine. This relationship is supported by consistent emission wavelengths recorded during measurements.

Table 1 compares different carbon nanodots (CNDs) used for detecting caffeine. It includes sulfur-doped CNDs [37], iron-doped Red CNDs [38] and glycine-derived CNDs (this work). While sulfur-doped CNDs and iron-doped CNDs have a narrow linear range but high sensitivity with a LOD of 0.05 μ M, in this study, glycine-derived CNDs have the broadest detection range and a LOD of 0.043 μ M, making them versatile for various caffeine detection applications. Moreover, utilizing glycine and sugars as starting materials reduces the potential for toxicity, making it particularly suitable for use in the food industry.

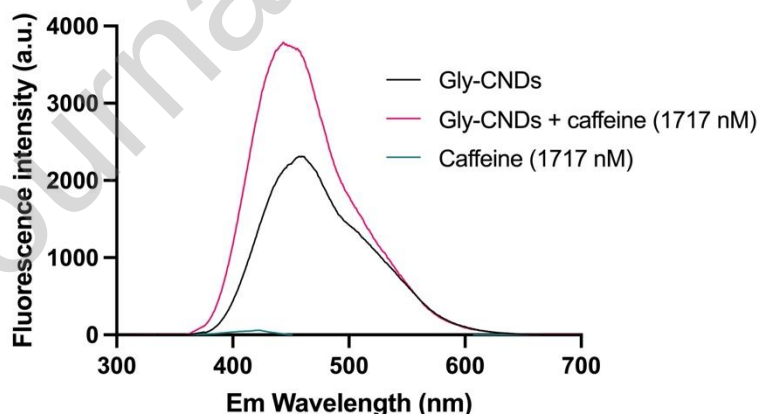


Figure 5. Emission spectra of carbon nanodots derived from glycine (Gly-CNDs) at the excitation wavelength 360 nm, including (1) Gly-CNDs with caffeine (1717 nM) added, (2) only Gly-CNDs without caffeine added, and (3) caffeine solution (1717 nM) without Gly-CNDs.

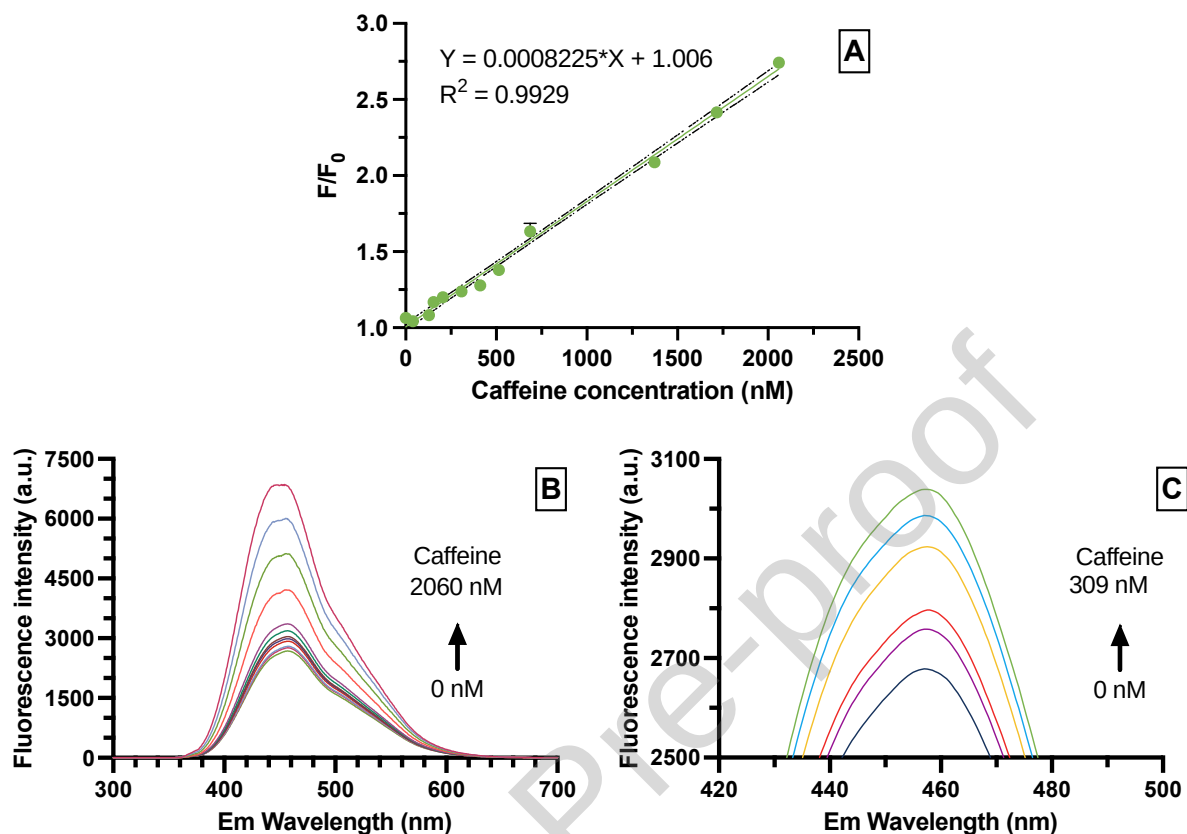


Figure 6. A, Plot of enhancing fluorescence efficiency of carbon nanodots derived from glycine (Gly-CNDs) against the caffeine concentration including 0, 39, 129, 154, 206, 309, 412, 514, 687, 1373, 1717, and 2060 nM. B, Emission spectra of Gly-CNDs with caffeine at various concentrations ranging from 0 to 2060 nM at the excitation wavelength at 360 nm. C, Emission spectra of Gly-CNDs with caffeine at various concentrations ranging from 0 to 309 nM at the excitation wavelength at 360 nm. All samples were measured in triplicate. Error bars expressed the difference of means. F and F_0 present the fluorescent intensity with and without caffeine.

Table 1. Comparison of different CNDs for the detection of caffeine

Types of CNDs	Precursors	Linear ranges (μM)	LOD (μM)	References
Sulfur-doped CNDs	Citric acid, sodium thiosulfate	0.2 μM to 70 μM .	0.05 μM	[37]
Fe-Doped Red Fluorescent CNDs	Neutral red, FeCl_3	1 to 40 $\mu\text{g/mL}$	0.024 $\mu\text{g/mL}$	[38]
Gly-CNDs	Glycine, sugars	50 to 2060 nM	0.0403 μM	This work

3.5. Proposed mechanisms for carbon nanodots derived from glycine (Gly-CNDs) fluorescence enhancement by caffeine

The sensing mechanism of Gly-CNDs towards caffeine is characterized by a notable enhancement in fluorescence intensity, as evidenced by the linear increase in F/F_0 with increasing caffeine concentration (**Figure 6A**). This suggests a direct interaction between Gly-CNDs and caffeine, leading to a quantifiable change in the nanodot's emissive properties. Characterization of the Gly-CNDs (**Figure 2**) reveals a spherical morphology with an average size of 4.2 ± 1.2 nm (**Figure 2A, B**), indicating a high surface area conducive to interactions. The presence of abundant surface functional groups, including hydroxyl, carboxyl, and nitrogen-containing moieties, is confirmed by FTIR spectroscopy (**Figure 2F**). These groups likely play a crucial role in the interaction with caffeine. Given the observed fluorescence enhancement, we propose that caffeine molecules interact with these surface functional groups through hydrogen bonding, potentially restricting vibrational or rotational freedom and reducing non-radiative decay pathways. Furthermore, the presence of graphitic domains, inferred from the Raman spectra (**Figure 2D**), suggests the possibility of π - π stacking interactions with the aromatic rings of caffeine. This interaction, along with potential electrostatic interactions between the nitrogen-containing groups on Gly-CNDs and polar regions of caffeine, could contribute to surface passivation or structural rigidification, thereby enhancing radiative decay and increasing fluorescence. The observed linear fluorescence enhancement (**Figure 6A**) and the concentration-dependent emission spectra (**Figure 6B, C**) further support a direct and quantifiable interaction.

3.5. Optimization of experimental conditions for caffeine detection using carbon nanodots derived from glycine (Gly-CNDs)

The optimal experimental conditions for utilizing carbon nanodots derived from glycine (Gly-CNDs) to detect caffeine content were investigated, which resulted in **Figure 7** highlighting the effects of pH, incubation time, and temperature on the fluorescence intensity of Gly-CNDs in the presence of caffeine (1717 nM). First, the stability of Gly-CNDs in the presence of caffeine (1717 nM) in different pH conditions was assessed across a pH range of 3.0 to 11.0, where pH 3.0 - 5.0 is the most suitable pH range to measure caffeine (**Figure 7A**). The fluorescence significantly decreases at pH levels beyond 7, reaching minimal values at pH 9, 10, and 11. These findings are illustrated in **Figure 7B**, showing a significant reduction in emission spectra as the pH increases. This pattern indicates that Gly-CNDs exhibit good stability and strong fluorescence in mildly acidic to neutral conditions, typical for many food products. The reduced fluorescence at higher pH suggests a change in surface chemistry or a quenching effect [39]. This is consistent with the reversible 'off-on' fluorescence switch observed in nitrogen-doped carbon dots (N-CDs) between strongly acidic and alkaline solutions [40].

Subsequently, the impact of different incubation times on the fluorescence intensity of Gly-CNDs with the addition of 1717 nM caffeine at 40°C (**Figure 7C**) was investigated. The results indicated that the fluorescence intensity peaked at 25 minutes, signifying that this is the optimal incubation time for the interaction between Gly-CNDs and caffeine. After this peak, a gradual decrease in fluorescence intensity was observed, suggesting potential photobleaching or saturation effects of the Gly-CNDs over longer incubation periods. Additionally, it was observed that the optimal temperature for maximum fluorescence intensity ranged from 40 to 50°C (**Figure 7D**). Increasing the temperature beyond this range resulted in a steady decline in fluorescence intensity, indicating that higher temperatures may have an adverse effect on the fluorescence properties of Gly-CNDs. This could be attributed to thermal quenching effects or destabilization of the carbon

nanodots at elevated temperatures [41]. In conclusion, the best conditions for detecting caffeine using Gly-CNDs derived from glycines are a pH of 5.0, an incubation time of 25 minutes, and an incubation temperature of 40°C. These conditions produce the highest fluorescence intensity, making caffeine detection more sensitive and accurate.

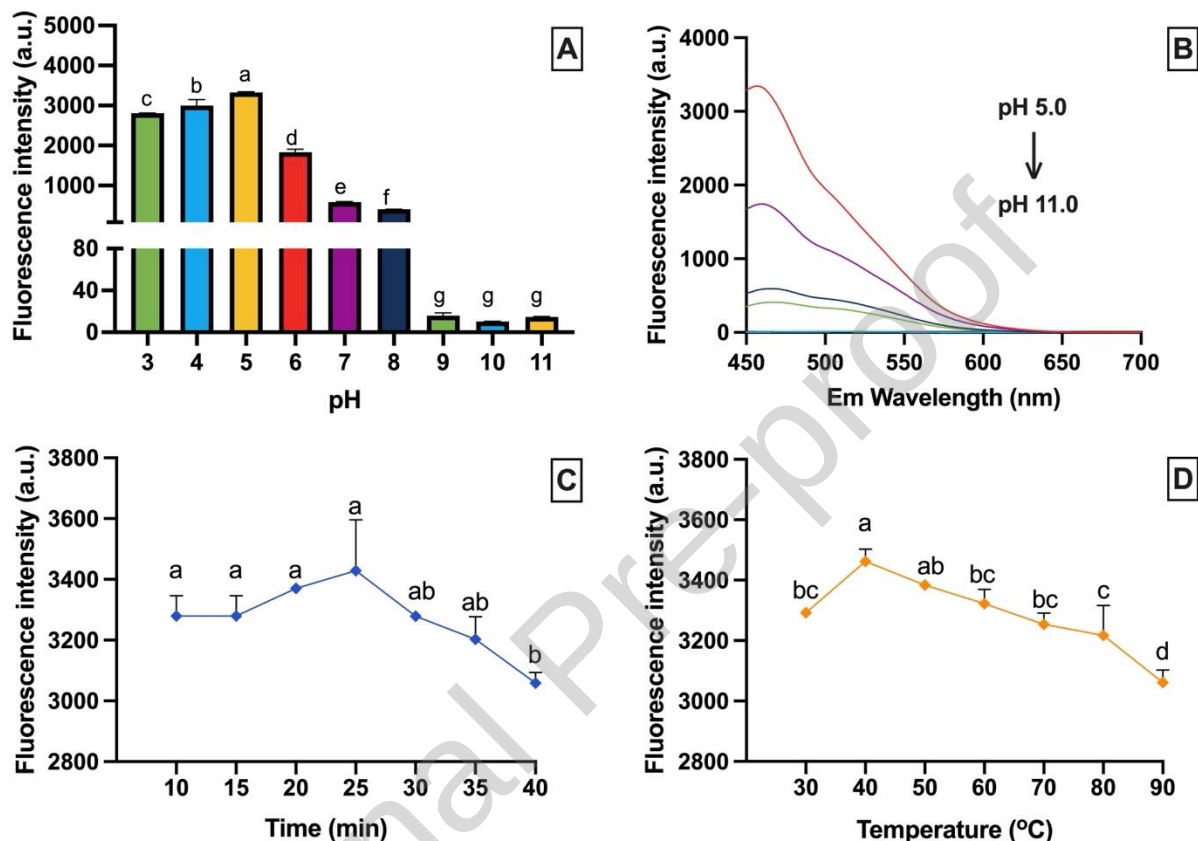


Figure 7. Optimum experiment conditions for detecting caffeine using carbon nanodots derived from glycine (Gly-CNDs). **A**, Stability in different pH conditions ranging from pH 3.0 to 11.00. **B**, the emission fluorescence spectra at the excitation wavelength 360 nm with pH ranging from 4.0 to 11.0. **C**, Fluorescence intensity over incubation time at 40°C. **D**, Fluorescence intensity at different incubation temperatures after 25 minutes. Caffeine (1717 nM) was used for all experiments. All samples were measured in triplicate. Error bars expressed the difference of means. Letters a- g indicate the significant differences at $p < 0.05$.

3.6. Detection of caffeine in commercial beverages and compared with HPLC method

Figure 8 evaluates the application of Gly-CNDs for measuring caffeine in actual beverage samples, comparing the results with high-performance liquid chromatography (HPLC) as a reference method. Three energy drinks were selected for this measurement including sample 1: HELL energy drink- classic boxing kings editions (HELL Magyarország Ltd., Hungary); sample 2: XIXO energy drink original flavor (XIXO Company Ltd., Hungary); sample 3: energy drink apple flavor (Hansen Natural Company, USA) along with three type of tea including sample 4: green tea (TenRen Co., Taiwan); sample 5: black tea (TenRen Co., Taiwan); sample 6: olong tea (Cau Dat farm

Co., Da Lat, Vietnam). In particular, one commercial fake coffee, which is coffee mixed with burnt corn, was collected from the Vietnam market (sample 7) to ensure that this method can be used to measure caffeine in coffee adulteration. **Figure 8A** displays the emission spectra of the energy drinks sample. The blue line represents the spectra of sample 1 without the presence of Gly-CNDs, while the red line represents the spectra of sample 1 with the addition of 75 ppm Gly-CNDs at an excitation wavelength of 360 nm. The spectrum with added Gly-CNDs (red line) exhibits a significant increase in fluorescence intensity, approximately eight times higher than the spectrum without Gly-CNDs (blue line). This marked enhancement confirms the sensitivity of Gly-CNDs to caffeine, as the Gly-CNDs interact with caffeine molecules, leading to fluorescence enhancement that can be detected and quantified. Furthermore, the caffeine concentration of three energy drink samples was measured using fluorescence-based and HPLC measurements (**Figure 8B**). There are no significant differences between the caffeine concentration of the three samples using the fluorescence-based measurements and HPLC results ($p > 0.05$). **Table 2** validates the recovery of caffeine concentration in the samples. The recovery ranged from $95.902 \pm 2.173\%$ to $102.703 \pm 1.834\%$, with the RSD% maintained from 1.272% to 1.968%. This demonstrates the effectiveness of Gly-CNDs as a reliable sensor for quantifying caffeine. This suggests that the Gly-CNDs can effectively be a sensing platform for detecting caffeine in real-world beverage samples.

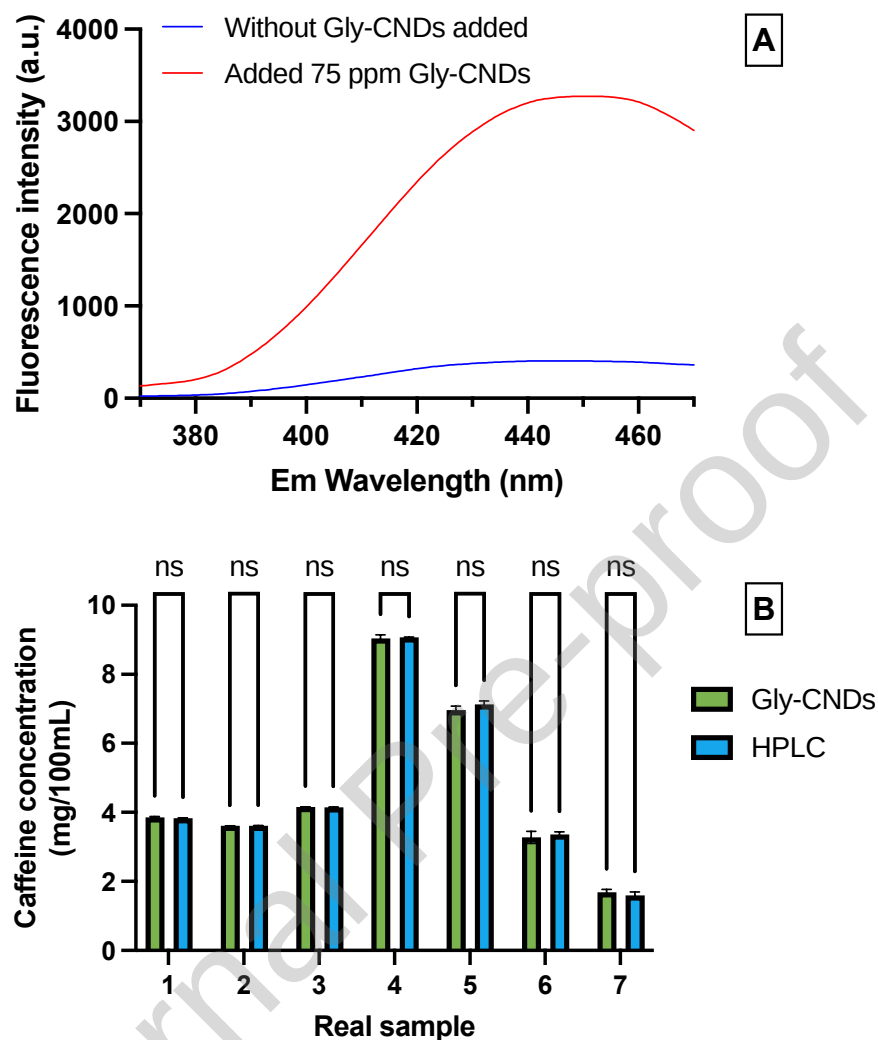


Figure 8. Measurement of caffeine in commercial beverages. **A**, the emission fluorescent spectra of sample 1 with and without Gly-CNDs were added. **B**. The caffeine concentration of three beverage samples was measured using Gly-CNDs with fluorescence spectrophotometer and HPLC methods. Three energy drinks were selected for this measurement, including **sample 1**: HELL energy drink- classic boxing kings editions (HELL Magyarország Ltd., Hungary); **sample 2**: XIXO energy drink original flavor (XIXO Company Ltd., Hungary); **sample 3**: energy drink apple flavor (Hansen Natural Company, USA); **sample 4**: green tea (TenRen Co., Taiwan); **sample 5**: black tea (TenRen Co., Taiwan); **sample 6**: olong tea (Cau Dat farm Co., Da Lat, Vietnam); **sample 7**: Commercial fake coffee (Coffee mixed with burnt corn from Vietnam market). All samples were measured in triplicate. Error bars expressed the difference of means. Letter a- l and indicate the significant differences at $p < 0.05$. ns is abbreviated as “not significant differences.”

Table 2. Recoveries of caffeine in beverages detected by the proposed method (n=5)

Sample	Added caffeine (mg)	Measured caffeine (mg)	Recovery (%)	RSD (%)*
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Energy drink apple flavor (Hansen Natural Company, USA)	0	-	-	-
	0.020	0.020 ± 0.001	100.017 ± 6.048	1.968
	0.100	0.103 ± 0.002	102.703 ± 1.834	1.272
	0.200	0.192 ± 0.004	95.902 ± 2.173	1.863

*RSD (%) means relative standard deviation

4- Conclusion

This study successfully demonstrates the potential of carbon nanodots derived from glycine (Gly-CNDs) as an effective tool for rapidly detecting caffeine in beverage samples. The synthesized Gly-CNDs, with an average size of 4.2 ± 1.2 nm, exhibited remarkable stability across various conditions, including different NaCl concentrations, time durations, and pH levels. The fluorescence-enhancing property of these carbon nanodots in the presence of caffeine enabled a highly sensitive detection method, with a linear response observed within the caffeine concentration range of 39 to 2060 nM with a low LOD of 36.5 nM. Additionally, the Gly-CNDs maintained their stability and fluorescence emission characteristics across a pH spectrum of 3.0 to 5.0, ensuring consistent performance in diverse sample acidic environments. Comparative analysis with the HPLC method revealed no significant variance in caffeine concentration measurements, confirming the accuracy and reliability of the Gly-CNDs-based fluorescence technique ($p > 0.05$). To our knowledge, this study is the first article using carbon nanodots derived from food-grade material (glycine and sugar) to rapidly detect caffeine in foods with the broadest detection range and lowest LOD. This research highlights the feasibility of utilizing Gly-CNDs from coffee brews as a fast, cost-effective, and reliable approach for caffeine detection, presenting a promising alternative for the food and beverage industries.

CRedit authorship contribution statement

Hassan El-Ramady: Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Jozsef Prokisch:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Duyen Nguyen Huu Huong:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

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Reference

- [1] A. Samoggia, T. Rezzaghi, The Consumption of Caffeine-Containing Products to Enhance Sports Performance: An Application of an Extended Model of the Theory of Planned Behavior, *Nutrients* 13 (2021) 344. <https://doi.org/10.3390/nu13020344>.
- [2] O.R. Portillo, A.C. Arévalo, Caffeine. A critical review of contemporary scientific literature, *RB* 7 (2022) 1–15. <https://doi.org/10.21931/RB/2022.07.03.16>.
- [3] H. Zhao, S. Wang, Y. Han, M. Yao, Y. Zhang, X. Zeng, Coffee consumption might be associated with lower potential risk and severity of metabolic syndrome: national health and nutrition examination survey 2003–2018, *Eur J Nutr* (2024). <https://doi.org/10.1007/s00394-024-03367-1>.
- [4] C. Willson, The clinical toxicology of caffeine: A review and case study, *Toxicology Reports* 5 (2018) 1140–1152. <https://doi.org/10.1016/j.toxrep.2018.11.002>.
- [5] M. Perez, I. Domínguez-López, A. López-Yerena, A. Vallverdú Queralt, Current strategies to guarantee the authenticity of coffee, *Critical Reviews in Food Science and Nutrition* 63 (2023) 539–554. <https://doi.org/10.1080/10408398.2021.1951651>.
- [6] W.L. Cheah, M. Fang, HPLC-Based Chemometric Analysis for Coffee Adulteration, *Foods* 9 (2020) 880. <https://doi.org/10.3390/foods9070880>.
- [7] B.L. Madison, W.J. Kozarek, C.P. Damo, High-Pressure Liquid Chromatography of Caffeine in Coffee, *Journal of AOAC INTERNATIONAL* 59 (1976) 1258–1261. <https://doi.org/10.1093/jaoac/59.6.1258>.
- [8] J.L. Torres Y Torres, S.L. Hiley, S.P. Lorimor, J.S. Rhoad, B.D. Caldwell, G.L. Zweerink, M. Ducey, Separation of Caffeine from Beverages and Analysis Using Thin-Layer Chromatography and Gas Chromatography–Mass Spectrometry, *J. Chem. Educ.* 92 (2015) 900–902. <https://doi.org/10.1021/ed500977r>.
- [9] Y. Zhang, L. Gao, S. Ma, T. Hu, Porous MB@Cd-MOF Obtained by Post-Modification: Self-Calibrated Fluorescent Turn-on Sensor for Highly Sensitive Detection of Carbaryl, *Crystal Growth & Design* 22 (2022) 2662–2669. <https://doi.org/10.1021/acs.cgd.2c00089>.
- [10] M. Saraban, A. Numnuam, N. Nontipichet, T. Kangkamano, P. Thavarungkul, P. Kanatharana, S. Khumngern, A disposable electrochemical caffeine sensor based on a screen-printed electrode modified with a copper-metal organic framework and functionalized multi-walled carbon nanotube nanocomposite, *New Journal of Chemistry* 48 (2024) 3638–3645.
- [11] W. Xu, T.-H. Kim, D. Zhai, J.C. Er, L. Zhang, A.A. Kale, B.K. Agrawalla, Y.-K. Cho, Y.-T. Chang, Make Caffeine Visible: a Fluorescent Caffeine “Traffic Light” Detector, *Sci Rep* 3 (2013) 2255. <https://doi.org/10.1038/srep02255>.
- [12] A. Yokokawa, H. Takano, H. Shimazaki, S. Ogawa, M. Fukae, H. Akiyama, S. Igarashi, T. Furihata, H. Shibasaki, Quantification of Melatonin, Caffeine, and Paraxanthine in Human Plasma Using Liquid Chromatography-Tandem Mass Spectrometry, *Chromatographia* 87 (2024) 167–174. <https://doi.org/10.1007/s10337-023-04308-6>.
- [13] X. Xu, R. Ray, Y. Gu, H.J. Ploehn, L. Gearheart, K. Raker, W.A. Scrivens, Electrophoretic Analysis and Purification of Fluorescent Single-Walled Carbon Nanotube Fragments, *J. Am. Chem. Soc.* 126 (2004) 12736–12737. <https://doi.org/10.1021/ja040082h>.
- [14] L. Lin, Y. Wang, Y. Xiao, X. Chen, Ratiometric fluorescence detection of riboflavin based on fluorescence resonance energy transfer from nitrogen and phosphorus co-doped carbon

- dots to riboflavin, *Anal Bioanal Chem* 411 (2019) 2803–2808.
<https://doi.org/10.1007/s00216-019-01725-1>.
- [15] E.L. Rossini, M.I. Milani, H.R. Pezza, Green synthesis of fluorescent carbon dots for determination of glucose in biofluids using a paper platform, *Talanta* 201 (2019) 503–510.
<https://doi.org/10.1016/j.talanta.2019.04.045>.
- [16] F.Y. Vadia, S. Ghosh, V.N. Mehta, S. Jha, N.I. Malek, T.J. Park, S.K. Kailasa, Fluorescence “Turn OFF-ON” detection of Fe³⁺ and propiconazole pesticide using blue emissive carbon dots from lemon peel, *Food Chemistry* 428 (2023) 136796.
<https://doi.org/10.1016/j.foodchem.2023.136796>.
- [17] S.S.J. Xavier, G. Siva, J. Annaraj, A.R. Kim, D.J. Yoo, G.G. Kumar, Sensitive and selective turn-off-on fluorescence detection of Hg²⁺ and cysteine using nitrogen doped carbon nanodots derived from citron and urine, *Sensors and Actuators B: Chemical* 259 (2018) 1133–1143.
<https://doi.org/10.1016/j.snb.2017.12.046>.
- [18] M. Algarra, M. Pérez-Martín, M. Cifuentes-Rueda, J. Jiménez-Jiménez, J.C.G. Esteves Da Silva, T.J. Badosz, E. Rodríguez-Castellón, J.T. López Navarrete, J. Casado, Carbon dots obtained using hydrothermal treatment of formaldehyde. Cell imaging in vitro, *Nanoscale* 6 (2014) 9071–9077. <https://doi.org/10.1039/C4NR01585A>.
- [19] H. Wang, Y. Xie, S. Liu, S. Cong, Y. Song, X. Xu, M. Tan, Presence of Fluorescent Carbon Nanoparticles in Baked Lamb: Their Properties and Potential Application for Sensors, *J. Agric. Food Chem.* 65 (2017) 7553–7559. <https://doi.org/10.1021/acs.jafc.7b02913>.
- [20] D. Nguyen, H. El-Ramady, J. Prokisch, Food safety aspects of carbon dots: a review, *Environ Chem Lett* (2024). <https://doi.org/10.1007/s10311-024-01779-3>.
- [21] M.P. Sk, A. Jaiswal, A. Paul, S.S. Ghosh, A. Chattopadhyay, Presence of Amorphous Carbon Nanoparticles in Food Caramels, *Sci Rep* 2 (2012) 383. <https://doi.org/10.1038/srep00383>.
- [22] A.I. Costa, P.D. Barata, B. Moraes, J.V. Prata, Carbon Dots from Coffee Grounds: Synthesis, Characterization, and Detection of Noxious Nitroanilines, *Chemosensors* 10 (2022) 113.
<https://doi.org/10.3390/chemosensors10030113>.
- [23] D.H. Nguyen, A. Muthu, H. El-Ramady, Á. Béni, J. Prokisch, Detection and formation of fluorescent carbon nanodots in coffee brews and its relationship with other compositions, *Journal of Food Composition and Analysis* (2024) 106347.
- [24] X. Song, L. Cao, S. Cong, Y. Song, M. Tan, Characterization of Endogenous Nanoparticles from Roasted Chicken Breasts, *J. Agric. Food Chem.* 66 (2018) 7522–7530.
<https://doi.org/10.1021/acs.jafc.8b01988>.
- [25] Y. Song, Y. Wu, H. Wang, S. Liu, L. Song, S. Li, M. Tan, Carbon quantum dots from roasted Atlantic salmon (*Salmo salar* L.): Formation, biodistribution and cytotoxicity, *Food Chemistry* 293 (2019) 387–395. <https://doi.org/10.1016/j.foodchem.2019.05.017>.
- [26] D. Nguyen, A. Muthu, H. El-Ramady, L. Daróczy, L. Nagy, S. Kéki, Á. Béni, I. Csarnovics, J. Prokisch, Optimization of extraction conditions to synthesize green carbon nanodots using the Maillard reaction, *Mater. Adv.* (2024) 10.1039/D4MA00037D.
<https://doi.org/10.1039/D4MA00037D>.
- [27] ISO 20481:2008, Coffee and coffee products Determination of the caffeine content using high performance liquid chromatography (HPLC) Reference method, (2017).
<https://www.iso.org/home.html>. (accessed April 23, 2024).

- [28] D. Li, X. Na, H. Wang, Y. Xie, S. Cong, Y. Song, X. Xu, B.-W. Zhu, M. Tan, Fluorescent Carbon Dots Derived from Maillard Reaction Products: Their Properties, Biodistribution, Cytotoxicity, and Antioxidant Activity, *J. Agric. Food Chem.* 66 (2018) 1569–1575. <https://doi.org/10.1021/acs.jafc.7b05643>.
- [29] H. Wang, W. Su, M. Tan, Endogenous Fluorescence Carbon Dots Derived from Food Items, *The Innovation* 1 (2020) 100009. <https://doi.org/10.1016/j.xinn.2020.04.009>.
- [30] W. Zhao, Z. Lu, F. Song, J. Han, Q. Zhang, Y. Cong, A. Lu, T. Gao, One-step electrolytic synthesis of nitrogen-doped carbon dots using graphite electrode in ionic liquid and their LED applications, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 712 (2025) 136459. <https://doi.org/10.1016/j.colsurfa.2025.136459>.
- [31] J. Groenewegen, The infrared spectrum of glycine chemisorbed by supported nickel, *Journal of Catalysis* 27 (1972) 369–373. [https://doi.org/10.1016/0021-9517\(72\)90172-8](https://doi.org/10.1016/0021-9517(72)90172-8).
- [32] X. Ma, Y. Ma, L. Dong, X. Chang, T. Yang, X. Cao, L. Feng, Z. Hu, T. Hu, Novel hydroxyl and carboxyl group-enriched nitrogen-doped carbon dots N-CDs as highly efficient corrosion inhibitors for carbon steel Q235 in 1 M HCl, *Inorganic Chemistry Communications* 175 (2025) 114167. <https://doi.org/10.1016/j.inoche.2025.114167>.
- [33] R.B. González-González, L.T. González, M. Madou, C. Leyva-Porras, S.O. Martinez-Chapa, A. Mendoza, Synthesis, Purification, and Characterization of Carbon Dots from Non-Activated and Activated Pyrolytic Carbon Black, *Nanomaterials* 12 (2022) 298. <https://doi.org/10.3390/nano12030298>.
- [34] A.C. Ferrari, S.E. Rodil, J. Robertson, Interpretation of infrared and Raman spectra of amorphous carbon nitrides, *Phys. Rev. B* 67 (2003) 155306. <https://doi.org/10.1103/PhysRevB.67.155306>.
- [35] A. Kolanowska, G. Dzido, M. Krzywiecki, M.M. Tomczyk, D. Łukowiec, S. Ruczka, S. Boncel, Carbon Quantum Dots from Amino Acids Revisited: Survey of Renewable Precursors toward High Quantum-Yield Blue and Green Fluorescence, *ACS Omega* 7 (2022) 41165–41176. <https://doi.org/10.1021/acsomega.2c04751>.
- [36] A.D. Shafagulina, A.A. Revina, N.P. Platonova, S.A. Borovikova, A.K. Buryak, The Use of Size-Exclusion Chromatography for Determining the Sizes of Metal Nanoparticles Synthesized in Reverse Micelles, *Colloid J* 81 (2019) 292–297. <https://doi.org/10.1134/S1061933X19030128>.
- [37] F. Nemati, M. Hosseini, R. Zare-Dorabei, F. Salehnia, M.R. Ganjali, Fluorescent turn on sensing of Caffeine in food sample based on sulfur-doped carbon quantum dots and optimization of process parameters through response surface methodology, *Sensors and Actuators B: Chemical* 273 (2018) 25–34. <https://doi.org/10.1016/j.snb.2018.05.163>.
- [38] H.O. Othman, Fe-Doped Red Fluorescent Carbon Dots for Caffeine Analysis in Energy Drinks Using a Paper-Based Sensor, *J Fluoresc* (2024). <https://doi.org/10.1007/s10895-024-04062-4>.
- [39] M.J. Głowacki, M. Ficek, M. Sawczak, A. Wcisło, R. Bogdanowicz, Fluorescence of nanodiamond cocktails: pH-induced effects through interactions with comestible liquids, *Food Chemistry* 381 (2022) 132206. <https://doi.org/10.1016/j.foodchem.2022.132206>.
- [40] Y. Liu, L. Zhou, Y. Li, R. Deng, H. Zhang, Facile synthesis of nitrogen-doped carbon dots with robust fluorescence in a strongly alkaline solution and a reversible fluorescence ‘off–on’

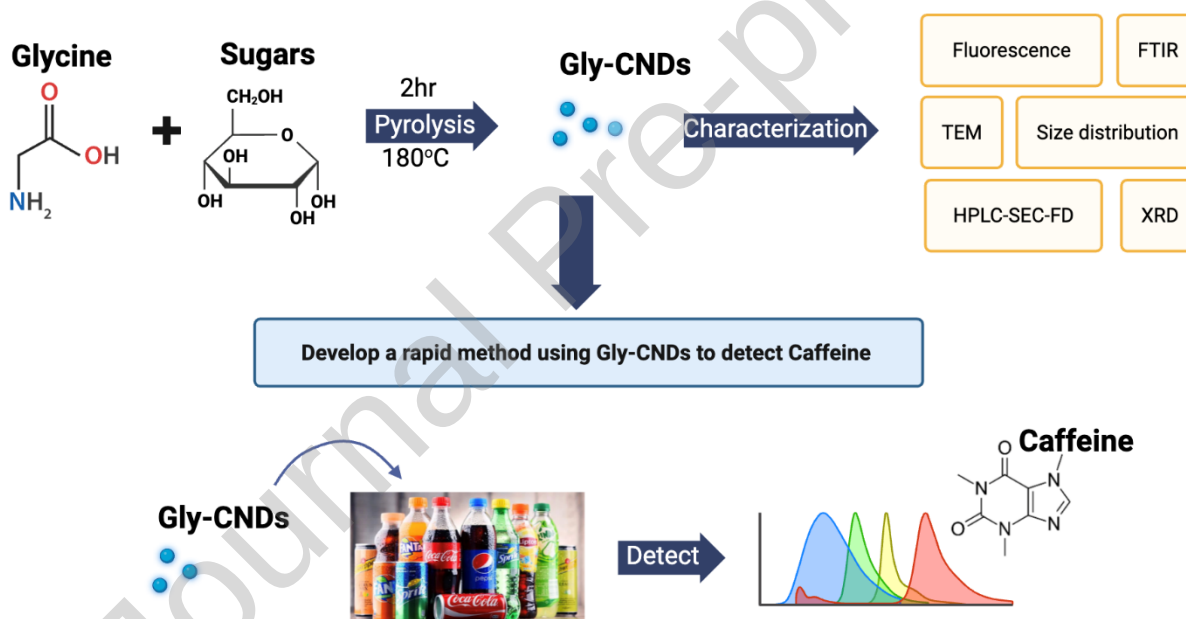
switch between strongly acidic and alkaline solutions, RSC Adv. 6 (2016) 108203–108208. <https://doi.org/10.1039/C6RA21994B>.

[41] P. Yu, X. Wen, Y.-R. Toh, J. Tang, Temperature-Dependent Fluorescence in Carbon Dots, J. Phys. Chem. C 116 (2012) 25552–25557. <https://doi.org/10.1021/jp307308z>.

Declaration of interests

☒ 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.

Graphical abstract



Highlights

- A fast and cost-effective technique for measuring caffeine in food and drinks.
- Carbon nanodots are derived from food-grade materials (glycine and sugars) to ensure food safety.
- Glycine-carbon nanodots, averaging 4.2 ± 1.2 nm, were stabilized under different conditions.
- The caffeine content closely aligns with the results obtained from the HPLC method.
- The optimal experimental conditions are an acidic pH.