Analyst

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: M. Wang, M. Wang, F. Zhang and X. Su, *Analyst*, 2020, DOI: 10.1039/D0AN01065K.

View Article Online
DOI: 10.1039/D0AN01065K

A ratiometric fluorescent biosensor for sensitive determination of α-glucosidase activity and acarbose based on N-doped carbon dots

Mengjun Wang, Mengke Wang, Feng Zhang, Xingguang Su*

College of Chemistry, Jilin University, Changchun, 130012, PR China

*Corresponding author

Tel.: +86-431-85168352

E-mail address: suxg@jlu.edu.cn (X. Su).

View Article Online
DOI: 10.1039/D0AN01065K

Abstract

In this work, a novel ratiometric fluorescent platform for α-glucosidase (α-glu) and its inhibitor was constructed based on N-doped carbon dots (N-CDs). The presence of α-glucosidase can catalyze the release of hydroquinone (HQ) from α-arbutin. Then, the generated HQ can be oxidized and copolymerized with polyethyleneimine (PEI) to form yellowish green fluorescence copolymer (P_{HQ-PEI}) with intense fluorescence emission at 510 nm. When the P_{HQ-PEI} was formed, blue fluorescence of N-CDs at 425 nm was decreased, whereas the fluorescence of P_{HQ-PEI} at 510 nm increased sharply as a result of the fluorescence resonance energy transfer (FRET) effect between N-CDs and P_{HQ-PEI}. However, in the presence of acarbose, the activity of α-glucosidase is inhibited, and α-arbutin can't be hydrolyzed to hydroquinone, leading to the fluorescence recovery of N-CDs at 425 nm and the fluorescence decreasing of P_{HQ-PEI} at 510 nm. The linear range from 0.2 to 1.6 mU mL^{-1} and 25-150 μmol L^{-1} were obtained for α-glucosidase and acarbose detection, respectively. And the detection limit (LOD) for α-glucosidase and acarbose were as low as 0.082 mU mL^{-1} and 14.5 μmol L^{-1}. Thus, a ratiometric fluorescent sensor with good sensitive and high specificity was established for α-glucosidase assay and satisfactory results were acquired in real sample determination.

Key Words: N-doped carbon dots; α-glucosidase; acarbose; ratiometric fluorescence sensor

View Article Online
DOI: 10.1039/D0AN01065K

1. Introduction

Carbon dots (CDs), are a kind of luminescent nanomaterials, have gained tremendous attention due to their noticeable properties including tunable fluorescence intensity, good water solubility, light stability and excellent biocompatibility [1-3]. In view of these distinctive features, carbon dots have been applied to various fields, such as photocatalysis, fluorescent sensor, drug delivery, and etc [4-8]. For example, Yao et al. revealed that the conjugation of magnetofluorescent CDs with different materials could achieve dual-modality cellular imaging and drug delivery [9]. Li et al. designed a "turn-off-on" fluorescent sensing platform for the assay of rutin and Al^{3+} on the basis of tiny-carbon dots [10]. Liu et al. developed a ratiometric fluorescence sensor for the determination of mercury based on the CDs and nanoclusters [11]. Some non-metal element-doped carbon dots, such as N-doped carbon dots, have become a topical subject of research due to them possess better electronic structure and chemical stability. These characteristics prompt they widely explored in the field of biosensing, solar cells [12], electrocatalysis [13], and etc. Doping CDs with nitrogen atoms can effectively adjust its surface structures and related fluorescence properties [14]. Yang et al. proved that emission spectrum of the N-doped CDs is tunable by adjusting the concentration of nitrogen atoms [15]. A ratiometric fluorescence sensor utilizing N-CQDs/Au NCs nanohybrid for carbendazim assay was successfully established. In addition, green-emitting N-CDs which are synthesized via one-pot method were applied as a multi-mode sensing platform towards Cr (VI), ascorbic acid and 2,4,6-trinitrophenol determination and bioimaging [16]. Therefore, the non-metal doped fluorescent nanomaterials with good optical properties could effectively enhanced the performance in biosensing and determination of biomolecules.

View Article Online
DOI: 10.1039/D0AN01065K

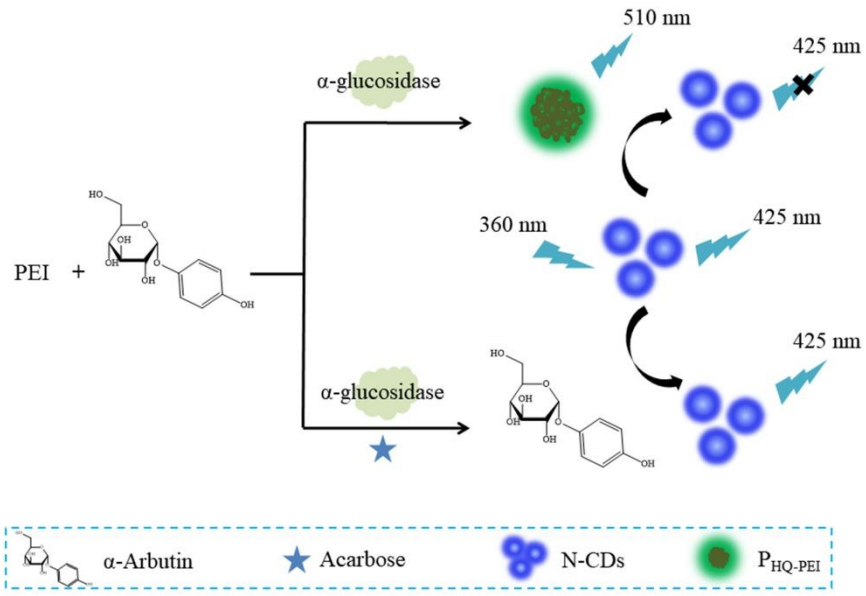
Diabetes mellitus is a common and complex immune disorder with feature of an abnormal blood sugar level [17, 18]. It has serious impact on human health, may damage to the eyes and nerves system, even led to diseases such as cardio-cerebrovascular disorders or renal failure. α-glucosidase, a hydrolytic enzyme that catalyzes the release of α-glucose from the non-reducing end of the substrate, is widely distributed in the apical brush border of intestinal epithelial cells and microorganisms [19-21]. It participates in the metabolism of sugar in the organism and plays an important role in maintaining normal physiological functions of the body. Moreover, acarbose, α-glucosidase inhibitors, could reversibly occupy the complexing site of α-glucosidase and sugar, and inhibit α-glucosidase catalyzing the degradation of polysaccharides to monosaccharides, thereby make the concentration of blood sugar within a stable level [22-24]. Thus, it is crucial to monitor the concentration of α-glucosidase because of excess or insufficient enzymes both influence the health of the organism. For example, Cheng et al proposed a turn-on ratiometric fluorescent probe that can quickly response to alkaline phosphatase and α-glucosidase based on the use of dual-color carbon dots [25]. Liu and co-workers established a fluorescent “turn off-on” nanoprobe based on $CuInS_2$ quantum dots monitoring the activity of β-glucosidase and its inhibitor [26]. Liu et al. utilized fluorescent polymer nanoparticles for highly sensitivity detection of β-glucosidase activity [27]. However, the detection limit (LOD) of these methods are relatively high. In addition, factors such as uneven distribution of fluorescent nanoparticles, photobleaching, and instrument variability may affect the accuracy and sensitivity of single-wavelength emission probes. Ratiometric fluorescent probes, by contrast, possess the ability of self-calibration by monitoring two or more emission peaks and keep high accuracy and inherent reliability in a

View Article Online
DOI: 10.1039/D0AN01065K

complex environment. Thus, it is necessary to construct a ratiometric fluorescence method to detect glucosidase activity accurately.

Herein, taking advantage of the features of high fluorescence intensity and good stability of $P_{HQ\text{-}PEI}$, we construct an efficient ratiometric fluorescence strategy for α-glucosidase activity and acarbose determination by using $P_{HQ\text{-}PEI}$ and blue-emitting N-CDs. The mechanism for α-glucosidase activity and acarbose assay was illustrated in Scheme 1. As we know, hydroquinone of α-arbutin hydrolysate can be rapidly oxidized to 1,4-benzoquinone in alkaline conditions. The yellow-green fluorescent polymer $P_{HQ\text{-}PEI}$ is obtained by the formation of quinone-imine bond between 1,4-benzoquinone and amino groups of polyethyleneimines (PEI) . Furthermore, varying the concentration of hydroquinone of α-arbutin hydrolysate, it is possible to modulate the degree of aggregation of $P_{HQ\text{-}PEI}$ and produce an obvious fluorescent response. With the increasing concentration of α-glucosidase, the formation of $P_{HQ\text{-}PEI}$ led to the fluorescence quenching of N-CDs at 425 nm due to the fluorescence resonance energy transfer (FRET) effect between N-CDs and $P_{HQ\text{-}PEI}$. However, in the presence of acarbose, α-glucosidase the activity of is inhibited, substrates α-arbutin can't be catalytically hydrolyzed to generate hydroquinone (HQ), making the fluorescence of $P_{HQ\text{-}PEI}$ quenched and the fluorescence of N-CDs restored again. Therefore, N-doped carbon dots-based ratiometric fluorescence sensor was constructed for the analysis of α-glucosidase and acarbose by measuring the fluorescence intensity ratio I_{510}/I_{425}.

View Article Online
DOI: 10.1039/D0AN01065K



Scheme 1. Schematic diagram of the ratiometric fluorescence strategy for α-glucosidase and acarbose assay in polymerization process.

2. Experimental

2.1. Material sand instruments

Related chemicals and instruments have been given in the Supporting Information (SI).

2.2. Synthesis of N-CDs

Incorporating adenosine as carbon and nitrogen source, the fluorescent N-CDs were synthesized by hydrothermal approach according to a reported method with a little modification [28]. In brief, aqueous solution of adenosine (10 mL, 5 mM) was put into a Teflon-lined autoclave (15 mL) and heated at 200 °C for 9.5 h. The solution turned from colorless to light yellow transparent liquid, indicating the formation of N-CDs. Then, the obtained mixture was centrifuged (16,00 rpm, 17 min) and stored at 4 °C for further experiments.

2.3. Fluorescence sensing of α-glucosidase and its inhibitor

For α-glucosidase assay, 500 μL α-glucosidase (with various final concentrations from 0 to 5

View Article Online
DOI: 10.1039/D0AN01065K

mU mL^{-1}), 100 μL of 0.8 mmol L^{-1} α-arbutin and 100 μL of 16 mmol L^{-1} PBS (pH = 6.4) were added into 2 mL calibrated test tube. After incubating at 37 ℃ for 50 min, 300 μL of 2 mg mL^{-1} PEI and 100 μL of 400 mmol L^{-1} PBS (pH = 8.7) were successively added to above solutions and incubated at 70 ℃ for 60 min. Then, 100 μL N-CDs was added to the obtained solution, and 800 μL deionized water was utilized to supplement the value to 2 mL. Fluorescence signals of the resulting solution were recorded by a fluorescence spectrometer in the range of 475 nm to 700 nm (λex = 360 nm).

For acarbose detection, 500 μL α-glucosidase (3.2 mU mL^{-1}), 50 μL varied amounts of acarbose, 50 μL of 32 mmol L^{-1} PBS (pH = 6.4) and 100 μL of 0.8 mmol L^{-1} α-arbutin were mixed thoroughly and incubated in 2 mL calibrated test tube for 50 min at 37 ℃. Then, 300 μL PEI (2 mg mL^{-1}) and 100 μL PBS (400 mmol L^{-1}, pH = 8.7) were added into the above solutions, separately. After incubating at 70 ℃ for 60 min, 100 μL N-CDs and 800 μL deionized water were added to the solution. Fluorescence signals of the resulting solution were recorded by a fluorescence spectrometer in the range of 475 nm to 700 nm (λex = 360 nm).

2.4. Assay of α-glucosidase in serum sample

The developed ratiometric fluorescent sensor was further applied to determination of α-glucosidase activity in serum sample, which was supplied by the Changchun China Japan Union Hospital. All experiments were performed in compliance with the relevant laws and institutional guidelines, and approved by the ethics committee at Jilin University. Informed consents were obtained from human participants of this study. Before preparing the spiked samples, all the blood samples need to be centrifuged at 11 000 rpm for 10 min to remove the precipitates and diluted 50-fold with deionized water for subsequent experiments. Then, different concentrations (0.4, 0.8,

View Article Online
DOI: 10.1039/D0AN01065K

1.2 mU mL^{-1}) of α-glucosidase were added into the above diluted serum sample. Following procedures for α-glucosidase determination were same as described in part 2.4 and corresponding fluorescent spectra spectrometer were taken under 360 nm excitation wavelength.

3. Results and discussion

3.1. Characterization of N-CDs

The structure and optical properties of the blue-emissive N-CDs were studied by transmission electron microscopy (TEM), Fourier transform infrared (FT-IR) spectroscopy, fluorescence and UV-vis absorption spectroscopy. TEM image showed that a large proportion of the N-CDs are spherical particles with good dispersion and approximately 4.66 nm in size (Fig.1a). To identify the functional groups on the surface of N-CDs, FT-IR spectra of the N-CDs was investigated and the results are illustrated in Fig.1b. The characteristic peaks at 3334 cm^{-1} and 3166 cm^{-1} were assigned to O-H and N-H bonds, respectively. The obvious peak at 1682 cm^{-1} and 1607 cm^{-1} corresponded to the stretch of C=O and N-H, respectively. The band at 1403 cm^{-1} was attributed to C-N bond and the peak located at around 1121 cm^{-1} was ascribed to C-O stretching. The FT-IR data proved that the surface of N-CDs possess abundant amino groups, carboxyl groups. From Figure 1c, it can be seen that an intense emission peak of N-CDs is located at 425 nm under 360 nm excitation. And a weak peak in UV absorption spectra centered at 327 nm could be observed, which was probably attributed to π–π* electronic transition of carbon sp2 domains [29]. From the Figure 1c insert, we can see that the N-CDs were light-yellow color under sunlight and exhibited intense blue fluorescence under UV irradiation (365 nm).

View Article Online
DOI: 10.1039/D0AN01065K

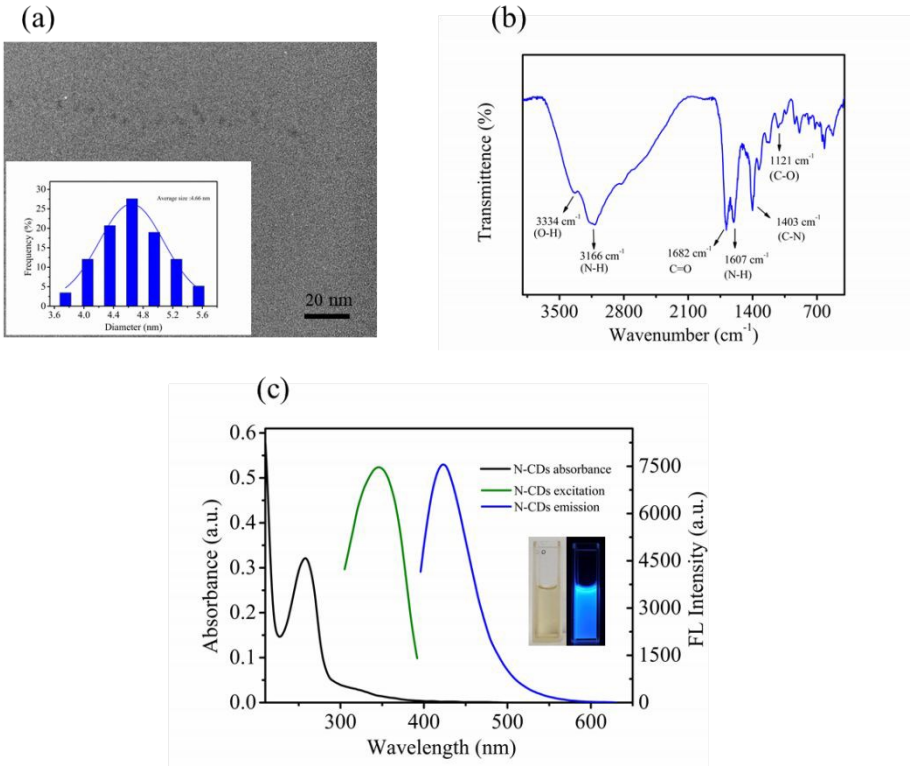


Fig. 1. (a) TEM image of N-CDs. The insert shows that the size distribution of N-CDs. (b) FT-IR spectra for N-CDs. (c) UV–vis absorption spectra (blank) and fluorescence excitation (green) and emission (blue) spectra of N-CDs. Insert showed the photographs of N-CDs under the sunlight (left) and 365 nm UV irradiation (right).

3.2. Mechanism for α-glucosidase and acarbose determination

The detection mechanism for α-glucosidase assay based on the enzymatic product hydroquinone-triggered in situ fluorescence generation of $P_{HQ\text{-}PEI}$ were shown in Scheme 1. In this sensing system, α-glucosidase catalyzes the release of hydroquinone from α-arbutin. The generated hydroquinone promotes the cross-linking of PEI, thereby forming a yellow-green fluorescence copolymer $P_{HQ\text{-}PEI}$ with emission at 510 nm. Firstly, to prove that the hydroquinone treated with PEI can form yellow-green fluorescence copolymer $P_{HQ\text{-}PEI}$, we studied the fluorescence spectra of the N-CDs/PEI system with different concentrations of hydroquinone. Fig. S1 showed that a new fluorescence peak at 510 nm appeared with the addition of hydroquinone,

View Article Online
DOI: 10.1039/D0AN01065K

and its fluorescence intensity increased with the increase of the hydroquinone concentration, indicating the fluorescence copolymer $P_{HQ\text{-}PEI}$ formed. From Fig. 2a, we can see that the maximum fluorescence emission and excitation peaks of fluorescent $P_{HQ\text{-}PEI}$ respectively concentrated at 380 nm and 510 nm. To study the feasibility of the N-CDs/$P_{HQ\text{-}PEI}$-based fluorescent sensing strategy for α-glucosidase assay, we conducted a series of tests to study the influence of related substances in the N-CDs/$P_{HQ\text{-}PEI}$ system and the results are illustrated in Fig. 2b. It can be seen that α-glucosidase, α-arbutin, PEI and the mixture of α-glucosidase and α-arbutin have little effect on the fluorescence of N-CDs. When the mixture of α-arbutin and α-glucosidase were added to PEI solution, an intense fluorescence emission peak at 510 nm could be observed due to the polymerization reaction between hydroquinone and PEI, and the formed $P_{HQ\text{-}PEI}$ could effectively quench the fluorescence of N-CDs. Upon addition of acarbose, the activity of α-glucosidase was inhibited, which leading to the fluorescence at 425 nm gradually increased and the fluorescence at 510 nm decreased owing to the lack of enough hydroquinone to oxidative polymerization with PEI. These results implied that acarbose and α-glucosidase activity could be detected by monitoring the value of I_{510}/I_{425}. Additionally, fluorescence quenching mechanism of the ratiometric fluorescent sensor was further investigated.

As displayed in Fig.2c, the absorbance peak of $P_{HQ\text{-}PEI}$ has an obvious overlap with the emission spectrum of N-CDs, indicating that there is an inner filter effect (IFE) or fluorescence resonance energy transfer (FRET) between the N-CDs and $P_{HQ\text{-}PEI}$. To further investigate the quenching mechanism, we conducted the fluorescent lifetime test and the results showed that the fluorescent lifetime of the N-CDs in the absence and presence of $P_{HQ\text{-}PEI}$ were 5.23 ns and 3.91 ns, respectively (Fig.2d). Therefore, we can conclude that there is a FRET effect between N-CDs and

View Article Online
DOI: 10.1039/D0AN01065K

$P_{HQ\text{-}PEI}$.

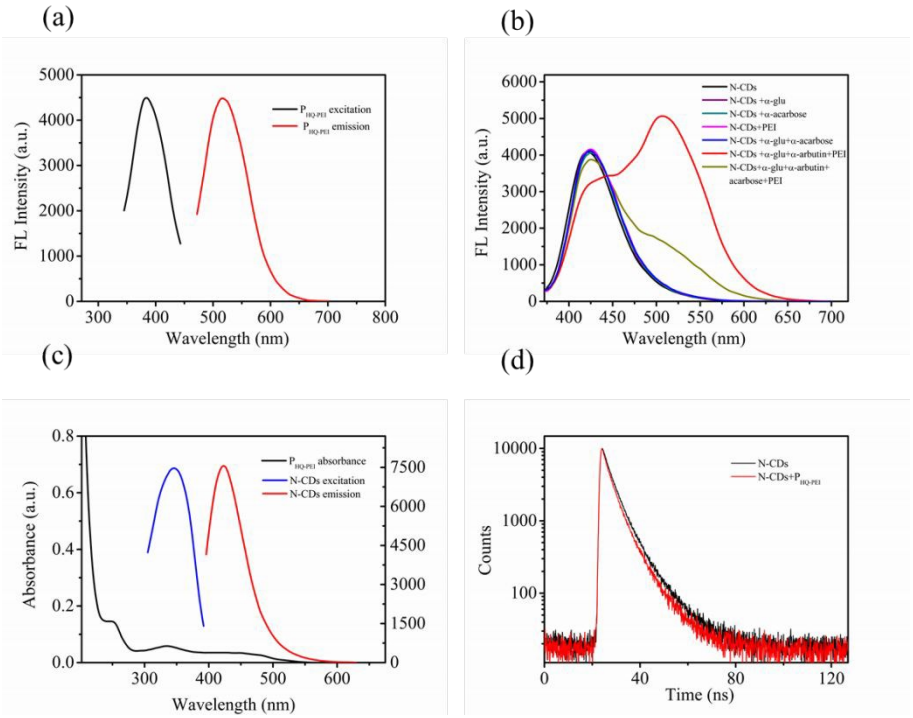


Fig. 2. (a) Fluorescence excitation (blank) and emission (red) spectra of $P_{HQ\text{-}PEI}$. (b) Fluorescence emission spectra of N-CDs; N-CDs+α-glu; N-CDs+α-arbutin; N-CDs+PEI; N-CDs+α-glu+α-arbutin; N-CDs+α-glu+α-arbutin+PEI; N-CDs+α-glu+α-acarbose+acarbose+PEI. (c) Fluorescence excitation and emission spectra of N-CDs and UV−vis absorption spectra of $P_{HQ\text{-}PEI}$. (d) Fluorescence lifetime spectra of N-CDs and N-CDs/$P_{HQ\text{-}PEI}$ system.

3.3. Optimization for α-glucosidase detection

For the construction of a high selective and sensitive biosensor for α-glucosidase activity detection, some important experimental factors such as reaction pH, temperature, PEI concentration, reaction time for polymerization reaction were optimized. Firstly, the effect of PEI concentration was investigated. Fig. 3a showed the fluorescence intensity ratio I_{510}/I_{425} increased to maximum at 2 mg mL^{-1} PEI and kept stable with increasing the concentration of PEI. Thus, 2 mg mL^{-1} PEI was selected for the further experiments. The fluorescence intensity ratio I_{510}/I_{425} at

View Article Online
DOI: 10.1039/D0AN01065K

different pH value and reaction temperature was also investigated. As shown in Fig. 3b-c, the fluorescent intensity ratio I_{510}/I_{425} reached maximal when pH was 8.7 and temperature was 70 ℃, so pH 8.7 and reaction temperature of 70 ℃ were chosen as the optimal polymerization reaction conditions. Furthermore, the maximum of fluorescence intensity ratio I_{510}/I_{425} was achieved at reaction time of 60 min and kept stable with further increasing the reaction time, which indicated that the produced hydroquinone completely oxidized polymerization with PEI and formed $P_{HQ\text{-}PEI}$ (Fig. 3d). The concentration of α-arbutin, enzymatic temperature, pH, and reaction time were further optimized for the enzyme-catalyze hydrolysis reaction. It is reported that neutral and slightly acidic condition is beneficial for α-glucosidase activity to reach the maximum value, so pH values ranging from 6 to 7 were chosen for further study. The results in Fig S2a displayed that the maximum fluorescent intensity ratio I_{510}/I_{425} could be achieved at pH 6.4. Enzyme reaction time is another key factor effecting the fluorescent intensity of $P_{HQ\text{-}PEI}$ Fig S2b showed that the fluorescent intensity ratio I_{510}/I_{425} increased with the increase of enzyme reaction time and reached a maximum at 50 min, and then decreased gradually after 50 min. Thus, 50 min was chosen as the optimal enzymatic reaction time. The effect of reaction temperature was investigated (Figure S2c) and 37 °C exhibited a better fluorescent intensity ratio response. The effect of α-arbutin concentration was further studied and the optimal concentration of 0.8 mmol L^{-1}(Figure S2d) was used for the further experiments.

View Article Online
DOI: 10.1039/D0AN01065K

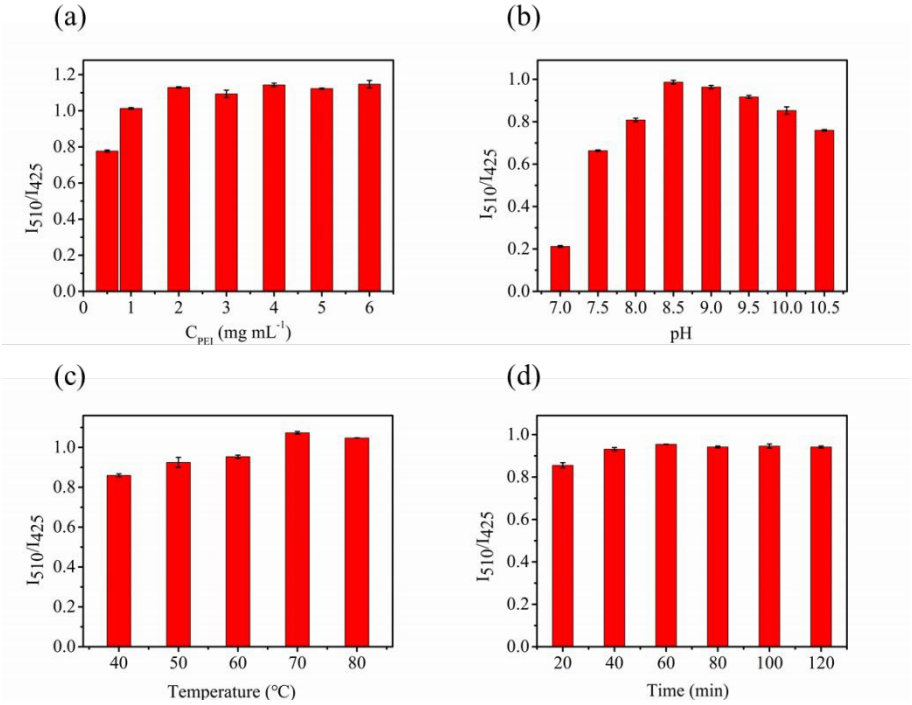


Fig. 3. Effect of PEI concentration (a), pH value (b), temperature (c) and reaction time (d) on the fluorescence intensity ratio I_{510}/I_{425} of N-CDs/$P_{HQ\text{-}PEI}$ system in the process of polymerization.

3.4. Assay of α-glucosidase and acarbose

Under the optimal conditions, the fluorescence spectra of N-CDs/$P_{HQ\text{-}PEI}$ system with various HQ concentrations were recorded in Fig. 4a-b. We can see that the fluorescence intensity at 425 nm decreased and meanwhile increased at 510 nm with increasing concentration of α-glucosidase (0-5 mU mL^{-1}).The plot of the fluorescence intensity ratio I_{510}/I_{425} responses towards α-glucosidase concentration (0.2-1.6 mU mL^{-1}) possessed a good linear relationship, and the linear equation was I_{510}/I_{425}=0.120 + 0.781[α-glucosidase] (mU mL^{-1}) (R^2 = 0.999). The limit of detection (LOD) for α-glucosidase was 0.082 mU mL^{-1} calculated from the equation of LOD=3 S_B/b (S_B represented the standard deviation (n=11) of the blanks; b represented the slope of calibration curve). Compared with other methods for α-glucosidase detection, as shown in Table S1, our sensing strategy exhibits superior performance in sensitivity and linear range.

View Article Online
DOI: 10.1039/D0AN01065K

It was noted that some specific inhibitors of α-glucosidase could decrease blood glucose and glycated hemoglobin concentration. Developing an inhibitor detection method with low-cost and high-accuracy is of great importance. To achieve enzymatic inhibitor screening, acarbose was chosen as a representative inhibitor of α-glucosidase to be investigated. As exhibited in Fig. 4c-d, with increasing the concentration of acarbose ranging from 25 μmol L^{-1} to 450 μmol L^{-1}, α-glucosidase activity was inhibited, and its ability to catalyze the hydrolysis of α-arbutin was reduced, resulting in the fluorescence intensity ratio I_{510}/I_{425} decreased. The obtained fitting linear equation could be expressed as $I_{510/I425}$= 0.995-0.004[acarbose] (μmol L^{-1}) (R^2 = 0.992), and corresponding LOD was as low as 14.5 μmol L^{-1}. Above results indicated that the N-CDs/$P_{HQ\text{-}PEI}$ sensing platform was feasible for the determination of acarbose activity. Furthermore, we investigated some other inhibitors of α-glucosidase, such as rutin, quercetin, quercitrin and isoquercitrin. Fig. S3 showed that the fluorescence of N-CDs and $P_{HQ\text{-}PEI}$ were both decreased when rutin, quercetin, quercitrin or isoquercitrin was introduced to the sensing system. According to previous report [30], flavonoids, such as quercetin, are usually colored and the UV-Vis spectra of most flavonoids display benzoyl band in the 240–280 nm range and the cinnamoyl band in the 320–385 nm range, which will largely overlap with the excitation peak of N-CDs, and result in the fluorescent quenching of N-CDs. So the fluorescence of N-CDs and $P_{HQ\text{-}PEI}$ were both decreased. Thus, we selected acarbose as a representative inhibitor of α-glucosidase in this study.

View Article Online
DOI: 10.1039/D0AN01065K

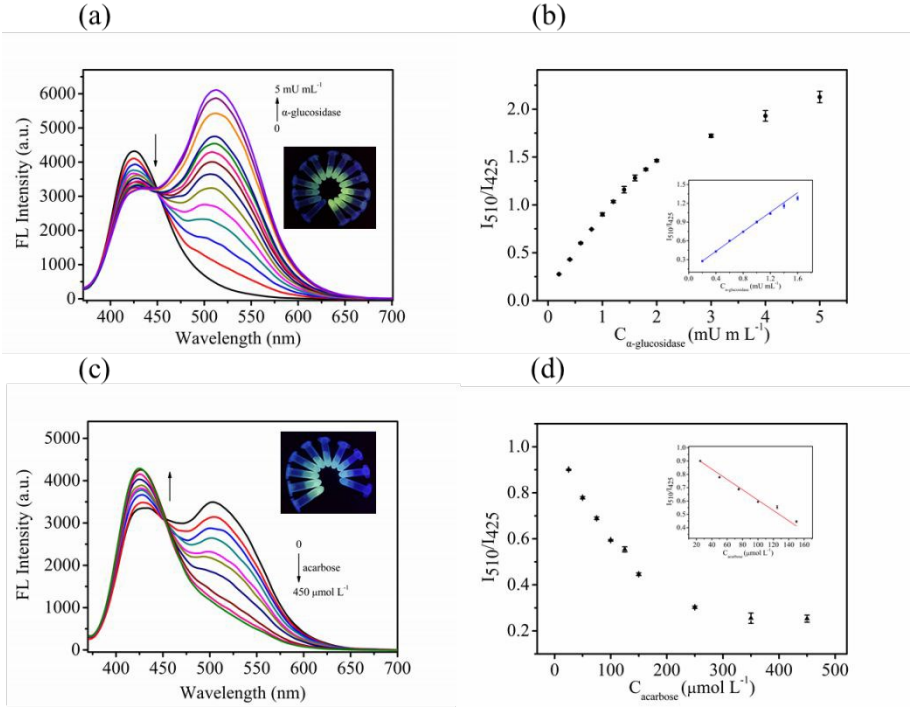


Fig. 4. (a) Fluorescent intensity ratio (I_{510}/I_{425}) response towards various α-glucosidase concentrations. Insert: corresponding digital images of the N-CDs/$P_{HQ\text{-}PEI}$ system with various α-glucosidase concentration under 365 nm UV light (from blue to green: 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.5, 3, 3.5, 4, 4.5 and 5 mU mL^{-1}). (b) Relationship between the fluorescent intensity ratio I_{510}/I_{425} and different α-glucosidase concentration. Inset: Plot of the fluorescent intensity ratio I_{510}/I_{425} of the N-CDs/$P_{HQ\text{-}PEI}$ system versus the concentration of α-glucosidase. (c) The fluorescent intensity ratio I_{510}/I_{425} response towards various acarbose concentrations. Insert: corresponding images of the N-CDs/$P_{HQ\text{-}PEI}$ system with various acarbose concentrations in the presence of α-glucosidase under UV light (from left to right: 25, 50, 75, 100, 125, 150, 250, 350, 450 μmol L^{-1}). (d) Relationship between the fluorescent intensity ratio I_{510}/I_{425} and different acarbose concentration. Inset: Plot of the fluorescent intensity ratio I_{510}/I_{425} of the N-CDs/$P_{HQ\text{-}PEI}$ system versus the concentration of acarbose.

3.5. Interference test

View Article Online
DOI: 10.1039/D0AN01065K

For a fluorescent sensing system, having high sensitivity is obviously important, but possess good selectivity is also vital for its applications in real samples. The selectivity of this ratiometric fluorescent sensor for α-glucosidase activity detection was estimated by monitoring its response to various interfering compounds, including trypsin (Try), papain (Pap), urease, lysozyme (Lys), ascorbic acid oxidase(AAOx), pepsin (Pep), hyaluronidase (HAase), cholesterol oxidase (COD), hemoglobin (HB), protamine (Pro), bovine serum albumin (BSA), and some inorganic salt ions (K^+, Na^+, Ca^{2+}). As illustrated in Fig. 5, the N-CDs/$P_{HQ\text{-}PEI}$ system has an obvious response towards α-glucosidase, while the fluorescent intensity ratio I_{510}/I_{425} exhibited no significant change after the addition of other interfering substances. The ratiometric fluorescent method possess satisfactory anti-interference ability for α-glucosidase activity detection.

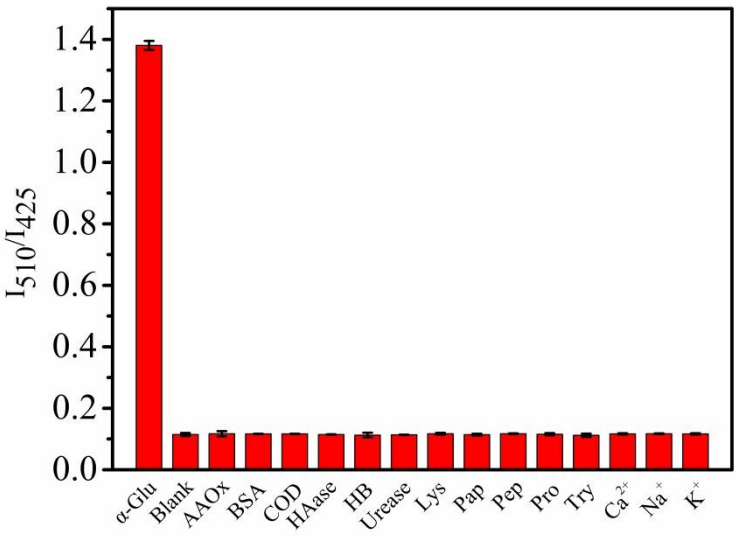


Fig. 5. Fluorescent intensity ratio I_{510}/I_{425} response towards α-glucosidase (1.6 mU mL^{-1}) and possible interfering substances. Conditions: 100 mmol L^{-1} ions (K^+, Na^+, Ca^{2+}); 5 μg mL^{-1} biomolecules (Pro, BSA, HB); 16 mU mL^{-1}bio-enzymes (HAase; Urease, Lys, AAOx, Pap, Pep, COD, Try); 2 mg mL^{-1} PEI; λ_{ex} = 360 nm; pH=6.4; 0.8 mmol L^{-1} α-Arbutin.

View Article Online
DOI: 10.1039/D0AN01065K

3.6. Real sample analysis

To study the feasibility and practicability of this ratiometric fluorescence sensor, standard addition method was adopted for the assay of α-glucosidase activity in human serum samples. As illustrated in Table S1, the recoveries of α-glucosidase in serum samples ranged from 95.0 to 107.5% with the relative standard deviations RSDs lower than 3%, which proved that the proposed ratiometric fluorescence method possesses good accuracy and high reliability for α-glucosidase assay in actual samples.

Table 1 Assay results for α-glucosidase determination in serum samples

Sample	Added (mU/mL)	Found (mU/mL)	Recovery (%)	RSD (%, n = 3)
	0			
1	0.40	0.43	107.5	0.56
2	0.80	0.83	103.7	1.09
3	1.20	1.14	95.0	1.91

4. Conclusion

In summary, a facile and sensitive ratiometric fluorescent sensor was established for α-glucosidase activity and acarbose detection based on FRET effect between N-CDs and $P_{HQ\text{-}PEI}$. Upon the addition of α-glucosidase to α-arbutin, the generated hydroquinone continuously oxidized and polymerized with PEI and formed $P_{HQ\text{-}PEI}$, which further quenching the fluorescence of N-CDs. However, the presence of acarbose would regulate the FRET effect by inhibiting α-glucosidase activity and the fluorescent at 425 nm restored with the florescence at 510 nm decreased. This ratiometric fluorescent sensor strategy exhibited excellent linear relationships and low LOD, satisfactory results in practical application for α-glucosidase activity detection.

View Article Online
DOI: 10.1039/D0AN01065K

Acknowledgments

This work is supported by the National Natural Science Foundation of China (No. 21775052 and No. 21575048), the Science and Technology Development project of Jilin province, China (No. 20180414013GH).

References

1. S. N. Baker and G. A. Baker, *Angew Chem Int Ed Engl*, 2010, **49**, 6726-6744.
2. S. Zhu, Q. Meng, L. Wang, J. Zhang, Y. Song, H. Jin, K. Zhang, H. Sun, H. Wang and B. Yang, *Angew Chem Int Ed Engl*, 2013, **52**, 3953-3957.
3. R. Liu, D. Wu, S. Liu, K. Koynov, W. Knoll and Q. Li, *Angew Chem Int Ed Engl*, 2009, **48**, 4598-4601.
4. J. Du, N. Xu, J. Fan, W. Sun and X. Peng, *Small*, 2019, **15**, e1805087.
5. X. Ye, Y. Xiang, Q. Wang, Z. Li and Z. Liu, *Small*, 2019, **15**, e1901673.
6. H. Liu, Y. Sun, Z. Li, J. Yang, A. A. Aryee, L. Qu, D. Du and Y. Lin, *Nanoscale*, 2019, **11**, 8458-8463.
7. Q. Y. Cai, J. Li, J. Ge, L. Zhang, Y. L. Hu, Z. H. Li and L. B. Qu, *Biosens Bioelectron*, 2015, **72**, 31-36.
8. G. Liu, J. Zhao, S. Wang, S. Lu, J. Sun and X. Yang, *Sensors and Actuators B: Chemical*, 2020, **306**.
9. Y. Y. Yao, G. Gedda, W. M. Girma, C. L. Yen, Y. C. Ling and J. Y. Chang, *ACS Appl Mater Interfaces*, 2017, **9**, 13887-13899.
10. H. Li, Y. Xu, L. Zhao, J. Ding, M. Chen, G. Chen, Y. Li and L. Ding, *Carbon*, 2019, **143**,

View Article Online
DOI: 10.1039/D0AN01065K

391-401.

11. W. Liu, X. Wang, Y. Wang, J. Li, D. Shen, Q. Kang and L. Chen, *Sensors and Actuators B: Chemical*, 2018, **262**, 810-817.

12. K. S. Lee, W. J. Lee, N. G. Park, S. O. Kim and J. H. Park, *Chem Commun (Camb)*, 2011, **47**, 4264-4266.

13. Y.-S. Chen, J.-H. Huang and C.-C. Chuang, *Carbon*, 2009, **47**, 3106-3112.

14. H. Qi, M. Teng, M. Liu, S. Liu, J. Li, H. Yu, C. Teng, Z. Huang, H. Liu, Q. Shao, A. Umar, T. Ding, Q. Gao and Z. Guo, *J Colloid Interface Sci*, 2019, **539**, 332-341.

15. Y. Yang, X. Xing, T. Zou, Z. Wang, R. Zhao, P. Hong, S. Peng, X. Zhang and Y. Wang, *J Hazard Mater*, 2019, **386**, 121958.

16. Y. Meng, Y. Jiao, Y. Zhang, Y. Li, Y. Gao, W. Lu, Y. Liu, S. Shuang and C. Dong, *Talanta*, 2020, **210**.

17. R. J. Perry, V. T. Samuel, K. F. Petersen and G. I. Shulman, *Nature*, 2014, **510**, 84-91.

18. A. E. Kitabchi, G. E. Umpierrez, J. M. Miles and J. N. Fisher, *Diabetes Care*, 2009, **32**, 1335-1343.

19. Z. Zeng, S. Mizukami and K. Kikuchi, *Anal Chem*, 2012, **84**, 9089-9095.

20. X. Huang, Kelly S. E. Tanaka, and Andrew J. Bennet, J. Am. Chem. Soc. 1997, 119, 11147-11154.

21. D. Wu, N. Hu, J. Liu, G. Fan, X. Li, J. Sun, C. Dai, Y. Suo, G. Li and Y. Wu, *Talanta*, 2018, **190**, 103-109.

22. C. Tang, Z. Qian, Y. Qian, Y. Huang, M. Zhao, H. Ao and H. Feng, *Sensors and Actuators B: Chemical*, 2017, **245**, 282-289.

View Article Online
DOI: 10.1039/D0AN01065K

23. J. Jiang, J. Li, J. Zhu, Y. Yu, G. Duan, L. Zhou and Y. Li, *Talanta*, 2020, **209**, 120514.

24. W. Kong, D. Wu, N. Hu, N. Li, C. Dai, X. Chen, Y. Suo, G. Li and Y. Wu, *Biosens Bioelectron*, 2018, **99**, 653-659.

25. X. Cheng, J. Xu, L. Wang, G. Xu, F. Wei, Y. Chai, Q. Hu and Y. Cen, *Microchimica Acta*, 2019, **186**, 818.

26. Z. Liu, Y. Tian, Y. Han, E. Bai, Y. Li, Z. Xu and S. Liu, *Microchimica Acta*, 2019, **186**, 806.

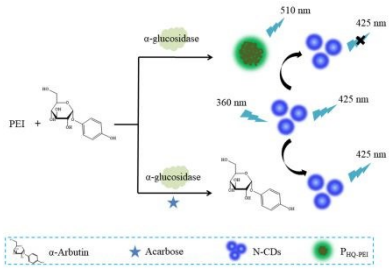
27. J. Liu, H. Bao, C. Liu, F. Wu and F. Gao, *ACS Applied Polymer Materials*, 2019, **1**, 3057-3063.

28. W. J. Zhang, S. G. Liu, L. Han, H. Q. Luo and N. B. Li, *Sensors and Actuators B: Chemical*, 2019, **283**, 215-221.

29. R. Wang, X. Wang and Y. Sun, *Sensors and Actuators B: Chemical*, 2017, **241**, 73-79.

30. M. M. Kasprzak, A. Erxleben and J. Ochocki, *RSC Advances*, 2015, **5**, 45853-45877.

View Article Online
DOI: 10.1039/D0AN01065K



A ratiometric fluorescent biosensor for the determination of α-glucosidase and acarbose was constructed based on the N-CDs/$P_{HQ\text{-}PEI}$ system.