Journal of Fluorescence (2019) 29:265–270
https://doi.org/10.1007/s10895-018-02336-2

ORIGINAL ARTICLE

3-Hydroxyphenylboronic Acid-Based Carbon Dot Sensors for Fructose Sensing

Diana M. A. Crista[1] · Guilherme P. C. Mello[1] · Olena Shevchuk[1] · Ricardo M. S. Sendão[1] · Eliana F. C. Simões[2] · João M. M. Leitão[2] · Luís Pinto da Silva[1,3] · Joaquim C. G. Esteves da Silva[1,3]

Received: 5 September 2018 / Accepted: 26 December 2018 / Published online: 5 January 2019

Abstract

The selective fluorescence sensing of fructose was achieved by fluorescence quenching of the emission of hydrothermal-synthesized carbon quantum dots prepared by 3-hydroxyphenylboronic acid. Quantification of fructose was possible in aqueous solutions with pH of 9 (Limit of Detection L_{OD} and Limit of Quantification L_{OQ} of 2.04 and 6.12 mM), by quenching of the emission at 376 nm and excitation ~380 nm with a linearity range of 0–150 mM. A Stern-Volmer constant (K_{SV}) of 2.11×10^{-2} mM^{-1} was obtained, while a fluorescent quantum yield of 31% was calculated. The sensitivity of this assay towards fructose was confirmed by comparison with other sugars (such as glucose, sucrose and lactose). Finally, the validity of the proposed assays was further demonstrated by performing recovery assays in different matrixes.

Keywords Carbon dots · Boronic acids · Fructose · Sensing · Fluorescence quenching

Introduction

Fructose is a sugar with high sweetness and is a major component of added sugars. However, excess intake of this sugar is associated with risk for metabolic disease. When compared with other sugars it shows ability to cause many diseases like intracellular ATP depletion, nucleotide turnover, endothelial dysfunction and oxidative stress [1, 2]. Furthermore, fructose provokes a rapid increase in the levels of uric acid [3], and diets rich in fructose are directly related to the epidemic growth of obesity [4]. In fact, sugar-sweetened beverages have become one of the primary sources of added sugars in daily diets [5, 6], which emphasizes the importance of fructose and its metabolism [7]. The ubiquity of fructose in modern diets also increases its relevance in diabetes, which is increasing at an alarming pace [5]. However, the fructose content in beverages and foods is not generally specified yet, though the total carbohydrate content is already done in many cases. Therefore, it is difficult to estimate the fructose consumption level at present [8] .

Given this, the ability to detect and quantify fructose is essential both in medical diagnostics [9] and in food and beverage industries [10]. Some of the approaches available for the detection of fructose and other sugars involve either gas/liquid chromatography [11–13] or capillary electrophoresis [14]. These methods are not ideal due to long-time of analysis and expensive instrumentation. Thus, it is of the utmost interest to develop efficient, accurate, simple and potentially automatable sensing methods for fructose. Some advance has been made in this direction with the development of Raman spectroscopic, enzymatic and non-enzymatic chemiluminescence-based methodologies [15–17], but they still require elaborated microscopy setups and characteristic periods of time [18].

Fluorescence is a very attractive sensing technique for fructose and other sugars due to its operational simplicity and high sensitivity [19, 20]. In fact, there has been significant effort in developing fluorescent molecular probes that have boronic

✉ Joaquim C. G. Esteves da Silva
jcsilva@fc.up.pt

[1] Chemistry Research Unit (CIQUP), Faculty of Sciences of University of Porto, R. Campo Alegre 697, 4169-007 Porto, Portugal

[2] Chemistry Research Unit (CIQUP), Faculdade de Farmácia da Universidade de Coimbra, Pólo das Ciências da Saúde, 3000-548 Coimbra, Portugal

[3] LACOMEPHI, GreenUPorto, Faculty of Sciences of University of Porto, R. Campo Alegre 697, 4169-007 Porto, Portugal

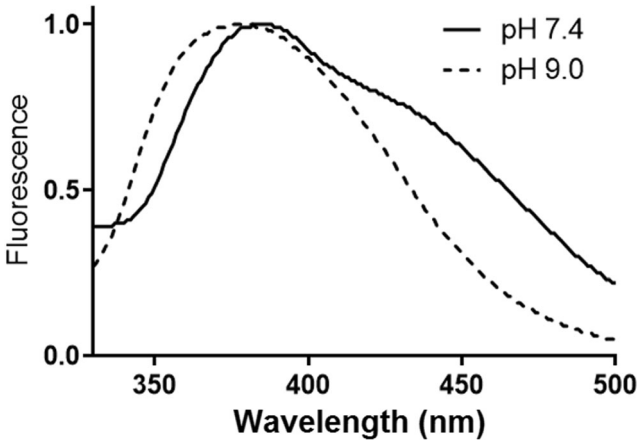

Fig. 1 Normalized fluorescent spectra obtained for 3-HPBA-based CDs, in aqueous solutions at pH 7.4 and 9, when excited at 310 nm

acid motifs [18, 21–25], due to their well-described ability to bind sugar molecules and form cyclic esters in neutral and basic solutions [26, 27]. Nevertheless, several of those fluorescent probes suffer from weak signaling responses [18]. To overcome this problem, some authors have added amine groups near to the boronic acid moieties to allow for photoinduced electron transfer (PET) [18, 28, 29], coupled dyes with high quantum yield (such as rhodamine and fluorescein) to the boronic acid moieties [30, 31], or created multi-dimensional sensors [25, 32, 33]. While some success was achieved with these different approaches, they require the preparation of different boronic acid receptors and sensing methods, which can be quite complex, and so, not very practical.

Herein, we describe a simple but selective and sensitive analytical procedure for the detection and quantification of fructose based on fluorescent carbon dots (CDs) synthesized hydrothermally from 3-hydroxyphenylboronic acid (3-HPBA). CDs are carbon-based nanoparticles with interesting optical and analytical properties [34–37]. They present high photo and chemical stability and biocompatibility, low

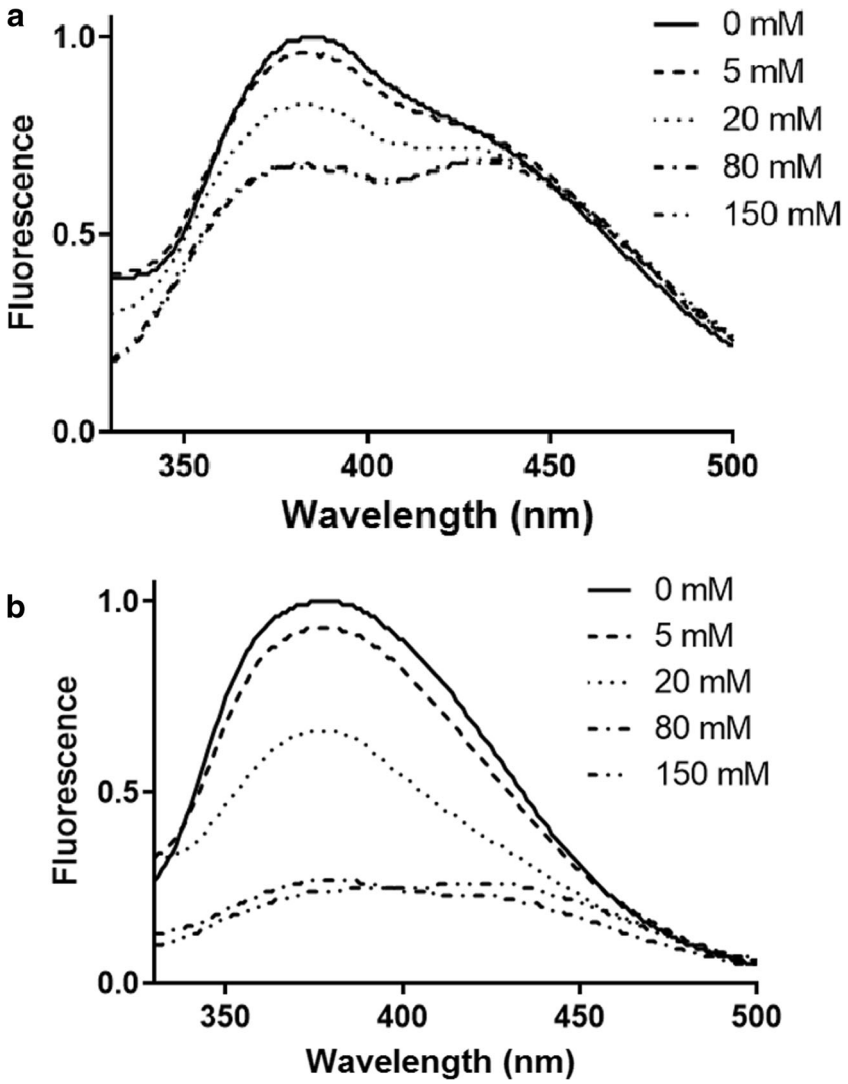

Fig. 2 Normalized fluorescence spectra of CDs with increasing fructose concentration (0–150 mM), in aqueous solutions at pH 7.4 (A) and 9 (B), when excited at 310 nm

toxicity, water solubility, and are easily functionalized. Moreover, they can be obtained from simple and green approaches, such as a hydrothermal [38, 39] and microwave [37, 40] methodologies.

The synthesized CDs show a relatively high quantum yield (Φ of 31%). The sensibility to fructose in different conditions (as different pH) were verified and their quantification ability was evaluated in standard solutions and in other matrixes. It was also analyzed the potential interference made by other sugars, such as glucose and sucrose. The Limit of Detection L_{OD} was calculated by measuring the fluorescence intensity equal to 3 times the SD of the blank ($n = 8$) divided by the slop of the calibration graph (Fig. 3), while the Limit of Quantification L_{OQ} was determined as 3 times the L_{OD}. The obtained CDs were revealed to be sensitive and selective sensors for fructose, with L_{OD} and L_{OQ} of 2.01 and 6.12 mM (respectively), in optimized conditions (pH 9).

Materials and Methods

Materials

3-Hydroxyphenylboronic acid ≥95%, fructose, glucose, sucrose, phosphate buffer, sodium carbonate (Na_2CO_3) and sodium hydrogen carbonate ($NaHCO_3$) from Sigma-Aldrich.

Synthesis of CDs

The CDs were prepared by a hydrothermal approach. Briefly, 0.05 g of 3-hydroxyphenylboronic acid was dissolved in 5 mL of deionized water and transferred to a Teflon reactor and heated to 200 °C in an oven for 2 h. After cooling to room temperature, the CDs' solution was centrifuged at 12,000 rpm for 10 min to remove suspended impurities. The CDs were preserved at 4 °C for further use.

Fructose Sensing Procedures in Aqueous Solution

Amounts of 100 μL of CDs and appropriated amount of phosphate buffer (pH 7.4) or carbonate (pH 9) prepared from Na_2CO_3 and $NaHCO_3$ buffer were placed in 500 μL Eppendorfs. Then, different amounts of fructose were added. The mixtures were mixed thoroughly, and their fluorescence spectra were recorded.

Fluorescence Quantum Yield

The quantum yield (Φ) was calculated by comparing the integrated luminescence intensities and the absorbance values of the synthesized CDs with the ones of tryptophan, with the following equation:

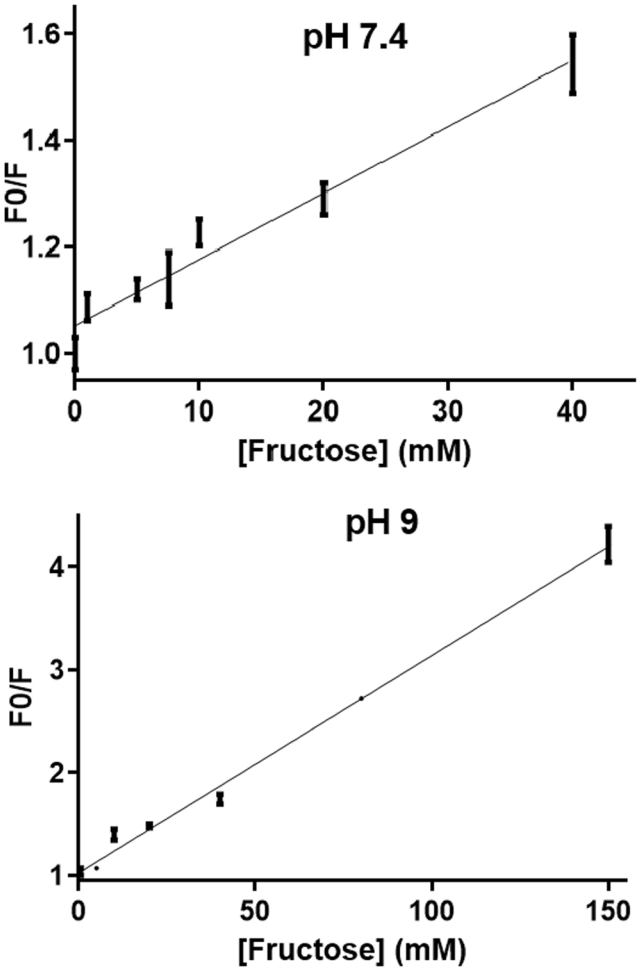

Fig. 3 Linear Stern-Volmer plots for the quenching induced by fructose on the fluorescence of 3-HPBA CDs, in aqueous solutions of different pH (7.4 and 9). The plots at pH 7.4 and 9 presented a R^2 value of 0.97 and 0.99, respectively

$$\Phi = \Phi_R \times \frac{Grad}{Grad_R} \times \frac{\eta^2}{\eta_R^2}$$

In the equation, Φ is the fluorescence quantum yield, *Grad* is the gradient from the plot of integrated fluorescence intensity versus absorbance and η is the refractive index. The subscript *R* refer to the reference fluorophore, tryptophan of known quantum yield, which is of 0.14 [41].

Instrumentation

Fluorescence spectra were obtained in a standard 10 mm fluorescence quartz cell and collected in a Horiba Jovin Yvon Fluoromax-4 spectrofluorimeter. The spectra were obtained with a 1 nm interval and slit widths of 5 nm. Dynamic light scattering (DLS) measurements were made in an Anton Paar, Litesizer™ 500. Absorbance measurements were made with an UV-3100PC Spectrophotometer. Quartz cells were used.

Results and Discussion

Characterization

Measurements by DLS determined the ζ potential by DLS of the CDs (−0.003 V) in aqueous solution (pH of ~5.6) to be of −0.003 V. Adjusting the pH to 7.4 and 9 of the aqueous solution containing the CDs changed the ζ potential to −0.021 and − 0.035 V, respectively, which is consistent with the ionization of the boronic acid moieties [26, 27]. Thus, the CDs can aggregate easily in aqueous solutions but suspend stably at pH 7.4 and 9.

The fluorescent spectra were obtained at pH 7.4 and 9 for centrifuged CDs and are presented in Fig. 1. While the spectra at both pH values show similar emission-maximum wavelength (of ~380 nm when excited at 310 nm), the spectrum is more sharp at pH 9. At neutral pH can be observed a shoulder at ~450 nm. A fluorescent quantum yield 31% was determined for the CDs, at pH 9.

Sensing of Fructose

As already mentioned, our aim is to use the obtained CDs as a fluorescent probe for the determination of fructose. It was analyzed the effected exerted by fructose addition on the fluorescent properties of CDs (Fig. 2), in aqueous solutions with pH of 7.4 and 9. A linear quenching effect was observed with addition of fructose at both pH values. Nevertheless, the degree of quenching is more pronounced at pH 9, having reached an 82.5% decrease in fluorescence intensity in the range of 0–250 mM for fructose. At pH 7, the maximum quenching was only of 33.0%. The quenching of CDs by fructose was expected due to the presence of boronic acid groups present in the 3-hydroxyphenylboronic acid precursor. More specifically, boronic acids are electron-deficient Lewis acids that can form reversible cyclic esters with the 1,2-diols of carbohydrates [18, 21–27]. The formation of this type of complex on the surface of the CD explains the change of its photophysical properties (in this case, the quenching induced by fructose). As for the selectivity toward fructose, it is expected given that boronic acids tend to exhibit inherent selectivity towards fructose [21].

The pH-dependence presented by the CDs toward fructose is also expected given that previous studies on boronic-diol binding have found that this reaction is more favorable upon the ionization of the boronic acid moiety, which should occur at more basic pH [18, 21–27]. In fact, our analysis of these CDs by DLS showed that their ζ potential is more negative at pH 9 than at pH 7.4.

Interestingly, at pH 7.4 quenching induced by fructose converts the shoulder at ~450 nm to a more well-resolved peak at fructose concentrations as low as 20 mM. As this second peak appears to not be responsive to fructose, it decreases the overall response of the CD to fructose. A similar peak is also seen at pH 9 but is only noticeable from fructose concentrations of 80 mM onward, and so, it does not have such significant impact on the response of the CD to the target sugar. This explains why the maximum quenching is significantly higher at basic pH than at neutral pH.

To analyze the quenching mechanism imposed by fructose, Stern-Volmer analysis was carried out and linear trends were obtained (Fig. 3). The reproducibility of the senor was good as shown in the error bars of Fig. 3, especially at pH 9. The Stern-Volmer constant (K_{SV}) for fructose was 2.11×10^{-2} mM^{-1} and

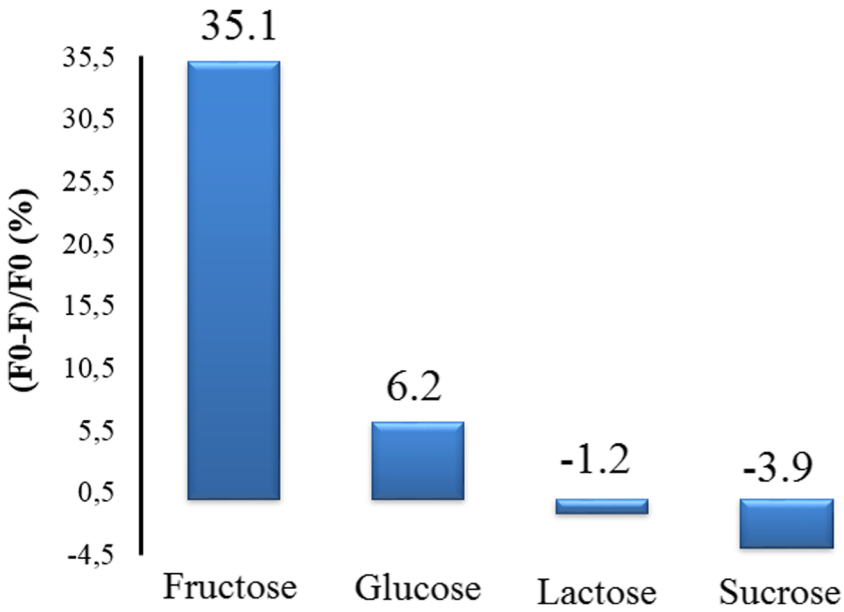

Fig. 4 Comparison of the response induced by different sugars on the fluorescence of the CDs in aqueous solution at pH 9. All sugars were present with a concentration of 30 mM

1.24×10^{-2} mM^{-1} at pH 9 and pH 7.4, respectively. It should be noted that while the CDs showed a good linear response to fructose between a concentration of 0 and 150 mM of this sugar at pH 9, at pH 7.4 a linear response was only found between a fructose concentration of 0–40 mM. The L_{OD} and L_{OQ} at pH 9 (with a relative standard deviation of 1.43%) was of 2.04 mM and 6.12 mM, respectively, while at pH 7.4 these parameters were of 5.18 and 15.54 mM (with a relative standard deviation of 6.05%). Thus, the proposed CDs behave better as fructose at pH 9 than at pH 7.4.

To ensure a good specificity for fructose by this method, it was measured the fluorescent response of the CDs in the presence of other sugars, such as glucose, sucrose and lactose (Fig. 4). We have only analyzed this response at pH 9, given that the best results were obtained at basic pH. Moreover, the different sugars were considered with a concentration of 30 mM. As expected, fructose elicited by far the highest degree of quenching (35.1%), followed by glucose. However, the response elicited by this sugar was quite smaller (6.2%). Both sucrose and lactose elicit almost negligible responses (between −1.2 and − 3.9%). Interestingly, the CDs responded to these latter sugars not by quenching but by fluorescence enhancement. In conclusion, glucose, lactose and sucrose produced only a limited response when compared with fructose, which indicates the specificity of these CDs for fructose.

Finally, we proceeded to further validate the proposed analytical approach by quantifying the amount of known fructose concentration (30 mM) to two different matrixes: tap water, and chamomile tea (Continente®, a Portuguese brand). We have obtained recovery values of 99.6% and 83.9%, respectively.

Conclusions

The CDs synthesized from 3-hydroyxphenylboronic acid with a hydrothermal approach showed blue emission characteristic of CDs with a relatively high fluorescent quantum yield (31%). More importantly, they can be used in fructose sensing due to a concentration-dependent quenching effect. Good quantification results were assessed in standard solutions and in other matrixes (tap water and chamomile tea). Other sugars (such as glucose, lactose and sucrose) showed quite limited interference in fructose quantification.

Optimization of the analytical assay was made by changing the pH from 7.4 to 9. We have found the assays to be more sensitive in pH 9 (L_{OD} and L_{OQ} of 2.01 and 6.12 mM) than in pH 7.4 (L_{OD} and L_{OQ} of 5.18 and 15.54), which can be attributed to a higher Stern-Volmer constant at higher pH (2.11×10^{-2} mM^{-1} versus 1.24×10^{-2} mM^{-1}). Moreover, at neutral pH the fluorescence spectrum is composed by fructose-responsive band at ~376 nm and an unresponsive shoulder at ~450 nm. The shoulder becomes a more-well defined peak at fructose concentrations as low as 20 mM, which decreases the response of the nano-sensor. At pH 9, the shoulder at ~450 only starts to become more well-resolved at concentrations of 80 mM and onwards. This explain why the linearity range of the CD is 0–150 mM at pH 9, and 0–40 mM at pH 7.4.

Acknowledgments This work was made in the framework of the project Sustainable Advanced Materials (NORTE-01-00145-FEDER- 000028), funded by "Fundo Europeu de Desenvolvimento Regional (FEDER)", through "Programa Operacional do Norte" (NORTE2020). Acknowledgment to project POCI- 01-0145-FEDER-006980, funded by FEDER through COMPETE2020, is also made. The Laboratory for Computational Modeling of Environmental Pollutants−Human Interactions (LACOMEPHI) is acknowledged. L.P. d.S. also acknowledges a post-doctoral grant funded by project Sustainable Advanced Materials (NORTE-01-00145-FEDER-000028). Projects PTDC/QEQ-QFI/0289/2014 and PTDC/QEQ-QAN/5955/ 2014 are also acknowledged. These projects are cofunded by FCT/MEC (PIDDAC) and by FEDER through "COMPETE− Programa Operacional Fatores de Competitividade" (COMPETE-POFC).

References

1. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, Ishimoto T, Sautin YY, Lanaspa MA (2013) Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes 62:3307–3315
2. Khitan Z, Kim DH (2013) Fructose: a key factor in the development of metabolic syndrome and hypertension. J Nutr Metab 2013:12
3. Nakagawa T, Tuttle KR, Short RA, Johnson RJ (2005) Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. Nat Clin Pract Nephrol 1:80–85
4. Bray GA, Nielsen SJ, Popkin BM (2004) Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 79:537–543
5. Popkin BM, Adair LS, Ng SW (2012) Global nutrition transition and the pandemic of obesity in developing countries. Nutr Rev 70: 3–21
6. Hu FB, Malik VS (2010) Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. Physiol Behav 100:47–54
7. Tappy L, Lê KA (2010) Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 90:23–46
8. Walker RW, Dumke KA, Goran MI (2014) Fructose content in popular beverages made with and without high-fructose corn syrup. Nutrition 30:928–935
9. Kawasaki T, Akanuma H, Yamanouchi T (2002) Increased fructose concentrations in blood and urine in patients with diabetes. Diabetes Care 25:353–357
10. Huang H, Yu H, Xu H, Ying Y (2008) Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: a review. J Food Eng 87:303–313
11. Wahjudi PN, Patterson ME, Lim S, Yee JK, Mao CS, Lee WN (2010) Measurement of glucose and fructose in clinical samples using gas chromatography/mass spectrometry. Clin Biochem 43: 198–207
12. Ma C, Sun Z, Chen C, Zhang L, Zhu S (2014) Simultaneous separation and determination of fructose sorbitol, glucose and sucrose in fruits by HPLC-ELSD. Food Chem 145:784–788

13. Cataldi TRI, Margiotta G, Zambonin CG (1998) Determination of sugars and alditols in food samples by HPAEC with integrated pulsed amperometric detection using alkaline eluents containing barium or strontium ions. Food Chem 62:109–115
14. Xiao Y, Li Y, Ying J, Tian Y, Xiao Y, Mei Z (2015) Determination of alditols by capillary electrophoresis with indirect laser-induced fluorescence detection. Food Chem 174:233–239
15. Ilaslan K, Boyaci IH, Topcu A (2015) Rapid analysis of glucose, fructose and sucrose contents of commercial soft drinks using Raman spectroscopy. Food Control 48:56–61
16. Alam AM, Kamruzzaman M, Dang TD, Lee SH, Kim YH, Kim GM (2012) Enzymeless determination of total sugar by luminol-tetrachloroaurate chemiluminescence on chip to analyze food samples. Anal Bioanal Chem 404:3165–3173
17. Curey TE, Salazar MA, Oliveira P, Javier J, Dennis PJ, Rao P, Shear JB (2002) Enzyme-based sensor arrays for rapid characterization of complex disaccharide solutions. Anal Biochem 303:42–48
18. Ashokkumar P, Bell J, Buurman M, Rurack K (2018) Analytical platform for sugar sensing in commercial beverages using a fluorescent BODIPY "light-up" probe. Sensors Actuators B Chem 256: 609–615
19. Kulmala S, Suomi J (2003) Current status of modern analytical luminescence methods. Anal Chim Acta 500:21–69
20. Zhai J, Pan T, Zhu J, Xu Y, Chen J, Xie Y, Qin Y (2012) Boronic acid functionalized boron dipyrromethene fluorescent probes: preparation, characterization, and saccharides sensing applications. Anal Chem 84:10214–10220
21. Wu X, Li Z, Chen XX, Fossey JS, James TD, Jiang YB (2013) Selective sensing of saccharides using simple boronic acids and their aggregates. Chem Soc Rev 42:8032–8048
22. Chapin BM, Metola P, Vankayala SL, Woodcock HL, Mooibroek TJ, Lynch VM, Larkin JD, Anslyn EV (2017) Disaggregation is a mechanism for emission turn-on of ortho-Aminomethylphenylboronic acid-based saccharide sensors. J Am Chem Soc 139:5568–5578
23. James TD, Sandanayake KRAS, Shinkai S (1996) Saccharide sensing with molecular receptors based on boronic acid. Angew Chem Int Ed Eng 35:1910–1922
24. Hansen JS, Christensen JB, Petersen JF, Hoeg-Jensen T, Norrild JC (2012) Arylboronic acids: a diabetic eye on glucose sensing. Sensors Actuators B Chem 161:45–79
25. Schiller A, Wessling RA, Singaram B (2007) A fluorescent sensor Array for saccharides based on Boronic acid appended Bipyridinium salts. Angew Chem Int Ed Eng 46:6457–6459
26. Ramsay WJ, Bayley H (2018) Single-molecule determination of the isomers of D-glucose and D-fructose that bind to Boronic acids. Angew Chem Int Ed Eng 57:2841–2845
27. Sun X, James TD, Anslyn EV (2018) Arresting "loose bolt" internal conversion from -B (OH)2 groups is the mechanism for emission turn-on in ortho-Aminomethylphenylboronic acid-based saccharide sensors. J Am Chem Soc 140:2348–2354
28. Qian S, Liang Y, Ma J, Zhang Y, Zhao J, Peng W (2015) Simple boric acid-based fluorescent focusing for sensing of glucose and glycoprotein via multipath moving supramolecular boundary electrophoresis chip. Sensors Actuators B Chem 220:1217–1223
29. James TD, Sandanayake KRAS, Shinkai S (1994) Novel photoinduced electron-transfer sensor for saccharides based on the interaction of boronic acid and amine. J Chem Soc Chem Commun:477–478
30. Lim S, Escobedo JO, Lowry M, Strongin RM (2011) Detecting specific saccharides via a single indicator. Chem Commun 47: 8295–8297
31. Elfeky SA, Flower SE, Masumoto N, D'Hooge F, Labarthe L, Chen WB, Len C, James TD, Fossey JS (2010) Diol appended quenchers for fluorescein Boronic acid. Chem Asian J 5:581–588
32. Schiller A, Vilozny B, Wessling RA, Singaram B (2008) Recognition of phosphor sugars and nucleotides with an array of boronic acid appended bipyridinium salts. Anal Chim Acta 627: 203–211
33. Edwards NY, Sager TW, McDevitt JT, Anslyn EV (2007) Boronic-acid based Peptidic receptors for pattern-based saccharide sensing in neutral aqueous media, an application in real-life samples. J Am Chem Soc 129:13575–13583
34. Zhou J, Zhou H, Tang J, Deng S, Yan F, Li W, Qu M (2017) Carbon dots doped with heteroatoms for fluorescent bioimaging: a review. Microchim Acta 184:343–368
35. Baker SN, Baker GA (2010) Luminescent carbon nanodots: emergent nanolights. Angew Chem Int Ed 49:6726–6744
36. Campos BB, Contreras-Cáceres R, Bandosz TJ, Jiménez-Jiménez J, Rodríguez-Castellón E, Esteves da Silva JCG, Algarra M (2017) Carbon dots coated with vitamin B12 as selective ratiometric nanosensor for phenolic carbofuran. Sensors Actuators B Chem 239:553–561
37. Simões EFC, Leitão JMM, Esteves da Silva JCG (2017) Sulfur and nitrogen co-doped carbon dots sensors for nitric oxide fluorescence quantification. Anal Chim Acta 960:117–122
38. Campos BB, Mutavdic D, Stankovic M, Radotic K, Lazaro-Martinez JM, Esteves da Silva JCG, Contreras-Cáceres R, Pino-Gonzalez MS, Rodriguez-Castellon E, Algarra M (2017) Thermo-responsive microgels based on encapsulated carbon quantum dots. New J Chem 41:4835–4832
39. Campos BB, Abellan C, Zougagh M, Jimenez-Jimenez J, Rodriguez-Castellon E, Esteves da Silva JCG, Rios A, Algarra M (2015) Fluorescent chemosensor for pyridine based on N-doped carbon dots. J Colloid Interface Sci 458:209–216
40. Simões EFC, Esteves da Silva JCG, Leitão JMM (2015) Peroxynitrite and nitric oxide fluorescence sensing by ethylenediamine doped carbon dots. Sensors Actuators B Chem 220:1043–1049
41. Kirby EP, Steiner RF (1970) Influence of solvent and temperature upon the fluorescence of indole derivatives. J Phys Chem 74:4480–4490