LETTER

The utilization of pH sensitive spirocyclic rhodamine dyes for monitoring D-fructose consumption during a fermentation process†
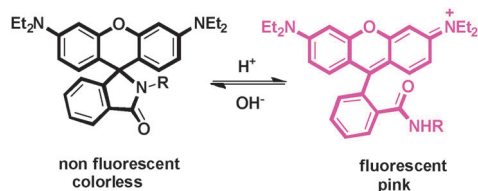
Erman Karakuş, Muhammed Üçüncü, Ritchie C. Eanes and Mustafa Emrullahoğlu*

The colorimetric and fluorometric detection of D-fructose was achieved by employing a two component sensing system composed of an arylboronic acid as the host molecule and a pH sensitive spirocyclic rhodamine dye as the indicator molecule.

Rhodamine dyes are well-known fluorophores with remarkable photophysical properties, such as long absorption and emission wavelengths, high fluorescence quantum yields and large absorption coefficients. The unique ring opening equilibrium of their spirocyclic derivatives has been widely utilized in the design of turn-on type fluorescent sensors.[1] The rhodamine spirolactam can exist in two isomeric forms, depending on the medium pH (Scheme 1). The rhodamine spirolactam in its closed form is non-fluorescent at neutral pH; however, the structure undergoes a change from the spirolactam to an open ring amide at lower pH (less than 5.0), resulting in a visibly colorful and highly fluorescent rhodamine amide.[2]
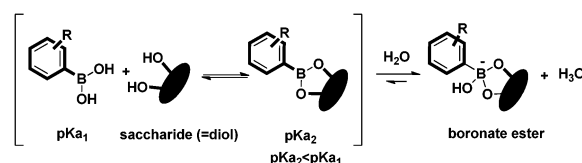
The ability of rhodamine based dyes to change their color and fluorescence in response to pH changes has been extensively exploited in monitoring the pH changes in various environments

Scheme 1 Two isomeric forms of rhodamine B spirolactam.

Department of Chemistry, Faculty of Science, İzmir Institute of Technology (IZTECH), Urla 35430, Izmir, Turkey. E-mail: mustafaemrullahoglu@iyte.edu.tr; Fax: +90 232 750 7509; Tel: +90 232 7507610
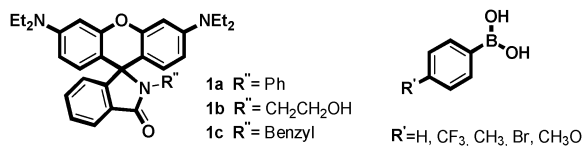
Scheme 2 Diol adduct of arylboronic acid.

(*i.e.* in solution, atmosphere and living cells).[2,3] Relying on their promising chemical and photophysical properties we envisioned that pH sensitive rhodamine dyes could serve as pH indicators in a saccharide sensing system, based on a boronic–saccharide affinity pair. It is well known that boronic acids show high affinities for diols.[4] A boronic acid reversibly forms a cyclic boronate ester with diols (Scheme 2), and in aqueous media the formation of the boronate ester is accompanied by an increase in hydronium ion concentration (*i.e.* lowered pH).[4]

It was our aim to develop a general sensing strategy that allows one to monitor the binding of a target saccharide to simple unmodified boronic acids both colorimetrically and fluorometrically. Considering the high sensitivity of spirocyclic rhodamine dyes to pH changes we designed a two component sensing system that employs an aryl boronic acid molecule as the host and a rhodamine B spirolactam dye as the signal reporter.

Practical examples of sensors that are based on this sensing principle have been reported recently.[5,6] The majority of the reported studies, however, have been focused on the colorimetric responses of the indicator dyes.[5a,b] Examples of detection methods that employ pH sensitive fluorophore indicators are quite rare.[6] In general, fluorometric detection methods are more sensitive than colorimetric detection methods in terms of both the detection limit and signal magnitude. In this regard, a method based on fluorometric detection might be well suited to meet the need for the highly efficient analysis of saccharide-containing samples.

Scheme 3 Spirocyclic rhodamine indicators (**1a–1c**) and arylboronic acid derivatives.
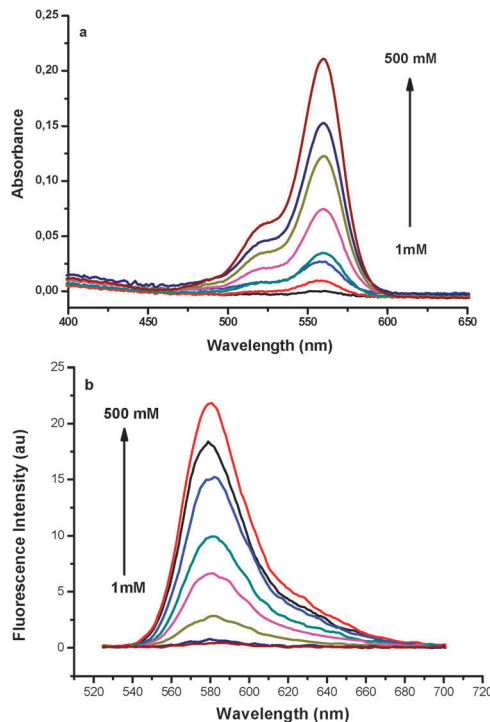

Fig. 1 (a) Absorption and (b) emission spectra of sensing system SS-1 in water–methanol (3:7, v/v) with increasing concentrations of D-fructose [0, 1.0, 5.0, 10, 25, 50, 100, 250, and 500 mM], λ_{ex}: 525 nm.

To the best of our knowledge, a strategy for specifically exploiting the use of rhodamine spirolactam dyes as pH indicators for a saccharide sensing system has not been presented in the scientific literature. Here we report a simple yet highly efficient method for a naked-eye as well as fluorometric detection of D-fructose employing a sensing system that utilizes pH sensitive rhodamine dyes as indicators. In this strategy the rhodamine spirolactam dye acts as a signal switcher, which is envisioned to turn-on when the target sugar is bound to arylboronic acid.

The pH indicator probe (**1a**) was prepared from rhodamine-B by using a "one-pot" synthetic procedure (POCl$_3$, reflux; then amine, NEt$_3$, CH$_3$CN, see ESI†). The resultant compound (**1a**) was colorless in solution, showed no absorption in the visible region, and was nonfluorescent, suggesting that the spirocyclic form of the rhodamine dye is the predominant form (Scheme 3).

In order to check the feasibility of our strategy, we first examined the binding between phenylboronic acid (PBA) and D-fructose in the presence of rhodamine (**1a**) as the signal reporter. D-Fructose was selected as the substrate to be detected in the optimization study as it is known to bind to PBA with high affinity.[7] The sensing system (SS-1) was prepared by mixing dye **1a** (25 μM) and PBA (50 mM) in an aqueous-methanolic solution. The water content in the semi-aqueous media was carefully inspected and a water–methanol (3:7, v/v) mixture was chosen as the reaction medium (Fig. S1, ESI†).

Upon the direct addition of D-fructose (100 mM, 2 equivalents with respect to arylboronic acid) to SS-1, the colorless and nonfluorescent solution instantly became pink in color and started to fluoresce under UV light. Fig. 1a shows the absorption spectrum of SS-1 upon addition of increasing concentrations of D-fructose. It can be seen that the solution of dye (**1a**) and PBA exhibited essentially no absorption in the visible region. However, upon addition of D-fructose, a new absorption band appeared at 558 nm. The effect of the added D-fructose on the fluorescence properties was then investigated. Fig. 1b shows how the fluorescence emission of the dye (**1a**) in SS-1 changes at 578 nm as a function of D-fructose. It can be seen that the solution of SS-1 displays no obvious spectral characteristics in the emission spectrum. When D-fructose was added to the solution (SS-1), a significant enhancement of fluorescence with an emission maximum at 578 nm was observed.

Encouraged by these results we synthesized two alternative rhodamine derivatives (**1b** and **1c**) and evaluated their optical performance and found that the fluorescence responses of dyes **1b** and **1c** were relatively weaker than dye **1a** (when used in SS-1) (Fig. S3, ESI†).[8]

With these results in mind, we further examined several commercially available arylboronic acid derivatives in an attempt to improve the effectiveness of the overall sensing process. The results showed p-CF$_3$-phenylboronic acid (p-CF3-PBA) to be the most efficient host among those tested (p-CH3-PBA, p-Br-PBA, p-CH3O-PBA and PBA) and that it caused the most dramatic change in acidity after complexation with D-fructose (Fig. S6, ESI†).[8] Moreover, in the absence of a boronic acid derivative, the addition of saccharides did not change the acidity of the media such that a spectroscopic response might be observed. Eventually, the sensing system employing p-CF3-PBA as the host molecule and **1a** as the indicator dye (SS-2) was discovered to be the most efficient.

In this sensing system, saccharides are indirectly detected by the creation of hydronium ions (Scheme 2) and the subsequent protonation of rhodamine spirolactam (Scheme 1), which gives an optical response. Accordingly, detection of saccharides should be dependent upon the absolute hydronium ion concentration as is true for any indicator and there would be an associated useful pH range for rhodamine spirolactam. Optimization studies revealed that both the initial and the final acidity of the media played an important role in the overall efficiency.[8]

We evaluated the effect of the solution acidity on the total efficiency of the sensing process. The fluorescence response of SS-2 to the addition of D-fructose under various pH conditions was recorded. We observed that the fluorescence response of SS-2 towards the addition of saccharides increases as the solution becomes more acidic, i.e. at pH 5.2. Below pH 5.0, SS-2 is not useful since even the dye (**1a**) itself responds to this acidity even before the addition of any sugar derivative (Fig. S2, ESI†).[8]
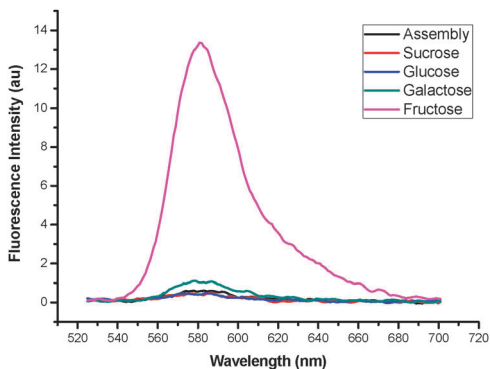
Fig. 2 Emission intensities of SS-2 (**1a**/*p*-CF$_3$-PBA (25 μM/5 mM)), towards the addition of D-fructose, D-glucose, D-galactose, and D-sucrose [10 mM] in water–methanol (3 : 7, v/v, pH 5.2).
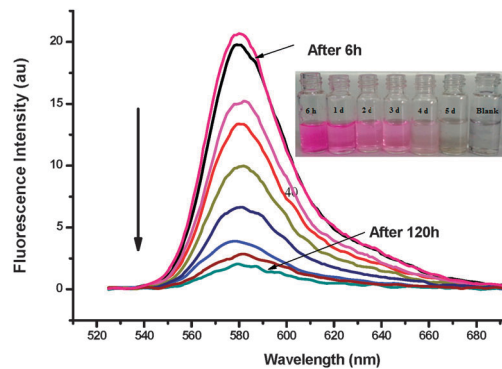

Fig. 3 Emission intensities of SS-2 (**1a**/*p*-CF$_3$-PBA (25 μM/50 mM) in water–methanol (3 : 7, v/v, pH 5.2)) throughout the consumption of D-fructose [100 mM]. Inset photograph: colorimetric monitoring of D-fructose consumption (0–120 hours).

On the other hand, at pH > 7.0, the ability of any boronic acid to decrease the pH after complexation with sugar was not enough in order to initiate a spectroscopic response (Fig. S2, ESI†).[8]

D-Fructose can be reliably seen to exhibit a fluorescence response at concentrations approaching 1 mM for solutions adjusted to pH 7.0 and at concentrations approaching 0.1 mM for solutions of pH 5.2. The fluorescence and absorption profiles of SS-2 upon the addition of increasing concentrations of D-fructose at pH 7.0 and 5.2 are depicted in Fig. S4 (ESI†).[8]

In order to validate the selectivity of the sensing system (SS-2), the spectroscopic response of SS-2 to the most common saccharides including D-glucose, D-galactose and D-sucrose was also investigated. Fig. 2 illustrates the fluorescence profile of SS-2 after the addition of D-fructose, D-glucose, D-galactose, and D-sucrose (10 mM of each). Under optimized conditions (**1a**/*p*-CF$_3$-PBA (25 μM/5 mM), water–methanol (3 : 7, v/v, pH 5.2)), only the addition of D-fructose (10 mM) resulted in a change in absorbance and fluorescence, whereas almost no spectroscopic response was observed for any other saccharide derivative indicating the exceptional selectivity of this sensing system to D-fructose.

Selective detection and monitoring of D-fructose throughout a fermentation process are of great importance for wine makers. In a wine fermentation process, yeasts convert most of the sugars such as D-fructose and D-glucose into alcohol and CO$_2$. D-Fructose is primarily responsible for the sweetness and characteristics of the wine, thus rapid and reliable methods for the routine analysis of D-fructose are highly demanded to achieve the desired taste.

Motivated by the obvious fluorescence response and the unique selectivity of the sensing system towards D-fructose we performed an experiment to show the utility of this sensing strategy for monitoring the consumption of D-fructose during fermentation. To this end, we prepared a solution of D-fructose (100 mM) in water to which wine yeast was added. The solution was maintained at 30 °C with continuous stirring for 120 hours under an inert atmosphere.

Samples were withdrawn periodically from the fermentation broth, and centrifuged to remove particulate matter. To prevent any discrepancy arising from the acidic by-products produced

via fermentation, the pH of each sample solution was adjusted to an optimum sensing condition (pH 5.2) by adding the required amount of NaOH solution. Then, each sample was subsequently subjected to the probe solution.

As displayed in Fig. 3, the fluorescence intensity decreased dramatically with time which is an indication that D-fructose was consumed throughout the fermentation process and after 120 hours its concentration dropped from about 100 mM to about 0.6 mM based on a standard calibration curve.[8] Moreover, the decrease in the concentration of D-fructose could be easily monitored by the naked eye (Fig. 3).

In summary, we have shown for the first time that spirocyclic rhodamine derivatives can be successfully utilized as pH indicators for monitoring pH lowering resulting from the complexation of arylboronic acid with diol containing compounds such as saccharides. We developed a simple yet highly efficient sensing strategy for the selective detection of D-fructose employing a two component sensing system which relies on the spectroscopic response of the spirocyclic rhodamine dyes to changes in acidity within the media. Among the tested saccharide derivatives, D-fructose can be selectively detected both colorimetrically and fluorometrically in an efficient manner. As a practical application we demonstrated the utility of this strategy for monitoring the consumption of D-fructose throughout a fermentation process. It is hoped that this sensing system may be further modified such that other target saccharides may be selectively sensed by adjusting the type of arylboronic acid and the rhodamine dye.

Experimental part

All reagents for synthesis were purchased from commercial suppliers (Aldrich and Merck) and used without further purification. Absolute methanol and distilled water were used throughout the experiment. The pH was recorded using a WTW InoLab pH 720 precision pH meter (Weilheim, Germany). UV absorption spectra were obtained on a Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using a Varian Cary Eclipse Fluorescence spectrophotometer. The slit width was 5.0 nm for both excitation and emission. The emission spectra

were integrated over the range 525 nm to 700 nm in 2.00 mm path length quartz cells (0.5 mL volume). All measurements were conducted at least in triplicate.

Rhodamine B derivatives (**1a**, **1b** and **1c**) were synthesized according to methods reported in the literature.[9]

Acknowledgements

Notes and references

1 For reviews see; (a) X. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev.*, 2012, **112**, 1910–1956; (b) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465–1472.

2 (a) W. Zhang, B. Tang, X. Liu, Y. Liu, K. Xu, J. Ma, L. Tong and G. Yang, *Analyst*, 2009, **134**, 367–371; (b) L. Yuan, W. Lin and Y. Feng, *Org. Biomol. Chem.*, 2011, **9**, 1723–1726.

3 (a) S. Wu, Z. Li, J. Han and S. Han, *Chem. Commun.*, 2011, **47**, 11276–11278; (b) Q. A. Best, R. Xu, M. E. McCarroll, L. Wang and D. J. Dyer, *Org. Lett.*, 2010, **12**, 3210–3221; (c) S. Kang, S. Kim, Y. K. Yang, S. Bae and J. Tae, *Tetrahedron Lett.*, 2009, **50**, 2010–2012.

4 (a) G. Springsteen and B. H. Wang, *Tetrahedron*, 2002, **58**, 5291–5300; (b) G. Springsteen, S. Deeter and B. H. Wang, *Tetrahedron*, 2004, **60**, 11205–11209; (c) R. Badugu, J. R. Lakowicz and C. D. Geddes, *Anal. Chem.*, 2004, **76**, 610–618; (d) G. Springsteen and B. H. Wang, *Chem. Commun.*, 2001, 1608–1609.

5 (a) J. W. Lee, J. S. Lee and Y. T. Chang, *Angew. Chem., Int. Ed.*, 2006, **45**, 6485–6487; (b) K. K. Ghosh, E. Yap, H. Kim, J. S. Lee and Y. T. Chang, *Chem. Commun.*, 2011, **47**, 4001–4003; (c) S. H. Lim, C. J. Musto, E. Park, W. X. Zhong and K. S. Suslick, *Org. Lett.*, 2008, **10**, 4405–4408.

6 Y. Kim, S. A. Hilderbrand, R. Weissleder and C. H. Tung, *Chem. Commun.*, 2007, 2299–2301.

7 E. V. Anslyn, *J. Am. Chem. Soc.*, 2010, **132**, 15833–15835.

8 See ESI†.

9 (a) X. Zhang, Y. Shiraishi and T. Hirai, *Tetrahedron Lett.*, 2007, **48**, 5455–5459; (b) Y. Zhao, X.-B. Zhang, Z.-X. Han, L. Qiao, C.-Y. Li, L.-X. Jian, G.-L. Shen and R.-Q. Yu, *Anal. Chem.*, 2009, **81**, 7022–7030; (c) A. Chatterjee, M. Santra, N. Won, S. Kim, J. K. Kim, S. B. Kim and K. H. Ahn, *J. Am. Chem. Soc.*, 2009, **131**, 2040–2041.