Analytica Chimica Acta xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis

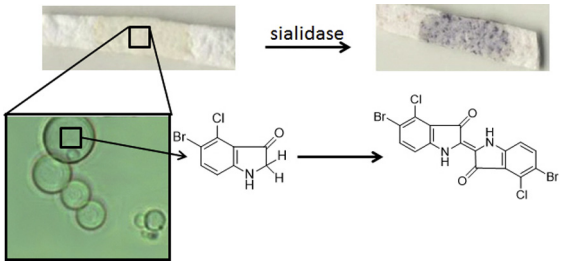
Yufen Zhang, Dominic Rochefort*

Département de chimie, Université de Montréal, CP6128 Succ. Centre-Ville, Montréal, Québec, Canada H3C 3J7

HIGHLIGHTS

- We demonstrate microencapsulation of reagents for determination of sialidase.
- We immobilized microcapsules on paper to fabricate a sensor for sialidase.
- The paper-based sensor has improved shelf-life time.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 May 2013
Received in revised form 8 August 2013
Accepted 16 September 2013
Available online xxx

Keywords:
Neuraminidase
Microcapsules
Bioactive paper strip
Bioactive paper
Colorimetric biosensor

ABSTRACT

In this contribution we present a sensitive colorimetric bioactive paper fabricated to determine sialidase-related diseases like bacterial vaginosis (BV) in a one-step and dry format spot assay with fast response and good storage stability. The paper was prepared by three simple steps. The first step involves preparation of poly(ethyleneimine) (PEI) microcapsules, the second step is to incubate positively charged microcapsules in negatively charged 5-bromo-4-chloro-3-indolyl-a-D-*N*-acetylneuraminic acid (BCIN) solution, a color enhancer nitro blue tetrazolium (NBT), and in the third step, paper was fabricated by incorporating incubated microcapsules into paper pulp. This paper changes color from white to dark purple in the presence of sialidase in as little as 6 min, and color could be enhanced with increased length of reaction time. In this reaction system, BCIN was the substrate for sialidase, NBT was the color enhancer, and PEI microcapsules acted as catalyst. The loading efficiency of BCIN was about 22.2%, and filtered BCIN solution could be reused for the next fabrication.

1. Introduction

Bacteria vaginosis (BV) is the most common vaginal infection in women of child-bearing age. Normal vaginal flora consisting of hydrogen peroxide-producing lactobacilli are replaced by *Gardnerella vaginalis*, *Mycoplasma hominis*, *Mobiluncus* species, which are anaerobic gram-negative rods in women with BV [1]. BV is strongly associated with gynecologic and obstetric complications including an increased risk for salpingitis, endometritis, pelvic inflammatory disease (PID), chorioamnionitis, premature rupture of membrane, and pre-term birth. BV is also associated with higher susceptibility to sexually transmitted pathogens, including HIV [2]. A number of studies have shown BV to be associated with elevated sialidase (also known as neuraminidase) activity, which is an abnormal condition of the vaginal ecosystem caused by a flora shift from the *Lactobacillus* species present in normal conditions to overgrowth of a variety of aerobic and anaerobic vaginal bacteria, including *G. vaginalis*, *Bacteroides*, *Prevotella*, *Mobilincus* and *Mycoplasma* species [3].

Many kinds of BV tests exist, the Amsel criteria [1,4–9] are the gold standard currently, which require the presence of at least three of four clinical signs set forth by Amsel et al.: (i) presence of abnormal vaginal discharge; (ii) elevated vaginal pH (>4.5); (iii) positive amine odor; (iv) presence of clue cells on gram stain or

* Corresponding author. Tel.: +1 514 343 6733; fax: +1 514 343 7586.
E-mail address: dominic.rochefort@umontreal.ca (D. Rochefort).

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.09.032

saline preparation of vaginal secretions. The Nugent gram stain criteria [5,7,10–12] measure specific gram-stained bacterial morphotypes. This method is to use a gram stained evaluation of vaginal smears with the Nugent criteria or score [12]: colorimetric pH test: 72% and 67%; Papanicolaou smear: 72% and 79% [13]. Consequently, organisms that do not gram stain, such as ureaplasmas and mycoplasmas, are not measured [7]. Some other detection methods, such as the Whiff test [7,14–17] and the Ison and Hay's criteria [18], were also developed. However, these conventional methods for diagnosing BV rely on an expert assessment and laboratory tests. The Nugent and Amsel criteria are considered as the most effective assessment for BV, but they are slow and accordingly dependent on specialist interpretation and laboratory equipment [19].

There has long been a need for a rapid, uncomplicated diagnostic test for BV. Since high levels of sialidase activity have been found in women with BV, colorimetric detection based on the detection of sialidase is becoming a promising method. But only the BVBlue assay [19–22] has been commercialized, it has a fast response (approx. 10 min), but it is not portable since it is a two-step liquid-phase chromogenic solution test kit. The active component for BVBlue assay is IBX-4041, which refers to the invention of US patent 2003/0162240 A1 [23]. The color reaction includes two steps. The optimal pH for the first hydrolysis step is 5.5–6, and the second color development step requires pH of ≥9. It is based on detection of the enzyme sialidase produced by pathogenic vaginal bacteria, and should be performed in a physician's office or a lab. This means patients cannot self-test at home. Since 2000, BCIN has been used as the substrate of sialidase for the detection of BV by Wiggins [24]. This method was first developed by some other authors, such as Reukov et al. [25] who immobilized BCIN onto nanocoated fibers. The portable biosensor shows very slow response, takes at least 2 h to light up, and BCIN loading efficiency is only about 18%. The reactions pathways that are involved in all of above assays were shown in Fig. 3, Reaction A. Sialidase in specimen catalyzed hydrolysis reaction of BCIN and produced light yellow color indoxyl isomers at pH 5.5–6, produced isomers were oxidized at higher pH 9 to generate visible blue color.

The method described in US Patent 7591978 B2 [2,3] is a dry format assay. Same substrate BCIN was assembled onto a sheet device with color enhancer of nitro blue tetrazolium (NBT) and some other compounds; while it gives response within a reasonable time, the device assembly method and detection step are complicated and expensive. Innovation of this method is the addition of color enhancer NBT, which decreases total reaction time. The reactions were illustrated in Fig. 3 (Reaction B). First step is the same as previous publications such as Wiggin's and Reukov's; hydrolysis reaction of BCIN catalyzes by sialidase at pH 5.5–6 to produce indoxyl isomers; indoxyl isomers are oxidized in the presence of NBT at pH 9 to generate dark purple precipitate. It can detect liquid flow as a lateral flow assay and running buffer solution is necessary for the sample preparation. Moreover, the assembly method is not compatible with industrial fabrication. Furthermore, storage stability, which is a very important factor in potential applications, was not discussed.

Here, we report a method of bioactive paper fabrication method for the detection of sialidase, which could be applied to BV diagnosis by women. PEI microcapsules soaked in BCIN and NBT solution were incorporated into paper pulp for the fabrication of bioactive paper. The paper can be used for the detection of sialidase-related diseases like BV. This kind of BV paper strips was resulted efficient in permitting to obtain a fast evaluation of the risk of pathologies related to the presence of sialidase with good storage stability. There is no sample preparation step and detection is in as little as 6 min, suspected patients can test by themselves before visiting a physician office. Such bioactive paper strip was expected to be used not only by women on a day-to-day basis, but also by a physician as a fast determination.

2. Materials and methods

2.1. Materials

BCIN was purchased from Toronto Research Chemicals Inc., NBT, PEI (50 wt% soln. in water, MW: 1300), sebacoyl chloride, cyclohexane, Span 85, and neuraminidase from *Clostridium perfringens* (*C. welchii*, Type V) were purchased from Sigma–Aldrich. Salts used for buffer preparation were purchased from A&C American Chemicals Ltd. and MilliQ water (18 MΩ cm) was used in the preparation of all solutions.

2.2. Preparation of microcapsules

PEI microcapsules were prepared by the mechanical method via interfacial polycondensation. The detailed method has been described by our group [26]. Briefly hydrophilic microdroplets of McIlvaine buffered PEI and Span 85 in cyclohexane (continuous phase) were crosslinked by sebacoyl chloride to form microcapsules.

2.3. Fabrication of bioactive paper

Neuraminidase (Sialidase, acetyl-neuraminyl hydrolase) can catalyze the hydrolysis of α2–3, α2–6, and α2–8 linked *N*-acetylneuraminic acid residues from glycol-proteins and oligosaccharides. BCIN is a known chromogenic substrate of neuraminidase [24], but the two-step color reaction requires different optimal pH. So it is not possible to make a one-step assay. Tetrazolium salts are known as highly sensitive color indicators in combination with

Table 1
Materials used for the preparation of four kinds of paper.

Paper	Pulp (g)	Microcapsules (g)	BCIN ($\times 10^{-7}$ mol)	NBT ($\times 10^{-7}$ mol)
1	1	1	3.1	7.1
2	1	1	3.1	3.6
3	1	1	3.1	0
4	1	0	3.1	7.1
5	1	0	In 6.88×10^{-8} mol BCIN + 1.58×10^{-7} mol NBT	

Table 2
Paper 1 components per square centimeter of paper.

Microcapsules (g cm^{-2} paper)	Wet pulp (g cm^{-2} paper)	BCIN (mol cm^{-2} paper)	NBT (mol cm^{-2} paper)	Loading efficiency
0.144	0.144	2.24×10^{-8}	5.10×10^{-8}	Assume 100%
0.144	0.144	5.28×10^{-9}	NA	Measured 22.2%

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032

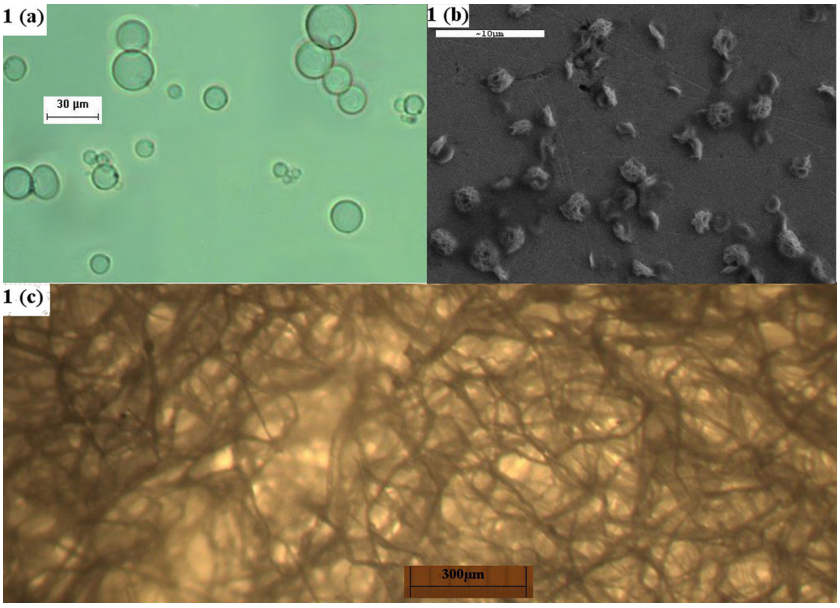


Fig. 1. Microcapsules in wet (a) and dried (b) format and microcapsules in paper (c). The wet microcapsules (a) and microcapsules in paper (c) were obtained from optical microscope; the dried format micrograph was obtained by SEM, and copper was used as the support.

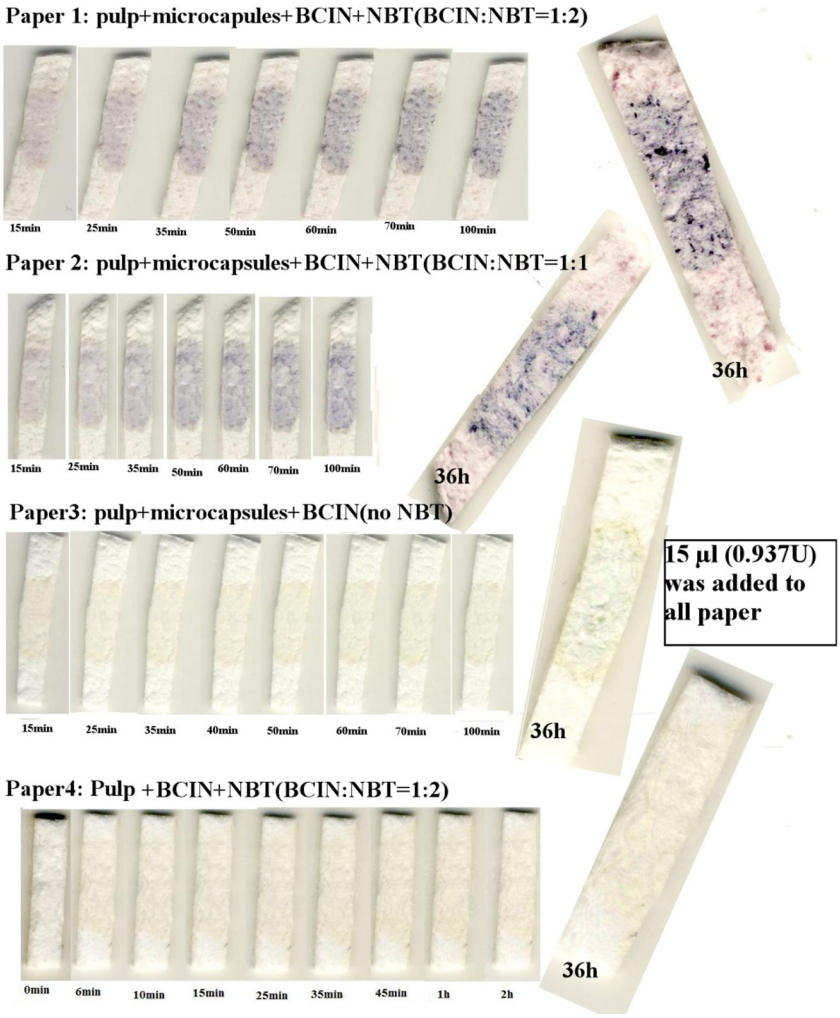


Fig. 2. The color reaction of four kinds of paper in the presence of sialidase. Papers 1 and 2 gave fast and significant color response in the presence of sialidase, which indicates the hydrolysis of BCIN and oxidation reaction of the intermediate accelerated by alkalization of NBT. Paper 3 showed a little bit of blue color after 2 h, and was easy to recognize the color at 36 h, which indicated the hydrolysis of BCIN in the presence of sialidase at pH 6. Paper 4 did not give any color response, which indicated there were no BCIN and NBT remaining in the paper.

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032

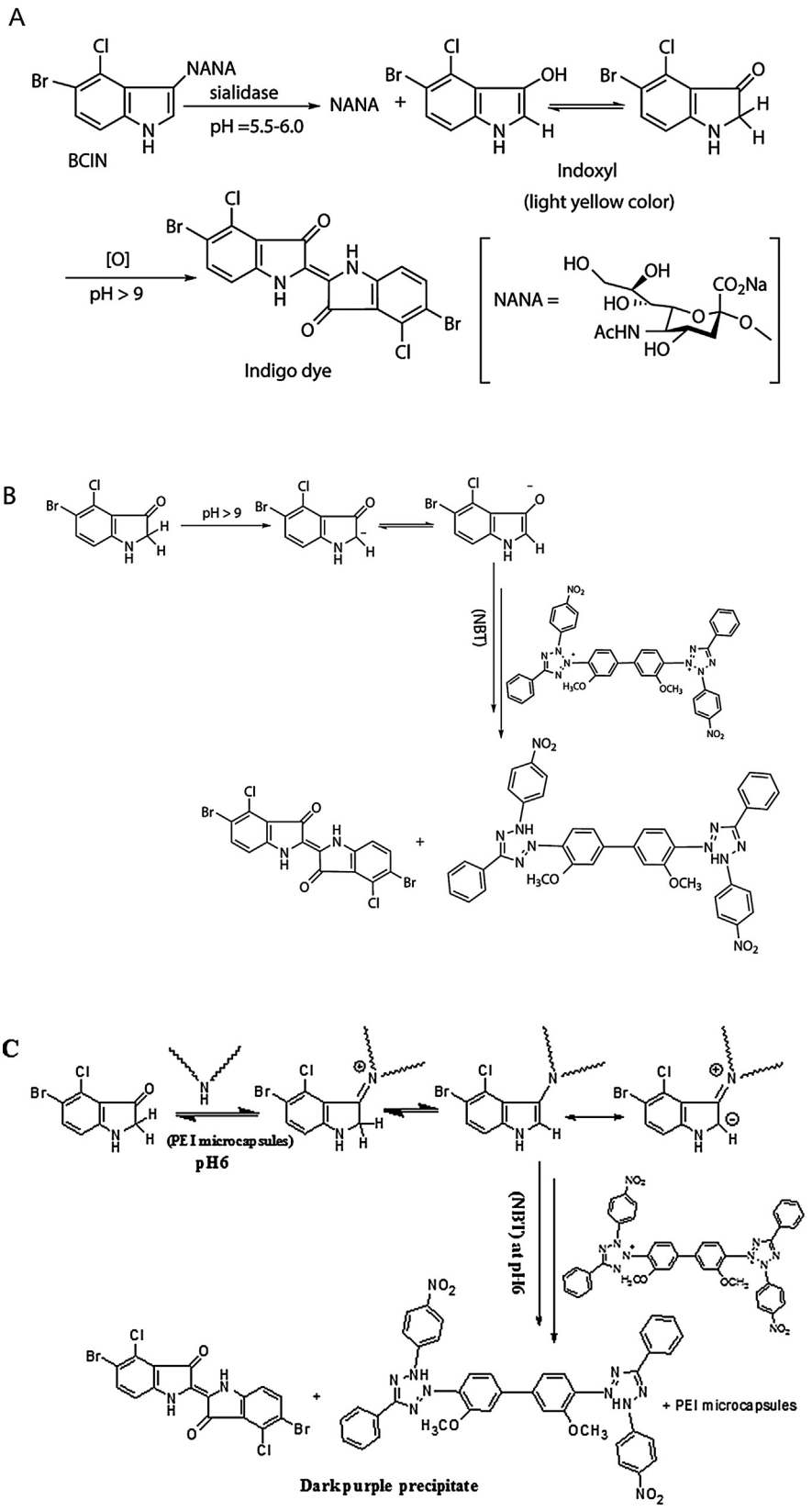


Fig. 3. Reaction mechanism of chromogenic substrate of sialidase. Reaction A: BCIN was hydrolyzed in the presence of sialidase at pH 5.5–6 to produce indoxyl, and then indoxyl isomers were oxidized to blue color indigo dye at higher pH (pH > 9); Reaction B: indoxyl (hydrolyzed monomer) was oxidized in the presence of NBT at higher pH (pH > 9) to form a dark purple precipitate; Reaction C: the redox reaction of indoxyl and NBT was catalyzed by secondary amino groups (on the membranes of PEI microcapsules) at the same pH.

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032

5-bromo-4-chloro-3-indolyl-phosphate for the detection of nucleic acid, protein, or glycoconjugates and are very common oxidizers in staining applications [27–30]. NBT was used here to oxidize the intermediate (halogenated indoxyl) of sialidase-catalyzed hydrolysis of BCIN and form dark purple product [3]. Then neuraminidase substrate was comprised by BCIN, NBT and PEI microcapsules for the fabrication of bioactive paper.

Four kinds of paper were fabricated with paper pulp. The materials used for their fabrication are listed in Table 1. A 0.01 M pH 6 phosphate buffer was used in paper fabrication. Samples were obtained in sheet at a ca. 95% consistency and without any retention agent used. Prior to handsheet formation, hard wood kraft pulp was suspended in water or buffer using a blade mixer for 16 h. Mixed suspensions were transferred to the top of Whatman No. 4 filter paper with pore size 20–25 μm in a Buchner filter funnel with diameter of 7 cm. In papers 1 and 2, microcapsules were soaked in BCIN and NBT solutions for 30 min, and then mixed with paper pulp. Microcapsules only were socked in BCIN solution for the preparation of paper 3. Paper 4 did not contain microcapsules; only the paper pulp was mixed with BCIN and NBT solutions. In all 4 cases, the microcapsules and paper pulp used for paper preparation were wet. The paper after filtration was dried by purging with nitrogen gas. Since filter solutions of papers 1–4 contained enough BCIN and NBT, which can be reused to soak new batch of microcapsules to fabricate new paper strips.

In order to visualize the function of PEI microcapsules, paper 5 was fabricated. Paper pulp was suspended in pH 6 buffer solution and the paper was prepared by filtration. Wet paper was dried by purging with nitrogen. This unmodified paper was soaked in BCIN and NBT solutions and dried again under a flow of nitrogen gas. All soaked reagents are remained in the paper, and then paper 5 contained the same amount of BCIN and NBT as calculated from paper 1 (see Tables 1 and 2).

The reaction taking place on the papers 1 and 2 required two steps. The first step was hydrolysis of BCIN catalyzed by sialidase in the specimen, and the second step is the oxidation of indoxyl isomers from hydrolysis reaction with redox indicator NBT in the presence of PEI microcapsules formed a dark purple colored products. Secondary amine groups on PEI membranes acted as a catalyst in the second step of the reaction (Reaction C, Fig. 3). Unlike the description in previous publications, this both steps could be performed at the same pH value.

For the determination of paper activity, a very small amount (2–20 μL) of diluted neuraminidase was directly added to small piece of paper, and then paper strips were scanned at different times (from time 0 to 36 h) and mean gray values of scanned images were taken by Photoshop. Mean color intensities of different images were calculated by mean gray values (mean color intensity = 256-mean gray value).

Neuraminidase was dissolved in 0.01 M pH 6 phosphate buffer, since the optimal pH for its catalyzed hydrolysis reaction is pH 5.5–6. Normally, the vagina is slightly acidic with pH of 3.8–4.2, but it was elevated for the patients with bacterial vaginosis, so the elevated vaginal flora is close to this pH value.

2.4. Loading efficiency of BCIN

BCIN loading efficiency on paper modified with microcapsules was evaluated using a UV–vis spectrophotometer by measuring the difference in concentration of the solution used for incubation. A calibration curve was made from the measurements of standard solutions of BCIN and the absorbance of the solution remaining after BCIN uptake was measured. The loading efficiency was obtained from comparison of BCIN concentration in the solution and original solution before paper preparation.

3. Results and discussion

3.1. Microcapsules in bioactive paper

PEI microcapsules [26] for the entrapment of large enzyme molecules were used here to soak up small molecules for the preparation of sialidase-sensitive paper. Photos of the microcapsules are shown in Fig. 1a (wet microcapsules viewed with an optical microscope) and b (dried microcapsules using a scanning electron microscope (SEM)). Dried microcapsules could be rehydrated many times. Fig. 1(c) represents fabricated paper containing microcapsules, in which microcapsules and their aggregates were fixed in the kraft hard wood paper pulp.

3.2. Different kinds of paper

Since PEI-SC microcapsules are positively charged and BCIN is negatively charged at pH 6, BCIN can absorb onto the surface of microcapsules due to the electrostatic interaction. Membranes of microcapsules are porous, which allows absorption of small molecules like BCIN. In addition, the pore size of the membranes allows the diffusion of small molecules [26], thus BCIN and NBT molecules can diffuse across the membranes and remained in the core of microcapsules and absorbed onto outer membranes of microcapsules due to electrostatic interaction. Microcapsules with mean size of 20 μm and their aggregates can be fixed into paper with pore size ranging from 0.01 to 10 μm. Therefore, papers 1 and 2 can retain BCIN and NBT molecules due to existence of PEI microcapsules. On the other hand, both paper pulp and BCIN are negatively charged at pH 6 and the size of BCIN and NBT molecules are much smaller than the pore size of paper (0.01–10 μm). Therefore, paper 4 without microcapsules lost its BCIN and NBT after filtration, and thus it did not show any color response to neuraminidase (Fig. 2).

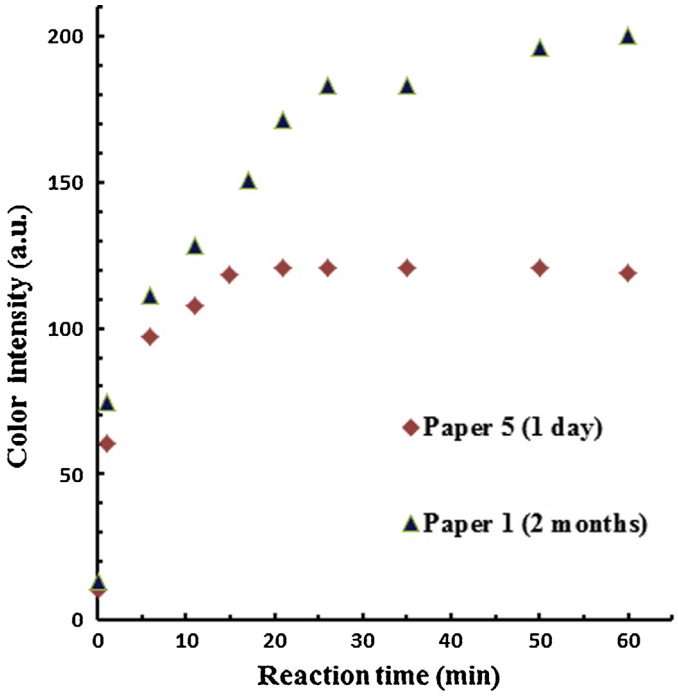


Fig. 4. The color response of papers 1 and 5 with different length of reaction time in the presence of 15 μL (0.937 UN) sialidase. Paper 1 was measured after 2 months of preparation, and has faster and intense response than paper 5 (1 day) due to the incorporation of microcapsules.

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032

Microcapsules were used for the fabrication of paper 3, so the substrate BCIN remained in it. The reaction mechanism is shown in Fig. 3, Reaction A. Since this is a one-step spot assay, only sialidase solution in pH 6 buffer was added to the paper strips. The second reaction step in reaction A was very slow. The color changes of paper 3 after adding neuraminidase are shown in Fig. 2, where the paper did not give a visible color within 100 min; only the photo at 36 h showed a little bit of green color. Since the second color development step requires higher pH and the intermediate (halogenated indoxyl) from the first hydrolysis step took a longer time to be oxidized at pH 6, so the color response is very slow. In addition, the amount of BCIN used here was very small (2.83×10^{-5} mg mm^{-2}), so the color appearing on paper 3 was very weak.

Both papers 1 and 2 gave fast and visible response to samples, but paper 1 was darker (Figs. 2 and 5) and displayed higher color intensity (Fig. 6) than paper 2. Table 1 shows that double the amount of NBT was used to prepare paper 1. The reaction scheme on papers 1 and 2 is illustrated in Fig. 3, Reactions A and C, showing that there is no need to change buffer pH. Indoxyl from the first step of reaction A was oxidized by NBT under the same condition, which was catalyzed by the amine-containing membranes of PEI microcapsules. For the same amount of intermediate (halogenated indoxyl), its oxidized dimer (indigo dye) was enhanced by double amount of oxidizer of NBT. Paper 1 gave a noticeable response at 6 min, which is faster than the patent reported by Dwir et al. [3]. They reported no optimal pH was found under which the overall reaction to yield the indigo dye proceeds fast enough to give a signal in less than 10 min. And their reported optimal pHs for two steps were 5.5–6 and ≥9, respectively, which was shown in Fig. 3, reactions A and B. The components of paper 1 per centimeter paper are listed in Table 2, BCIN used in paper 1 strips with the size of 2 mm × 5 mm was much less than it in assembled card in Dwir's patent [3] with width of 4 mm (2.83×10^{-4} mg in our strip vs. 3.53×10^{-2} mg in Dwir's patent), but response was faster than it, since this colorimetric reaction was catalyzed by PEI microcapsules (Reaction C). Fig. 4 shows the activity of papers 1 and 5 by adding of the same amount of neuraminidase. For the hydrolysis of BCIN, both papers did not have a significant difference, but paper 1 was getting darker than paper 5 (as expected by Reactions A and B at pH 6) after 10 min, and showed remarkably darker color with increased length of reaction time. The same amount of BCIN and NBT were loaded onto both papers, but paper 1 contained microcapsules and paper 5 was prepared only by paper pulp. It was found that papers 1 and 5 displayed different color intensities in the presence of the same amount of sialidase (Fig. 4), which indicated that the combination of microcapsules efficiently accelerated this color

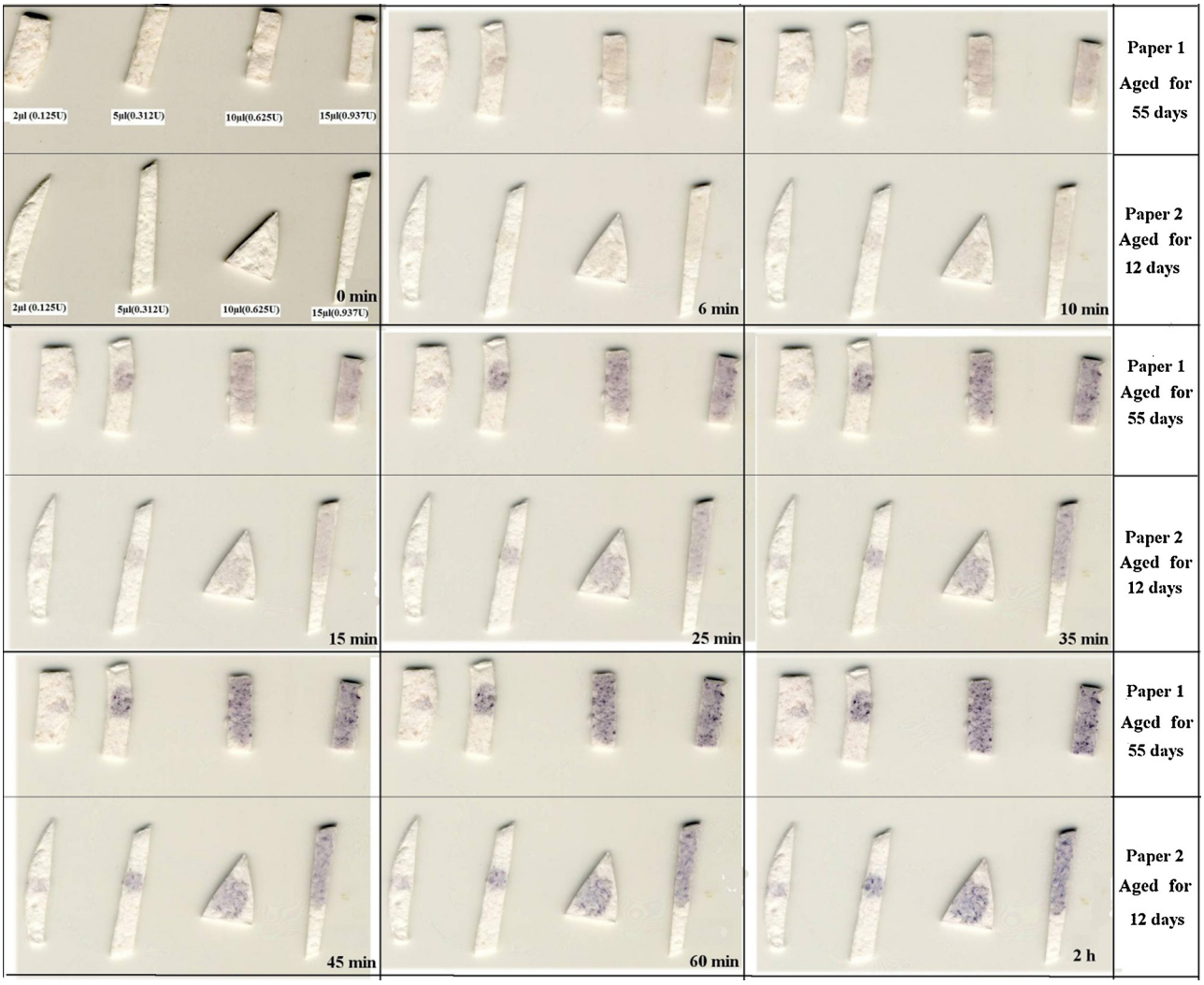


Fig. 5. The color response of papers 1 and 2 with different lengths of reaction time in the presence of various amount of sialidase (2 μL, 5 μL, 10 μL, 15 μL). Paper 1 was measured after 55 days of preparation, and had faster response than paper 2 (12 days) due to the high amount of NBT. Both paper strips can give visible dark purple color response in the presence of 5 μL (0.312 UN) sialidase. The noticeable color was developed in as little as 6 min.

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032

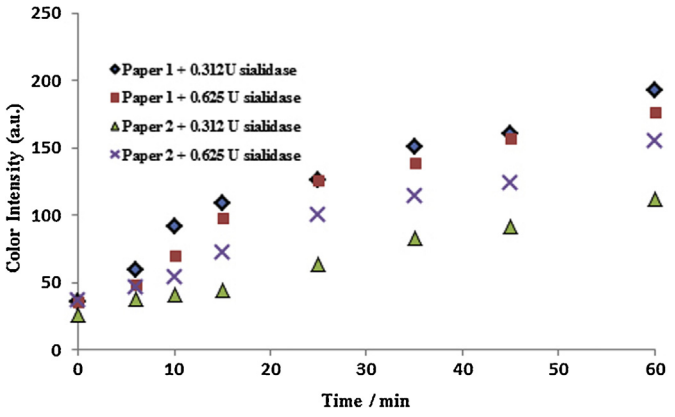


Fig. 6. Mean color intensity of papers 1 and 2 in the presence of sialidase at different time length.

reaction. In practice, paper 1 has better visibility than paper 5 due to its uneven intensity generated by PEI microcapsules and their aggregates containing high concentrations of BCIN and NBT. Paper 5 displayed the most uniform color since it did not contain microcapsules. By comparison with all kinds of paper strips prepared, paper 1 showed the fastest response and most intense color.

NBT in papers 1 and 2 could react with the microcapsules to form a little bit of pink product at room temperature, which was shown in Fig. 2 (papers 1 and 2 at 36 h). But it was much slower than the enzymatic reaction. Therefore, it does not really affect detection of sialidase. The rest part on papers 3 and 4 did not change to pink after 36 h, since NBT was not used for the fabrication of paper 3, and paper 4 did not contain microcapsules, so NBT was not retained in the paper after filtration.

3.3. The same paper with different amounts of sialidase

Papers 1 and 2 changed their color with different lengths of reaction time in the presence of various amount of sialidase (Fig. 5). Paper 1 was measured after 55 days of preparation, and had a little bit faster response than paper 2 (12 days) due to the high amount of NBT. Papers 1 and 2 showed noticeable color change by adding of 2 μL (0.125 UN) sialidase and a visible dark purple color appeared in the presence of 5 μL (0.312 UN) sialidase. With the addition of 10 μL (0.625 UN) sialidase, both papers displayed significant dark purple color change.

Fig. 6 shows the intensity comparison of papers 1 and 2 in the presence of 5 μL and 10 μL sialidase at different time length, which clearly indicated paper 1 have higher color intensity than paper 2 due to higher amount of color enhancer NBT. Paper 1 can give visible color in 6 min, which is faster than paper 2 (15 min).

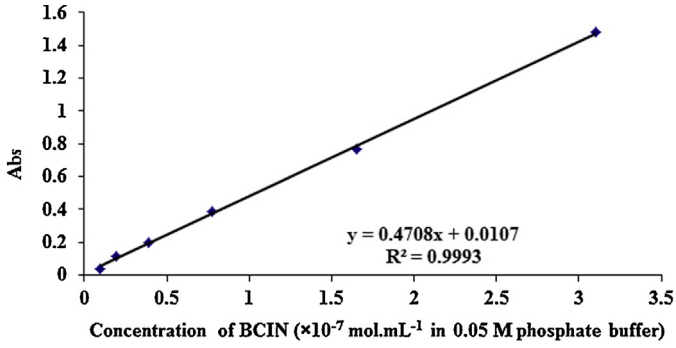


Fig. 7. External standard curve of BCIN solutions. The measurements were conducted by UV–vis absorbance at wavelength of 280 nm. Original concentration of BCIN was used in paper preparation was 3.1×10^{-7} mol mL^{-1} in 0.05 M phosphate buffer. And other solutions were obtained from different dilutions.

3.4. Loading efficiency of BCIN

The standard curve of BCIN is shown in Fig. 7. The loading efficiency of BCIN was calculated to be 22.2%. To date, paper 1 was kept for 13 months at −18 °C and it still works well. A putative explanation for this is that PEI microcapsules can prolong the storage stability of BCIN.

4. Conclusions

A colorimetric biosensor for the detection of neuraminidase was successfully fabricated though incorporating PEI microcapsules containing BCIN and NBT into paper pulp. The portable paper strips could be easily stored at −18 °C since BCIN is sensitive to temperature. Paper strips kept the same activity over a minimum of 12 months. The existence of PEI microcapsules improves visibility of color reaction. This colorimetric spot assay did not require any sample preparation step, and low amounts of sample (less than 10 μL) can be directly added to small pieces of the bioactive paper. The noticeable response time was in as little as 6 min, and the dark purple color can be developed by increasing the reaction time. NBT and microcapsules enhanced the paper color by accelerating the second step of the reaction of BCIN and sialidase, which made it possible to have trace amounts of BCIN in paper giving significant color response in the presence of sialidase. In practice, there is no expertise requirement for this simple one-step test, thus it could be used by women in the privacy of their home. Since the paper fabrication step is compatible with industrial application, this method could be potentially commercialized. Some further studies, such as paper response to different types of sialidase and real specimens from patients are envisioned in future work.

Acknowledgements

The authors thank the financial support from the SENTINEL Research Network on Bioactive Paper and from the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

[1] R. Amsel, P.A. Totten, C.A. Spiegel, K.C.S. Chen, D. Eschenbach, K.K. Holmes, Am. J. Med. 74 (1983) 14.
[2] O.S. Dwir, T. Karen, F. Fish, US Patent 20080038766, 2008.
[3] O.S. Dwir, T. Karen, F. Fish, US Patent 007591978 B2, 2009.
[4] D.H. Yan, Z. Lu, J.R. Su, Chin. Med. J. 122 (2009) 2748.
[5] S.R.R. de Figueiredo Leite, M.M.R. de Amorim, W.B. Calabria, T.N. de Figueiredo Leite, V.S. de Oliveira, J.A.A. Ferreira Junior, R.A. de Alencar Ximenes, Rev. Bras. Ginecol. Obstet. 32 (2010) 82.
[6] V. Dadhwal, R. Hariprasad, S. Mittal, A. Kapil, Arch. Gynecol. Obstet. 281 (2010) 101.
[7] C.L. Haggerty, P.A. Totten, M. Ferris, D.H. Martin, S. Hoferka, S.G. Astete, R. Ondondo, J. Norori, R.B. Ness, Sex. Transm. Infect. 85 (2009) 242.
[8] J.L. Thomason, S.M. Gelbart, R.J. Anderson, A.K. Walt, P.J. Osypowski, F.F. Broekhuizen, Am. J. Obstet. Gynecol. 162 (1990) 155.
[9] J.L. Munoz Bellido, J.E. Garcia Sanchez, J.A. Garcia-Rodriguez, Enferm. Infecc. Microbiol. Clin. 10 (1992) 340.
[10] J.R. Schwebke, S.L. Hillier, J.D. Sobel, J.A. McGregor, R.L. Sweet, Obstet. Gynecol. 88 (1996) 573.
[11] M.R. Joesoef, S.L. Hillier, S. Josodiwondo, M. Linnan, J. Clin. Microbiol. 29 (1991) 1730.
[12] R.P. Nugent, M.A. Krohn, S.L. Hillier, J. Clin. Microbiol. 29 (1991) 297.
[13] S. Yen, M.A. Shafer, J. Moncada, C.J. Campbell, S.D. Flinn, C.B. Boyer, Obstet. Gynecol. 102 (2003) 927.
[14] J. Thulkar, A. Kriplani, N. Agarwal, J. Indian, Med. Res. 131 (2010) 445.
[15] A.G. Rouse, K.M. Gil, K. Davis, Arch. Gynecol. Obstet. 279 (2009) 545.
[16] G. Giacomini, D. Reali, D. Vita, S. Barahmandpour, A. Dacunto, Diagn. Cytopathol. 3 (1987) 198.
[17] M.S. Nelson, J. Am, Emerg. Med. 5 (1987) 488.

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032

[18] A.M. Roberton, R. Wiggins, P.J. Horner, R. Greenwood, T. Crowley, A. Fernandes, M. Berry, A.P. Corfield, J. Clin. Microbiol. 43 (2005) 5504.
[19] L. Myziuk, B. Romanowski, S.C. Johnson, J. Clin. Microbiol. 41 (2003) 1925.
[20] F.R. Jones, G. Miller, N. Gadea, R. Meza, S. Leon, J. Perez, A.G. Lescano, J. Pajuelo, C.F. Caceres, J.D. Klausner, T.J. Coates, N.C.H.S. Prevent, Int. J. STD AIDS 18 (2007) 188.
[21] P. Sumeksri, C. Koprasert, S. Panichkul, J. Med. Assoc. Thai. 88 (Suppl. 3) (2005) S7.
[22] C.S. Bradshaw, A.N. Morton, S.M. Garland, L.B. Horvath, I. Kuzevska, C.K. Fairley, J. Clin. Microbiol. 43 (2005) 1304.
[23] S.C. Johnson, M. Luo, A. Saeed, US Patent 2003/0162240 A1, 2003.
[24] R. Wiggins, T. Crowley, P.J. Horner, P.W. Soothill, M.R. Millar, A.P. Corfield, J. Clin. Microbiol. 38 (2000) 3096.
[25] V. Reukov, A. Vertegel, O. Burtovyy, K. Kornev, I. Luzinov, P. Miller, Mater. Sci. Eng. C, Biomimetic Supramol. Syst. 29 (2009) 669.
[26] Y. Zhang, D. Rochefort, J. Microencapsulation 27 (2010) 703.
[27] E. Seidler, Histochem. J. 12 (1980) 619.
[28] V.A. DeBari, M.A. Needle, A. Prado, Histochem. Cell Biol. 56 (1982) 155.
[29] R.W. Horobin, Histochem. J. 14 (1982) 301.
[30] P.M. Marcuse, Obstet. Gynecol. 11 (1958) 707.

Please cite this article in press as: Y. Zhang, D. Rochefort, Fast and effective paper based sensor for self-diagnosis of bacterial vaginosis, Anal. Chim. Acta (2013), http://dx.doi.org/10.1016/j.aca.2013.09.032