Asian Journal of Chemistry; Vol. 26, No. 14 (2014), 4330-4334

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16408

Paper-Based Microfluidic Device for Determination of Nitrite in Pickled Vegetables

CHUNXIU XU, MINGHUA ZHONG, YUNYING WU, LONGFEI CAI*, JIANI HONG and YUNA HUANG

Department of Chemistry, Hanshan Normal University, Chaozhou 521041, P.R China

*Corresponding author: E-mail: cailf@hstc.edu.cn

Received: 23 September 2013; *Accepted*: 7 November 2013; *Published online*: 5 July 2014; AJC-15468

Nitrite is a carcinogen which has been detected in various foods including pickled vegetables, cured meat and fish products. Development of cost effective, simple and easy-to- use method for determination of nitrite in foods is thus highly desirable, especially for those foods that traditionally contains high contents of nitrite. In this work, a paper-based microfluidic analytical method was proposed for the determination of nitrite in pickled vegetables. Determination of nitrite was based on the chromogenic reaction between nitrite and mixed chromogenic reagents on a paper-based microfluidic device. This method offers advantages of easy fabrication, low reagents/sample consumption, ease of use, fast analysis speed and low cost, which may have potential in food safety testing for household use.

Keywords: Paper-based microfluidics, Nitrite, Determination, Pickled vegetables.

INTRODUCTION

Processing of pickled vegetables is popular and the pickled vegetables have been one of the traditional dishes in Chinese families from ancient times. During the pickles processing, however, not only various nutrients including vitamin C and vitamin B1 were lost, but also nitrite is produced. Nitrite has been assigned as a carcinogen to human by the International Agency for Research on Cancer (IARC). The acceptable daily intake recommended by the World Health Organization is less than 8 mg nitrite for an adult of 60 kg. Consequently, detection of nitrite in foods is of great importance. Various analytical methods including spectrophotometry[1,2], fluorimetry[3,4], chemiluminescence[5], electrochemistry[6,7], gas chromatography[8], liquid chromatography[9] and capillary electrophoresis[10] have been used to determine the nitrite ions in foods and other samples. However, these methods usually need some specialized analytical instruments and professional personnel, which make the nitrite detection for household use impossible.

Recently, Microfluidic chips or lab on a chip are emerging as a robust platform for performing micro chemical and biological experiments[11-14]. Microfluidic chips, owing to the advantages of low consumption, fast analysis speed and portability, is recognized as one of the most potential analytical tools for household use. However, to the best of our knowledge, few microfluidic analytical products have been really developed and applied to the real samples especially for household use. This may be partially due to the time/cost-consuming fabrication process of microanalytical systems on the materials of silicon, glass and polymers[15-17]. Recent developed paper-based microfluidics was provided as a low cost and effective alternative, which make use of simple techniques such as the photolithography[18], wax printing[19], inkjet printing[20] and wax drawing[21] to pattern the microfluidic design on a tiny piece of filter paper. The paper-based microfluidic devices are featured as easy fabrication, low reagent/sample consumption, low cost and ease of use. These features may make the paper-based microfluidic analytical devices suitable for household use.

In this work, we presented a simple method for determination of nitrite in foods by paper-based microfluidic analytical devices. The paper-based microfluidic devices were fabricated by inkjet printing followed by wax drawing. The determination of nitrite was based on the chromogenic reaction between the nitrite and the mixed chromogenic reagent. The images of colorimetric assay could be captured by a digital camera or mobile phone, the gray intensity was obtained by the Image J software and was used to calculate the nitrite contents in food. All of the operations are simple and cost effective, which could be applied to the determination of nitrite in foods such as pickled vegetables by the common families without professional skills in analytical chemistry.

EXPERIMENTAL

All chemicals used were of analytical grade unless mentioned otherwise. Doubly deionized water was used throughout. 4 g/L sulfanilic acid solution was prepared by dissolving 0.4 g of sulfanilic acid and diluted to 100 mL with 20 % hydrochloric acid. 0.4 g/L N-(1-naphthyl) ethylenediamine was prepared by dissolving 0.1 g of N-(1-naphthyl) ethylene-

diamine and diluted to 250 mL with water. The mixed chromogenic reagent was prepared by mixing the 4 g/L sulfanilic acid solution and 0.4 g/L N-(1-naphthyl) ethylenediamine with a ratio of 7:3. The mixed solution contains 2.8 g/L sulfanilic acid and 0.12 g/L N-(1-naphthyl) ethylenediamine. A stock standard NO_2^- solution of 1 mg/mL was prepared by dissolving 0.1500 g of sodium nitrite and diluted to 100 mL with water. The working standard NO_2^- solutions were prepared by appropriate dilution of the stock solution.

An inkjet printer (Canon Pixma ip1000) was used to transfer the channel design to the filter paper (102, Hangzhou Xinhua Paper Limited, Hangzhou, China). A wax pen was used to pattern the microfluidic channels on the filter paper. A micro heating plate (YH-946B, Guangzhou Yihua Electronic Equipment Co., Ltd., Guangzhou, China) was used to heat the wax patterned in the paper. A digital camera (Canon IXUS9515, Japan) or a mobile phone was used to capture the images of colorimetric assay performed on the paper-based microfluidic device. The Image J software was used to calculate the gray intensity in each detection unit.

Fabrication of the paper-based microfluidic device: The paper-based microfluidic device was fabricated as described elsewhere[21]. Briefly, the channel configuration was designed by the Corel DRAW X3 software and was printed on a A4 sized filter paper, then the pattern was painted along the printed configuration with a wax pen by hand (Fig. 1). The patterned device was then put on a heating plate (135 °C) for 1 min, such that the wax was melt and penetrate into the paper to form a hydrophobic wax wall[22]. This allowed the liquids flow inside the edges of the wax walls.

Analytical procedure: 20 µL of mixed chromogenic reagent was first spotted on the central unit and allowed to dry for 10 min. Because the six distribution channels have the same length and width and the six detection units have the same diameter, the mixed chromogenic reagents on the central unit would flow equally along the six channels into the detection units. Thus, the six detection units would contain the same amounts of chromogenic reagents. Then, 1 µL of five standard solutions containing different concentrations of nitrite as well as the sample solution were spotted onto the detection units (one solution for each unit). The device was allowed to dry for 10 min such that the nitrite reacts with the mixed chromogenic reagent, forming a red-colored chemical in the detection units. The images in the detection units were captured and the gray intensity was calculated with the Image J software for quantitative analysis of nitrite content.

RESULTS AND DISCUSSION

We determined the volume of reagents and sample required for analysis. Although the smaller channel and detection units may be beneficial to reduce the sample and reagent consumptions, relatively large channels (2 mm wide and 5 mm long) and detection units (6 mm diameter) were designed such that the chip configuration could be readily painted using a wax pen by hand along the printed pattern. Furthermore, dispensing less than 0.2 µL is difficult with a standard micropipette. The reagent and sample volume required to wet the entire detection units were determined by spotting 0.4-1.5 µL of blue food dye solution onto the detection units. As shown in Fig. 2a, 0.4-0.8 µL of reagent solution cannot completely wet the entire detection units, whereas 1.2 µL would spread out of the detection unit. 1 µL reagent or sample solution was thus selected to spot onto the detection units in the recommended procedure. The volume of mixed solution required to wet the entire chip

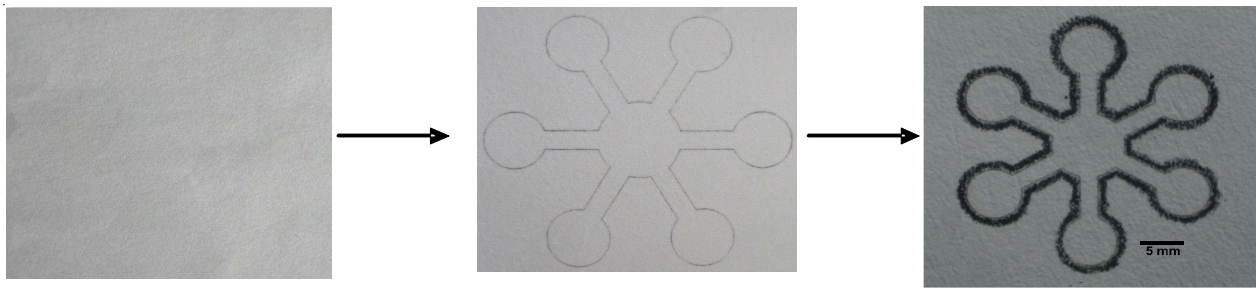

Fig. 1. Fabrication of paper-based microfluidic chip, (a) Filter paper before the pattern was printed, (b) Filter paper with a pattern printed by an inkjet printer, (c) Filter paper after the pattern was painted by a wax pen along the pattern printed in (b)

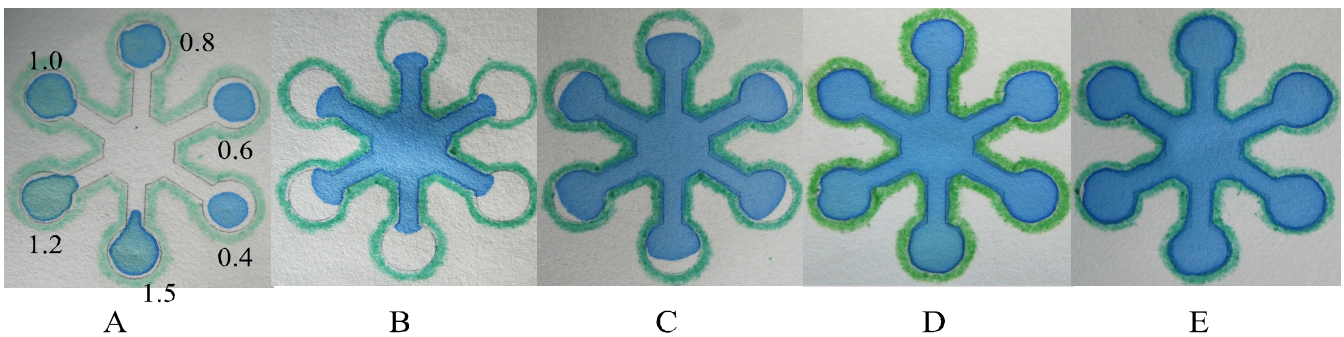

Fig. 2. (a) Paper-based microfluidic device with 0.4-1.5 µL food dye solution spotted onto the detection units, (b-e) Paper-based microfluidic device with 10 (b), 15 (c), 20 (d) and 25 µL (e) of blue food dye solution spotted onto the central unit

channel and detection units was determined by dropping 10-25 µL of the same dye solution into the central unit. As shown in Fig. 2b-e, 20 µL of dye solution is required to wet the entire device, thus 20 µL mixed chromogenic solution was spotted onto the central unit in the recommended procedure.

Spotting of reagents: In our preliminary experiment, the two chromogenic reagents, sulfanilic acid solution and N-(1-naphthyl) ethylenediamine solution were not mixed before being spotted onto the central unit. In other words, sulfanilic acid solution and N-(1-naphthyl) ethylenediamine solution were spotted onto the central units sequentially. As shown in Fig. 3a, the chromogenic reactions were not occurred at the whole detection units. This may be attributed to the fact that the chromogenic reagents were added respectively rather than spotted together onto the central unit. As the N-(1-naphthyl) ethylenediamine was added onto the central unit, it will flow along the channel into the detection units, thus the dried sulfanilic acid spotted previously on the device was dissolved and flow to the detection units. Consequently, the chromogenic reactions were occurred at the interface between sulfanilic acid and N-(1-naphthyl) ethylenediamine in the detection units after the nitrite solution was spotted onto the detection units. The non-uniform distribution of the reaction product in the detection units offers difficulties in selection of measured area when using the Image J software to measure the gray intensities. To address this issue, the sulfanilic acid solution and N-(1-naphthyl) ethylenediamine solution were mixed prior to spotting onto the central unit. By spotting the mixed solution onto the central unit followed by spotting the nitrite solution onto the detection units, the red-colored reaction product was distributed uniformly in the detection units (Fig. 3b).

Effect of chromogenic reagents concentration on the chromogenic reaction: The effects of sulfanilic acid concentration and N-(1-naphthyl) ethylenediamine in the mixed solution on the chromogenic reaction were investigated. To optimize the sulfanilic acid concentration, 0.4-3.2 g/L sulfanilic acid were prepared in the mixed solution with the N-(1-naphthyl) ethylenediamine concentration maintaining constant as 0.12 g/L. As shown in Fig. 4a, the colour intensity increased with the sulfanilic acid concentrations in the range of 0.4-2.8 g/L, whereas the color intensity maintain constant with the increased sulfanilic concentrations when the sulfanilic acid concentration is higher than 2.8 g/L. Therefore, 2.8 g/L sulfanilic acid was selected in the recommended procedure.

The effect of N-(1-naphthyl) ethylenediamine concentration in the range of 0.04-0.24 g/L on the color intensity was studied by maintaining the sulfanilic acid concentration constant as 2.8 g/L. As shown in Fig. 4b, the colour intensity maintains constant in the range of 0.04-0.24 g/L N-(1-naphthyl) ethylenediamine. In this work, 0.12 g/L N-(1-naphthyl) ethylenediamine was selected in the recommended procedure.

Effect of reaction time: The effect of reaction time in the range of 1-20 min on the chromogenic reaction was investigated. The colour intensity in the reaction unit increased with the time in the range of 1-15 min, whereas the colour intensity maintains constant with a reaction time higher than 15 min. Thus 15 min was selected as the reaction time in the recommended procedure.

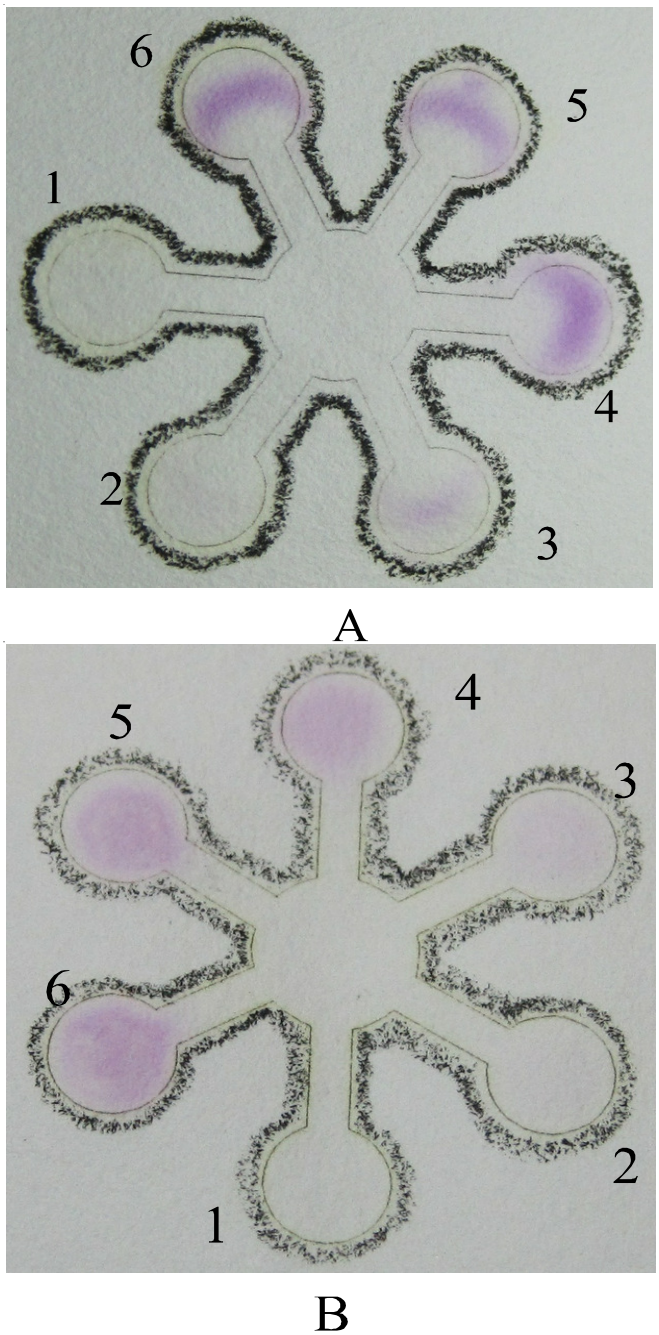

Fig. 3 (a) Colorimetric assay by spotting sulfanilic acid and N-(1-naphthyl) ethylenediamine onto the central unit, respectively, (b) Colorimetric assay by spotting the mixed reagents (sulfanilic acid and N-(1-naphthyl) ethylenediamine) onto the central unit. 0 (1), 10 (2), 20 (3), 30 (4), 40 (5) and 50 (6) µg/mL nitrite solution were added onto the detection units, respectively

Sample analysis: A suitable amount of pickled vegetables about 10 g was accurately weighed and smashed in a mortar. The smashed pickled vegetable was transferred into a beaker and then 100 mL water was added to extract the nitrite for 3 h. The extracts were filtered and diluted to 100 mL in a volumetric flask. The extracts was then determined as the analytical procedure described above. The image of the colorimetric assay was captured with a camera or mobile phone and stored in JPEG format (Fig. 5a). The image was then inverted and the mean gray values in the detection units were obtained by subtracting the blank value. Data were imported into origin

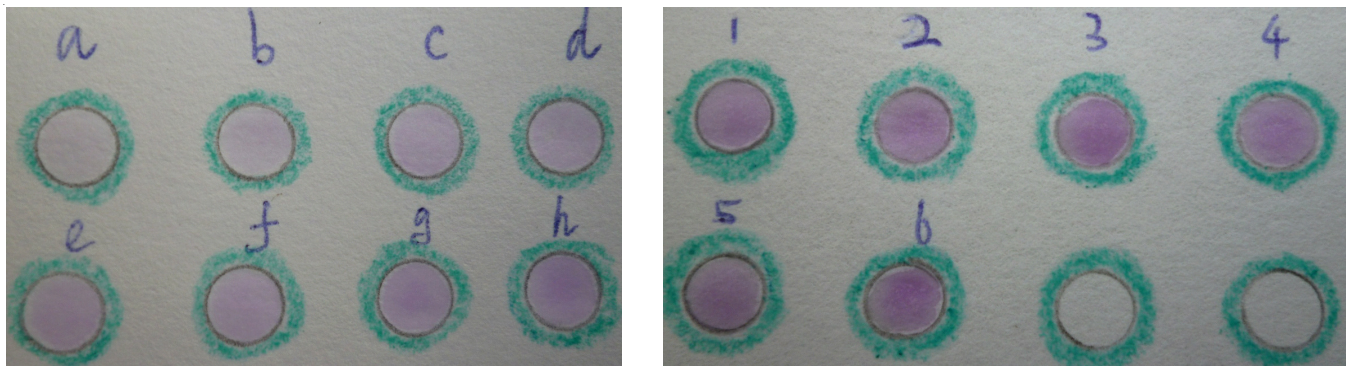

Fig. 4 (a) Photograph of colorimetric assay obtained with 0.4 (a), 0.8 (b), 1.2 (c), 1.6 (d), 2.0 (e), 2.4 (f), 2.8 (g) and 3.2 g/L (h) sulfanilic acid in the mixed chromogenic reagents, (b) Photograph of colorimetric assay obtained with 0.04 (1), 0.08 (2), 0.12 (3), 0.16 (4), 0.20 (5) and 0.24 g/L (6) N-(1-naphthyl) ethylenediamine in the mixed chromogenic reagents. The diameter of the microreactor unit: 6 mm; Volume of mixed chromogenic reagents and nitrite solution: 1.0 µL; CNO_2^-: 50 µg/mL

(version 7.5) to obtain a linear correlation between gray intensity, GI and nitrite concentration, $C_{NO_2^-}$. The linear correlation between the gray value and nitrite concentration

$$GI = 0.3813C_{NO_2^-} - 0.22 \quad (1)$$

was obtained with a correlation coefficient of 0.979 (Fig. 5b). The relative standard deviation of this method was 2.9 % by determining 60 µg/mL nitrite solution six times. The concentration of nitrite in the extract of pickled vegetable was 11.6 µg/mL calculated from the gray value in the sample detection unit and linear equation above. The content of nitrite in pickled vegetable was 1.16 ×102 µg/g according to the following equation:

$$w = \frac{CV}{m} \quad (2)$$

where w (µg/g) is the content of nitrite in pickled vegetable, C (µg/mL) is the concentration of nitrite in the extract of pickled vegetable, V (mL) is the volume of extract and m (g) is the weighed mass of pickled vegetable. The nitrite content (1.16 ×102 µg/g) in pickled vegetables determined by this method compared favorably with that (1.25 × 102 µg/g) measured by a standard method[23], demonstrating that this paper-based microfluidic analytical method is reliable for the determination of nitrite in pickled vegetables.

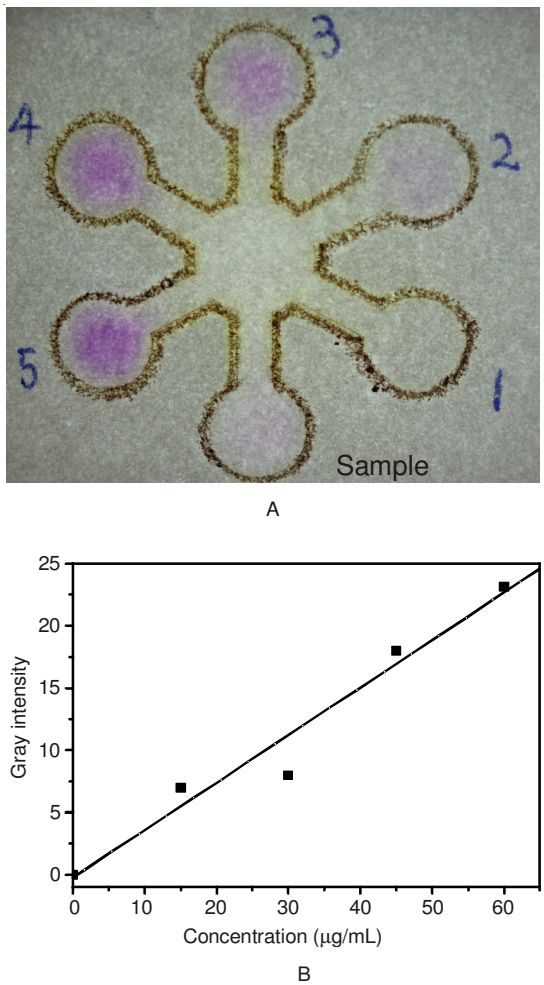

Fig. 5. (a) Image showing results of nitrite assay on a paper-based microfluidic device. Nitrite standard solutions spotted onto detection units of no. 1-5 are 0, 15, 30, 45 and 60 µg/mL, respectively. Sample solution was spotted onto the remaining detection unit, (b) Gray intensity varies as a function of nitrite concentration obtained from data of (a)

Conclusion

We described a method for determination of nitrite content in pickled vegetables by paper-based microfluidic devices. This method has advantages of simplicity, fast analysis speed and low cost, which could be operated easily by common person even those who haven't professional skills in analytical chemistry. Although the pickled vegetables were selected as sample in this work, this method could also be used to determine the nitrite contents in other foods. The disadvantage of this work is the limited sensitivity, which may pose difficulties in a wide range of applications especially when the nitrite content is at low level. The low sensitivity is the common disadvantage that the paper-based microfluidic devices shared when the determination is based on the chromogenic reactions. This limitation could be addressed by development of the pre-concentration strategies on the paper-based microfluidic analytical devices, or by developing other sensitive detection methods for paper-based microfluidic devices, for example the fluorescence detection. These strategies and methods for enhancing the sensitivity of paper-based microfluidic analytical method are ongoing in our research group.

ACKNOWLEDGEMENTS

This work was supported by the Guangdong Provincial Natural Science Foundation of China (Gant S2013010012046, S2012040007274 and S2011040002246) and the Research Start-up Fund of Hanshan Normal University (Grant QD20120521).

REFERENCES

1. I.T. Bae, R.L. Barbour and D.A. Scherson, *Anal. Chem*., **69**, 249 (1997).
2. I.M.P.L.V.O. Ferreira, J.L.F.C. Lima, M.C.B.S.M. Montenegro, R.P. Olmos and A. Rios, *Analyst*, **121**, 1393 (1996).
3. Y.X. Guo, Q.F. Zhang, X.C. Shangguang and G.D. Zhen, *Spectrochim. Acta A*, **101**, 107 (2013).
4. N.Q. Jie, Z.K. Si, J.H. Yang, Z. Miao, X.R. Huang, Q. Zhang and Z.Q. Song, *Microchem. J*., **55**, 351 (1997).
5. D.Y. He, Z.J. Zhang, Y. Huang and Y.F. Hu, *Food Chem*., **101**, 667 (2007).
6. X.H. Chen, C.M. Ruan, J.L. Kong and J.Q. Deng, *Anal. Chim. Acta*, **382**, 189 (1999).
7. B. Unnikrishnan, P.L. Ru, S.M. Chen and V. Mani, *Sens. Actuators B Chem*., **177**, 887 (2013).
8. S. Yurchenko and U. Mölder, *Food Chem*., **96**, 325 (2006).
9. J. Kieber and P.J. Seaton, *Anal. Chem*., **67**, 3261 (1995).
10. X. Wang, E. Adams and A. Van Schepdael, *Talanta*, **97**, 142 (2012).
11. L.-F. Cai, Y. Zhu, G.-S. Du and Q. Fang, *Anal. Chem*., **84**, 446 (2012).
12. G.M. Walker, J.Q. Sai, A. Richmond, M. Stremler, C.Y. Chung and J.P. Wikswo, *Lab Chip*, **5**, 611 (2005).
13. S. Gotz, T. Revermann and U. Karst, *Lab Chip*, **7**, 93 (2007).
14. A. Huebner, L.F. Olguin, D. Bratton, G. Whyte, W.T.S. Huck, A.J. de Mello, J.B. Edel, C. Abell and F. Hollfelder, *Anal. Chem*., **80**, 3890 (2008).
15. Z.J. Jia, Q. Fang and Z.L. Fang, *Anal. Chem*., **76**, 5597 (2004).
16. G.M. Whitesides, E. Ostuni, S. Takayama, X.Y. Jiang and D.E. Ingber, *Annu. Rev. Biomed. Eng*., **3**, 335 (2001).
17. C.G. Khan Malek, *Anal. Bioanal. Chem*., **385**, 1362 (2006).
18. A.W. Martinez, S.T. Phillips, M.J. Butte and G.M. Whitesides, *Angew. Chem. Int. Ed*., **46**, 1318 (2007).
19. K.M. Schilling, A.L. Lepore, J.A. Kurian and A.W. Martinez, *Anal. Chem*., **84**, 1579 (2012).
20. X. Li, J.F. Tian, G. Garnier and W. Shen, *Colloid Surf. B*, **76**, 564 (2010).
21. Y. Lu, W.W. Shi, L. Jiang, J.H. Qin and B.C. Lin, *Electrophoresis*, **30**, 1497 (2009).
22. L.F. Cai, Y.Y. Wu, C.X. Xu and Z.F. Chen, *J. Chem. Educ*., **90**, 232 (2013).
23. Z. Kang, Food Analysis and Detection, Light Industry Press, Beijing, China (2006).