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Automated chemiluminescence immunoassay for a nonionic surfactant using a recycled spinning-pausing controlled washing procedure on a compact disc-type microfluidic platform

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ABSTRACT

A fully automated and integrated chemiluminescence immunoassay, carried out on a compact disc (CD)-type microfluidic platform, for the detection of alkylphenol polyethoxylates (APnEOs) is described. The pattern of the CD-type microchip was designed so as to permit the sequential solution delivery of the sample solution, the washing solution and the luminol solution, which are required in the chemiluminescence immunoassay process, along with a designed rotation program for spinning the CD-type microchip. The procedure for flowing the washing solution, the volume of which was limited on the CD-type microchip, was optimized by using a recycled spinning-pausing rotation program to overcome the non-specific adsorption of the horseradish peroxidase labeled APnEOs at the detection area. The detection limit of the immunoassay is about 10 ppb.

1. Introduction

Research on analytical techniques using CD-type or centrifugal platforms has progressed in last decades [1–6], and has resulted in an increasing recognition of the potential of the technique for use in biochemical analyses. Various laboratory functionalities such as sampling, mixing, metering, reaction and detection can be integrated on a CD-type microchip, and many analytical processes such as immunoassays, nucleic acid assays, and flow analyses can be accomplished using specifically designed CD-type microfluidic platforms. The solutions, which are located in corresponding reservoirs, are delivered by the centrifugal force generated from the rotation of the disc through the microchannel. This delivery system does not require any additional components such as pumps, sample tubes as well as switch valves. An analytical system fabricated on a CD-type microfluidic platform represents the latest innovation in the development of flow based analytical chemistry, since, as opposed to many other techniques, the solutions can be delivered without pumping systems, compared with common microchips.

Polydimethylsiloxane (PDMS) is one of the most widely used materials for fabricating a CD-type microfluidic platform as well as a common microchip, due to its unique properties such as excellent transparency, good thermal and oxidative stability, ease of fabrication and the fact that sealing can be accomplished with various materials. However, the hydrophobic property of PDMS continues to pose a barrier that needs to be overcome ifa analytical process on a PDMS-based CD-type microfluidic platform is to ne generally applied, since the non-specific adsorption of proteins on the surface of the microchannel walls can occur, due to the fact that the hydrophobicity of PDMS can influence the quantification of analytes [7]. In order to decrease the adsorption of proteins, many attempts have been made to modify the surface of the PDMS-based microchip from hydrophobic to hydrophilic through plasma treatment and silanization [8–12], chemical vapor deposition [13,14], curing with co-polymers [15–18], polymer grafting [19–32] as well as modification with nanoparticles [33–35]. Although the physical adsorption of proteins can be decreased to a certain degree compared with the bare PDMS surface, the procedure used for the surface modification makes the technique more complicated, which introduces additional difficulties associated with the preparation of the microchip. As a result, the modified layers are sometimes unstable and recycling becomes limited.

In our previous study [36], we carried out a semi-batch chemiluminescence immunoassay for the determination of a type of widely used nonionic surfactants, namely, alkylphenol polyethoxylates (APnEOs) using a CD-type microchip. A series of biodegradation products produced from these surfactants are suspected to act as endocrine disrupters, and there a number of methods for the determination of APnEOs have been reported, including chromatography [37–47], ELISA [48], sequential injection

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analytical (SIA) systems [49] and related techniques. Since the most of them are somewhat time-consuming and tedious, in order to develop a more rapid and simpler analytical method for APnEOs, we proposed a chemiluminescence immunoassay on a CD-type microchip for APnEOs in our previous paper [36]. However, one problem is that the washing efficiency for removing the non-specifically adsorbed HRP-labeled APnEOs (abbreviated as HRP-APnEOs) from the detection area is very low. Because of this, it was necessary to carry out the washing operation separately in a test tube and it was not possible to integrate the entire analytical process on the CD-type microchip.

In this study, a specifically designed rotation program was used to deliver the washing solution, thus improving washing efficiency by using a limited volume (~250 μL). This permits the entire chemiluminescence immunoassay process to be carried out on one CD-type microchip. The proposed method has the advantages of integration, miniaturization, simplicity of operation, portability and it is low-cost. This is the first report concerning improving washing efficiency by controlling the flow of the washing solution on a CD-type microchip. The developed analytical system has the potential for use in monitoring APnEOs as well as other low molecular weight analytes which can also be detected by chemiluminescence immunoassay methodology in environmental water samples.

2. Experimental

2.1. Design of CD-type microfluidic platform

Fig. 1 shows the 1/6 part of the CD-type microfluidic platform. The reservoirs are used for storing the solutions, and are linked to the detection area by microchannels. Fig. 2(a) shows the initial state of the microfluidic platform. A sample solution (containing HRP-APnEOs at a constant concentration and APnEOs at different concentrations), a washing solution (pH 8.4 Tris–HCl buffer containing a surfactant, Tween 20 at 0.1 wt%) and a luminol solution (containing *p*-iodophenol, hydrogen peroxide) are placed in reservoirs 1, 2 and 3 respectively. A U-turn shaped microchannel functions as the detection area located downstream, where an anti-APnEOs antibody immobilized magnetic microbeads was previously inserted and fixed by a permanent magnet imbedded in the substrate platform.

The chemiluminescence immunoassay for APnEOs involves 3 steps. In the 1st step, the sample solution in reservoir 1 flows as the result of the centrifugal force caused by the rotation of the microfluidic platform, and a part of the sample solution is maintained in the detection area due to the equal actions of the centrifugal force at the two ends of the solution in the U-shaped microchannel. The competitive immunoreaction between the HRP-APnEOs and the free APnEOs in the bulk solution and the anti-APnEOs antibody immobilized on the surface of magnetic microbeads occurs in the detection area, as shown in Fig. 2(b).

In the 2nd step, the washing solution in reservoir 2 flows across the detection area to remove HRP-APnEOs that are non-specifically adsorbed on the surface of the magnetic microbeads and the PDMS walls of the microchannel, as shown in Fig. 2(c). In this research, in order to increase washing efficiency for the non-specifically adsorbed HRP-APnEOs, the washing solution in reservoir 2 is allowed to flow by a recycled spinning-pausing rotation program, details of which are discussed below.

In the 3rd step of the chemiluminescence immunoassay, the luminol solution in reservoir 3 flows across the detection area by the rotation of the platform. Similarly a part of the luminol solution is retained in the U-turn shaped microchannel, and due to the activity of the HRP enzyme, the luminol in the solution is oxidized to the excited state, which will transfer to the ground state immediately and emit a chemiluminescence signal at the same time, as shown in Fig. 2(d). The intensity of the chemiluminescence signal can be used for quantification, since it is proportional to the amount of the HRP-APnEOs-antibody complex on the surface of the magnetic microbeads which is determined by the concentration of the free APnEOs in the sample solution.

As has been explained in detail in many previous reports, as well as in the Supporting information of this report, the rotation speed required for the delivery of the solution in a certain reservoir is determined by a series of parameters such as the distance between the outlet of the reservoir and the center of the platform, the cross sectional size of the microchannel, the height of the solution in the reservoir in the radial direction, the surface property of the solution and so on. In this research, in order to for the sample solution, the washing solution and the luminol solution to sequentially flow through the detection area, the related parameters mentioned above of each reservoir must be adjusted (see Supporting information). To observe the conditions of the solution in reservoirs 1, 2 and 3 during the rotation of the CD-type microchip, 44 μL and 250 μL of 0.5 mM Rose Bengal solutions (0.1% Tween 20) were placed in reservoirs 1 and 2 respectively, and a 12 μL aliquot of the 0.5 mM Rose Bengal solution without Tween 20 was placed in reservoir 3. The CD-type microchip was set on a turn table (fabricated by Kyushu Keisoku Co., Ltd., Japan), and its speed of rotation was controlled by the applied voltage using the LabView software program. A high speed CCD camera (VW-Z1, Keyence, Co., Japan) was used to observe the real-time status of the solutions on the microchip and to judge the rotation speed required for the delivery of each solution. The relationship between the rotation speed and the applied voltage was preliminarily calibrated using a stroboscope.

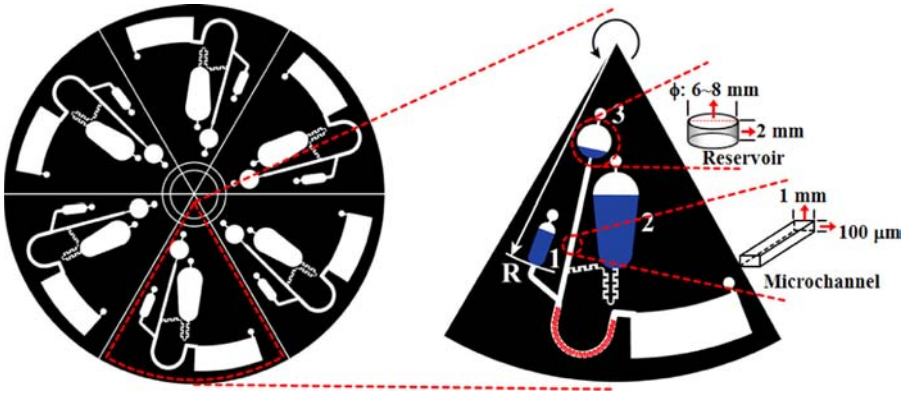

Fig. 1. Design of the CD-type microfluidic platform.

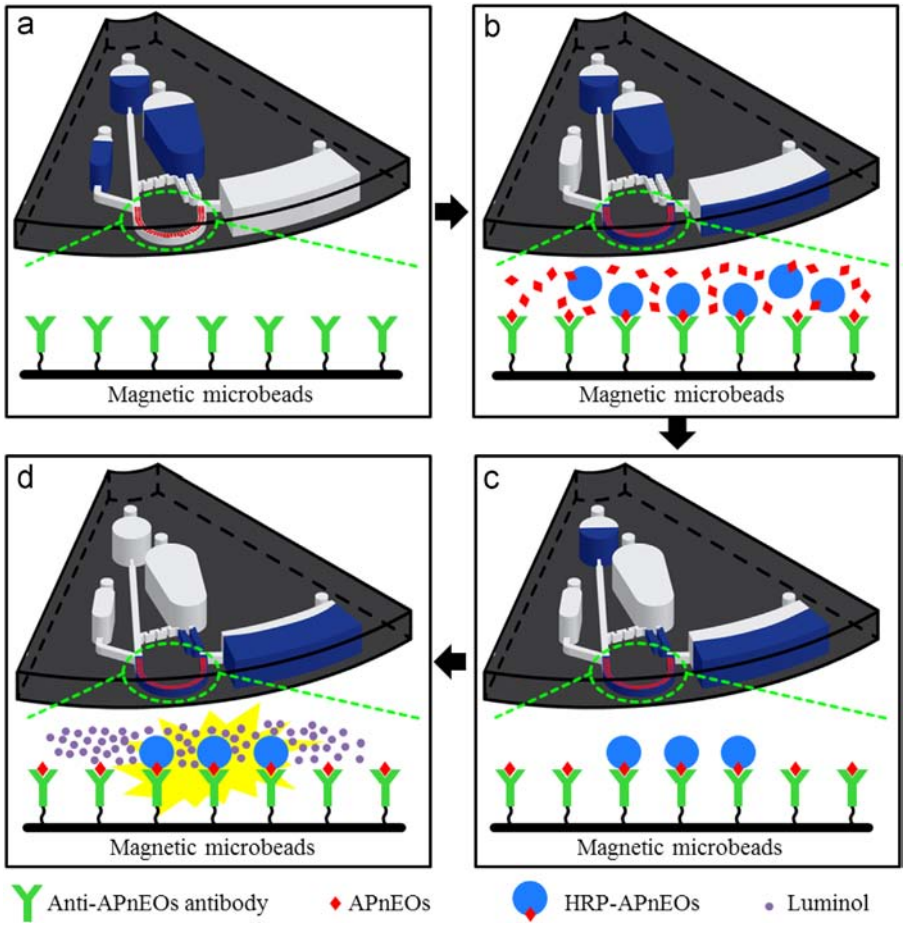

Fig. 2. Chemiluminescence immunoassay process for APnEOs on a CD-type microfluidic platform.

2.2. *Recycled spinning-pausing controlled washing procedure for enhancing the washing efficiency*

In our previous work [36], a semi-batch chemiluminescence immunoassay for APnEOs on a CD-type microchip was carried out. Namely, after the immunoreaction of an anti-APnEOs antibody on the magnetic microbeads with APnEOs and HRP-APnEOs in a test tube, the magnetic microbeads were washed with a buffer solution containing Tween 20. The resulting magnetic microbeads were then placed in a downstream reservoir on a CD-type microfluidic platform, and a luminol solution was allowed to flow to the microbeads from an upstream reservoir by rotating the microchip. The chemiluminescence signal was observed, and the APnEOs was then quantified from the chemiluminescence signal.

In this work, in order to fully integrate the reported chemiluminescence immunoassay, the efficiency by the washing solution was increased to reduce the amount of the adsorbed HRP-APnEOs at the detection area. A recycled spinning-pausing rotation program was designed to permit the washing solution in reservoir 2 to flow, step by step, across the detection area, so that the adsorption–desorption balance of the HRP-APnEOs is repeatedly shifted to the desorption side and the surface concentration of the adsorbed HRP-APnEOs will be continuously decreased.

As shown in Fig. 3, after flowing the sample solution and performing the competitive immunoreaction, the washing solution is delivered to the detection area by executing a recycled spinning-pausing rotation program. In the first spinning step, the rotation speed is first increased to 668 rpm within 5 s, and is then maintained for 2 s at 668 rpm, and then decreased back to 0 rpm within 2 s. In this short period, a small volume of the washing solution will be allowed to flow through the detection area, so that the desorbed HRP-APnEOs will flow out from the detection area. During the period of pausing 1, a new adsorption–desorption balance of the HRP-APnEOs will be built up at the detection area, and the concentration of the HRP-APnEOs adsorbed on the surface (HRP-APnEOs) will be decreased. Similarly in the following, second spinning step, another small volume of the washing solution is allowed to flow through the detection area and to flow out the desorbed HRP-APnEOs. In the period of the following pausing step 2, a new adsorption–desorption balance will be built up and the desorbed HRP-APnEOs will flow out at spinning step 3. During the repeated recycled spinning and pausing steps the concentration of adsorbed HRP-APnEOs will be gradually decreased at the detection area.

In order to optimize the washing procedure, 5 different rotation programs including different numbers of spinning-pausing cycles (2, 9, 17, 25 and 30) were designed by adjusting the rotation speed and maintaining the time period for the spinning steps. Detailed information regarding these programs can be found in the Supporting nformation. For evaluating the washing efficiency of each recycled spinning-pausing rotation program, 44 μL of an HRP-APnEOs solution (500 ppb, 0.1% Tween 20), 250 μL of the washing solution (pH 8.4 Tris–HCl buffer, 0.1% Tween 20) and 12 μL of the luminol solution were placed in reservoirs 1, 2 and 3, respectively, and in the detection area, a 0.4 mg sample of bare magnetic microbeads was previously loaded to monitor the effect of only the physical adsorption of the HRP-APnEOs. The CD-type microchip was set on the turn table, and rotated up to a speed of 518 rpm, which was then maintained at the same speed for 10 min. Under these conditions, the HRP-APnEOs solution in

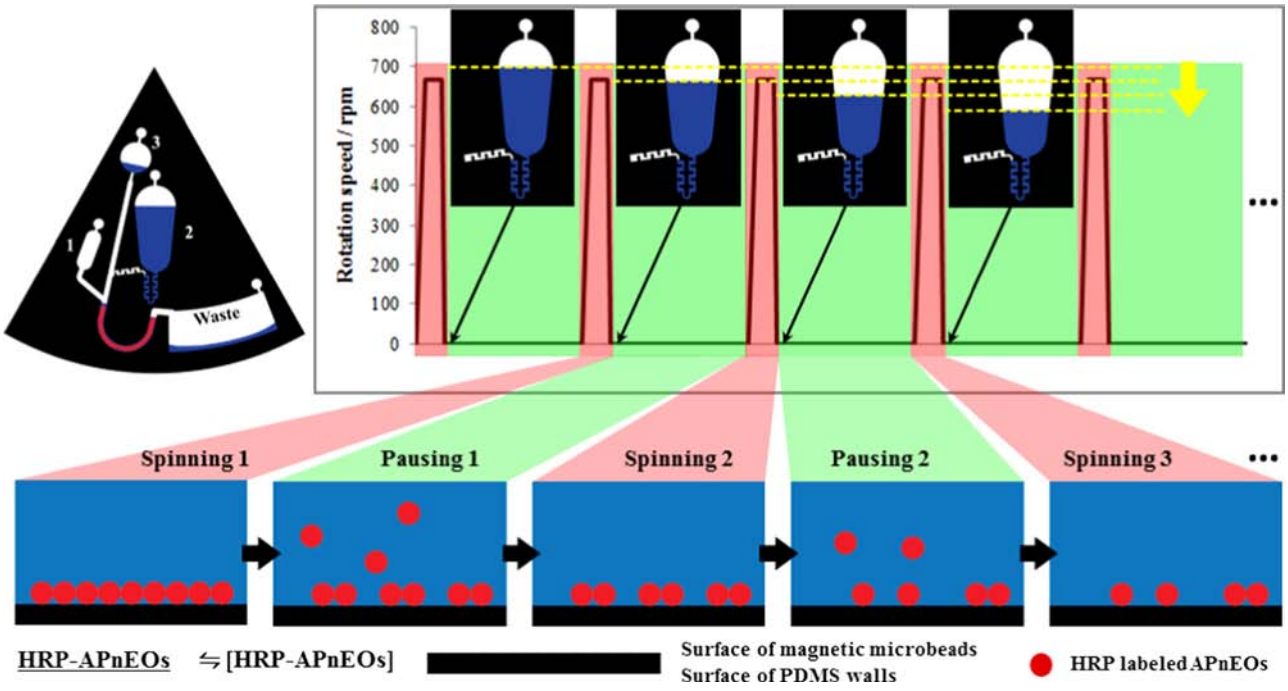

Fig. 3. Schematic diagram of removing the physically adsorbed HRP-APnEOs by using the recycled spinning-pausing rotation program.

reservoir 1 was allowed to flow through the detection area and a part of the solution was allowed to remain in the U-turn shaped detection area. The rotation speed of the microchip was then adjusted according to each designed recycled spinning-pausing rotation program for the stepwise flow of the washing solution. After finishing the washing step, the luminol solution was allowed to flow to the detection area at a rotation speed of 1568 rpm. The chemiluminescence light was emitted due to the reaction of luminol with the physically adsorbed HRP-APnEOs at the detection area. A lower chemiluminescence intensity indicates a better washing efficiency for removing non-specifically adsorbed HRP-APnEOs.

2.3. Chemiluminescence immunoassay for APnEOs on CD-type microfluidic platform

In order to carry out a chemiluminescence immunoassay for APnEOs on the CD-type microchip, a 44 μL volume of sample solution ([HRP-APnEOs]=500 ppb and [APnEOs]=0, 1, 10, 50, 100, 500 and 1000 ppb, respectively, in pH 8.4 Tris–HCl buffer containing 0.1% Tween 20), a 250 μL volume of the washing solution (pH 8.4 Tris–HCl buffer containing 0.1% Tween 20) and a 12 μL volume of the luminol solution (0.5 mM luminol, 0.4 mM *p*-iodophenol and 4 mM H_2O_2) were placed in reservoirs 1, 2 and 3, respectively on the CD-type microchip. Anti-APnEOs antibody immobilized magnetic microbeads (d=30 μm), prepared by the same method as reported in our previous study [36], was previously placed in the detection area and fixed by a permanent magnet imbedded in the substrate platform.

The CD-type microchip was set on the turn table, and was rotated to s speed of 518 rpm and maintained at this speed for 10 min to permit the competitive immunoreaction to proceed. After the immunoreaction, the rotation speed of the microchip was adjusted according to the optimized recycled spinning-pausing rotation program for the flow of the washing solution in reservoir 2 to physically remove adsorbed HRP-APnEOs from the detection area, the luminol solution in reservoir 3 was allowed to flow under a rotation speed of 1568 rpm for chemiluminescence detection. The intensity of the chemiluminescence signal was proportional to the amount of HRP-APnEOs-anti-APnEOs antibody complex on the surface of the magnetic microbeads, which was determined by the concentration of the free APnEOs in the sample solution, thus permitting the APnEOs to be quantified by chemiluminescence detection.

3. Results and discussion

3.1. Automated sequential solution delivery on CD-type microfluidic platform

A 0.5 mM Rose Bengal solution (red colored) was placed in reservoirs 1, 2 and 3 for the visual observation of the status of the solutions on the CD-type microchip during the rotation. A high-speed CCD camera was used for monitoring the flow of the solutions on the CD-type microchip, and some still-frames of the solutions in reservoirs 1, 2 and 3 under the different rotation speeds together with the rotation program are shown in Fig. 4. Fig. 4(a) shows the initial state of the CD-type microfluidic platform before rotation, in which 44 μL and 250 μL of the 0.5 mM Rose Bengal solutions (0.1% Tween 20) were placed in reservoirs 1 and 2, respectively, and a 12 μL volume of the 0.5 mM Rose Bengal solution without Tween 20 was placed in reservoir 3. The rotation speed of the microchip was first increased up to 518 rpm and then maintained at that speed for 10 min. During this period, the Rose Bengal solution in reservoir 1 flowed through the detection area, as shown in Fig. 4(b), and the red color of the solution in reservoir 1 disappeared as observed by the high-speed CCD camera. In a period of 10 min, the Rose Bengal solution was maintained at the detection area, and this time interval corresponded to the process of the immunoreaction in the present immunoassay.

After the Rose Bengal solution had completely flowed out, another Rose Bengal solution in reservoir 2, which corresponds to the washing solution for the immunoassay, was started to flow according to the designed recycled spinning-pausing rotation program. During this operation, a 250 μL volume of the Rose Bengal solution in reservoir 2 was flowed stepwise through the

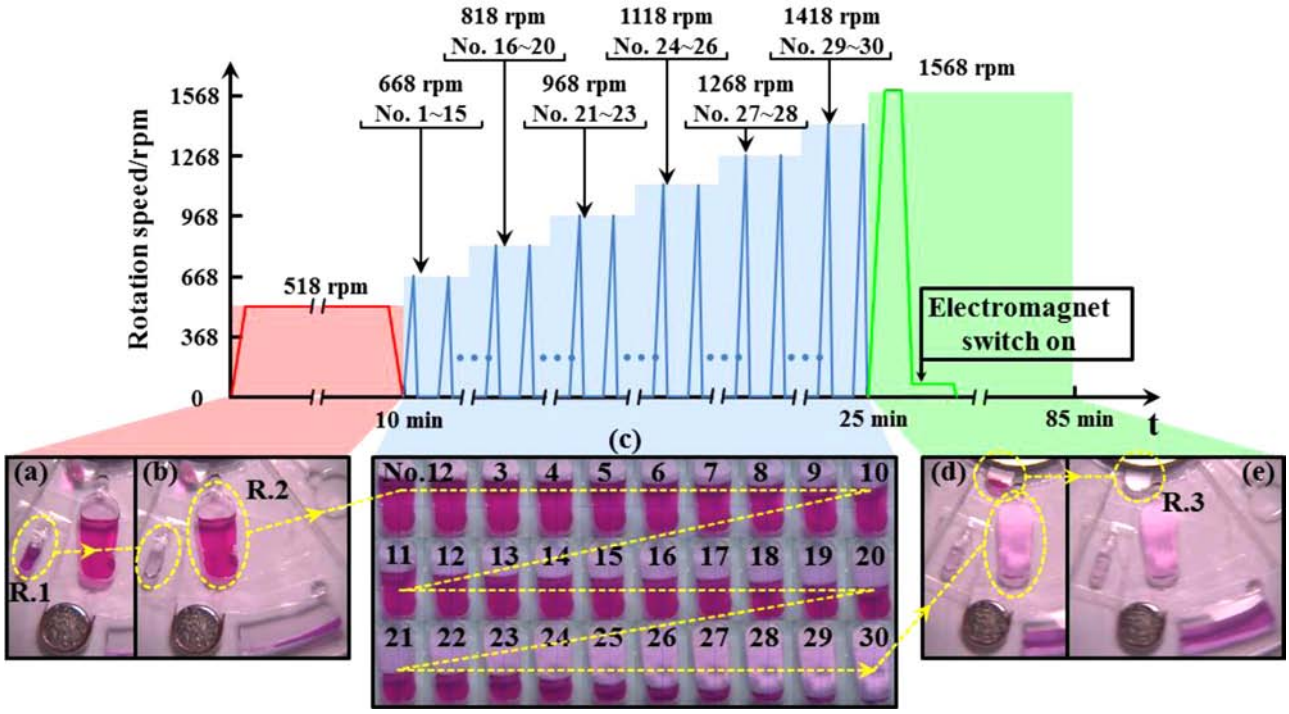

Fig. 4. Automated sequential delivery of the solutions in reservoirs 1, 2 and 3 on the designed CD-type microchip.

detection area by each triangle-shaped spinning step, while the flow of the solution was stopped during the pausing steps. The liquid level of the Rose Bengal solution in reservoir 2, which was observed by the CCD camera, was decreased, as shown in Fig. 4(c). Along with the decrease in the liquid level of the solution in reservoir 2, the required rotation speed in the spinning steps was increased in order to generate sufficient centrifugal force, and after 30 spinning-pausing cycles, all of the 250 μL Rose Bengal solution was allowed to flow out and the time was adjusted to 15 min. This operation for the delivery of the 250 μL Rose Bengal solution (0.1% Tween 20) was adopted as the washing step in the present immunoassay.

As shown in Fig. 4(d), after the 250 μL Rose Bengal solution in reservoir 2 had completely flowed out, the 12 μL of the Rose Bengal solution containing no Tween 20 was still maintained in reservoir 3. This solution in reservoir 3 corresponds to the luminol solution in the present immunoassay. When the rotation speed of the microfluidic platform was increased up to 1568 rpm and maintained at the same speed for 2 min, the solution in reservoir 3 was delivered to the detection area, as shown in Fig. 4(e). As shown in the rotation program in Fig. 4, the rotation speed was decreased to 60 rpm after being maintained at 1568 rpm for 2 min, and an electromagnet which was located under the detector was switched on in order to stop the rotation and the detection area is adjusted so as to be precisely aligned beneath the detector position. For the present immunoassay, the chemiluminescence signal is monitored for 60 min after the rotation stops.

3.2. *Evaluation of the washing efficiency with different recycled spinning-pausing rotation programs*

In order to evaluate the performance of the stepwise delivery of the washing solution in reservoir 2 driven by the 5 designed rotation programs with different numbers of alternative spinning-pausing cycles, video frames of the solution in reservoir 2 during the pausing steps under each rotation program were recorded using a CCD camera, and the results are included in Supporting information. As one example, a still-frame of the stepwise delivery of the washing solution by 30 recycled spinning-pausing steps is shown in Fig. 4(c).

The average volume of the washing solution delivered to the detection area by each spinning step in each rotation program was estimated from the area of the still-frames of reservoir 2, details of which are included in Supporting information. In these cases, the total volume of the washing solution was kept constant at 250 μL, and the average volume of the washing solution delivered by each spinning step in all cases is larger than the volume of the solution phase in the detection area (about 3 μL), indicating that the washing solution was completely replaced, which is beneficial for the removal of the physically adsorbed HRP-APnEOs at the detection area.

As described in experimental Section 2.2, the washing efficiency was evaluated by chemiluminescence detection by flowing a luminol solution after washing the detection area, in which a 500 ppb HRP-APnEOs solution was preliminarily flowed. As shown in Fig. 5, the intensity of the chemiluminescence signal decreased with increasing number of spinning-pausing cycles in the rotation program. Since the intensity of the signal was proportional to the amount of physically adsorbed HRP-APnEOs at the detection area, a higher signal intensity is indicative of a lower washing efficiency of the corresponding rotation program. By using the rotation program which includes 30 spinning-pausing cycles, the intensity of the chemiluminescence signal reached the background level, indicating an excellent washing efficiency. Therefore in the following experiments for preparing a calibration curve of APnEOs, a rotation program comprised of 30 spinning-pausing cycles was used as the washing step in the present immunoassay.

3.3. *Quantification of the chemiluminescence immunoassay for APnEOs*

In this work, the chemiluminescence immunoassay for APnEOs including sequential steps of immunoreaction, washing and chemiluminescence detection was carried out on a CD-type microfluidic platform. Namely, the sample solutions containing APnEOs (0–1000 ppb) and the HRP-APnEOs at a concentration of

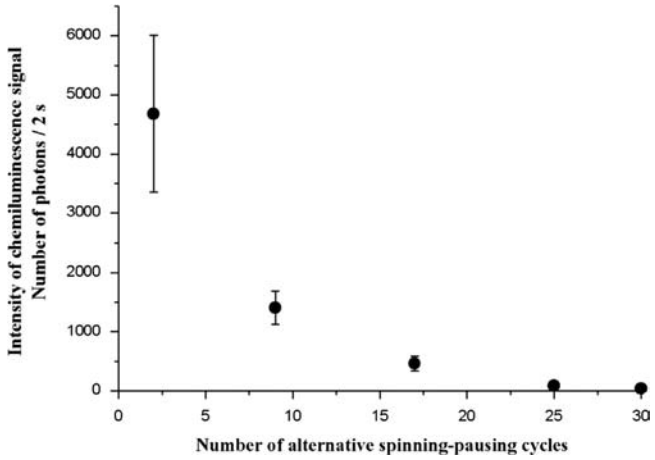

Fig. 5. Comparison of the intensity of the chemiluminescence signals after washing procedures by executing the 5 different recycled spinning-pausing rotation programs.

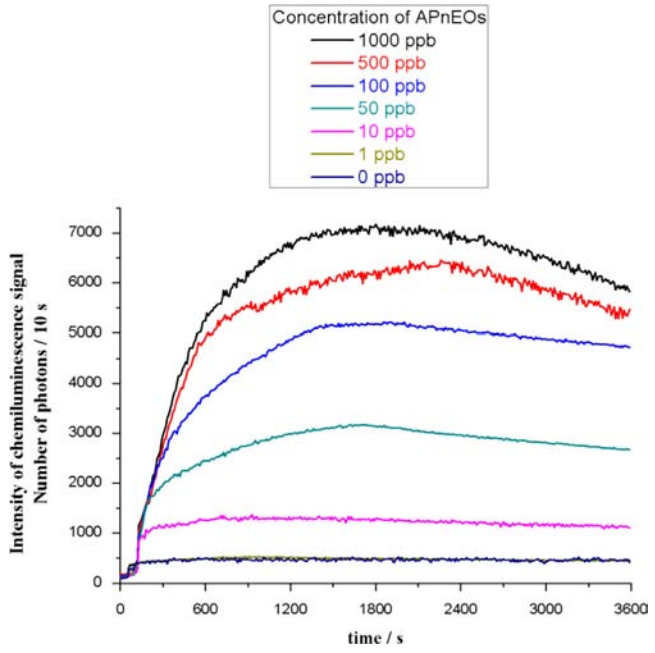

Fig. 6. Chemiluminescence signals for a series of sample solutions of different free APnEOs concentrations.

500 ppb, the washing solution (pH 8.4 Tris–HCl buffer containing 0.1% Tween 20) and the 0.5 mM luminol solution containing *p*-iodophenol, hydrogen peroxide were placed in reservoirs 1, 2 and 3, respectively, on the CD-type microchip, and the resulting microchip was rotated according to the program shown in Fig. 4. The time-courses for the chemiluminescence signals resulting from the reaction between luminol and the HRP-APnEOs-antibody complex on the surface of magnetic microbeads are shown in Fig. 6. The chemiluminescence signals were monitored for 60 min after starting the rotation for the third step, when the luminol solution reached the detection area. Each data point is the average of 3 measurements for the same sample, and the error bar is shown in the figure. The error bars in the present immunoassay range from 3% to 14%. This is due to the fact that the chemiluminescence immunoassay process includes a multi-step procedure. Indeed, when the chemiluminescence signals were obtained by a simple reaction of a luminol solution in the reservoir with the HRP immobilized at the detection area, the error bars were within 5%.

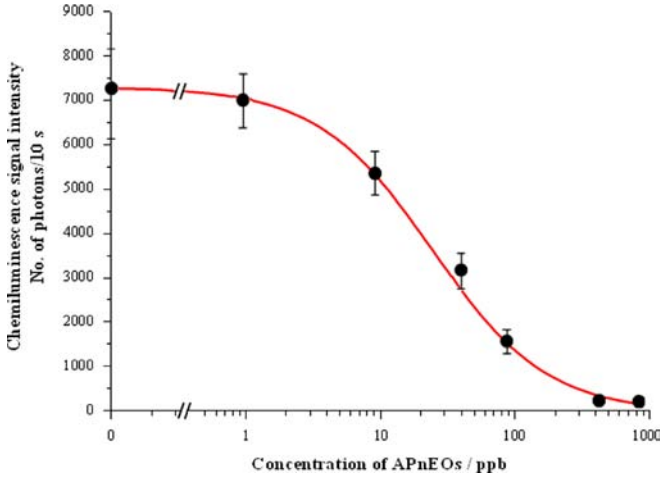

Fig. 7. Calibration curve for APnEOs obtained by the chemiluminescence immunoassay on the CD-type microfluidic platform. The curve is calculated by using Eq. (1) for fitting the observed data.

In our previous study [49], a theoretical equation describing the ratio between the HRP-APnEOs-anti-APnEOs antibody complex and the total anti-APnEOs antibody immobilized on the magnetic microbeads was derived and is expressed as follows:

$$[\mathrm{Ab-HRP-APnEOs}]/[\mathrm{Ab}]^{\mathrm{T}} = K_2 C_{\mathrm{HRP-APnEOs}}/(1 + K_1 C_{\mathrm{APnEOs}} + K_2 C_{\mathrm{HRP-APnEOs}}) \quad (1)$$

where $[\mathrm{Ab}]^{\mathrm{T}}$ and [Ab-HRP-APnEOs] denote the total surface concentration of the anti-APnEOs antibody immobilized on the magnetic microbeads, and the surface concentration of the antibody-HRP-APnEOs complex. K_1 and K_2 are the binding constants between the antibody immobilized on the microbeads with the APnEOs and HRP-APnEOs. C_{APnEOs} and $C_{\mathrm{HRP\text{-}APnEOs}}$ can be assumed to be the initial concentration of the APnEOs and HRP-APnEOs in the sample solution, because these values remain nearly unchanged when considering the large difference between the amount of the anti-APnEOs antibody immobilized on the magnetic microbeads and the amount of the APnEOs and the HRP-APnEOs molecules in the solution.

In the competitive immunoreaction between the free APnEOs and HRP-APnEOs in the solution with the anti-APnEOs antibody immobilized on the magnetic microbeads, the ratio between the HRP-APnEOs-antibody complex ([Ab-HRP-APnEOs]) and the total concentration of the antibody ($[\mathrm{Ab}]^{\mathrm{T}}$) is determined by the concentration of the free APnEOs in the sample solutions, since the concentration of the HRP-APnEOs is maintained constant. The intensity of the chemiluminescence signal is proportional to the amount of HRP-APnEOs on the magnetic microbeads, which is to say, the intensity of the chemiluminescence signal is proportional to the ratio, $[\mathrm{Ab\text{-}HRP\text{-}APnEOs}]/[\mathrm{Ab}]^{\mathrm{T}}$, so that the experimental calibration curve for the APnEOs can be fitted according to the theoretical expression as a function of the concentration of APnEOs in the sample solutions using K_1 and K_2 as parameters and $C_{\mathrm{HRP\,\cdot\,APnEOs}}$ of 1.2×10^{-8} M (500 ppb), as shown in Fig. 7. The fitted calibration curve is a typical sigmoidal curve, and the IC_{80} value was calculated to be about 10 ppb which is similar to that reported in our previous research [36,49] and is comparable to the value reported by Goda et al. [48] using an ELISA method. According to our previous report dealing with a chemiluminescence immunoassay for the same analyte in the SIA system [49], when river water samples were spiked with 20 ppb APnEOs, a good recovery (100% ± 10%) was obtained. Due to the fact that the same procedure of chemiluminescence immunoassay for APnEOs is applied in this study, the proposed analytical system can be concluded to show an analytical performance similar or superior to that in our previous study [49].

4. Conclusion

We describe a fully integrated and automated chemiluminescence immunoassay for APnEOs on a CD-type microfluidic platform, where the sample solution, the washing solution and the luminol solution are placed in reservoirs and delivered sequentially to the detection area by rotating the CD-type microchip according to a predetermined rotation program. Compared with our previous report on a semi-batch chemiluminescence immunoassay for the same analyte, we were able to successfully overcome the effect of the non-specific adsorption of HRP-APnEOs by developing a recycled spinning-pausing rotation program in order to increase washing efficiency. The analytical performance of the proposed method was evaluated and a reasonable calibration curve for APnEOs was obtained. The detection limit was found to be similar to that obtained in the sequential injection analytical (SIA) system [49] as well as our previous semi-batch chemiluminescence immunoassay method [36]. Further work is currently under the way for monitoring APnEOs in actual, environmental samples, such as the tap water or river water.

Improvements to the proposed method with the respect to reducing the detection time, simultaneous multi-sample detection on a single chip and so on are currently in progress by evaluating the use of electrogenerated chemiluminescence detection. The proposed method is universal for the analysis of small molecular analytes in which a competitive immunoassay is used, and it has the potential for use in the monitoring of environmental waters as well as sewage effluents in waste-water-treatment plants.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.06.075.

References

[1] C.T. Schembri, T.L. Burd, A.R. Kopf-Sill, L.R. Shea, B. Braynin, J. Autom. Chem. 17 (1995) 99–104.
[2] M.J. Madou, J. Florkey, Chem. Rev. 100 (2000) 2679–2692.
[3] I.H.A. Badr, R.D. Johnson, M.J. Madou, L.G. Bachas, Anal. Chem. 74 (2002) 5569–5575.
[4] L.G. Puckett, E. Dikici, S. Lai, M.J. Madou, L.G. Bachas, S. Daunert, Anal. Chem. 76 (2004) 7263–7268.
[5] S. Lai, S. Wang, J. Luo, L.J. Lee, S.-T Yang, M.J. Madou, Anal. Chem. 76 (2004) 1832–1837.
[6] S. Morais, J. Carrascosa, D. Mira, R. Puchades, Á. Maquieira, Anal. Chem. 79 (2007) 7628–7635.
[7] H. Chen, M.A. Brook, H Sheardown, Biomaterials 25 (2004) 2273–2282.
[8] J.G. Alauzun, S. Young, R. D'Souza, L. Liu, M.A. Brook, H.D. Sheardown, Biomaterials 31 (2010) 3471–3478.
[9] H. Chen, Z. Zhang, Y. Chen, M.A. Brook, H. Sheardown, Biomaterials 26 (2005) 2391–2399.
[10] M. Farrell, S. Beaudoin, Colloids Surf. B81 (2010) 468–475.
[11] L. Yang, L. Li, Q. Tu, L. Ren, Y. Zhang, X. Wang, Z. Zhang, W. Liu, L. Xin, J. Wang, Anal. Chem. 82 (2010) 6430–6439.
[12] Z. Zhang, X. Feng, F. Xu, X. Hu, P. Li, B.-F. Liu, Anal. Methods 5 (2013) 4694–4700.
[13] B. Bhushan, D. Hansford, K.K. Lee, J. Vac. Sci. Technol. A24 (2006) 1197–1202.
[14] T. Tugulu, H.-A Klok, Macromol. Symp. 279 (2009) 103–109.
[15] K. Ishihara, B. Ando, M. Takai, Nanobiotechnol 3 (2007) 83–88.
[16] Z. Wu, K. Hjort, Lab Chip 9 (2009) 1500–1503.
[17] J. Zhou, H. Yan, K. Ren, W. Dai, H. Wu, Anal. Chem. 81 (2009) 6627–6632.
[18] E. Holczer, Z. Fekete, P. Fürjes, Mater. Sci. Forum 729 (2013) 361–366.
[19] L.B. Carneiro, J. Ferreira, M.J.L. Santos, J.P. Monteiro, E.M. Girotto, Appl. Surf. Sci. 257 (2011) 10514–10519.
[20] N.D. Smet, M. Rymarczyk-Machal, E. Schacht, J. Biomater. Sci. 20 (2009) 2039–2053.
[21] M. Farrell, S. Beaudoin, Colloids Surf. B81 (2010) 468–475.
[22] A. Geissler, M.-F Vallat, P. Fioux, J.-S. Thomann, B. Frisch, J.-C Voegel, J. Hemmerlé, P. Schaaf, V. Roucoules, Plasma Process. Polym. 7 (2010) 64–77.
[23] T. Goda, T. Konno, M. Takai, T. Moro, K. Ishihara, Biomaterials 27 (2006) 5151–5160.
[24] T. Goda, R. Matsuno, T. Konno, M. Takai, K. Ishihara, Colloids Surf. B63 (2008) 64–72.
[25] W. Lin, J. Zhang, Z. Wang, S. Chen, Acta Biomater. 7 (2011) 2053–2059.
[26] S. Sugiura, J.-I Edahiro, K. Sumaru, T. Kanamori, Colloids Surf. B63 (2008) 301–305.
[27] Q. Tu, J.-C. Wang, R. Liu, J. He, Y. Zhang, S. Shen, J. Xu, J. Liu, M.-S. Yuan, J. Wang, Colloids Surf. B102 (2013) 361–370.
[28] A.-J. Wang, J.-J Xu, H.-Y. Chen, J. Chromatogr. A 1147 (2007) 120–126.
[29] Z. Wu, W. Tong, W. Jiang, X. Liu, Y. Wang, H. Chen, Colloids Surf. B96 (2012) 37–43.
[30] P.Y. Yeh, Z. Zhang, M. Lin, X. Cao, Langmuir 28 (2012) 16227–16236.
[31] X. Zhao, Y. Su, Y. Li, R. Zhang, J. Zhao, Z. Jiang, J. Membr. Sci. 450 (2014) 111–123.
[32] C. Zilio, L. Sola, F. Damin, L. Faggioni, M. Chiari, Biomed. Microdevices 16 (2014) 107–114.
[33] H.-J. Bai, M.-L Shao, H.-L Gou, J.-J Xu, H.-Y. Chen, Langmuir 25 (2009) 10402–10407.
[34] D.-H. Fan, S.-W Yuan, Y.-M. Shen, Colloids Surf. B75 (2010) 608–611.
[35] Q. Zhang, J.-J Xu, Y. Liu, H.-Y. Chen, Lab Chip 8 (2008) 352–357.
[36] S. Guo, K. Nakano, H. Nakajima, K. Uchiyama, A. Hemmi, Y. Yamasaki, S. Morooka, R. Ishimatsu, T. Imato, Pure Appl. Chem. 84 (2012) 2027–2043.
[37] R. Espejo, K. Valter, M. Simona, Y. Janin, P. Arrizabalaga, J. Chromatogr. A 976 (2002) 335–343.
[38] C.Y. Cheng, W.R. Li, J.W. Chang, H.C. Wu, W.H. Ding, J. Chromatogr. A 1127 (2006) 246–253.
[39] V. Andreu, E. Ferrer, J.L. Rubio, G. Font, Y. Pico, Sci. Total Environ. 378 (2007) 124–129.
[40] P.A. Lara-Martin, A. Gomez-Parra, E. Gonzalez-Mazo, J. Chromatogr. A 1137 (2006) 188–197.
[41] M. Cantero, S. Rubio, D. Perez-Bendito, J. Chromatogr. A 1046 (2004) 147–153.
[42] Y.K.K. Koh, T.Y. Chiu, A.R. Boobis, E. Cartmell, S.J.T. Pollard, M.D. Scrimshaw, J. N Lester, Chemosphere 73 (2008) 551–556.
[43] S. Banerjee, Anal. Chem. 57 (1985) 2590–2592.
[44] A. Marcomini, S. Capri, W. Giger, J. Chromatogr. 403 (1987) 243–252.
[45] A. Marcomini, W. Giger, Anal. Chem. 59 (1987) 1709–1715.
[46] J.F. Liu, X. Liang, G.B. Jiang, Y.Q. Cai, Q.X. Zhou, G.G. Liu, J. Sep. Sci. 26 (2003) 823–828.
[47] T. Takasu, A. Iles, K. Hasebe, Anal. Bioanal. Chem. 372 (2002) 554–561.
[48] Y. Goda, A. Kobayashi, S. Fujimoto, Y. Toyoda, K. Miyagawa, M. Ike, M. Fujita, Water Res. 38 (2004) 4323–4330.
[49] R. Zhang, H. Nakajima, N. Soh, K. Nakano, T. Masadome, K. Nagata, K. Sakamoto, T. Imato, Anal. Chim. Acta 600 (2007) 105–113.