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Bead-Based Mesofluidic System for Residue Analysis of Chloramphenicol

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A highly selective and sensitive mesofluidic immunoassay system based on competitive immunoassay in polydimethylsiloxane (PDMS) channels was developed. This immunoassay system was successfully applied to quantificationally detect chloramphenicol (CAP) in animal foods. The glass beads (∅ 250 μm) were amino-silane modified, covalently precoated with chloramphenicol succinate, and then infused into the microchannels (∅ 300 μm); the CAP molecules of samples or standards in flow solution competed for CAP antibody with the CAP immobilized on the beads. The CAP antigen−antibody complex anchored on the beads was probed by Cy5-labeled secondary antibody, and the fluorescence intensities of beads were employed to determine the concentration of CAP. In this system, the detection limit of CAP is 0.008 μg/L. The method reveals good recovery rates from 90 to 108% and coefficients of variance (CV) from 4.72 to 6.52%. The experimental results demonstrate that the bead-based mesofluidic system has high sensitivity and excellent performance. Indeed, this system can readily be operated automatically and expanded for multicomponent analysis. It is therefore an attractive alternative to conventional immunoassays in routine supervised domain application for contamination in foods or the environment.

KEYWORDS: Mesofluidic; immunoassay; glass bead; chloramphenicol; residue detection

INTRODUCTION

Chloramphenicol (CAP) is an effective broad-spectrum antibiotic previously widely used in veterinary medicine for treatment of serious infections. However, the clinical application of CAP is prohibited in animal production due to its toxic and allergic reactions (*1–4*). To safeguard the public health, many countries and organizations over the world such as the European Community have banned strictly the use of CAP in food-producing animals, and the maximum residue levels (MRLs) have been legislated as zero tolerance (*3*). Therefore, it should be highly desirable to develop a sensitive and selective residue detection method that can provide simple, practical routine determination of CAP for food samples. There have been several methods employed for effectively monitoring and detecting CAP residues in different levels of sensitivity, selectivity and other characteristics in animal foods such as a microbiological method (*5*), chromatography measurements (*6–8*), immunological methods (*9, 10*), and biosensor and microarray technologies (*10–12*). Either low sensitivity, sample capacity, or the time-consuming property limited the microbiological and chromatographic methods' wide application as screening methods. The immunoassay is well-known as one of the most important analytical methods and is widely used in clinical diagnoses and biochemical studies for its high selectivity and sufficiently sensitivity. However, the conventional immunoassay requires a relatively long assay time, troublesome liquid-handling procedures, and large reagent consumption.

Over the past decade, to improve the immunoassay method, much effort has been devoted toward miniaturization and high throughput of the immunoassay system such as microarray technologies (*12–15*) and continued development of the micro total analysis system (μTAS) concept such as laboratory-on-chip (*16*). So far, microfluidic or mesofluidic chips have been developed and applied to various biological and chemical processes, taking advantage of automation, large surface to volume ratios, low solvent consumption, sensitivity, short separation time, miniaturization, and portability, and they can be controlled by the fluid velocity (*17–25*).

Herein, we present a novel highly sensitive and selective mesofluidic method to analyze CAP residue in food samples that are based on the competitive immunoassay on beads in PDMS channels. Microfluidic (∅ < 1 μm) and mesofluidic (∅ > 100 μm) analytical systems are becoming very popular in chemical and biomedical applications, the main difference between them being the scale of the channels. The mesofluidic immunoassay reveals better fluidics-control and maneuverability compared with the microfluidic system. It allows accurate control of required conditions on beads and flow of reagents to facilitate the immunoreactions using a peristaltic pump. The competitive immunoassay was carried on the different beads in PDMS channels, making it suitable for high throughput and

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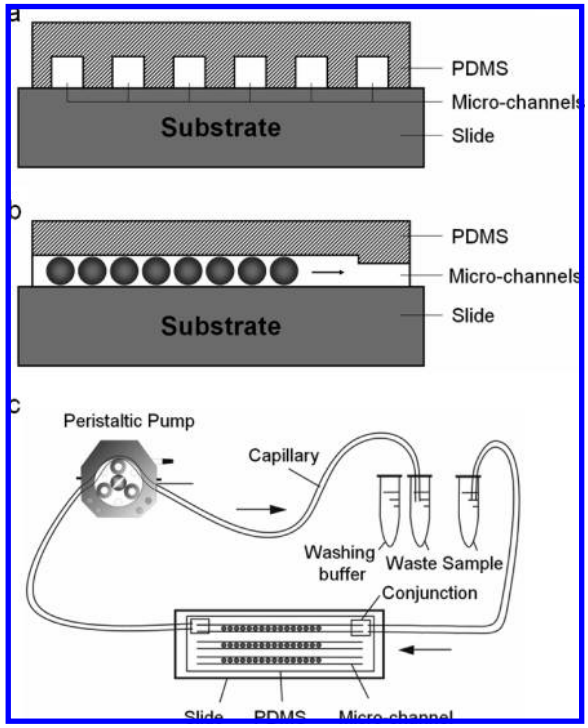

Figure 1. Scheme of mesofluidic immunoassay system: (**a**) left view of microchannels formed by PDMS and slides; (**b**) cutaway view of microchannels full of glass beads; (**c**) fabrication scheme of mesofluidic immunoassay system.

parallel analysis in food samples (**Figure 1**). The glass beads (∅ 250 μm) were amino-silane modified, covalently precoated with chloramphenicol succinate, and then infused into the microchannels (∅ 300 μm); the CAP molecules of samples or standards in flow solution competed for CAP antibody with the CAP immobilized on the beads. The CAP antigen−antibody complex anchored on the beads was probed by Cy5-labeled secondary antibody, and the fluorescence intensities of beads were used to determine the concentration of CAP. The overall assay can be completed within only about 30 min, even including the preconcentration process.

MATERIALS AND METHODS

Materials. The glass beads (average diameter = 250 μm), 2,4,6-trinitrobenzene sulfuric acid solution (TNBS, 5% w/v in H_2O), 3-aminopropyl trimethoxysilane (APTMS, 97%), chloramphenicol sodium succinate, and the standard of chloramphenicol were purchased from Sigma-Aldrich (St. Louis, MO); the monoclonal antibody (mAb) to chloramphenicol was purchased from Biodesign (Monrovia, ME). Cy5-conjugated affinity-purified goat anti-mouse IgG from Rockland (Burlingame, PA) with a fluorochrome/protein (F/P) labeling ratio of 11.2 was used as secondary antibody. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was bought from Pierce (Rockford, IL) and 2-(*N*-morpholino)ethanesulfonic acid (MES) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). PDMS was from Dow Corning (Midland, MI). All other chemicals and solvents were purchased from Sigma (St. Louis, MO), unless stated otherwise, and used without further purification. All solutions were prepared in deionized and sterilized water.

Glass Beads Modification. On the basis of our former work (*26*), 100 mg of the glass beads was first thoroughly washed by ethanol to clean out organic compounds and then hydroxylated overnight by 6 M HCl with gentle shaking at room temperature (RT). The hydroxyl-glass beads were extensively washed by distilled water until neutral and dried under vacuum for 2 h at 110 °C. Subsequently, the hydroxyl-glass beads were derivated with APTMS in anhydrous toluene. Silanization was carried out with gentle shaking at RT for 4 h. After three washings with anhydrous toluene, the aminopropyl-glass beads were dried overnight under vacuum at 110 °C and stored at RT for future use. The amino-functionalized beads were tested to ensure the successful silanization reaction. The amount of active amino groups was investigated by color reaction using TNBS, which was described by Janolino and Swaisgood (*27*) with some modification. Briefly, 20 μL of 0.01 M phosphate-buffered saline (PBS, 0.01 mol/L phosphate buffer containing 0.8% NaCl at pH 7.4) and 5 μL of 5% aqueous TNBS were added to 1 mg of aminopropyl-glass beads and reacted for 2 h at RT. After reaction, the beads were washed by distilled water and examined for their color. A color change to orange from transparent indicated that silanization was successful.

CAP Immobilization. Two milligrams of aminopropyl-glass beads was immersed in chloramphenicol succinate solution in 100 μL of 0.2 M MES buffer (pH 4.5). Fresh EDC solutions (10 and 100 mg/mL, prepared in 0.1 M, pH 6.5, MES buffer in 15 min before the reaction) as coupling agents were added during the immobilization; the addition amount of EDC was changed with time, being 15 μL (10 mg/mL) at 0 min, 7.5 μL (100 mg/mL) at 60 min, and 7.5 μL (100 mg/mL) at 90 min, with the final concentration of 7 mg/mL. The reaction took place with gently shaking for 120 min at RT, and then washing with 0.01 M PBST (PBS with 0.05% Tween 20) once and PBS twice was performed.

Fabrication Process. The mesofluidic immunoassay system is schematically shown in **Figure 1c**; the core component was the mesofluidic reaction chamber based on glass beads in PDMS channels. A silicon wafer with a pattern made of SU-8 by photolithography was used to cast the PDMS mesofluidic mold. PDMS was mixed well with curing agent at a ratio of 10:1 (w/w), and then the mixture was poured onto the silicon wafer, which had been fumigated by fluoroalkyl silanes. Subsequently, the master with the PDMS was then placed in a vacuum desiccator for approximately 15 min to help remove air bubbles from the PDMS that were introduced during the stirring in of the curing agent. Then the master with PDMS was removed from the desiccators and placed on an 80 °C hot plate for 1 h to cure. After the PDMS cured on the master, it was lifted off the master and cut to form mesochannels with the depth of 300 μm. Then the PDMS device was bonded to a glass slide using an oxygen plasma bonder. Inside the plasma bonder, the bonding surfaces of the slide (25 mm × 75 mm × 1.0 mm) and the PDMS chip were exposed to high-energy plasma, which strips away electrons on the surface. This causes the surfaces to become hydrophilic. When these two hydrophilic surfaces come into contact, they form a strong bond. The integrated mesofluidic chamber device was fabricated by sealing with a glass slide, which contained access holes for connection of mesofluidic fittings (**Figure 1a**,**b**). The entire process was performed according to the reported literature methods (*28, 29*).

The following fabrication process was system assembling. The system included a programmable multichannel peristaltic pump (Masterflex L/S, Vernon Hills, IL), which gave the driving force, the mesofluidic chamber device where the immunoassay took place, a reagent handling model, and some conjunction capillaries (**Figure 1c**). The capillaries were connected to the inlet and outlet of the mesochannels using epoxy resin as adhesive.

Analytical Procedures. The entire analysis process in the mesofluidic system, including bead loading, bead blocking, washing, and reagent injection, was automatically carried out in parallel with a programmable multichannel peristaltic pump. First, the CAP-modified beads were infused into the microchannels with PBS for driving. After that, the inlet capillary was connected to the sample reservoir, and the outlet capillary was connected to the waste reservoir through the peristaltic pump. The inner wall of the microchannels and the glass beads were blocked by 0.1% BSA solution for 10 min, and then the blocking solution was washed away by PBST and PBS buffer sequentially. The mixtures of samples and CAP antibody were emitted into the microchannels, the system was kept at 37 °C for several minutes, and the liquid was kept swinging in a little range. After reaction, PBST and PBS were infused again into the microchannels successively to wash the beads. Subsequently, the secondary antibody was introduced into the microchannels, and another incubation time was needed for the secondary antibody recognizing the antibody−antigen

complex on the surface of the beads. At last, the beads were washed again with PBST and PBS thoroughly. The flow rate of the solution was adjusted to 1.5 μL/min for the antigen−antibody and antibody−secondary antibody reaction and to 10 μL/min for the washing process using PBST and PBS. Washing followed with each step was carried out in this process, 1 min with PBST and 2 min with PBS.

Signal Recording and Data Analysis. After immunoreaction, three beads selected randomly from every microchannel were scanned and imaged on a laser confocal scanner Axon 4000B (Axon Instruments, Foster City, CA) with 5 μm resolution using a Cy5 optical filter. The laser power and photomultiplier tube voltage (PMT) were set to gain optimum signal intensities. The original 16-bit tiff images were quantified with Genepix software 5.0 (Axon Instruments, Foster City, CA).

Sample Preparation. The pretreatment of the samples, including homogenization, centrifugation, and extraction, followed the instruction of chloramphenicol ELISA kit (R-Biopharm, Darmstadt, Germany). For milk sample preparation, milk was centrifuged for 15 min at 5000 rpm. The layer between the upper lipid layer and above the protein pellet was collected, and 5 μL of the collection was used for analysis. For meat sample preparation, a reasonable amount of sample was homogenized first, 3 g of homogenized sample mixed with 3 mL of distilled water and 6 mL of ethyl acetate in a centrifuge tube, and then the tube was shaken intensely for 10 min using a mechanical shaker. The tube was then centrifuged for 10 min at 5000 rpm at room temperature. After that, 4 mL of ethyl acetate supernatant was transferred into a new tube and reduced to dryness at 60 °C under a weak nitrogen flow. One milliliter of an isooctane/chloroform (2:3) mixture was added to dissolve the dried residue, and 0.5 mL of buffer 1 provided in the chloramphenicol ELISA kit was added. The tube was shaken intensely for 1 min and centrifuged at 5000 rpm for 10 min. Five microliters of the aqueous layer was used for analysis.

RESULTS AND DISCUSSION

Optimization of Glass Bead Modification. Silanization was the crucial step of chemical modification of glass beads for probe immobilization. Several different silanization solvents such as anhydrous ethanol, acetone, and anhydrous toluene were tested. Among all of these reagents, APTMS with anhydrous toluene as solvent revealed the best results. Furthermore, the influence of APTMS concentration in anhydrous toluene was also studied. The TNBS test results showed the orange color increased from 1% concentration to 2% concentration of APTMS used in the silanization; however, the color decreased gradually from 2% to 10%. Therefore, 2% APTMS in anhydrous toluene was the best choice for the silanization solution.

CAP Immobilization. The surface concentration of CAP immobilized at the solid surface played an important role in capturing antibody. Five different CAP concentrations of the coating solution were studied in the reaction processes of immobilization. **Figure 2** shows the effect of the coating CAP concentration. As expected, the fluorescence intensity increased with the coating concentration when it was <4 mg/mL; however, the fluorescence intensity decreased when the coating concentration of CAP increased to >4 mg/mL. We have noted that, when the coating concentration of CAP increased to >4 mg/mL, turbidity appeared immediately after EDC added, and the jelly prevented the interaction between the CAP and the aminopropyl beads. Therefore, to overcome this disadvantage and improve the efficiency of CAP immobilization, the coating CAP was added in batches, each batch with 4 mg/mL and repeated three times. After immobilization, to eliminate high background noise, the active beads and the wall of the microchannels were blocked by keeping them immersed in PBS with 0.1% BSA as a blocking agent for 10 min.

Influence of Immunoreaction Conditions. Every immunoassay involves a combination of antibody and antigen interactions, and appropriate primary and secondary antibody concentrations depend on each antibody's specific activity and specificity for its antigen, as well as the amount of antigen present in the sample. Concentrations of the antigen and primary and secondary antibodies that are too high or too low can impair both sensitivity and linear range, increase variations, and cause a variety of undesirable results. Optimization was necessary for the proposed immunoassay system including the concentration of the competitive antigen and primary and secondary antibodies. The optimizations of primary and secondary antibodies were established by chessboard method, and the results are shown in **Figure 3**. **Figure 3a** shows the fluorescence intensity increase with the concentration of anti-CAP at the different concentrations of CAP: anti-CAP concentration neither too high nor too low got poor selectivity; however, when the concentration was 10 mg/L, the selectivity was good and excellent detection fluorescence intensity was achieved. Secondary antibody in this study was used only for indicating the antigen−antibody complex anchored on the bead surface; therefore, good fluorescence signal value is the main consideration for optimization of secondary antibody concentration. The detection fluorescence intensity was also no longer increased when the concentration of Cy5-labeled secondary antibody went to 2 mg/L. On the basis of comprehensive consideration of selectivity and sensitivity, the optimum concentrations of antibody and secondary antibody were 10 and 2 mg/L, respectively.

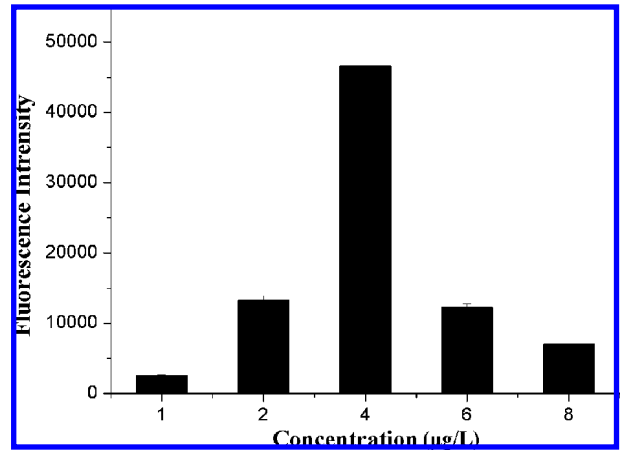

Figure 2. Comparison of the effect of different coating CAP concentrations from 1 to 8 μg/L.

Influence of Incubation Times. The immunoreaction time is also an important parameter affecting the outcome of an immunoassay, especially in fast detection, for the incubation duration is given for the molecules to interact with each other and form a stable immunocomplex. There were two incubation durations in the proposed system, antigen−antibody reaction and antibody−secondary antibody reaction, respectively. The incubation times of immunoassay in mesofluidic channels were investigated under the optimum conditions. The reagents were injected into a customized mesofluidic chamber. The time when the bead surface was completely exposed to the reagent flow was controlled by the fluid velocity and bidirectional liquid movement. The results shown in **Figure 4** demonstrate that the reaction in PDMS channels takes only approximately 5 min to achieve equilibrium. The reaction times required in the mesofluidic immunoassay system were remarkably reduced compared with those of former immunoassays such as ELISA, which needed 30 min per step. This was mainly attributed to the liquid-swing in channels to enhance the transport and reaction between the antigen immobilized on the beads and the antibodies in the liquid.

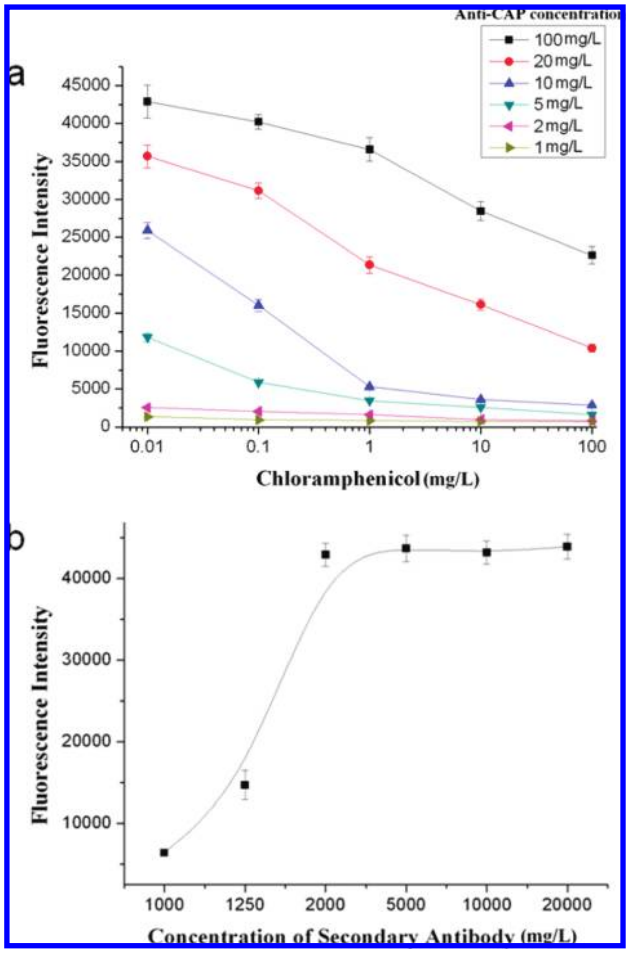

Figure 3. Optimizations of primary and secondary antibody: (**a**) optimizations of anti-CAP concentration (mg/L): (**b**) optimizations of Cy5-labeled secondary antibody concentration (μg/L).

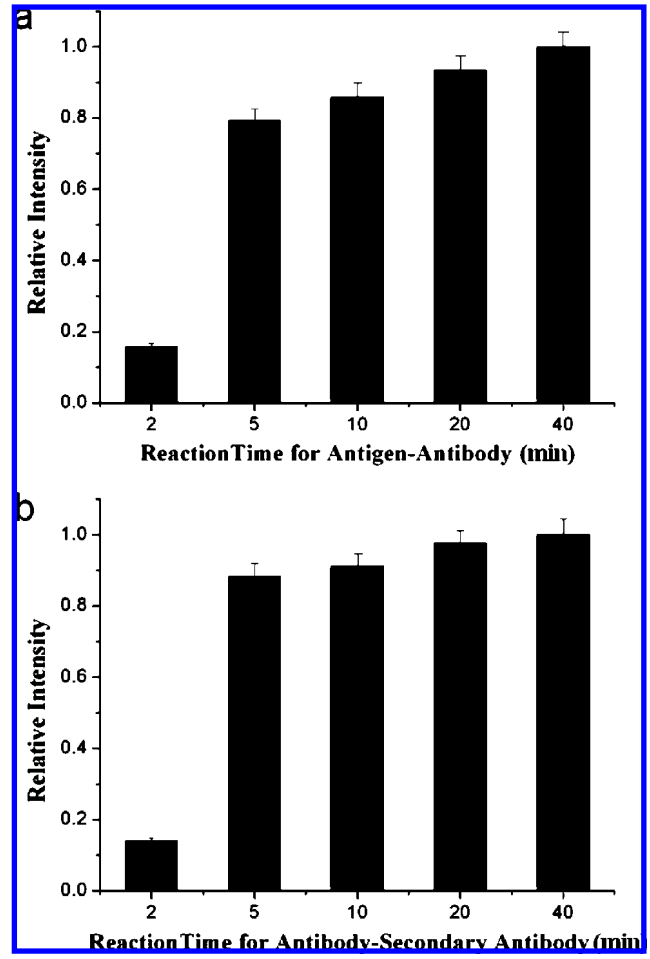

Figure 4. Immunoreaction time optimization: (**a**) comparison of the effect of different CAP antigen−antibody reaction times; (**b**) comparison of the effect of different antibody−secondary antibody reaction times.

Calibration Curves, Detection Limit, Recovery, and Reproducibility Studies. CAP standards with concentrations of 0.01−100 μg/L in 10-fold were used as competitive antigens for calibration curve preparation. The immunoassay was carried out under the optimum conditions described above, and the detection fluorescence intensity of each point of the calibration curve was obtained. Each point of the calibration curve corresponded to the mean value from three replicates, and the relative intensity obtained by the fluorescence intensity of standards divided by the fluorescence intensity of the zero standard. The images and the calibration curve are shown in **Figure 5**. The detection limit for CAP was 0.008 μg/L on the basis of the usual 3σ definition as 3 times the standard deviation of the blank sample. The linear range was from 0.01 to 1.0 μg/L. The recoveries were determined by spiking 0.05 and 0.2 μg/L CAP in six blank samples, respectively. The results are shown in **Table 1**. The method reveals good recovery rates from 90 to 108% and coefficients of variance (CV) from 4.72 to 6.52%. These values are in accordance with the required accuracy in trace analysis.

Sample Determination. To assess the applicability and validity of the method on the real samples, 40 blind samples from milk and 40 from meats were parallel determined by the mesofluidic immunoassay and chloramphenicol ELISA kit from R-Biopharm. Among the 80 samples, 18 samples were assigned as positive (>0.05 μg/L) by mesofluidic against 17 positive samples by ELISA. It must be pointed out that no "real positive sample" (>0.3 μg/L) was found in these samples. Here, to verify the usefulness of this system, we set the positive standard as 0.05 μg/L artificially. **Figure 6** presents the scatter plot of the determined concentrations of CAP in samples by these two methods. The values of the two methods were highly correlated (r = 0.91). It is observed that the positive coincidence rate between the results obtained by these two methods was 100%, and the negative coincidence rate was 98.4%.

In this paper, an integrated mesofluidic system, comprising a bead-based mesofluidic reaction chamber device and a peristaltic pump, was developed for the immunoassay. **Table 2** presents the advantages of this method compared with the other methods used for CAP detection. The system provides a platform for injection, transport, and manipulation of the ligand-modified beads in PDMS channels to facilitate the immunoreactions. As a proof-of-principle experiment, a practical application for the mesofluidic system is confirmed by the ability to determine CAP residue in food samples. The multistep reactions can be implemented on the surface of beads in PDMS channels. The capture and preconcentration of beads is possible in the system. It is known that the procedures of preconcentration of the analytes are very important in antibiotic residue analysis in real samples. This system can implement preconcentration by keeping the sample solution circulation flow. More importantly, the entire assay time could be reduced to only approximately 30 min. Compared with the microtiter plate format, the use of a mesofluidic chip reduced sample and reagent consumption from 100 to 5 μL. This method is fast, has high sensitivity, and can be automated to carry out parallel and high-throughput

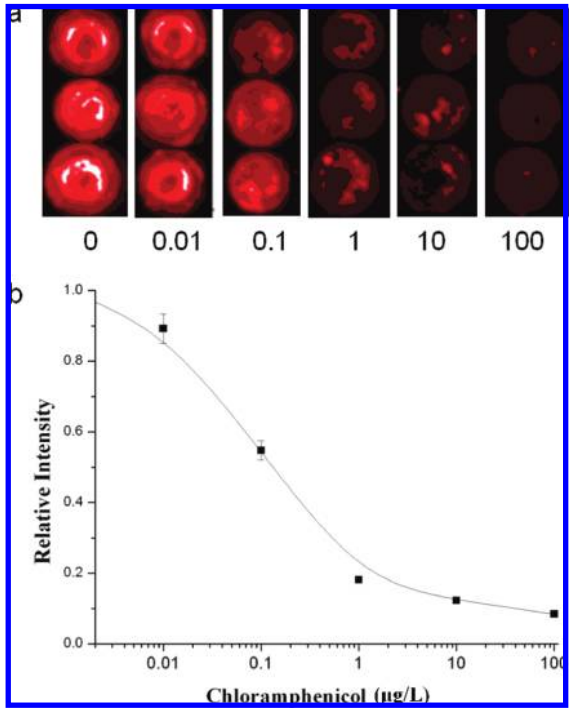

Figure 5. (**a**) Image of the laser scanned result. From left to right, the concentration of CAP standard was increased 10-fold with 0 μg/L as blank control. (**b**) Calibration curves for CAP. Using the mean detection fluorescence intensity of three replicates as the one in this concentration, then they were divided by the intensity that was obtained by the blank control to gain the relative intensity. The calibration curves were obtained with the relative intensity against antibiotic concentration (μg/L).

Table 1. Spiked Experiment Results of Recoveries ($n = 6$) and Repeatability

spiked (μg/L)	detected (μg/L)	recoveries (%)	spiked (μg/L)	detected (μg/L)	recoveries (%)
0.05	0.052	104	0.2	0.212	106
0.05	0.048	98	0.2	0.187	93.5
0.05	0.047	94	0.2	0.193	96.5
0.05	0.054	108	0.2	0.207	103.5
0.05	0.052	104	0.2	0.194	97
0.05	0.046	92	0.2	0.197	98.5
av	0.0498	100	av	0.198	96
CV	6.52%		CV	4.72%	

assay. The mesobeads improved the assay sensitivity by increasing the active capture area and also decreased the variability and incubation times. The function of preconcentration improved the assay sensitivity, especially at low concentrations of analytes. Moreover, the automation of the mesofluidic system could be further improved by integrating the fluorescence detection device in the system. Indeed, the use of multiplex different ligand-modified beads in mesofluidic devices should open up new routes to flexible, high-throughput approaches for bioanalysis.

In conclusion, we developed a simple and fast mesofluidic system based on beads for chloramphenicol detection. It takes only approximately 30 min to determine the concentration of CAP, including bead infusing, immunoreactions, and washing steps through the process simply by controlling the peristaltic pump. These operations were much easier than the liquid-handling operations in the conventional assay and caused relatively small variation in operations. The detection limit of chloramphenicol is 0.008 μg/L. The method reveals good recovery rates from 90 to 108% and coefficients of variance

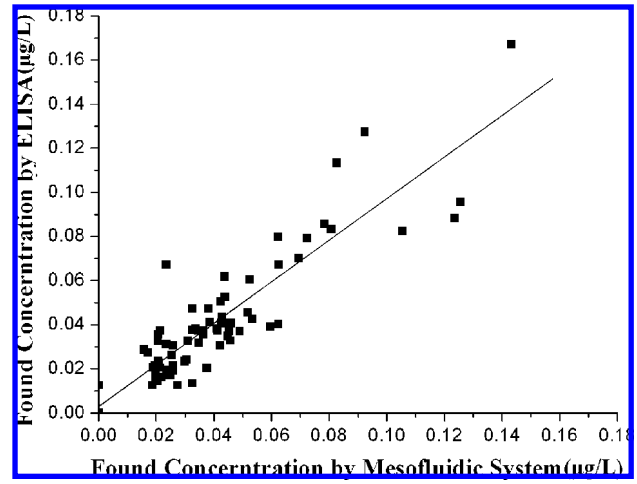

Figure 6. Comparison of the found concentration (μg/L) obtained from the mesofluidic system and the ELISA for the quantification of the chloramphenicol residue in 80 samples. The values of two methods were highly correlated ($r = 0.91$).

Table 2. Comparison of Chloramphenicol Detection with Different Methods

method	detection limit (μg/L)	detection index	operation difficulty	time consumed
microbiological	10	single	low	>2 days
instrumental	0.01−0.1	partly multicomponent	high	2−3 h
ELISA	0.02	single	low	2−3 h
mesofluidic system	0.008	extensible multicomponent	medium	0.5−1 h

(CV) from 4.72 to 6.52%. These values are much better than the required accuracy in trace analysis, and the accuracy and quick response should be suitable for customs, entry−exit inspection, and quarantine, quality control, or food safety supervised domain applications. The proposed method should be able to be extended to detection of not only more kinds of veterinary drug residues but also various substances including pigments, toxins, pesticides, and chemical additives in foodstuffs.

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LITERATURE CITED

(1) Campbell, S. G.; Mageau, P. R.; Schwab, B.; Johnston, W. R. Detection and quantitation of chloramphenicol by competitive enzyme-linked immunoassay. *Antimicrob. Agents Chemother.* **1984**, *2*, 205–211.

(2) Maciej, J. B.; Huda, H.; Eid, A. E.; Zuhour, I.; Mohammed, A. T. Rapid determination of chloramphenicol and its glucuronide in food products by liquid chromatography−electrospray negatie ionization tandem mass spectrometry. *J. Chromatogr., B* **2004**, *807*, 343–356.

(3) Commission Regulation (EEC) 2377/90. *Off. J. Eur. Communities* **1990**, *L224*, 1−8.

(4) Shi, X. Z.; Wu, A. B.; Zheng, S. L.; Li, R. X.; Zhang, D. B. Molecularly imprinted polymer microspheres for solid-phase extraction of chloramphenicol residues in foods. *J. Chromatogr., B* **2007**, *850*, 24–30.

(5) Thomas, J. L.; Francis, P. T.; John, G. B.; Sherwood, L. G. Rapid microbiological assay for chloramphenicol and tetracyclines. *Antimicrob. Agents Chemother.* **1976**, *9*, 874–878.

(6) Huang, J. F.; Zhang, H. J.; Feng, Y. Q. Chloramphenicol extraction from honey, milk, and eggs using polymer monolith microextraction followed by liquid chromatography−mass spectrometry determination. *J. Agric. Food Chem.* **2006**, *54*, 9279–9286.

(7) Shen, H. Y.; Jiang, H. L. Screening, determination and confirmation of chloramphenicol in seafood, meat and honey using ELISA, HPLC-UVD, GC-ECD, GC-MS-EI-SIM and GCMS-NCI-SIM methods. *Anal. Chim. Acta* **2005**, *535*, 33–41.

(8) Forti, A. F.; Campana, G.; Simonella, A.; Multari, M.; Scortichini, G. Determination of chloramphenicol in honey by liquid chromatography−tandem mass spectrometry. *Anal. Chim. Acta* **2005**, *529*, 257–263.

(9) Strasser, A.; Dietrich, R.; Usleber, E.; Märtlbauer, E. Immunochemical rapid test for multiresidue analysis of antimicrobial drugs in milk using monoclonal antibodies and hapten−glucose oxidase conjugates. *Anal. Chim. Acta* **2003**, *495*, 11–19.

(10) Terence, F.; Grace, M.; rew, C.; Christopher, E. Characterisation of antibodies to chloramphenicol, produced in different species by enzyme-linked immunosorbent assay and biosensor technologies. *Anal. Chim. Acta* **2007**, *592*, 51–57.

(11) Park, I. S.; Kim, D. K.; Adanyi, N.; Varadi, M.; Kim, N. Development of a direct-binding chloramphenicol sensor based on thiol or sulfide mediated self-assembled antibody monolayers. *Biosens. Bioelectron.* **2004**, *19*, 667–674.

(12) Zuo, P.; Ye, B. C. Small molecule microarrays for drug residue detection in foodstuffs. *J. Agric. Food Chem.* **2006**, *54*, 6978–6983.

(13) Bertram, G. K.; Angelika, S.; Richard, D.; Erwin, M.; Reinhard, N.; Michael, G. W. Automated microarray system for the simultaneous detection of antibiotics in milk. *Anal. Chem.* **2004**, *76*, 646–654.

(14) Ye, B. C.; Li, S.; Zuo, P.; Li, X. H. Simultaneous detection of sulfamethazine, streptomycin, and tylosin in milk by microplate-array based SMM-FIA. *Food Chem.* **2008**, *106*, 797–803.

(15) Zuo, P.; Ye, B. C. A novel immobilization strategy using oligonucleotide as linker for small molecule microarrays construction. *Biosens. Bioelectron.* **2008**, *23*, 1694–1700.

(16) Jonathan, W.; Marco, B.; Sven, T.; Andreas, M. Micro Total Analysis Systems: latest achievements. *Anal. Chem.* **2008**, *80*, 4403–4419.

(17) Nae, Y. L.; Yoonsun, Y.; Youn, S. K.; Sungsu, P. Microfluidic immunoassay platform using antibody-immobilized glass beads and its application for detection of *Escherichia coli* O157:H7. *Bull. Korean Chem. Soc.* **2006**, *27*, 479–483.

(18) Kiichi, S.; Manabu, T.; Tamao, O.; Hiroko, K.; Takeshi, O.; Masayuki, N.; Takehiko, K. Integration of an immunosorbent assay system: analysis of secretory human immunoglobulin A on polystyrene beads in a microchip. *Anal. Chem.* **2000**, *72*, 1144–1147.

(19) Cesaro-Tadic, S.; Dernick, G.; Juncker, D.; Buurman, G.; Kropshofer, H.; Michel, B.; Fattinger, C.; Delamarche, E. High-sensitivity miniaturized immunoassays for tumor necrosis factor alpha using microfluidic systems. *Lab Chip* **2004**, *4*, 563–569.

(20) Kiichi, S.; Manabu, T.; Hiroko, K.; Takehiko, K. Determination of carcinoembryonic antigen in human sera by integrated bead-bed immunoassay in a microchip for cancer diagnosis. *Anal. Chem.* **2001**, *73*, 1213–1218.

(21) Kiichi, S.; Maho, Y.; Hiroko, T.; Manabu, T.; Hiroko, K.; Takehiko, K. Microchip-based immunoassay system with branching multichannels for simultaneous determination of interferon-γ. *Electrophoresis* **2002**, *23*, 734–739.

(22) Kiichi, S.; Maho, Y.; Tomokazu, H.; Manabu, T.; Hiroko, K.; Takehiko, K. Microchip-based enzyme-linked immunosorbent assay (microELISA) system with thermal lens detection. *Lab Chip* **2004**, *4*, 570–575.

(23) Vincent, L.; Samuel, K. S.; George, M. W. Reagent-loaded cartridges for valveless and automated fluid delivery in microfluidic devices. *Anal. Chem.* **2005**, *77*, 64–71.

(24) Kazuo, H.; Masaki, O.; Kae, S.; Mizuo, M. Power-free sequential injection for microchip immunoassay toward point-of-care testing. *Lab Chip* **2006**, *6*, 236–241.

(25) Luo, C. X.; Fu, Q.; Li, H.; Xu, L. P.; Sun, M. H.; Ouyang, Q.; Chen, Y.; Ji, H. PDMS microfludic device for optical detection of protein immunoassay using gold nanoparticles. *Lab Chip* **2005**, *5*, 726–729.

(26) Sheng, H.; Ye, B. C. Different strategies of covalent attachment of oligonucleotide probe onto glass beads and the hybridization properties. *Appl. Biochem. Biotechnol.* **2008**, DOI: 10.1007/s12010-008-8245-9.

(27) Janolino, V. G.; Swaisgood, H. E. Analysis and optimization of methods using water-soluble carbodiimide for immobilization of biochemicals to porous glass. *Biotechnol. Bioeng.* **1982**, *24*, 1069–1080.

(28) Duffy, D. C.; McDonald, J. C.; Schuelle, O. J. A.; Whitesides, G. M. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* **1998**, *70*, 4974–4984.

(29) Effenhauser, C. S.; Bruin, G. J. M.; Paulus, A.; Ehrat, M. Integrated capillary electrophoresis on flexible silicone microdevices: analysis of DNA restriction fragments and detection of single DNA molecules on microchips. *Anal. Chem.* **1997**, *69*, 3451–3457.

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