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Multicomponent mesofluidic system for the detection of veterinary drug residues based on competitive immunoassay

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ABSTRACT

An automated multicomponent mesofluidic system (MCMS) based on biorecognitions carried out on meso-scale glass beads in polydimethylsiloxane (PDMS) channels was developed. The constructed MCMS consisted of five modules: a bead introduction module, a bioreaction module, a solution handling module, a liquid driving module, and a signal collection module. The integration of these modules enables the assay to be automated and reduces it to a one-step protocol. The MCMS has successfully been applied toward the detection of veterinary drug residues in animal-derived foods. The drug antigen-coated beads (ϕ250 μm) were arrayed in the PDMS channels (ϕ300 μm). The competitive immunoassay was then carried out on the surface of the glass beads. After washing, the Cy3-labeled secondary antibody was introduced to probe the antigen–antibody complex anchored to the beads. The fluorescence intensity of each bead was measured and used to determine the residual drug concentration. The MCMS is highly sensitive, with its detection limits ranging from 0.02 (salbutamol) to 3.5 μg/L (sulfamethazine), and has a short assay time of 45 min or less. The experimental results demonstrate that the MCMS proves to be an economic, efficient, and sensitive platform for multicomponent detection of compound residues for contamination in foods or the environment.

Microarrays are powerful tools that allow the simultaneous analysis of a large number of analytes. The development of solid-surface and flowthrough microarray technology has revolutionized the fields of analytical chemistry, biochemistry, and medicinal chemistry [1,2]. On the flowthrough microarray side, the micro-total analysis system (μTAS),[1] also called lab-on-a-chip (LOC) or microfluidic analytical system, is well studied and widely used. However, the recently developed micro-sequential injection system with a lab-on-valve (LOV) unit, also known as a mesofluidic system (with a fluidic channel diameter of the 10 μm–1 mm scale), is an excellent alternative to μTAS as it minimizes the required sampling volumes and sample pretreatments, as well as facilitates novel applications [3,4]. The mesofluidic system plays a very important role in the miniaturization of analytical systems, and in developing replacement techniques for labor-intensive manual operations [5]. Sample and reagent consumption, waste production, and sample preparation times are considerably lower than the same values in batch-mode operations. The mesofluidic system is much easier to operate than microfluidic chip systems and instruments, as its structure is much simpler than its microfluidic equivalents. In previous work, a mesofluidic system for the detection of chloramphenicol has been developed [6].

An attractive feature of the mesofluidic system incorporating glass beads described in the present work is the possibility of multiplexed detection of several analytes within a single channel. The number of beads in the channel represents the throughput analysis capacity (analogous to spots on a microarray). The novel system reported here is based on a competitive immunoassay on the surface of the beads situated in the polydimethylsiloxane (PDMS) meso-channels, making it a suitable tool for the high throughput and simultaneous analysis of multiple drug residues.

Low-molecular-weight compounds are widely used as animal drugs, food additives, and pesticides, added to food products, either directly or indirectly, to maximize productivity and profits. Residues of these compounds in food products, however, have been shown to be detrimental to human health. For instance, ractopamine (RAC), clenbuterol (CL), and salbutamol (SAL), used as β-adrenergic agonist growth promoters, increase feeding efficiency and leanness, and promote animal growth. However, in recent

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[1] Abbreviations used: APTMS, 3-aminopropyltrimethoxysilane; BDE, 1,4-butanediol diglycidyl ether; BSA, bovine serum albumin; CAP, chloramphenicol; CL, clenbuterol; GA, glutaraldehyde; LOC, lab-on-a-chip; MCMS, multicomponent mesofluidic system; OVA, ovalbumin; PDITC, 1,4-phenylene diisothiocyanate; PDMS, polydimethylsiloxane; RAC, ractopamine; SA, succinic anhydride; SAL, salbutamol; SM_2, sulfamethazin; μTAS, micro-total analysis system; TNBS, 2,4,6-trinitrobenzene sulfuric acid; TPAL, terephthalaldehyde.

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years these compounds have been banned as animal feed additives worldwide as they were shown to be very toxic to humans, and have been associated with a series of severe food poisoning outbreaks. Chloramphenicol (CAP) and sulfamethazin (SM_2) are broad spectrum antibiotics ubiquitously used for treating bacterial diseases in veterinary medicine, in aquaculture practices, and as food additives. However, they have the potential to cause serious toxic effects in humans and animals when administered orally or by injection, with nontarget effects such as bone marrow depression, aplastic anemia, hypoplastic anemia, thrombocytopenia, and granulocytopenia being reported [7]. China, the European Commission, and the United States, among other countries, have imposed a strict ban or important restrictions on the use of these five veterinary drugs in food-producing animals, and base the restrictions on Maximum Residue Limits (MRLs). The quantitative analysis of such compounds is therefore essential in safeguarding public health, preventing illicit use, and facilitating government regulation and surveillance. In order to comply with these strict regulations, many analytical methods have been validated to assay residues of these compounds, all with different levels of sensitivity and selectivity, as well as other characterization techniques for animal foods, such as instrumental measurements, immunological methods, and microarrays [8–11]. Instrumental measurements, however, are limited not only by their low screening efficiency and complicated operation procedures, but also by their high cost long assay times. They have therefore only been used as reference methods to confirm test results. Based on the limits of available techniques, it is highly desirable to develop a sensitive and selective drug detection method that can provide simple, practical, and high-throughput routine detection and quantification of drug residues in food samples.

Materials and methods

Materials

The glass beads (average diameter of 250 μm), 2,4,6-trinitrobenzene sulfuric acid solution (TNBS, 5% w/v in H_2O), 3-aminopropyltrimethoxysilane (APTMS, 97%), 3-(2,3-epoxypropoxy)propyltri methoxysilane (GTPS, 95%), glutaraldehyde solution (GA, 25%), terephthalaldehyde (TPAL), 1,4-phenylene diisothiocyanate (PDITC), 1,4-butanediol diglycidyl ether (BDE, 95%), succinic anhydride (SA), chloramphenicol sodium succinate, and the five drug standards were purchased from Sigma-Aldrich (St. Louis, MO). The five monoclonal antibodies (mAb) were obtained from Meridian (Saco, ME). Cy3-conjugated affinity-purified goat anti-mouse IgG from Rockland (Burlingame, PA) was used as the secondary antibody. PDMS was supplied by Dow Corning (Midland, MI). All other chemicals and solvents, unless stated otherwise, were purchased from Sigma-Aldrich, and used without further purification.

Experimental setup and process description

A schematic of the mesofluidic system is shown in Scheme 1. The core system component is the mesofluidic reaction chamber, which is based on glass beads situated in PDMS channels. A silicon wafer with a SU-8 pattern made by photolithography was used to cast the PDMS mesofluidic mold. The PDMS base was mixed with a curing agent in a ratio of 10/1 (w/w), and the mixture was poured onto the silicon wafer, which had been fumigated with fluoroalkyl silanes. The silicon wafer was then placed in a vacuum desiccator for approximately 15 min to remove air bubbles from the PDMS that were introduced during the mixing. Once the silicon wafer master with PDMS had been degassed it was removed from the desiccator and placed on an 80 °C hot plate for 1 h to cure. The cured PDMS was then lifted off the wafer and cut to form 300 μm-deep mesochannels. The PDMS device was then bonded to a glass slide (25 × 75 × 1.0 mm) using an oxygen plasma bonder. Inside the plasma bonder, the bonding surfaces of the slide and the PDMS chip were exposed to high-energy plasma, which strips away electrons from the surfaces causing 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 using a glass slide containing access holes for the connection of mesofluidic fittings to form a seal. The entire process was performed according to methods previously reported in the literature [6].

The overall assembled MCMS system included a programmable multichannel peristaltic pump (Masterflex L/S, Vernon Hills, IL), which provided the driving force to the mesofluidic chamber device in which the immunoassay was carried out. An epoxy resin was used as an adhesive to form fluidic joints.

The ELISA-like reactions on the surface of the glass beads in the mesochannels are shown in Scheme 1. The drug molecules or their BSA (bovine serum albumin) or OVA (ovalbumin) conjugates were immobilized on the bead surface as probes. The residue molecules of samples or standard solutions compete for the corresponding antibody with the probes immobilized on the beads. Following this reaction, free antigen and antibodies are washed away, leaving only the antigen–antibody complexes adsorbed on the surface of the glass beads. A Cy3-labeled secondary antibody was then used to indicate the antigen–antibody complex anchored to the glass beads. The fluorescence intensity of Cy3 indirectly determined the concentration of residue drugs in samples or standard solutions.

Glass bead modifications

For epoxy-silane modifications, the glass beads (500 mg) were cleaned by rinsing in 1.5 mL of 6 M HCl solution overnight with gently shaking at 55 °C. The beads were then extensively washed with distilled water until pH became neutral, and dried under vacuum at 110 °C for 2 h. The resulting beads (hydroxyl beads) were immersed in a 6% solution of GPTS in anhydrous toluene and left with gentle shaking at ambient temperature for 12 h. The silanized beads were then washed five times with anhydrous toluene, and dried at 60 °C in a vacuum for 3 h. The beads were then stored at room temperature, and named GPTS beads [12].

For aminosilane modifications, the dry hydroxyl beads were mixed with 2% APTMS, and shaken for 5 h at 37 °C. They were then washed five times with anhydrous toluene, and dried as previously described for the GPTS beads. The quantification of active amino groups on the bead surfaces was performed by the color reaction of TNBS [13]. The resulting aminopropyl beads were then further modified to introduce five different functional groups on their surfaces [14]. The five functional beads and the modification conditions are as follows. For GA beads, aminopropyl beads were immersed in a 5% aqueous GA solution (500 μL) and stirred for 30 min at room temperature [15]. For TPAL beads, aminopropyl beads were immersed in a 1 mL acetone solution containing 10 mmol/L TPAL, and then stirred for 90 min at 37 °C. For PDITC beads, aminopropyl beads (10 mg) were rinsed in dimethylformamide solution containing 10% pyridine and 0.2% PDITC (500 μL), and shaken for 2 h at room temperature [13]. For BDE beads, aminopropyl beads (10 mg) were added to a 500 μL aqueous BDE solution, and incubated overnight at 37 °C. For SA beads, aminopropyl beads (10 mg) were immersed in 10% solution of SA in 0.1 M sodium acetate, pH 4.5 (500 μL). The beads were shaken overnight at room temperature [6]. All the resulting beads were washed using the corresponding solvent, and dried under vacuum at room temperature. Schematic illustrations of bead treatment methods are shown in Supporting information.