An Integrated Microfluidic Chip for Rapid Methanol Detection

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Abstract: A widely-available CO_2 laser scriber is used to perform direct-writing ablation on a poly(methyl methacrylate) (PMMA) substrate to create a microfluidic chip for the rapid detection of methanol. The microfluidic designs are created using commercial layout software and are converted into the command signals required to drive the laser scriber in such a way as to reproduce the desired microchannel configuration on the surface of a PMMA substrate. Experimental results indicate that, using the proposed integrated microfluidic chip, linearity expression R^2 can reach 0.9972 when using 2 unit methanol oxidase (MOX) and basic fuchsin to detect methanol. The proposed device is thus a valuable tool for rapid methanol detection, with its micro mixer system providing a simple yet effective solution for mixing problems in the field of micro-total-analysis-systems.

Keywords: Methanol; CO_2 laser; microfluidic chip; PMMA

Introduction

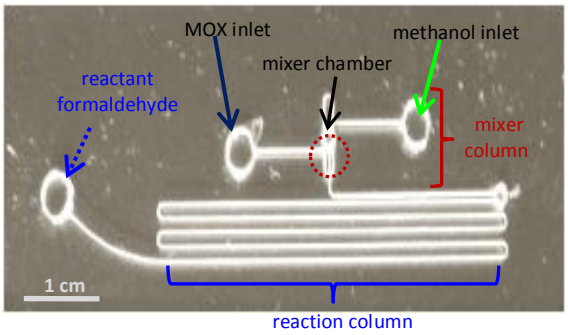
In recent decades, microsystem technologies have attracted significant interest among microfluidics researchers for the development of biochemical applications in food safety inspection and quality control [1, 2], drug discovery [3-5], environmental monitoring [6, 7], and biomedicines [8-10]. Several functional microfluidic devices have been developed to perform a variety of tasks, including sample pre-treatment and injection, species mixing, polymerase chain reaction, and cell/particle separation and counting [11-17]. Microfluidic devices present numerous crucial advantages, including reduced sample and reagent use, enhanced efficiency, improved sensitivity, shorter processing times, lower power consumption, greater portability, lower fabrication and operating costs, and the potential for integration with other miniaturized devices.

Recently, numerous groups have investigated methods for integrated microfluidic devices [18-20] for a wide range of applications in chemical and biological analysis. Lin *et al* [21] presented a rapid microfluidic mixer using a freeze-quenching technique, providing a useful means of trapping meta-stable intermediates populated during rapid chemical or biochemical reactions. Fu and Lin [22] presented a novel DNA digestion system using periodic electrokinetic driving forces and a high performance microfluidic mixer.

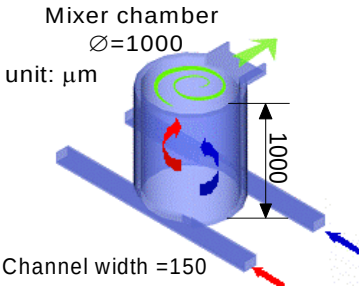
Methanol poisoning is usually caused by incorrect use of wood alcohol. However, other types of alcohol contain even higher concentrations of methanol (e.g., general alcohol and that found in fruit wine). According to Taiwan's Department of Alcoholic Beverage Control Standards, the methanol content in general alcohol is 1000 ppm, while wine contains 3000 ppm (in terms of pure ethanol). Current common methanol detection methods include gas chromatography detection (GC) and

potassium permanganate oxidation (commonly referred to as the traditional method) [23, 24]. While GC detection is fast and highly accurate, but required equipment and materials are expensive, making it unsuitable for use in testing devices for the general population or small businesses. The traditional test method, meanwhile, suffers from low accuracy due to the dissimilar reactive property that non-specific potassium permanganate oxidation has on methanol versus distilled alcohol. Some researchers have used methanol oxidase (MOX) in place of non-specific potassium permanganate to form formaldehyde via the methanol oxidation process, followed by mixing with basic fuchsin for colorimetry [25, 26].

Washing and drying testing equipment is a time-consuming process, while quartz glass for sample testing in spectrophotometers is expensive. This study presents a fast, cost-effective and microfluidic method for disposable methanol detection applications. The detection process is simple: a micro syringe-pump injects methanol and MOX into the microchip which is allowed to stand momentarily; magenta & HCl are added and the entire microchip is directly inserted into the spectrophotometer. The present study used a CO_2 laser system to ablate PMMA substrates, using a defocusing laser beam method to fabricate the integrated microfluidic chip. Figure 1 presents a schematic illustration and a photograph of the rapid detection of methanol using the integrated microfluidic chip, which features three entrances for the injection of methanol, enzyme, magenta, and HCl. All of the reagents were mixed and reacted in the rectangular reservoir, and then tested using the UV spectrophotometer. Experimental results indicate that the linear expression R^2 reaches 0.9972 using the proposed integrated microfluidic chip, as opposed to 0.9985 with the traditional method using the mixture of two-unit methanol oxidase (MOX) and basic fuchsin (BF, Schiff method) for the detection of various concentrations of methanol.



(a)



(b)

Figure 1. (a) photograph of methanol rapid detection microfluidic chip, and (b) schematic illustration of micro mixer-chamber geometry.

Experimental setup

Figure 2 presents the experimental setup for PMMA microchannel fabrication. The CO_2 laser (V-12, Laser Pro Venus laser system) has a maximum output power of 12 W, an output beam diameter of 3.5 mm at the aperture, a beam divergence (full angle) of 4 mrad, and a wavelength of 10.6 μm. The beam scanning speed was programmable over a range of 5 to 500 mm/s. The sample was mounted on a 300×210 mm^2 X-Y plane workspace driven by a DC servo control system. The substrate was secured on a platform that can move in the Z-axis. The microfluidic pattern was designed using commercially-available computer software (CorelDraw

Ting-Fu Hong graduated in Mechanical Engineering from the National Cheng-Kung University (Taiwan) in 1996. He was awarded M.S. degree in Metallic and Ceramic Materials from the University of Manchester Institute of Science and Technology (UMIST, UK) in 2001 and Ph.D. in Materials Science from the University of London (UK) in 2006. His graduate studies were focused on the characterisation techniques of porous materials and the development of biomaterials. He is currently an assistant professor in the Department of Materials Engineering at National Pingtung University of Science and Technology. His research interests are in functional surface treatments on metallic biomaterials, synthesis of micro-nano metallic and ceramic particles, and MEMS fabrication technologies.

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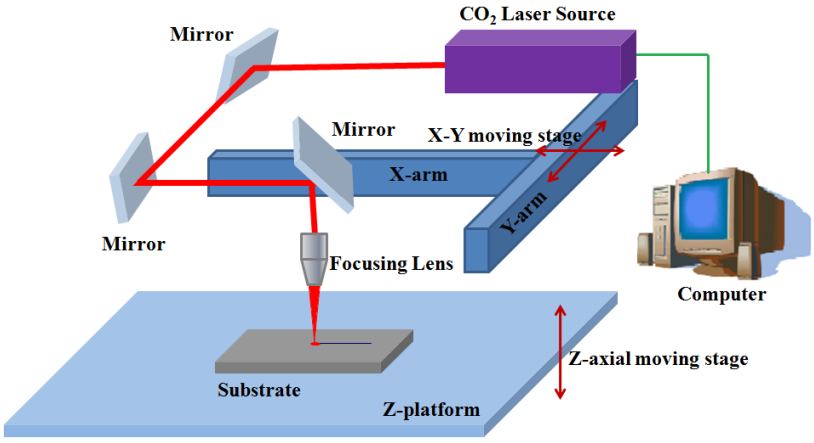

Figure 2. Schematic illustration of CO_2 laser system used to pattern PMMA substrates.

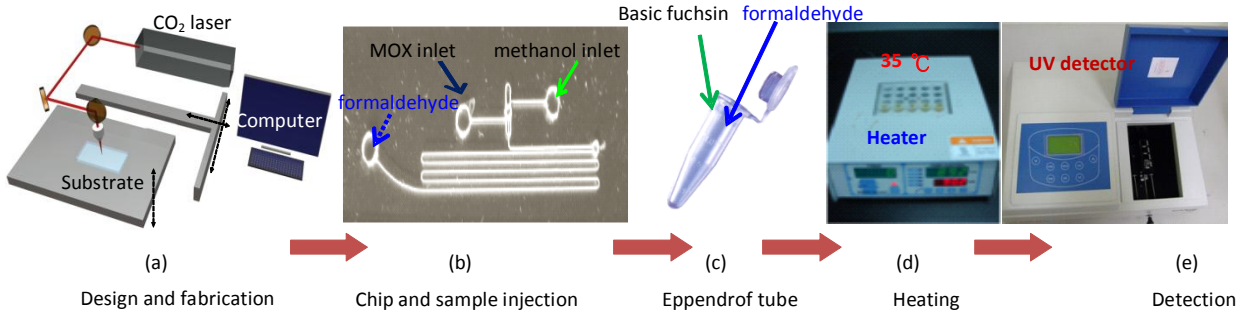

Figure 3. Schematic illustration showing major steps in chip fabrication and methanol concentration detection procedures: (a) design and fabrication; (b) chip and sample injection; (c) Eppendrof tube; (d) heating; (e) detection.

and AutoCAD). It was then translated into computer-controlled motion while the laser beam was used to etch the substrate. In the experiments, the laser power was set between 1.2 and 6 W, and the scanning speed varied between 50 and 300 mm/s. Experiments were focused on fabricated channels at low laser intensity using the laser beam defocusing method (the defocused laser beam ablation method is described in detail in [27]). The resulting channel widths ranged from 100 to 200 μm, while the channel depths ranged from 30 to 300 μm. Meanwhile, the upper substrate of the microfluidic device was formed by drilling holes using the CO_2 laser etched microscope slide. Following micromachining, the two plates were joined using a thermal bonding process performed in a hot embossing machine at a temperature of 105°C for 20 minutes and a pressure of 5 kg/cm^2.

Figure 3 presents the experimental procedure steps of the method for integrated rapid microfluidic detection of methanol concentrations. The sample reagents included methanol, alcohol oxidase, basic fuchsin, HCl, PBS, and DI water. Reagent preparation entailed: (1) methanol concentrations ranging from 10~30 ppm prepared by mixing methanol and DI water in appropriate quantities; (2) 67.5 ml of PBS and 100 μl alcohol oxidase mixed into two units; (3) 0.5 g basic fuchsin and 100°C DI water mixed with heating and stirring, followed by 1.5 g of anhydrous sodium sulfite dissolved in 10 ml DI water and mixed with fuchsin, along with 1 ml of sulfuric acid in a concentration of 36 N and 10 ml hydrochloric acid in a concentration of 36 N, cooled to room temperature and then diluted to 100 ml; and (4) diluted HCl in a concentration of 36 N to 1 N. In this experiment, the microchannel, micromixer, reservoir on the PMMA microchip, and substrate cutting were fabricated using CO_2 laser machining processes and sealed by a hot-press bonding technique (see Figure 3(a), 3(b)). In Figure 3(c), a methanol detection chip is installed in the experimental platform to achieve a reaction. The reactant of formaldehyde was then inserted into the eppendrof tube and mixed with basic fuchsin. Methanol and MOX were injected into the microchip using a syringe pump, mixed in an ultrasonic cleaner for 5 min and then maintained at a constant 25°C for approximately 25 min. The basic fuchsin and HCl were then increased in the microchip and maintained at a constant 35°C (see Figure 3(d)). The vortex effect enables MOX and methanol to become a uniform mixture in the micromixer, and MOX reacts with methanol to form formaldehyde, which can be written as

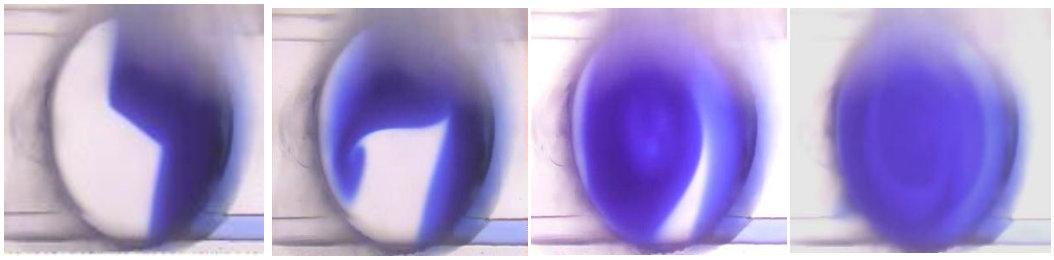

Figure 4. Experimental images showing flow rotation effect induced in the micro-mixer's micro-chamber at Re = 4 after (a) 2 s, (b) 4 s, (c) 7 s, and (d) 10 s.

$CH_3OH+O_2 \xrightarrow{MOX} HCOH+H_2O_2$. The syringe pump was then turned off and the microfluidic chip was removed then turned off and the microfluidic chip was removed from the experimental platform. The detection chip was then inserted into a spectrophotometer (Model U-2000, Tokyo, Japan) to observe the corresponding absorption spectrum (see Figure 3(e)).

Results and discussion

In the micro reaction experiment, a microscope (E-400, Nikon, Japan) and image capture card (DVD PKB, V-gear, Taiwan) were used to capture images to determine mixing efficiency in the chip's mixing chamber. To generate a high-efficiency mixing effect from the vortex in the micro-mixer, syringe pumps were used to inject a solution with blue dye and DI water at an optimal flow rate.

Figure 4 presents a series of experimental images showing the evolution of the flow rotation effect within the circular microchamber with blue dye injected into the micro-mixer with a Reynolds number of Re = 4. Detailed descriptions of the mixing effect and mixing mechanisms of circular microfluidic mixers can be found in [28]. The images show that, when the DI water and a solution containing blue dye are initially loaded into the microchamber, the species concentration distribution exhibits two distinct regions. However, the driving force is sufficient to establish a rotational flow effect in the microchamber, and thus a 3-D vortex is formed within 10 seconds (see Figure 4(d)). Analysis of the outlet region results shows that a mixing ratio of 95.8% is obtained for a Reynolds number of Re = 4. Figure 5 shows that the vortex structure results in an effective mixing of the two species at even low Reynolds number values. Moreover, the mixing performance is found to improve as the Reynolds number increases due to the corresponding increase in the intensity of the vortex structure. Overall, the results show that the optimal mixing ratio (~95%) is obtained at a Reynolds number greater than or equal to 4, which was used as the value for all remaining experiments and simulations.

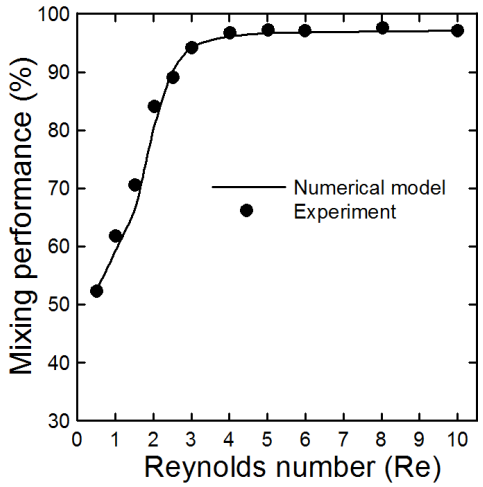

Figure 5. Micro-chamber mixing ratio given Reynolds numbers in the range of Re=0.5~10.

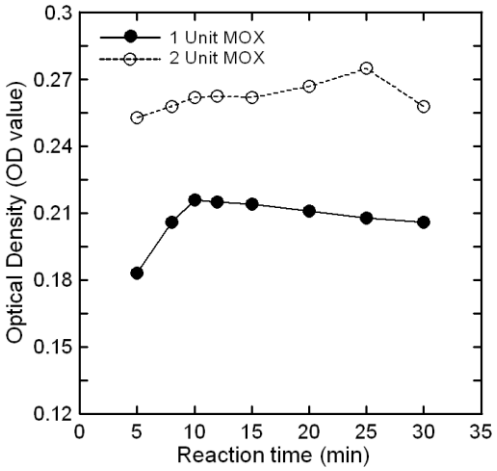

Figure 6. Minimum time required for different units of MOX with corresponding reaction times.

Figure 6 presents the optimized operating conditions for the proposed methanol detection system under different units of MOX with corresponding reaction times, at a methanol concentration of 30 ppm and Re = 4. The result indicates that a higher unit MOX (that is, the two-unit MOX in Figure 6) can result in a faster reaction (approximately 10 min), as well as an optical density suitable for the MOX-methanol reaction. Figure 7(a) presents the detected methanol concentration results using a traditional large-scale

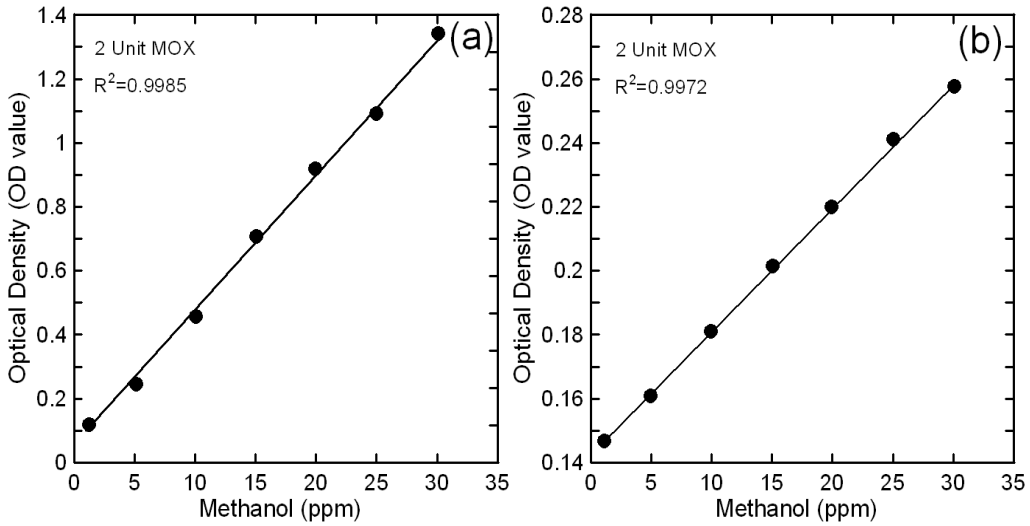

Figure 7. OD value of methanol detection results for (a) a conventional tube system and (b) an integrated microfluidic system.

system for methanol samples, with concentrations ranging from 10~30 ppm. The final methanol reactant was detected using a conventional UV spectrophotometer to detect the optical density (OD) value. The R square and relationship were determined to be R^2 = 0.9985 and Y = 0.0581X – 0.4641, respectively. Figure 7(b) presents the detected methanol concentration results using the proposed integrated microfluidic system for methanol samples with concentrations ranging from 10~30 ppm. The R square and relationship were determined to be R^2 = 0.9972 and Y = 0.0081X – 0.0442, respectively. In this instance, a rapid and thorough mixing was achieved in the mixing and reaction column of the proposed microfluidic chip, such that 30 min of reaction time was adequate for completing methanol detection. Thus the proposed integrated microfluidic system is able to obtain a larger range of the methanol concentration detection. The result confirms that the proposed integrated microfluidic system is an effective, accurate, and rapid method for methanol detection.

Conclusion

This paper presents a low-cost, time-saving, and high-performance integrated microfluidic system for detecting methanol concentrations. The microchip is fabricated on a polymethyl-methacrylate (PMMA) substrate, thus providing advantages including easy fabrication, favorable biocompatibility, and excellent light transmission characteristics. An experimental platform was created for multiple reaction processes. The optimal microchannel geometry design miniaturizes the pressure drop and the uniform flow rate completes the methanol and enzyme reaction to form formaldehyde. Using a distinct microchip size for various UV spectrophotometers, the proposed detection method can provide a low-cost alternative to expensive quartz detection chips. A series of experimental tests compared the performance of the traditional concentration detection method and the proposed integrated microfluidic system concentration detection method for methanol samples with concentrations ranging from 10~30 ppm. The correlation coefficient obtained when plotting the absorbance against the methanol concentration had a value of R^2 = 0.9985 for the traditional detection method, and R^2 = 0.9972 for the integrated microfluidic detection method. The proposed rapid integrated methanol detection microchip is a post-processing chip. After combining the proposed microchip with various analyses for liquor, alcohol, synthetic alcohol, industrial alcohol, and methanol, the proposed integrated detection method will be used to develop a microchip to separate methanol.

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