A COMBINATORIAL MULTICOMPONENT PLUG MIXER FOR SYSTEMS CHEMISTRY

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ABSTRACT

We report the construction and testing of a combinatorial multicomponent plug mixer (CMPM) chip that generates a large number of mix ratios. The CMPM chip has been designed to study ribonucleotide reductase (RNR) protein-protein/protein-ligand interaction networks. The 4-component chip is capable of 5400 different combinations in a 30 plug cycle. CMPM chips were tested producing fluorescent dye and dihydrofolate reductase NADPH/MX mixtures with plug lengths of 2 mm.

KEYWORDS: Chemical signals, CMPM, PDMS, RNR

INTRODUCTION

Systems chemistry deals with the emergent properties of interacting chemical systems or networks [1]. These properties result from the interaction between the components in a complex network. The study of such phenomena requires the generation of a large number of mix ratios in an efficient manner with minimal sample consumption. In this paper we report a combinatorial multicomponent plug mixer (CMPM) microfluidic chip which is capable of mixing four different components in a wide range of ratios. The CMPM chip has been designed to study complex ribonucleotide reductase (RNR) protein-protein/protein-ligand interaction networks reconstituted *in vitro*. The RNR system is critical to dNTP/DNA production and agents used clinically to treat cancer (e.g. hydroxyurea and gemcitabine).

CHIP DESIGN AND FABRICATION

Figure 1(a) shows a block diagram of the multicomponent mixer chip. The

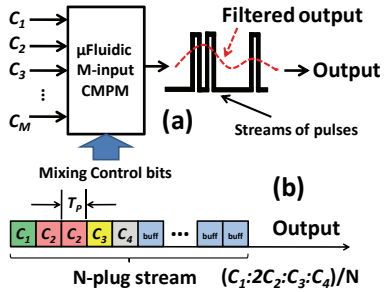

Figure 1. (a) The CMPM accepts a vector of multiple analyte flows and generates repeating streams of analyte plugs that control the mixture composition. (b) Illustration of a $(C_1:2C_2:C_3:C_4)/N$ mixture formation.

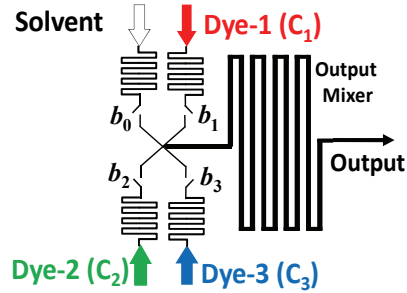

Figure 2. Schematic of four reagent CMPM. The multiplexer routes a series of discrete plugs to the output flow resistor that averages the plugs yielding the desired output mixture concentration.

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CMPM accepts a vector of M reactive reagents ($C_1,C_2,C_3,\ ...,\ C_M$) input flows and generates a continuous flow exit consisting of a stream of analyte plugs which rapidly mix by dispersion (i.e. sequential segmentation [2]) followed by chemical equilibrium. The equilibrium mixture is next analyzed by either fluorescence analysis or mass spectrometry. The initial constitution of the mixture is set by repeating N-combinations of short analyte plugs carried by a buffer flow. Each plug has equal time duration T_P. Figure 1(b) shows an illustrative example of the generation of $(C_1{:}2C_2{:}C_3{:}C_4)/N$ mixture. For a mixture stream with N plugs and M reagents (inclusive of buffer), the number of possible mix combinations is $C(N+M-1,N)=(N+M-1)!/N!/(M-1)!$ Periodic plug streams with 20 plugs can thus generate billions of combinations with this simple scheme. Figure 2 shows a schematic of a four-reagent CMPM. The plug output flow is directed to a long capillary/storage region where the

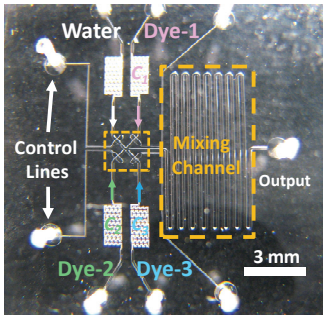

Figure 3. Four-reagent CMPM chip implemented with two-level PDMS technology. The chip dimensions are 1.5×1.8 cm².

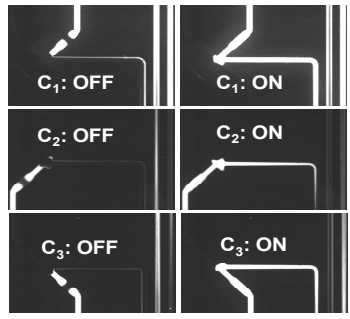

Figure 4. Fluorescence images of the three valves injecting plugs. The plug duration was 100 ms.

mixture can be incubated. Figure 3 shows a photograph of the chip implemented using two-level PDMS technology [3] with through-level vias [2]. The channel dimensions were 16×125 μm². The chip measures 1.5×1.8 cm².

EXPERIMENTS

The chip was tested using three water soluble fluorescent dyes: clear blue (IFWB-C0), fluorescent red (IFWB-C7) and fluorescent yellow-green (IFWB-C8) from Risk Reactor (Ca) mixed in H_2O at 50:1000, 1:1000 and 1:1000 ratios, respectively. The concentration of the output dye mixture was measured using an Olympus MVX10 fluorescence imaging microscope. Figure 4 shows photographs of the chip in operation loading dye plugs into the exit channel at T_P= 100 ms at average flow velocity of 2.0 cm/s, with 2 mm unit plug length. Figure 5 shows results a few measured exit concentration scans for a 10 plug, 20 cycle example with 64 different dye combinations recorded ~6 cm downstream from the multiplexers. Figure 6 shows a three dimensional scatter plot of the recorded dye mixtures. The decrease in the amplitude for C_2 was the result of dye interaction with C_1.

The chip was next used to perform initial experiments of the titration of dihydrofolate reductase with (fluorescent) NADPH [4] (nicotinamide adenine dinucleotide phosphate) in a stopped-flow fluorescence configuration. Dihydrofolate reductase catalyzes the NADPH-dependent reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-

tetrahydrofolate(THF). The enzyme is essential for thymidylate biosynthesis and hence for DNA synthesis. Figure 7 shows the recorded NADPH fluorescence traces using a DAPI blue filter and Olympus MVX 10 microscope.

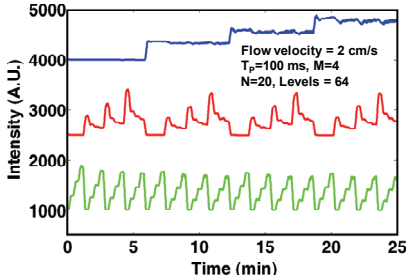

Figure 5. Composition of exit mixture for each dye averaged by the output flow resistor at ~6 cm downstream from the flow multiplexers. The fluorescence data was recorded at 2.5 images/s representing a 64-level sweep.

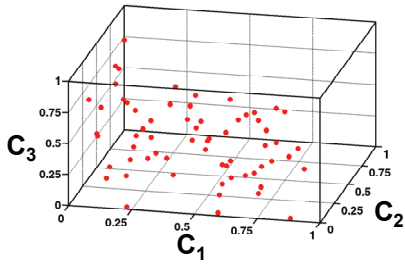

Figure 6. Three-dimensional scatter plot of the dye mixture combinations generated by the CMPM. Many more combinations can be generated with a larger number of plugs N.

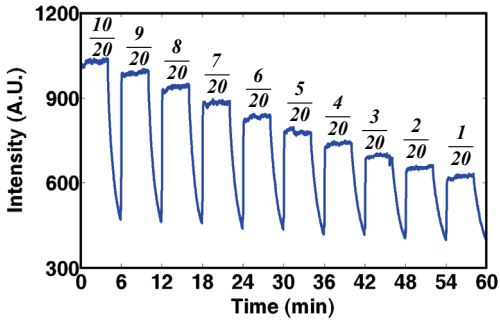

Figure 7. Titration of dihydrofolate reductase, MTX/R and NADPH [4] at different NADPH: protein-enzyme ratios. The protein solution was made by mixing 1 ml reaction buffer, 1.4 µl S-DHFR and 1 µl of MTX. The base (corresponding to 1/20) volume and different concentration of NADPH was 0.8 µl and 5 mM. The fluorescence data was recorded at 10 images/s.

CONCLUSIONS

We demonstrated a microfluidic combinatorial mixer chip for systems chemistry studies capable of generating thousands (4 inputs, 30 plugs) to billions (20 inputs, 20 plugs) of mix ratios.

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