A COMBINATORIAL MULTICOMPONENT PLUG MIXER FOR SYSTEMS CHEMISTRY

F. Azizi1, Q. Wan2, T. Radivoyevitch3, C. Dealwis2 and C. H. Mastrangelo1

Departments of Electrical Engineering1, Pharmacology2 and Bioststistics3
Case Western Reserve University, USA.

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](image1.png)

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](image2.png)

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.
CMPM accepts a vector of $M$ reactive reagents ($C_1, C_2, C_3, \ldots, C_M$) 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 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$_2$O 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.

REFERENCES