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EVALUATION OF MAGNETIC BEADS AGITATION PERFORMANCE OPERATED BY MULTI-LAYERED FLAT COILS

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Abstract: We have previously proposed a magnetic bead handling mechanism for micro-total-analysis-systems [1-2] and have developed a palmtop-sized biochemical analysis system [3]. Appropriate agitation is one of the crucial issues in micro-meter-sized fluidic systems. We investigated the agitation produced by using multilayered flat coils and magnetic beads. The beads were magnetically agitated inside the droplet by changing the external magnetic field distribution, and the agitation performance was evaluated by using an enzymatic reaction. The reaction efficiency increased linearly with an increase in the reaction time and was more than four times as efficient with agitation than without agitation.

Keyword: magnetic beads, micro-TAS, agitation, flat coil

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1. INTRODUCTION

Various micro-total-analysis-systems (μ-TAS), in which the mechanical and electrical components are integrated on the same chip, have been developed by using micro-electro-mechanical-systems technologies. Such systems are expected to reduce the amount of reagent solutions required and the analysis time and to enable the on-site monitoring of chemicals. Generally, most systems need complicated mechanical fluidic devices, such as valves and pumps, for handling solutions. Therefore, the size of the system can become large, even if the fluid channels and reactors are integrated onto microchips [1-3].

We have previously proposed a novel type of magnetic bead handling mechanism for a μ-TAS [4-6]. The proposed device does not need mechanical fluidic devices and can collect and rinse target samples effectively. Lehmann et al. recently developed a unique magnetic bead handling method based on a planar coil and showed that their system could perform two-dimensional manipulations on chips [7-8]. We recently miniaturized it to a palmtop-size system [9] and reported magnetic bead-cluster operation. Adequate agitation is one of the crucial issues for micro-meter-sized fluidic systems. We, therefore, evaluated the agitation produced by using magnetic beads and multilayered flat coils.

2. OPERATION OF PALMTOP-SIZED SYSTEM

Figure 1 shows a previously developed rotary-drive palmtop-sized analysis system. The system consists of a multi-well chip, two small magnets and another magnet coupled with multi-layered flat coils on a rotary table, and a stepping motor and its controller. The two magnets are used to transport beads from one droplet to another, and the magnet with the flat coils is used to agitate beads inside the droplet. We placed a droplet containing magnetic beads, which acted as a sample carrier, in the first well. The operation sequence is as follows.

Transportation

(1) The transporting magnet (T1) collects the dispersed magnetic beads in the droplet.
(2) The magnetic beads are selectively extracted, and then they are transported toward the next droplet along the bent channel by magnet (T1).
(3) The beads are trapped at the sidewall in the bent channel. After that, magnet (T2) transports the beads, and the bead finally fuses with the next droplet (2nd Droplet).

Agitation

(1) Beads are agitated by using multi-layered flat coils and magnets. The position of the beads in the droplet is controlled by the magnetic field distribution. Agitation is, therefore, produced by

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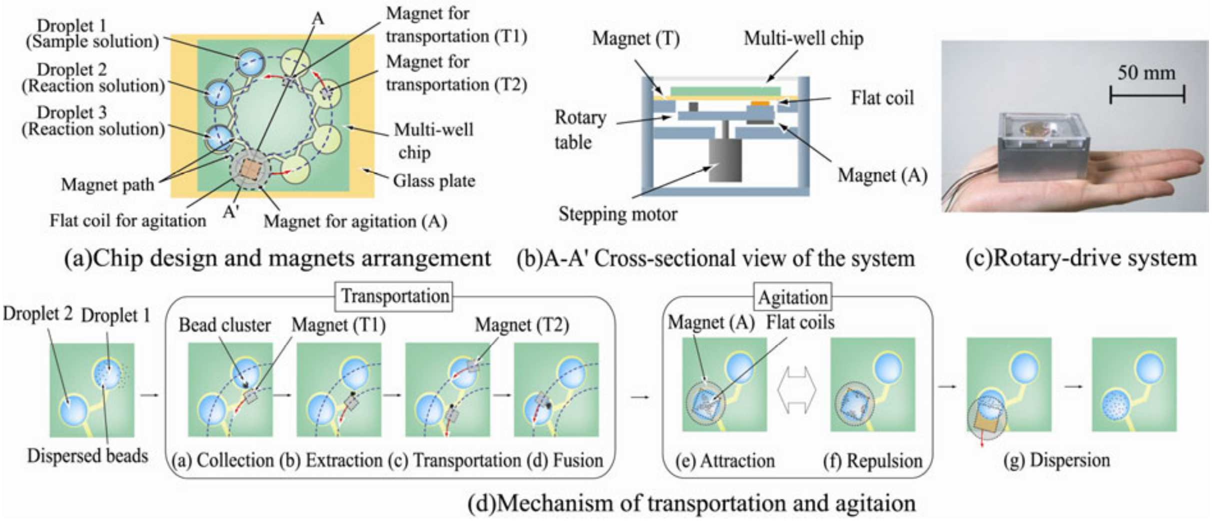

Fig.1 Schematic view of developed system

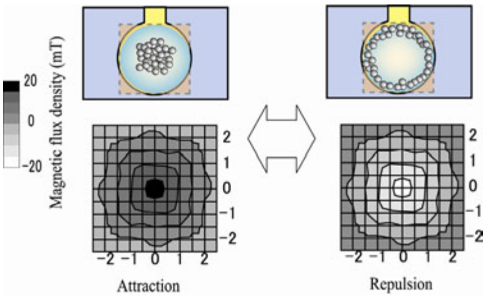

Fig.2 Magnetic-field distribution produced by using three-layered flat coil

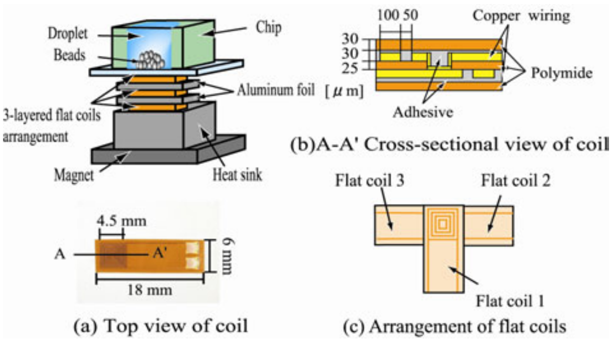

Fig.3 Agitation operation

changing the field distribution using the flat coil, as shown in Fig. 2.

(2) The beads are redispersed into the droplet until further rotational motion.

Multi-step biochemical reaction is performed by doing these operations in turn in the counter clockwise directions.

3. EXPERIMENT

Agitation devices

We fabricated a new agitation device to evaluate its performance, as shown in Fig. 3. We used a two-layered flat coil in a polyimide film produced by Fujikura Ltd., Japan, as shown in Fig. 3(b). Square-shaped coils were formed on both sides of the polyimide film to increase the number of turns (14 turns). The copper-wiring dimension had a width of 100 μm and a height of 30 μm, and the space between the windings was 50 μm. We piled the flat coils on top of each other to form a tri-laminar structure to enlarge the magnetic force.

Heat release

The magnetic force to agitate the beads was proportional to the current applied to the coil. A high current, in the order of several hundred mA, is required for the device to operate correctly. However, this generates heat and increases the temperature in the droplet. The polyimide film used as the coil substrate has excellent electrical and thermal insulation properties. We, therefore, inserted pieces of Al foil between the flats coils to effectively release the heat. The droplet temperature decreased 7-8^{o}C when the same driving current was used with the Al foil inserted. We also confirmed that we were able to keep the temperature less than 35^{o}C at the appropriate driving currents (less than 1.2 A), as shown in Fig. 4.

Reaction efficiency

We conducted an enzyme reaction with and without agitation to evaluate the reaction efficiency. We used alkaline phosphatase (AP) as the enzyme and p-nitrophenyl phosphate (pNPP) as the substrate. The AP decomposed the pNPP, and the color of the pNPP solution changed to

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yellow. The enzymatic reaction activity of the AP was detected by measuring the absorbance at a wavelength of 405 nm. We used commercially available abivin-modified magnetic beads (Dynabeads) and enzymatically labeled the beads with AP by using the chemical bond between the avidin and biotin molecules.

We initially investigated the reaction's dependency on the amount of pNPP substrate present to decide the amount of the substrate required to evaluate the effect of agitation. We used a two-well chip and placed a droplet containing the AP labeled magnetic beads and the substrate into either well. The beads were collected and extracted from the original droplet, and then transferred and fused into the substrate droplet by a magnet. The reaction time was 10 min. As shown in Fig. 5, the absorbance value increased linearly with an increase in substrate and became constant when it was larger than 1 mg/ml. Based on this result, we decided to use a substrate density of 10 mg/ml to evaluate the effects of agitation.

We performed an enzymatic reaction by manipulating the AP-labeled magnetic beads selectively, as shown in Fig. 6. Two droplets were initially placed in each well. The beads were collected and extracted from the original droplet, and then transferred and fused into the substrate droplet by the magnet. The beads were then agitated by changing the magnetic field distribution, and finally a NaOH solution was added to stop the reaction. The absorbance increased linearly with an increase in the reaction time with and without agitation. The absorbance value of the agitated sample was more than four times the absorbance of the unagitated sample, as shown in Fig. 7. The agitation frequency was 0.1 Hz, and continuous agitation was performed during the reaction.

We then investigated the reaction's dependency on agitation time. The agitation times varied from 1.0 min to 10 min for the 10 min reaction time. We also used two different agitation waveforms, as shown in Fig. 8. The absorbance increased linearly with an increase in the ratio between the agitation/reaction times. The absorbance did not depend on the agitation waveform. This is because magnetic beads used were poorly dispersed. We think that the reaction performance will be improved by using much smaller magnetic beads having excellent dispersibility.

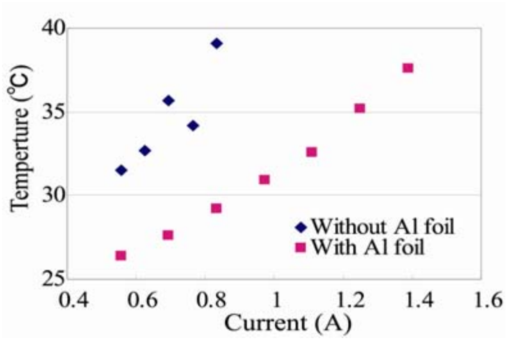

Fig.4 Relationship between temperature inside droplet and applied current

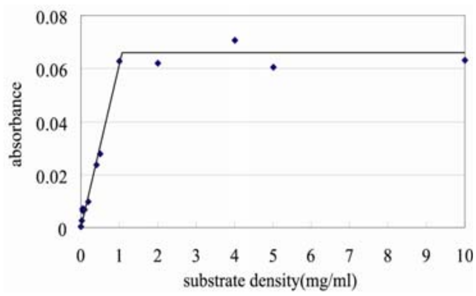

Fig.5 Relationship between absorbance of enzymatic reacted pNPP and concentration of pNPP

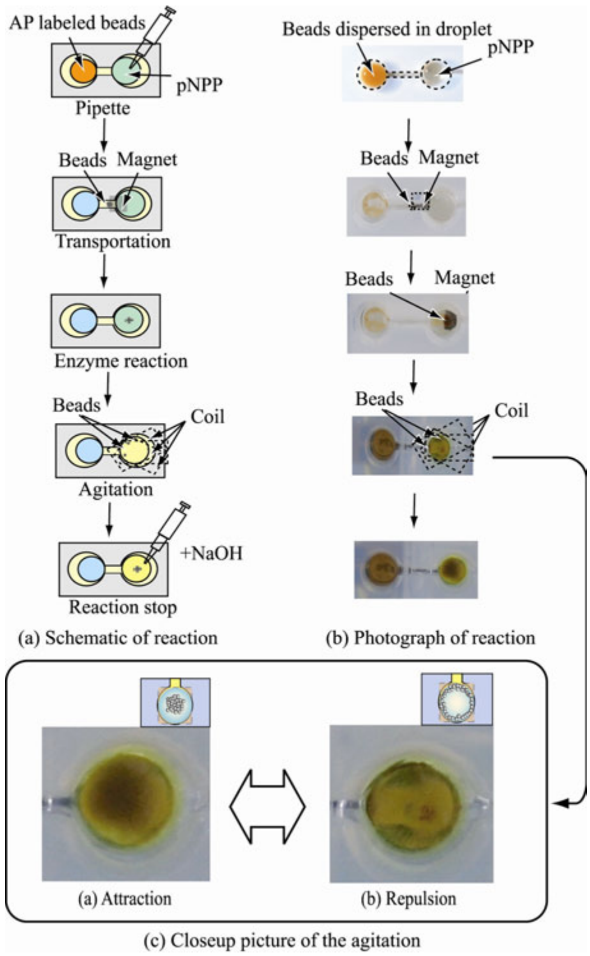

Fig.6 Reaction sequences

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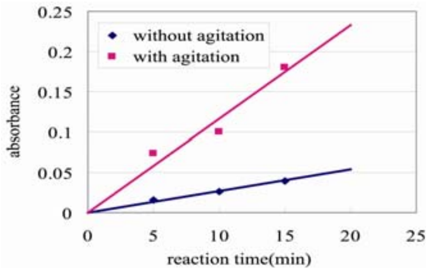

Fig.7 Relationship between time and reaction efficiency

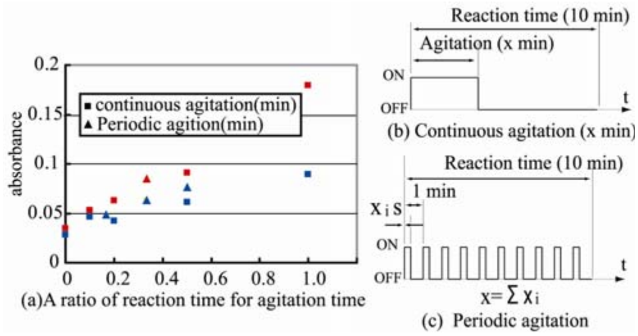

Fig.8 Reaction dependency on agitation time

4. CONCLUSION

We investigated the performance of agitation produced using multilayered flat coils and magnetic beads. The obtained results are summarized as follows.

(1) The temperature in the droplet was kept less than 35°C at the appropriate agitation driving currents by adding heat-sink foil between the flat-coils.

(2) The beads were magnetically agitated inside the droplet by changing the external magnetic field distribution.

(3) The reaction efficiency increased linearly with an increase in the reaction time and was more than four times as efficient with agitation than without the agitation.

5. ACKNOWLEDGMENTS

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