Development of Micro Particles Separation Device with Piezo-Ceramic Vibrator

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Abstract:

During hemanalysis, it is necessary to separate blood cells from whole blood. Many blood separation methods, for example, centrifugation and filtering, are in practical use. However, the use of these methods involves problems from the perspectives of processing speed and processing volume. We develop new types of blood separation devices that use piezo-ceramic vibrators. The first device uses a capillary. One end of the capillary is fixed to the device frame, and the other is fixed to a piezo-ceramic vibrator. The vibrator transmits bending waves to the capillary. This device can process only a small amount of solution; therefore, it is not suitable for hemanalysis. In order to solve this problem, we developed a second device; this device has a pair of thin glass plates with a small gap as a substitute for the capillary used in the first device. These devices are based on the fact that particles heavier than water move toward transverse velocity antinodes while those lighter than water move toward velocity nodes. In this report, we demonstrate the high-speed separation of silica microbeads and 50-vol% glycerol water by using these devices. The first device can separate the abovementioned solution within 3 min while the second can separate it within 1 min. Both devices are driven by a rectangular wave of 15 to 20 Vpp. Furthermore, it has been confirmed that red blood cells are separated from diluted whole blood using the first device within approximately 1 min. These devices have transparency, so they can compose as the analysis system with the chemical analyzer easily.

1. INTRODUCTION

Blood is a valuable source to get various information because it rounds our whole bodies. Though the elements of blood are usually keep within the constant range, the changes take place when abnormalities are found in our bodies. Therefore, when blood is examined, not only the sickness of blood but also various information on our bodies is obtained. By the blood examination, it is possible to detect a lot of diseases such as the lifestyle diseases and treat it early stage, and many people receive the inspection for sick prevention and a healthy check in recent years. However, it takes about one week by the results are obtained when inspecting in a small medical agency.

Then, in the measuring instrument industry of medical, there are high expectations of developing the device that can be measured at once in place of diagnosis and treatment without allowing the patient to wait for many days in the blood test. By the way, there is a problem that the red blood cells becomes the interfering substance of the blood test, it is necessary to separate the red blood cells from the whole blood.

Nowadays, many blood separation methods are researched and developed. For example, centrifugation, filtration, separation by microchannel[1], blood cells trapping by dielectrophoretic force[2], and so on. The traditional methods (e.g., centrifugation, filtration) have many disadvantages, and most of new developing devices are aimed to usage in micro total analysis systems (μTAS). These devices can separate blood cells from whole blood speedy and absolutely, however, they are not suitable for massively separation.

We aimed to vibration type blood separation system, and developed the prototype devices. This method uses the phenomenon that the particles are collected at the transverse velocity antinodes. In this report, the principles of vibration type particle separation method are described first, after that, development and performance evaluation of 2 prototype devices are described. These devices can separate particles from the suspension liquid, and they will be counted on novel blood separation devices in the future.

2. BLOOD CELLS SEPARATION

The whole blood is consists of red blood cells, white blood cells, platelet and blood plasma. The blood plasma includes water, blood proteins and a small mount of fat, sugar, mineral salts. At the hematologic test, the blood plasma has very important health markers, for example, glucose, cholesterol and so on. But the blood cells interfere with the reactions at these tests, and they become the causes of noise. Therefore, removal of blood cells from whole blood is important for the accurate test. Nowadays, various blood cells separation methods are used. Table 1 shows the features of these methods.

Table 1 Features of blood cells separation methods

	Centrifugal method	Filtering method
Advantage	Massively separation at one time	High-speed separation
Disadvantage	Low-speed separation Big-size package Complicated operation	Small separation volume Strong negative pressure Destructions of RBC

One of the blood cells separation methods is the centrifugation. This method uses the centrifugal force for the separation of mixture, and the rotation speed extend to

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several thousands rpm. The high-density particles in the mixture sink to bottom of centrifugal tubes by the force, and the solvent move to upper side. The advantage of this method is that it can separate a large amount of blood at one time. However, it needs about 10 minutes in case of hemanalysis, and the centrifugal machine has relatively big package. Furthermore, it needs a certain level of blood volume and complicated procedures.

Another method is the filtration. This method uses the porous membrane or nonwoven material, the particles are trapped by these filters. The advantage of this method is high-speed separation, for example, "Plasmafilter PF" manufactured by Fujifilm medical Co., Ltd. can separate blood cells and blood plasma in about 1 minute by using of negative pressure. Though, these pores are plugged up by the particles easily, for that reason, the filtration is not suitable for the massive separation. Additionally, the increase of negative pressure for speeding up is a frequent cause of the destruction of red blood cells.

Presently, various blood separation methods are researched and developed. These methods use sedimentation of blood cells by hydrodynamic separation[1,3-6], capture the blood cells using microfilters or microchannels of taper toward the tip[1,7,8]. Furthermore, there is a technique using of dielctrophoresis for cell handling[9], and the blood separation and the blood cells handling by using of this technique are carried out[2,10].

Meanwhile, the particle that has larger density than solution is trapped by the acoustic wave at inside of the capillary.

3. VIBRATION-TYPE BLOOD CELLS SEPARATION DEVICE

The acoustic radiation inside a capillary are usually performed by aligning a transducer into a microchannel from one end to excite longitudinal modes of acoustic field through the tube[11]. Another method is to use concave transducers to generate acoustic field to trap particles at the focus limiting the concentration area[12,13].

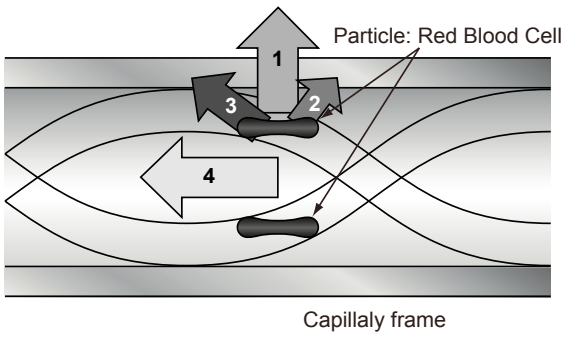


Figure 1 Pull of particle to the transverse velocity antinodes[14].

The particles are collected at the places that have maximum transverse velocity. At inside of a capillary, the particles are given the force towards antinodes by the inertial motion. Figure 1 shows the schematic diagram of vibration type particle separation principle. In this figure, the force given to the particle toward the capillary frame (arrow 1) will contain the two components of direction 2 and 3. The capillary do not have effective space for movement of direction 1, however particle has the little resistance to move in direction 3, which will give a net motion through direction 4 after a complete up and down cycle[14].

4. SEPARATION DEVICE WITH CAPILLARY

4.1 Simulated Blood

The simulated blood that was used in this experiment, the silica bead (Corefront Co., Ltd., diameter: 5 μm, specific gravity: 1.9 g/mL) was used as the simulated red blood cells. The particle density of the solution include this beads is diluted to 1.052 mg/mL by 50 w% glycerin aqueous solution. This particle density of this diluted solution is as same as the red blood cell density of human blood, and the viscosity of 50 w% glycerin aqueous solution is similar to the viscosity of whole blood.

4.2 Materials and Method

Figure 2 shows the schematic diagram of experimental system. The piezo-ceramic vibrator is driven by the waveform boosted by a power amplifier, and the waveform was generated by the function generator.

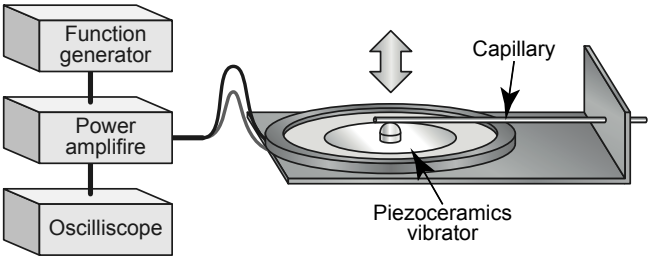


Figure 2 Schematic diagram of experimental system.

The photo image of prototype separation device is shown in figure 3. The piezo-ceramic vibrator was "Ceramitone" VSB50EWH0301B, purchased from Murata Co., Ltd. That was the bimorph type piezo-ceramic speaker, and that had wide-band frequency characteristics. The capillary was purchased from Charles Supper Company, its dimension was as follows, outer diameter: 800 μm, inner diameter: 760 μm, length: 80 mm. The one end of the capillary was fixed on the center of piezo-ceramic vibrator, and the other end was fixed to metal frame of the device. All components were glued by epoxy bond.

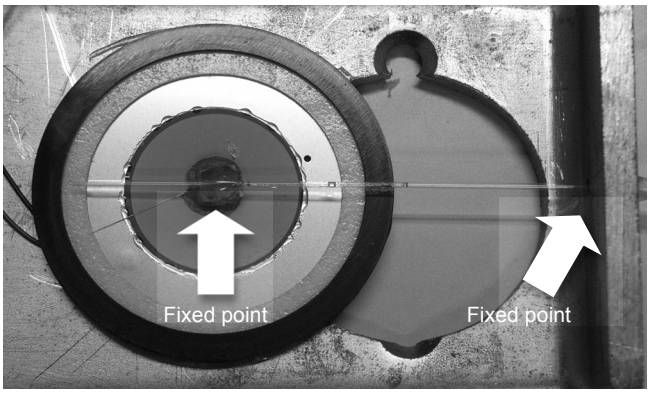


Figure 3 Prototype capillary type separation device.

4.3 Experiment

Before particle separation experiment, the measurement of resonant frequency of the system by displacement determination was carried out. From the results of this experiment, it was confirmed that the resonant frequencies of this system were 350 Hz, 1450 Hz and 6000 Hz.

At first, the applied frequency and applied waveform were selected by measurement of separation ability and separation time. After that, the experiment about effects by the measurement conditions were carried out, and the conditions were distance between fixed points, sample solution viscosity and applied voltage.

4.3.1 Selection of applied frequency and waveform

For selection of measurement conditions, the experiments about separation by frequency and separation by waveform were carried out. From the results about resonant frequency, the above-mentioned frequencies were applied to the piezoceramic vibrator, and their separation abilities were evaluated. By using of the selected frequency, the selection of applied waveform was carried out.

4.3.2 Effects by distance between fixed points

In this system, the optimization of capillary movement is important for efficient separation. One of the optimization factors is capillary length, and the length has the decisive influence on displacement and velocity of transverse motion. The experiment that confirmed the effects of distance between fixed points was carried out. The experimental conditions were 40, 50 and 60 mm. In this experiment, the separation time was measured.

4.3.3 Effects by viscosity of sample solution

The effects by viscosity of sample solution were not discussed before. In this subsection, the influence that the viscosity of sample gives to the separation speed is confirmed. The distance between fixed points were 50mm and 60mm, the glycerin concentration of solvent were 0, 10, 30 and 50 w%.

4.3.4 Effects by applied voltage

At the system using of the piezo-ceramic actuator, when the large displacement is needed, the higher voltage are applied. In this subsection, the relationship between particle concentration and separate time at 10 V and 20 V were evaluated.

4.4 Results and Discussions

4.4.1 Selection of applied frequency and waveform

Table 2 shows the separation results by applied frequency. According to these results, the only device that applied 1450 Hz could separate beads and solution. In the condition of 350 Hz applied, it was clearly that the displacement of vibrator side capillary end was largest from measurement of resonant frequency, but it did not have separation ability. This results means the frequency of 350 Hz was too slow to separate the particles. On the other hand, when 6000 Hz was applied to the vibrator, the device did not have separation ability too. This means the displacement was not enough to separate the particles. Therefore, it became clearly that 1450 Hz was the suitable frequency for separation.

Table 2 Separation results by applied voltage

Applied Frequency / Hz	350	1450	6000
Separation	×	○	×

The separation time by applied waveform is shown in table 3. From these results, square wave could separate fastest of these 3 types of waveform. The square wave has a lot of harmonics, therefore, this result had roots in the displacement of square wave applied was largest of other conditions. From above-mentioned results, the square wave of 1450 Hz was selected as applied waveform.

Table 3 Separation time by applied waveform

Applied waveform	Sine wave	Saw wave	Square wave
Separation time / sec	23	21	17

4.4.2 Effects by distance between fixed points

The relationship between the concentration of particles and separation time by distance between fixed points is shown in figure 4. In this figure, it was observed that the device with the longest distance had the fastest separation speed at the high-concentrated particle region. The longest distance leads the largest displacement of transverse motion at the antinode. This fact means the large displacement of transverse motion can deliver the high-speed separation ability.

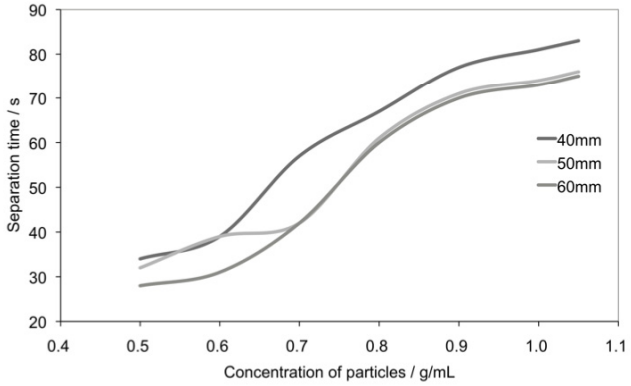


Figure 4 Relationship between concentration of particles and separation time by the distance between fixed point.

4.4.3 Effects by viscosity of sample solution

Figure 5 shows the relationship between concentration of particles and separation time by viscosity of sample solution. In this figure, it was confirmed that the high viscosity led the low separation ability. In case of 50 w% glycerin was used, the device with the 50 mm distance of fixed points could not separate the sample solution. This means the viscosity of sample solution has the potent influence to separation ability.

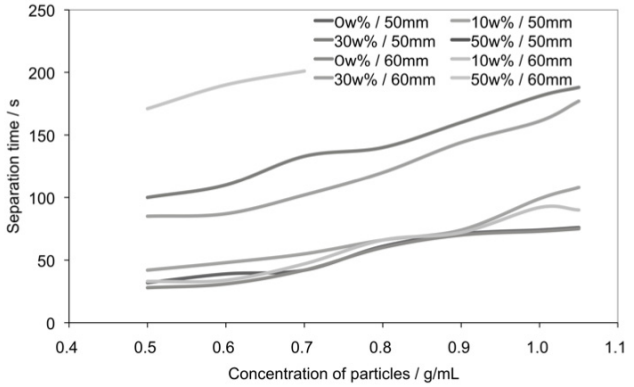


Figure 5 Relationship between concentration of particles and separation time by the viscosity of sample solution.

4.4.4 Effects by applied voltage

Figure 6 shows the relationship between concentration of particles and separation time by applied voltage. The higher voltage led the larger displacement of piezoceramic vibrator for granted. Therefore, higher voltage led the larger displacement of transverse motion at antinode, too. Furthermore, larger displacement of antinode led the high velocity movement. In the condition of applied voltage: 20 V_{PP} and distance between fixed points: 60 mm, the device could separate the particles from the suspension liquid of 1.05 g/mL. This value is similar to human whole blood, and the viscosity of suspension liquid is similar to whole blood, too. From these facts, this device will be expected as the human blood separation device.

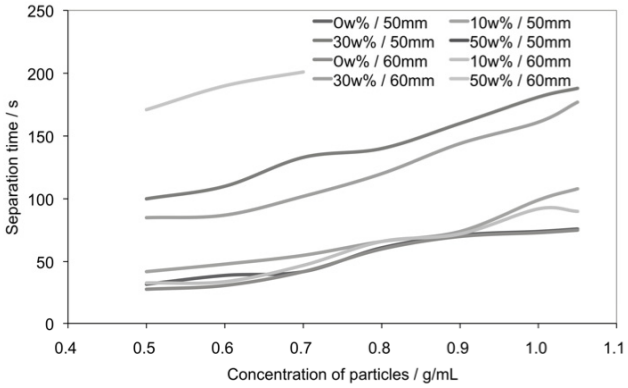
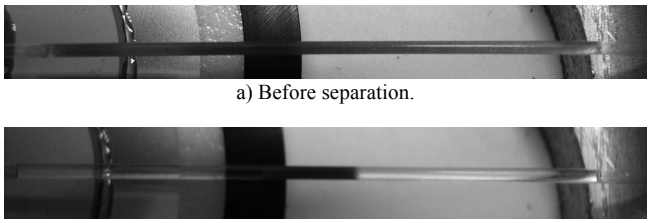


Figure 6 Relationship between concentration of particles and separation time by the applied voltage.

The photo images of before and after particle separation experiment are shown in figure 7. The collection of the particles at the antinode (center of both fixed points) was confirmed.



a) Before separation.

b) After separation.

Figure 7 Photo images before/after actuation. (Capillary type device)

5. SEPARATION DEVICE WITH GLASS PLATE

5.1 Increase of Separation Volume

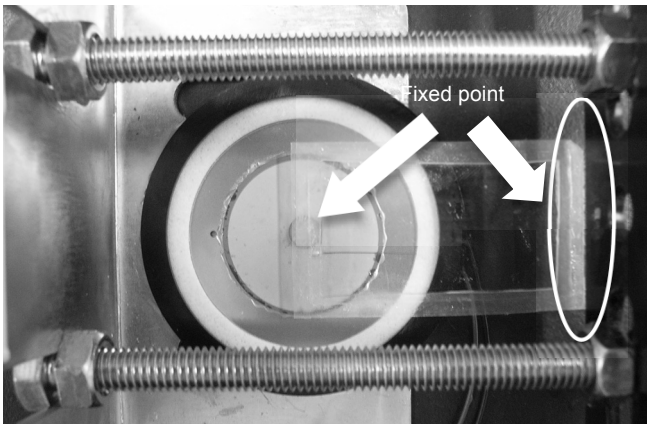


Figure 8 Photo image of prototype plates type device

As shown in preceding chapter, the vibration-type particle separation device with capillary could separate particles from suspension liquid. However, the volume of separable solution was only 27.2 μL, this volume is not enough to multiple test at the biochemical examine of human blood. Therefore, massively separation ability is desired. This separation method needs the displacement limit of transverse direction, the cavity will be able to extend to non-transverse direction.

From above-mentioned inference, the separation device with the cavity of rectangular parallelepiped was suggested. The photo image of the prototype device with above-referenced cavity is shown in figure 8. This cavity was constructed according to figure 9. This unit was consisted of two glass thin plate and thin silicone rubber with rectangular window. These components were glued tightly. The calculational volume of this cavity was 262.5 μL. Figure 10 shows the schematic diagram of the prototype device system.

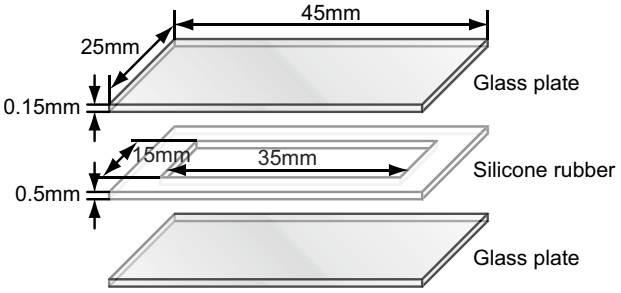


Figure 9 Construction of separation unit

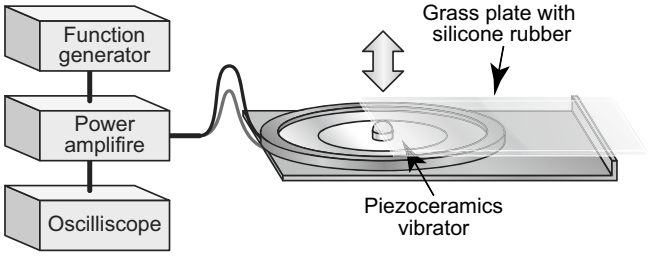


Figure 10 Prototype plates type device.

The separation unit with the rectangular parallelepiped cavity was fixed at center of piezoceramic vibrator and frame of the device. The distance between the fixed points was 35 mm, same as length of the cavity.

5.2 Experiment

For evaluation of the new type particle separation device with the rectangular parallelepiped cavity, the experiment of the particle separation was carried out. The conditions of experiment were mentioned as follows.

1) Sample solution was simulated blood referred to subsection 4.1.
2) Applied waveform was rectangular wave.
3) Applied frequency was 1450 Hz, according to measurement of resonant frequency of this device.
4) Applied voltage was 20 V_{PP}.

The experiment continued until the particle movement stopped.

5.3 Results and Discussions

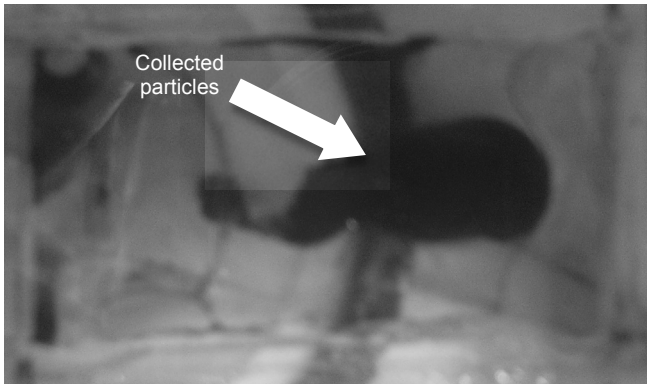


Figure 11 Photo Image of after actuation.

Figure 11 shows the photo image of after separation experiment. The particles were separated from the simulated blood, in 4 minutes. The collection area was near the center of cavity, but the clarification of initiate relationship between the collection area and the movement of separation unit needs the numerical analysis (e.g., FEM). This device did not optimize of resonant frequency or the unit shape, when the optimization of these terms progressed, this device will have higher separation ability. From this figure, there was enough particle inexistent area for sensor chip installation. In the future, the combination of this device and sensor chip will be realization.

6. CONCLUSION

In this report, we proposed the vibration-type blood separation device, and the two types of prototype separation devices were demonstrated. The ability of separation system was evaluated. The following knowledge was acquired.

1) The vibration-type separation device that used capillary as separation cavity was manufactured by way of trial and evaluated its performance. This device could separate the simulated blood in conditions as follows, applied waveform: square wave, applied voltage: 20 V_{PP}, applied frequency: 1450 Hz.

2) For increase of separable solution, the device that used the cavity of rectangular parallelepiped constructed by glass-silicone rubber-glass structure was developed and evaluated of its separation ability. This device had the ability of massively separation ability. This device will be hopeful as excellent separation device, depending on its optimization in the future.

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