Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Hiroyuki Nakazawa[ab]; Takenori Tanimura[ac]; Zenzo Tamura[a]
[a] FACULTY OF PHARMACEUTICAL SCIENCES UNIVERSITY OF TOKYO, TOKYO, JAPAN [b] Department of Radiology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan [c] Toyama Medical and Pharmaceutical University, Japan

To cite this Article Nakazawa, Hiroyuki , Tanimura, Takenori and Tamura, Zenzo(1978) 'A Device for Countercurrent Distribution of Particles by an Aqueous Polymer Two-Phase System', Separation Science and Technology, 13: 9, 745 — 752
To link to this Article: DOI: 10.1080/01496397808057125
URL: http://dx.doi.org/10.1080/01496397808057125

SEPARATION SCIENCE AND TECHNOLOGY, 13(9), pp. 745–752, 1978

A Device for Countercurrent Distribution of Particles by an Aqueous Polymer Two-Phase System

HIROYUKI NAKAZAWA,* TAKENORI TANIMURA,† and ZENZO TAMURA

FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF TOKYO
HONGO 7–3–1, BUNKYO-KU, TOKYO, JAPAN

Abstract

A new device for countercurrent distribution using an aqueous polymer two-phase system was developed. The device consists of a rotating column separated into many loculi in which steel balls are placed as stirrers, and a motor is used to rotate the column at two selected speeds alternately. At the lower speed of rotation with little centrifugal force, the steel balls roll down along the surface of column wall and promote effective mixing, while at the higher speed of rotation the centrifugal force keeps the balls still and accomplishes phase separation.

The distribution behavior of cells or cell particles by the device is demonstrated with red blood cells from rat.

INTRODUCTION

One of the essential steps in experimental chemistry is the purification and separation of materials. For this purpose, various kinds of chromatography have been developed. However, the usual types of chromatography are not applicable to the separation of cells and particles.

*Present address: Department of Radiology, Tokyo Metropolitan Institute of Medical Science, Honkomagome 3–18–22, Bunkyo-ku, Tokyo, Japan.

†Present address: Toyama Medical and Pharmaceutical University, Sugiya, Toyama-shi, Toyama Prefecture, Japan.

Albertsson developed a technique for partitioning between aqueous polymer two-phase systems where the biological activities of cells and particles were retained during separation due to the extremely small interfacial tensions (*1*, *2*). However, when the system was applied to countercurrent distribution with Craig's machine (*3*, *4*), it required impractical long times for phase separation, because these phases are highly viscous and have small difference in density.

To minimize the time for phase separation in multistage partitioning with aqueous polymer two-phase systems, Albertsson developed thin-layer countercurrent distribution (*5*, *6*). On the other hand, Ito et al. introduced countercurrent chromatography to achieve highly efficient partitioning between two immiscible phases in all-liquid systems (*7–9*). Countercurrent chromatography has been applied for aqueous polymer two-phase systems, and the possibility of chromatographic separation of cells and macromolecules without loss of biological activities was demonstrated (*10–12*).

We have developed a device to achieve efficient partitioning and phase separation in alternately applied centrifugal forces and to transfer the distributed cells by intermittent flow of the top phase. The efficiency of the device was examined with the distribution of red blood cells from rat.

EXPERIMENTAL

Materials and Methods

Materials

Dextran T 500, $M_w = 5 \times 10^5$, was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Polyethylene glycol with $M_n = 6 \times 10^3$ was obtained from Union Carbide, New York, as Carbowax. Other reagents were of analytical grade and the water was doubly distilled. Trypsin was purchased from Sigma Chemical Co., U.S.A.

Preparation of Phase System

The aqueous polymer two-phase system used in the present study contained 5% (w/w) dextran, 4% (w/w) polyethylene glycol, and 0.11 *M* sodium phosphate buffer, pH 7.4. The phases were throughly equilibrated at 4°C and were separated into the top and bottom phases before use.

Preparation of Red Blood Cell Suspension

Rat blood samples were obtained by the amputation of the carotid artery and collected in acid–citrate–dextrose solution (ACD). The cells were washed three times with phosphate-buffered saline (PBS) and packed by centrifuging at 1500 rpm for 10 min. They were suspended in an equal volume of 0.11 *M* sodium phosphate buffer, pH 7.4.

Preparation of Trypsin-Treated Cell Suspension

Packed cells (1500 rpm for 10 min) were treated with double their volume of 0.01% (w/v) trypsin in PBS at 37°C for 30 min, followed by three washings with 0.11 *M* sodium phosphate buffer, pH 7.4, and suspended in an equal volume of the buffer.

Determination of Partition Rate of Cells

Equal volumes (5 ml) of top and bottom phase of aqueous polymer were poured into calibrated test tubes. Fifty microliters of red blood cell suspension were delivered into each tube. The test tubes were inverted gently several times for mixing, and the phases were allowed to settle for exactly 20 min. At the end of this time the volume of each phase was measured and a 1-ml aliquot was carefully pipetted from the top phase. Four milliliters of distilled water was added to lyse the cells. The hemoglobin concentration was determined by measuring the absorbance at 540 nm on a Hitachi 101 Spectrophotometer. The experiments were carried out at room temperature.

The partition ratio was expressed as the fraction of total cells (in percent) which was found in the top phase.

Determination of Electrophoretic Mobility of Cells

Electrophoretic mobility of the cells was determined at 25°C in the cylindrical chamber of a cell electrophoresis instrument from Sugiura Laboratory, Tokyo. The electrophoretic medium (*13*) consisted of 0.015 *M* Tris-(hydroxymethyl)-aminomethane, 0.01 *M* glucose, 0.04 *M* potassium acetate, 0.24 *M* glycine, and 0.03 *M* sucrose adjusted to pH 7.2 using acetic acid. Each value of the cells was obtained by timing the traverse of 12.5 μm, with subsequent reversal of polarity of the electrodes, at a constant current (0.3 mA). The resulting time pairs were averaged if they did not differ by more than 10%. Velocity determinations

were made on at least 20 cells. The electrophoretic mobility of red blood cells from Donryu rat (-1.150 μm/sec/V/cm) was used as a standard for determining the electrophoretic mobility of the sample cells which is given by the following formula (*14*):

$$\text{Electrophoretic mobility } (\mu\text{m/sec/V/cm}) = -1.150 \times \frac{T_r}{T_s}$$

where T_r = time (in seconds) for traverse of 12.5 μm determined for red blood cells from Donryu rat, and T_s = time (in seconds) for traverse by sample cells.

Distribution Device

Components of a Distribution Device

A schematic diagram of the distribution device is illustrated in Fig. 1. The distribution column used in this experiment was made by placing Teflon disks (Chuo-Packing Co., Tokyo) into the Junflon tubing (Junkosha Co., Tokyo). The Teflon disk had a wall thickness of 1 mm, a diameter of 7 or 9 mm, and had holes along a central circle as shown in Fig. 1. Three to five steel balls (1.5 mm in diameter) were placed in each loculus to achieve efficient mixing. The column was placed in a cylinder made of brass to fix the rotating axis horizontally at the center. The connections to the column were made of Teflon tubing (Kyowa Seimitsu Co., Tokyo) with 0.5 mm i.d. The rotation speed of the column was varied up to a maximum of 1200 rpm by a synchronous motor. Figure 2 is a schematic drawing of the rotating seal.

Operation of Distribution Column

The column was first held in a vertical position and filled with the bottom phase by a peristaltic pump (Vario perpex pump, LKB Instru-

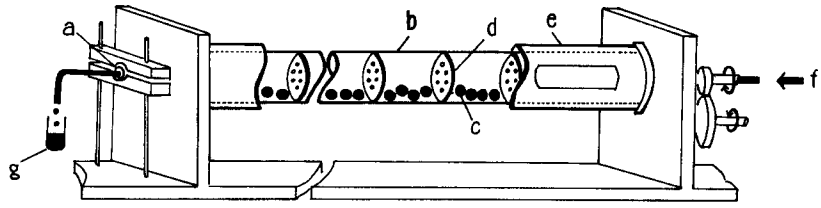

FIG. 1. Diagram of distribution device: (a) rotating seal, (b) column, (c) steel ball, (d) Teflon disk, (e) column holder, (f) pump, (g) test tube.

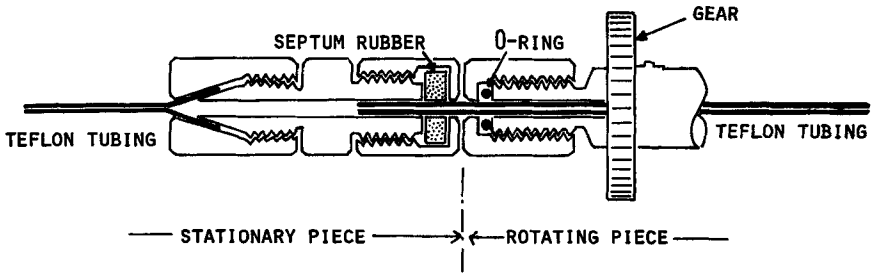

FIG. 2. Schematic drawing of rotating seal.

ments, Sweden). When all air bubbles were removed, the column was placed inside the cylinder. The top phase was fed into the first loculus and 100 μl of cell suspension were introduced into the first loculus by a syringe. After application of the sample solution, the mixing was started by rotating the column at a speed of 100 to 250 rpm, as illustrated in Fig. 3(A). At this speed of rotation, the balls in each loculus rolled along the wall and promoted mixing effectively. After mixing for 0.5 to 1.0 min, the speed of rotation was increased to about 1000 rpm. At this rotational speed the steel balls were pinned to the wall by centrifugal force and did not roll. The top phase moved to the axis of rotation while the bottom phase moved to the outer part of the loculi, and phase separation was accomplished (Fig. 3B). Then a portion of top phase equivalent to the amount present in the first loculus was fed into the column, transfering this phase from the first loculus to its neighbor through the holes in the disk (Fig. 3C). The processes of mixing, separation, and phase transfer were repeated automatically by a control unit.

The temperature during the run was held at 4°C. Each aliquot of effluent from the rotating seal was collected in a tube. The red blood cells

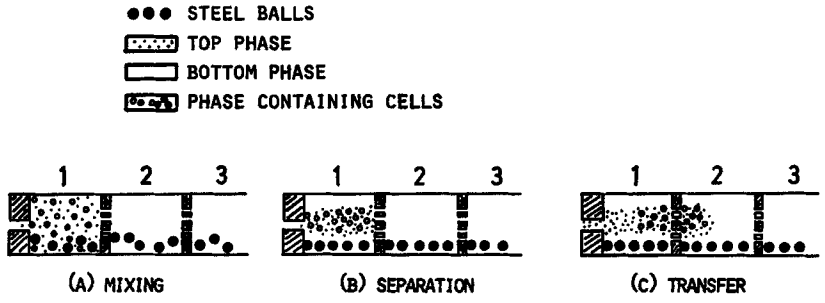

FIG. 3. Schematic illustration of distribution column.

obtained from each tube were lysed by adding an appropriate volume of distilled water, and the concentration of hemoglobin in each lysate was determined by the absorbance at 540 nm.

RESULTS AND DISCUSSION

The place, size, and number of holes were designed to minimize the mixing of liquid phases between loculi and to hold adequate amounts of the stationary phase in each loculus. Holes of 0.5 to 1.0 mm diameter in the Teflon disks are sufficiently large to allow free passage of the phases, but sufficiently small to prevent mixing of liquid phases between loculi.

In this experiment the connections of the column were made with Teflon tubing of 0.5 mm i.d., which allows the free movement of polymer and red blood cells without clogging.

To examine the partitioning and phase separation in the device, red blood cells of rat were chosen as a model. The red blood cells provide the following advantages: (1) they are visible in the distribution column,

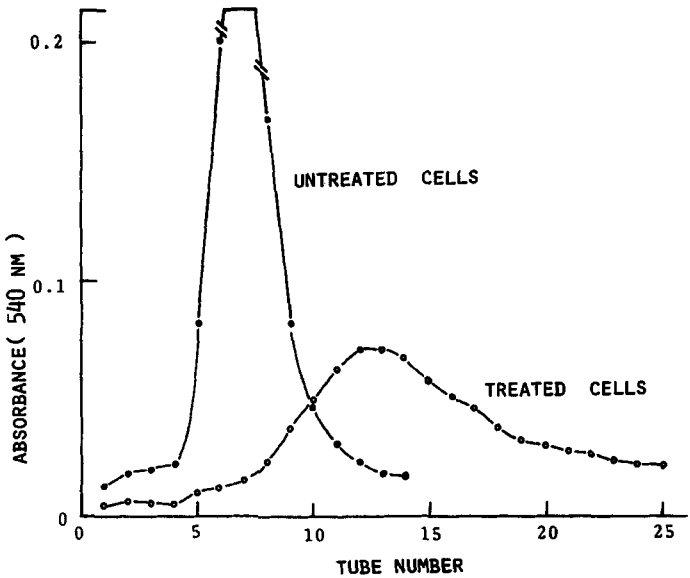

FIG. 4. Elution patterns of trypsin-treated and -untreated red blood cells from mature rat. The device had a cycle consisting of mixing for 30 sec at 140 rpm, a separating period for 120 sec at 860 rpm, and then transfer. In the transfer, the top phase was delivered by a peristaltic pump at a flow rate of 0.075 ml/min for 75 sec. The distribution column has 13 loculi, which were made by placing Teflon disks (7 mm diameter) at 8 mm intervals throughout the tube.

(2) they are relatively resistant to forming aggregates and lysis, and (3) the fundamental behavior of the cells in aqueous polymer two-phase systems is known (*15*, *16*).

Figure 4 shows the superimposed elution patterns of trypsin-treated and untreated cells from a mature rat. The prolonged elution time of the former peak indicated a decrease of the partition ratio of the cells from 81 to 51%. The trypsin treatment also markedly reduces the electrophoretic mobility of the cells; the delayed elution of trypsin-treated cells from the column can be presumed to be due to a decrease of the surface charge of red blood cells (*17*).

Figure 5 shows the elution pattern of red blood cells from a week old rat. The elution curve was unsymmetrical and broader than that of mature rat cells, indicating that the red blood cells of the young rat were heterogenous compared to those of the mature rat. The electrophoretic mobilities of cells collected in different fractions are listed in Table 1. The cells eluted earlier showed higher electrophoretic mobility.

The present device is easily constructed and simple to operate.

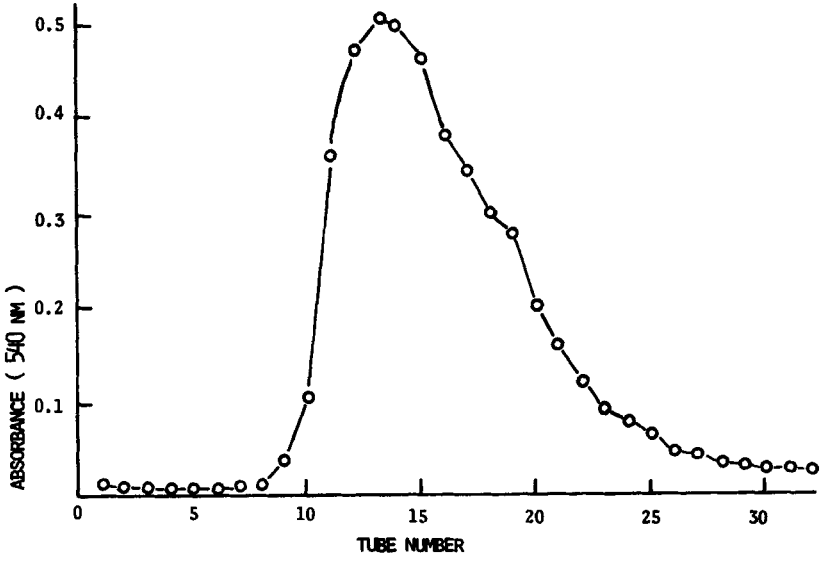

FIG. 5. Elution pattern of red blood cells from newborn rat. The device had a cycle consisting of mixing for 30 sec at 150 rpm, a separating period for 120 sec at 860 rpm, and then transfer. In the transfer, the top phase was delivered by a peristaltic pump at a flow rate of 0.075 ml/min for 90 sec. The distribution column has 20 loculi, which were made by placing Teflon disks (9 mm diameter) at 8 mm intervals.

TABLE 1

Electrophoretic Mobility of Eluted Cells from Distribution Column in Fig. 5[a]

Subject	Electrophoretic mobility (μm/sec/V/cm)
Before countercurrent distribution	$-0.951 + 0.014$
After countercurrent distribution:	
Fraction no. 10	-0.955 ± 0.014
Fraction no. 11–13	-0.922 ± 0.007
Fraction no. 20	-0.882 ± 0.009
Retained on the column	-0.825 ± 0.021

[a] Values are presented as the mean $\pm$ standard deviation. Red blood cells from a newborn rat were distributed by the column consisting of 20 loculi.

Acknowledgments

We are greatly indebted to Dr. Iwaguchi, The Tokyo Metropolitan Institute of Medical Science, for help and advice in determining the electrophoretic mobility of the cells. We are also very grateful to Dr. Kabasawa, Nihon University, for a useful suggestion toward developing the device.

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Received by editor March 10, 1978