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Multistage magnetic particle separator

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Abstract

Significant motion of a particle suspended in a fluid in a magnetic field requires steep gradients and hence short migration distances. Recognizing this constraint a magnetic particle separator was designed to classify particles on the basis of magnetophoretic mobility. Multiple short migration paths were achieved by using a multistage extractor, which draws particles out of a feed cuvette using an increasing magnetic driving force at each stage. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Commercial methods are available for the separation of magnetic from non-magnetic particles [1–4], and such binary separations have been applied, for example, to living or fixed cells tagged with magnetic microparticles [5,6]. Despite three decades of research on magnetic microparticles [7], very little has been done to achieve classification of particles in proportion to their degree of magnetization. Significant motion of a particle suspended in a fluid in a magnetic field requires steep gradients and hence short migration distances [8]. Recognizing this constraint a magnetic particle separator was designed to classify particles on the basis of

magnetophoretic mobility, defined as

$$\mu_m = v_m/|\nabla B^2|,$$

where v_m is instantaneous velocity in a magnetic field B per unit driving force ∇B^2 [9,10]. Multiple short migration paths were achieved by using a multistage extractor [11] which draws particles out of a feed cuvette using increasing magnetic driving force at each stage. Thus, particles with high magnetophoretic mobility are captured first, and particles with low magnetophoretic mobility are captured last. The multistage magnetic separator can thus classify cells according to number of receptors when they are labeled with suitably liganded magnetic particles, since magnetophoretic mobility is proportional to the number of magnetic particles per cell [10]. It is also possible to classify magnetic microparticle carriers [12] according to magnetophoretic mobility, an important variable in carrier function in many cases [13]. To date, this

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separation has been previously achieved only in flowing suspensions in a strong field gradient [14].

The implementation of this method has been accomplished by constructing and testing instruments, currently named MAGSEP, that first align all particles that respond to a magnetic field at a fixed distance below a levitating magnet having a flat pole piece. Several such levitating magnets are used in sequence to collect particles of varying magnetophoretic mobility. The purpose of this article is to introduce and describe this instrumentation.

2. Principle of multistage magnetic separation

Each stage of the MAGSEP device selects particles of different magnetophoretic mobility ranges. The particles collected in each of the stages will thus have a different mobility distribution. The low-magnetic field strengths will select particles of higher mobility, whereas the higher magnetic field strengths will select for lower mobilities. Therefore, each stage will contain a magnetophoretic mobility cutoff, based on the magnetic field strength of the capture magnet and the dwell time of the capture.

Fig. 1 is a schematic representation of a multistage electromagnetic separator showing comparison with a hypothetical magnetic chromatography column. The MAGSEP device utilizes a step-wise rotary distribution and containment system which selects, isolates and stores particles of different magnetophoretic mobilities. Thus, cells are separated according to the quantity of ligand on their surfaces.

Fig. 2 is a diagram showing a single stage of the magnetic separation process whereby cells that bind magnetic beads are drawn along the gradient toward the pole. The illustration shows a magnetic source, ‘holding magnet’ either permanent or electromagnetic, at the top of the upper, inverted cuvette which produces a magnetic field gradient that causes movement of magnetized particles in accordance with their magnetophoretic mobility. For example, all separands attached to magnetized particles such as cells or proteins may be drawn into the upper half-cavity of a multistage separator from a uniform suspension, while

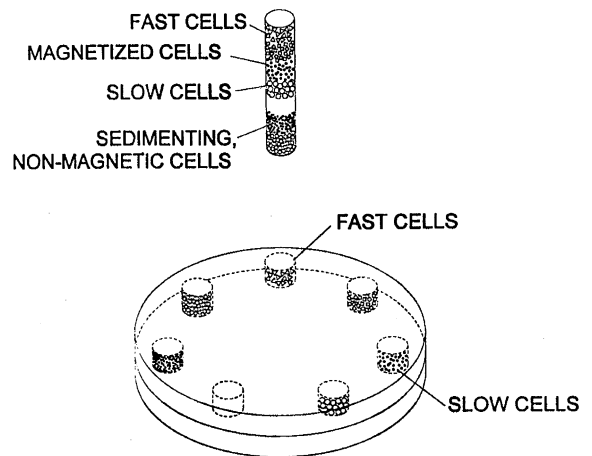


Fig. 1. MAGSEP achieves the equivalent of a magnetic separation column realizing that a column would require an unreasonably strong magnet with a gradient that could not be sustained more than a few centimeters.

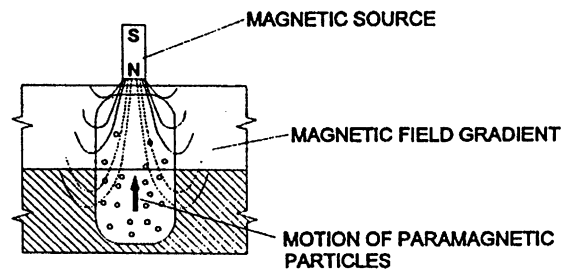


Fig. 2. Diagram illustrating the principle of magnetic capture of particles in a single stage of MAGSEP.

non-magnetic separands remain distributed in the lower cavity. Non-magnetic particles are allowed to settle during the capture. In this diagram, however, particles are distributed throughout the lower cavity and do not travel a uniform distance before capture. To solve this problem a ‘translating electromagnet’ (Fig. 3) aligns the particles in the lower cuvette. The field strengths of both the translating electromagnet and the holding magnet can be varied during the separation process. Separated particles are then removed, in their suspending fluid, from the upper cuvette through a horizontal port that is also used when filling the cuvette with receiving fluid.

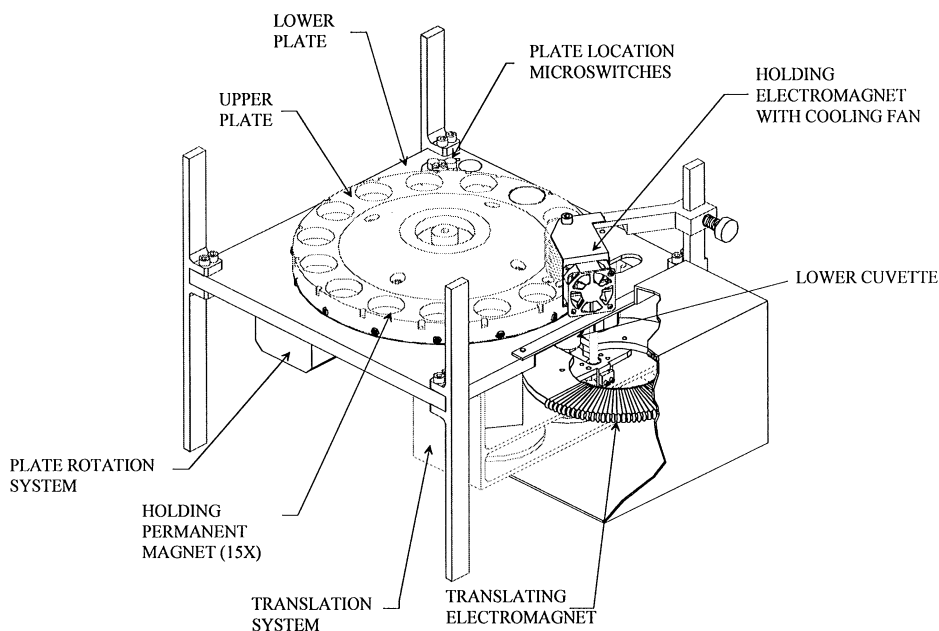


Fig. 3. Laboratory MAGSEP unit (details are described in the text).

3. MAGSEP instruments

Fig. 3 is a perspective view of an existing example of a multistage electromagnetic separator. This MAGSEP unit consists of a stationary lower plate supported by legs and an upper plate that contains a number of upper collection cuvettes that can be moved into selected fluid communication with the single cuvette in the lower plate. A seal is formed between the plates with a sealant such as a grease, wax, or other lubricating and/or sealing constituent. Fig. 3 also shows a translating electromagnet, its translation system, a holding magnet, of which there are 15 permanent magnets in this example, a holding electromagnet with cooling fan, a plate rotation system (motor with a worm gear), and a plate location microswitch. The holding electromagnet is used to extend the range of holding-magnet field strengths.

A commercial unit has also been constructed and is mounted onto a housing which includes a power switch, 110VAC connector, RS232 communications port, indicator lights, cooling fan, computer and software in which the status of the power, translating electromagnet, holding magnet and

plate rotation are indicated with a Graphics User Interface (GUI).

The upper and lower plates bolt together through a tapered roller bearing that allows the plates to rotate with respect to one another. The lapped interface between the plates provides a seal separating the fluids. With a sealant, as mentioned above, this mechanical arrangement prevents sample leakage and maintains sterility. The lower cuvette can be aligned with as many as 15 upper cuvette stations during processing. A two-phase stepping motor rotates the upper plate by driving the rotation system that engages an internal gear mounted to the underside of the upper plate.

The translating electromagnet aligns particles at a specific height in the lower cuvette prior to their being attracted by a holding magnet above the upper cuvette. The translating electromagnet is mounted to a motorized translation system that translates the electromagnet upward along the lower cuvette. A programmed amount of current is sent to the electromagnet creating a magnetic field across the lower cuvette. The translating electromagnet field strength can be programmed from 0 to 1400 g (measured at the poleface), or other

selected ranges. The electromagnet translation system moves the electromagnet up and down the lower cuvette. The translation rates can be programmed to range from 5 to 2000 $\mu\text{m/s}$ or other values selected to match the rate of accumulation and/or sedimentation of the sample particles. The translating electromagnet is switched off for the subsequent separation steps.

The holding magnet assembly captures particles that migrate into each upper cuvette within a specified time interval under the driving force of the holding magnet, thereby holding only particles within a specified mobility range. In the example in Fig. 3 the holding electromagnet assembly consists of an electromagnet mounted on an arm that is suspended above a cavity for a permanent magnet, in turn above a receiving cuvette. Thus, the electromagnet and/or permanent magnets can be used together to establish the captured mobility range, and a small permanent magnet can be used to prevent particle sedimentation after capture.

As shown in Fig. 4, one configuration of a translating electromagnet consists of a steel core with 818 windings of 26-gage copper magnet wire formed in a disk having an air gap. It receives current ranging from 0 to 2.16 A from the electronics box.

4. The capture of particles at each stage

MAGSEP was designed to separate magnetically susceptible materials suspended in fluids. An imple-

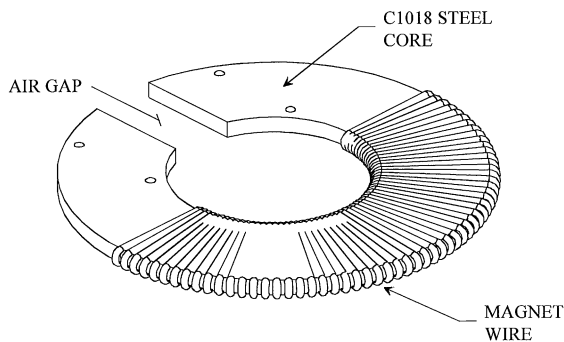


Fig. 4. One of the translating electromagnet configurations: 818 windings of 26 G. Copper wire with an air gap that accommodates the sample feed cuvette.

mentation of the configuration shown in Fig. 3 is as follows:

The upper plate and lower plate are set to the fill position ('half stepped'), and the fluid samples are filled into the upper and lower cuvettes. The upper cuvette rotates into position above the lower cuvette aligning the upper and lower cuvettes. The translating electromagnet energizes to a programmed current level and translates from the bottom of the lower cuvette to a position below the interface of the plates. The translating electromagnet is then de-energized, and the holding electromagnet is energized to a programmed current level pulling particles within a specified mobility range into the top of the captured upper collection cuvette. Finally, the holding electromagnet is de-energized leaving the permanent holding magnet to keep the collected sample particles in the top cuvette while the upper plate rotates thereby capturing the sample of the collected particles. Approximately, 4–5% of the fluid volume in the upper cavity mixes by convective transfer with the lower cuvette fluid during this capture process. Each step can be preprogrammed to vary or remain the same for upto 15 particle-capture cycles using up to 15 cuvettes. This process is readily understood by following it stepwise as illustrated in Figs. 5–8.

Fig. 5 is a cross-section of the plate assembly showing the bottom plate engaged with the upper plate in alignment with a sample cuvette and an upper collection cuvette and the holding electromagnet on the arm and the holding permanent magnet in its well above the upper cuvette. This

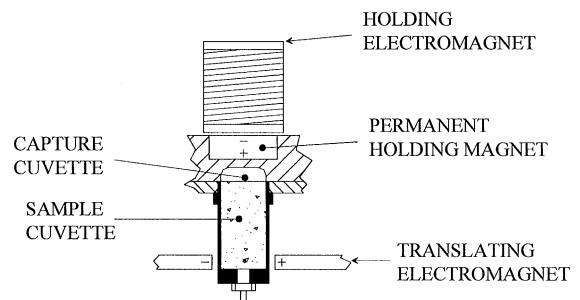


Fig. 5. Cross-section of a plate assembly showing lower and one upper cuvette engaged. Also illustrates nomenclature.

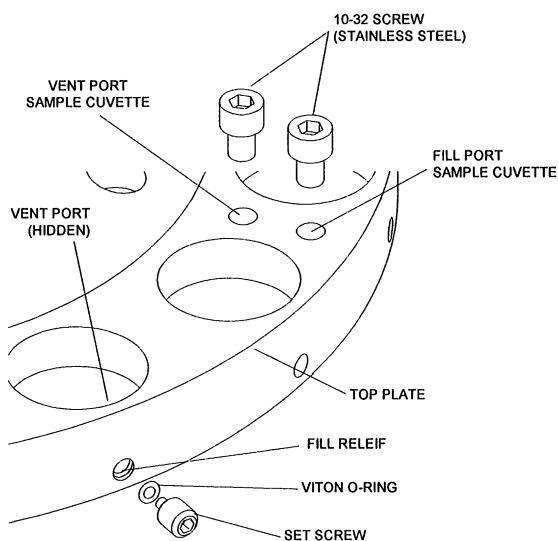


Fig. 6. Details of filling ports and plugs of the upper and lower cuvettes.

figure defines the terms that will be used in the description that follows.

Fig. 6 shows the details of the filling of the upper and lower cuvettes. The filling ports of the upper cuvettes are closed with plastic set screws, and the fill port and vent of the lower, sample cuvette is closed with stainless-steel cap screws.

In Fig. 5, the permanent holding magnet sits in a well above the upper, collecting, cuvette, and it is 'boosted' by the holding electromagnet, which serves each upper cuvette, in turn, at a specified field strength.

Fig. 7a shows the initial step, in which the filling port within the top plate is in fluid contact with the lower sample feed cuvette. The top plate is bolted to the bottom plate with a clamping bolt that allows the top plate to rotate while being sealed. The sample cuvette is attached to an opening in the bottom plate. This allows the collection cuvette to be rotated as shown in Fig. 7b over the sample cuvette, thus allowing particles in the sample cuvette to be transferred to the collection cuvette.

As shown in Fig. 7b, the top plate rotates with respect to the bottom plate and the sample cuvette to a 'full step' position. The translating electromagnet energizes and moves upward toward the plate

interface as depicted in Fig. 7c showing initiation of particle alignment in the sample cuvette.

Fig. 7d shows the final position of the translating electromagnet and the capture of particles at which time the translating electromagnet stops and deenergizes, and the holding electromagnet energizes and field couples with the permanent magnet. Finally, as shown in Fig. 7e, the top plate is rotated and sealed off to capture a selected fraction of the particles as the process sample.

Fig. 8 is a graph depicting the translating magnet field strength of a MAGSEP unit such as described in Fig. 3. As shown in Fig. 3, the programmable holding electromagnet is used to pull the sample past the plate interface and into the top of the upper cuvette. The permanent magnet is used to keep the captured sample at the top of the capture cuvette, thereby preventing it from falling into the plate interface and becoming trapped between the plates. The permanent magnet size and materials can be varied to provide a variety of field strengths.

5. Test experiment

Fig. 9 is a graph showing the results of a separation experiment separating magnetic from non-magnetic microparticles by the multistage magnetophoresis process. The experiment began with a mixture containing 90% 1–2 μm Magnetic spheres (Aminospheres, Polysciences) and 10% 6.0 μm non-magnetic spheres (Interfacial Dynamics Corporation) suspended in aqueous buffer. Six cavities were equipped with magnets ranging from 10 to 375 mT field at the pole face. Gradients were estimated using field measurements at 2.54 cm and converted to mT/m. Dwell time at each cavity was 15 min, and travel distance was on average 3 mm. From these data, a magnetophoretic mobility was estimated for each of the seven cavities, as given on the accompanying graph.

It is seen that 80.1% of the magnetic particles were all captured in cuvette #6, corresponding to a mobility of 0.6 mm/Ns, where only 2.8% of the non-magnetic particles were captured. The 'purity' of the magnetic spheres went from 90% to 99.6%.

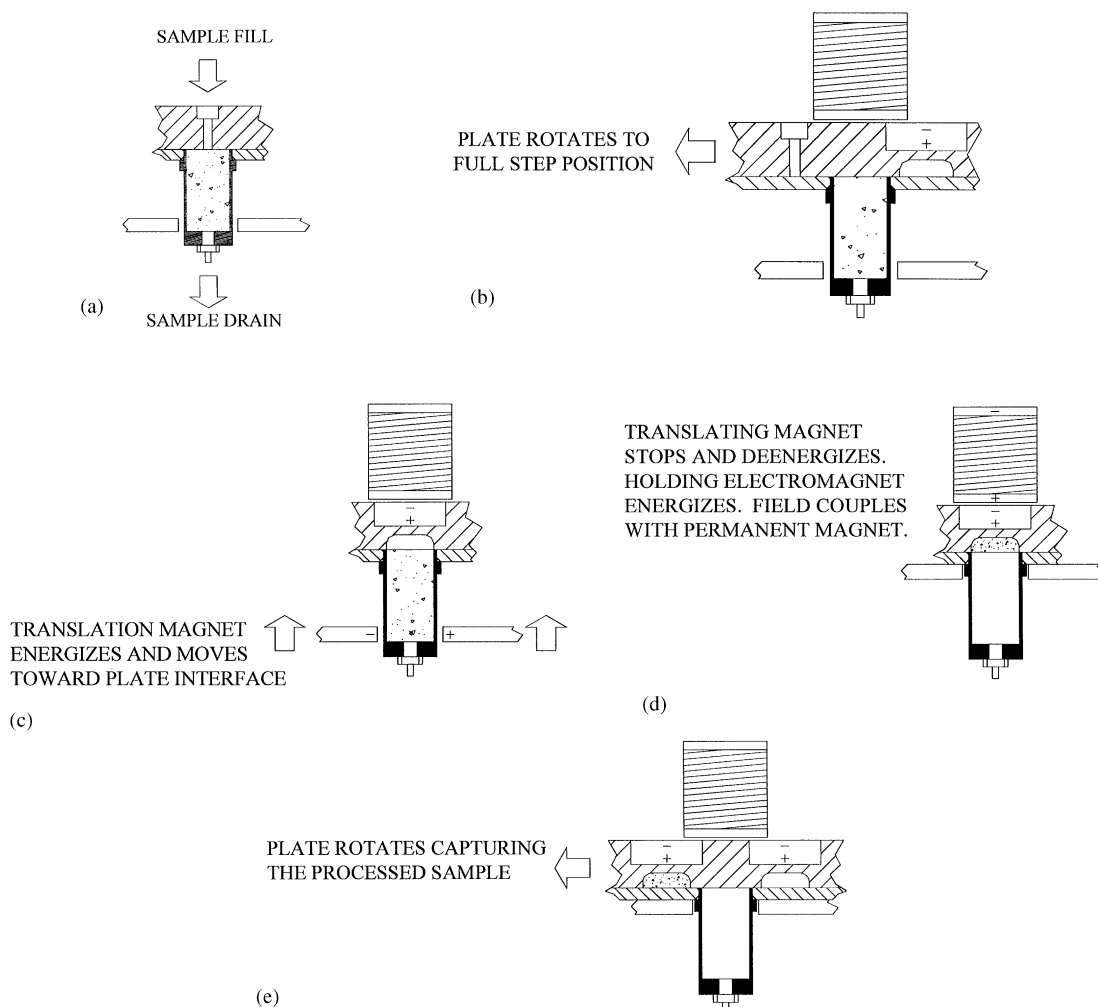


Fig. 7. (a) Filling the sample cuvette through a port in the top plate. (b) Rotation of the top plate seals sample into the sample cuvette. (c) Initiation of particle alignment in the sample feed cuvette. The translating magnet is at the bottom being prepared to rise and sweep up magnetic particles together. (d) Completion of particle alignment in the sample feed cuvette; the translating magnet is at the top of its travel; it can be switched off, and the holding-magnet field can be applied. (e) Rotating the top plate after collection seals the collected sample in a top cuvette.

6. Alternative modes of operation

The MAGSEP could also be used as a means of ‘Magnetic Chromatography’. Capture could be ‘isocratic’, wherein magnets in all of the stages have equal strength, or ‘gradient’ wherein magnets at increasing stage numbers have increasing field strength. In the latter case, in a typical application the first stage would have no magnet and no upper

cavity and would serve the purpose of homogenizing the cell mixture by stirring just before the beginning of transfers. The second stage would have no magnet and would serve the purpose of adding magnetic particles to the cell suspension from a low-volume upper cavity, mixing them together, and allowing them to react. The third stage would have a very weak magnet in the upper cavity, which would have similar volume to the lower cavity, and

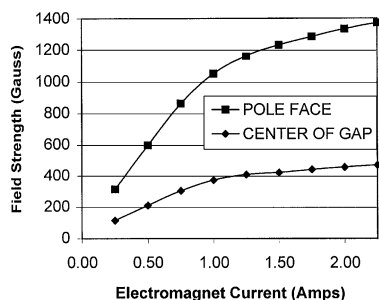


Fig. 8. Field versus current for a translating magnet. Squares: at the pole face; diamonds: at the center of the interface between plates.

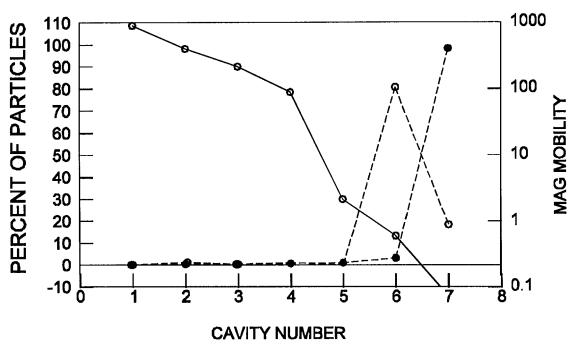


Fig. 9. Particle count distribution after a partial separation of magnetized and non-magnetized particles. Circles: magnetic microspheres; black dots: non-magnetic spheres; line-circles: magnetophoretic mobility.

would attract only the most highly magnetized cells, namely those with the most receptors for the magnetic ligand. The fourth stage would have a stronger magnet than does the third in its upper compartment and would attract more weakly magnetized cells, etc. until, at the final-but-one stage the strongest magnet of all would capture the cells with the least receptors. The final stage would also have no magnet, and the feed cuvette would contain any remaining completely unmagnetized cells after the final transfer.

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