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S. Chethana[a]; Ganapathi Patil[b]; M. C. Madhusudhan[c]; K. S. M. S. Raghavarao[c]
[a] Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, IISc campus, Bangalore, India [b] Department of Chemical Engineering, Padmasri Dr. B. V. Raju Institute of Technology, Narsapur, Medak Dist, AP, India [c] Department of Food Engineering, Central Food Technological Research Institute (CFTRI), Council of Scientific and Industrial Research, Mysore, India

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S. Chethana,[1] Ganapathi Patil,[2] M. C. Madhusudhan,[3] and K. S. M. S. Raghavarao[3]

[1]Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, IISc campus, Bangalore, India
[2]Department of Chemical Engineering, Padmasri Dr. B. V. Raju Institute of Technology, Narsapur, Medak Dist, AP, India
[3]Department of Food Engineering, Central Food Technological Research Institute (CFTRI), Council of Scientific and Industrial Research, Mysore, India

Abstract: Electrophoretic separation is a leading method for resolving mixtures of charged macromolecules or cells. In the present work, a combination of free electrophoresis and multistage extraction called Electrophoretic Counter Current Extractor was explored. A simple mathematical model was developed to describe the mass and heat transfer during the electrophoretic separation. In order to validate the model and also to test the extractor, the extraction of fixed human Red Blood Cells and Latex particles of different sizes suspended in phosphate buffer, was performed at different electric field strengths. The experimental results were found to agree reasonably well with those predicted by the model.

Keywords: Counter current, electrophoresis, electrophoretic mobility, extraction, separation

INTRODUCTION

Electrophoresis is a leading method for resolving mixtures of charged proteins, nucleic acids, or cells. Electrophoretic separation of proteins without gels (free electrophoresis) has been a long-standing goal of

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Address correspondence to K. S. M. S. Raghavarao, Department of Food Engineering, Central Food Technological Research Institute (CFTRI), Council of Scientific and Industrial Research, Mysore-570020, India, Ph: +91-821-2513910; Fax: +91-821-2517233, E-mail: fed@cftri.res.in

separation research (1,2). These separations are influenced either separately or jointly by factors such as the size (or molecular weight), shape, secondary structure, density, and charge of the macromolecule or cell. A marked correlation between the distribution of the cells and relative electrophoretic mobility led to the conclusion that the surface charge is a primary determinant in the cell separation (3,4).

Electrophoretic methods are most widely used high resolution techniques for the analytical and preparative separations (5). Electrophoresis involves the migration of electrically charged molecules in solutions due to an electric field applied. The continuous and simultaneous separation and fractionation of samples by free-flow electrophoresis (FFE) has both analytical and preparative applications (6). Free flow electrophoresis utilizes differences in electrophoretic mobility rather than density to separate cells or sub cellular organelles or molecules.

There are several kinds of cell electrophoresis. The most important are free-flow electrophoresis, scaled-up free-flow electrophoresis, and column electrophoresis (7). Among these free electrophoresis could enjoy more widespread use, because it is a high-resolution separation method that does not require adsorption to solid media and the subsequent solids handling. Furthermore, it can handle particles/cells as well as solutes (macromolecules) alike. Some of the applications of free electrophoresis include the separation of different cells of peripheral blood and bone marrow in hematological and immunological research with potential therapeutic applications (8), and the separation of proteins from body fluids, tissue extracts, and fermentation broth in biotechnology (9). FFE was successfully used to separate great variety of charged species from low molecular ions upto proteins, membranes, the plasma membrane vesicles from the secretory vesicles (10), and cells (11). However, the optimization of process parameters which influence the separation were needed for the design and development of the method for getting a high resolution.

In spite of its positive applications, free electrophoresis has never gained popularity as a preparative or an industrial separation method owing to the gravity dependent characteristics like thermal convection, electro-osmosis, particle sedimentation, droplet sedimentation, particle aggregation, and electro-hydrodynamic zone distortion.

In order to achieve the required scale-up, scientists and engineers resort to flowing methods (2). Scale-up using electrophoresis is hindered by ohmic heating, which denatures the labile bio-molecules or cells. Another type of mixing problem encountered in free electrophoresis, although less critical compared to Ohmic heating, is the mixing caused by the gas release at the electrodes. This problem can be addressed by employing non-gassing Palladium (Pd) electrode (12) (as in the present work) or membrane-separated electrodes (13) or the more complicated

membrane based system of Tulp et al. (14). Poggel and Melin studied the separation of two dyes (methyl orange and brilliant black) and proteins (Cytochrome C and BSA) with a new free flow zonal electrophoresis device at different conditions, shown the possibility for the continuous operation and method to avoid the thermal convection (15) using a separation volume of less than 20 ml.

A score of methods has been developed to effect free electrophoresis (16). These methods can be broadly divided into static and flowing methods, neither of which has satisfactory capacity for application as a manufacturing tool. Batch and continuous methods have also been developed. In almost all cases maximum sample input rates have been of the order of a few ml/h.

The efficiency of free-flow electrophoretic cell separation can be enhanced by combining with other physical methods (7). In the present work, an electrophoretic extractor, which is a combination of free electrophoresis and multistage extraction, called Electrophoretic Counter Current Extractor (ECCE) was employed for cells/particles separation. This extractor has the advantage of overcoming the problems of free electrophoresis (low convection, less Ohmic heating which causes the denaturation of biomolecules, Lower gas release, high extraction efficiency) and also being a multistage can be used continuously. Mathematical models for this process were developed and experiments for extractions of human Red Blood Cells and Latex particles were performed to verify the predictions of the models by collecting and counting particles in each cavity after fractionation.

MATHEMATICAL MODELING OF ECC EXTRACTION

In an Electrophoretic Counter Current Extractor (ECCE) apparatus, the separation of bioparticles having different electrophoretic mobility is achieved by contacting the buffer solutions of top chambers with bottom chambers containing the bioparticles and then applying an electric field (E) at regular, predetermined intervals. The transfer of electrophoresis technology to Counter Current Extractor involves the evaluation in terms of joule heating, electric field development, and its ability to transfer particles. Modeling studies were undertaken in these areas, and these were followed by experimental studies.

Field Assisted Accelerated Migration

Electrophoretic extraction of cells is a rate process (not an equilibrium process) and the particles having higher electrophoretic mobility are separated ahead of those having relatively lower mobility (17). The physical

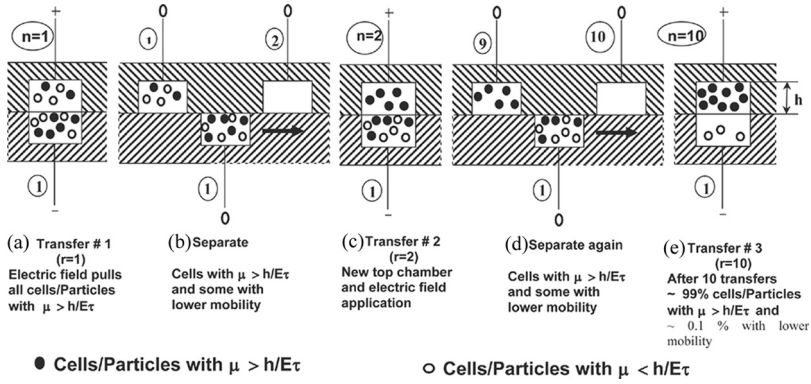

Figure 1. Physical description of multistage extraction of cells/particles.

description of the multistage extraction is shown in Fig. 1. An ECCE apparatus has "n" extraction stages. Let us consider a situation in which all particles of electrophoretic mobility μ_E are initially in one (first) bottom chamber. In the first extraction step, the top and bottom chambers of stage 1 are brought into interfacial contact with each other. Then the field of preselected intensity (E) is switched on. Cells having negative mobility move to the top chambers (having an anode). After applying the field for a predetermined time period (τ), the field is switched off. The bottom cavity is moved to a position to be in contact with a new top cavity having buffer. This process is repeated as many times as necessary to achieve the desired separation. Even when one type of particle is used, the particles usually have an approximately normal electrophoretic mobility distribution and not a single value. However, the average mobility can be estimated under actual conditions.

The quantity of bioparticles initially ($t = 0$) present in the first bottom chamber is denoted by "N". Now let us consider one chamber, whose total depth is "H" and radius is "R" with the bioparticles suspended in buffer solution filling the chamber. When a vertical electric field is applied, the bioparticles move upward as a slug, due to their electrophoretic mobility. Their velocity will be proportional to the applied field. In other words

$$dy/dt \propto E \qquad \text{or} \qquad dy/dt = \mu_E E \tag{1}$$

where the proportionality constant μ_E is electrophoretic mobility and its magnitude is decided by the surface charge of the bioparticles.

Integrating the above equation between the limits $y = 0$ to "h" and $t = 0$ to "τ" results in

$$h = \mu_E E \tau \tag{2}$$

so that a bioparticle of mobilty μ_E moves a distance h in an electric field of intensity E applied for a time of τ. It is obvious that if E is increased τ can be decreased to achieve the same distance of migration and vice-versa, under otherwise similar conditions.

The bioparticles are randomly distributed in space over the volume of the bottom chamber. However, the time required for an individual particle to move into the top chamber increases as its distance from the top surface of the bottom chamber increases, under a given set of experimental conditions of E and τ. That is, the ratio of these heights (h/H) gives the relative number of bioparticles that migrate to the top chamber at any given set of E and τ. To calculate the absolute number of particles transferred to the top chamber, the ratio of the distance to the top of the chamber to the total height "H" has to be multiplied by the concentration of the particles (i.e. number of particles per unit volume, the drag forces as well as gravitational forces are too small to be considered) since the ratio of the heights is nothing but the ratio of the volumes of the chamber corresponding to the location considered.

Therefore, if it were desired to capture the particles with mobility μ_E, located at distance "h" from the top surface of bottom chamber, the number of bioparticles that would migrate during a single step is

$$m = (h/H)(N) = (\mu_E E\tau/H)(N) \tag{3}$$

where $N = (c)(\pi R^2 H)$, "c" is the cell concentration (cells/ml) and $\pi R^2 H$ is the volume of the chamber and $h = \mu_E E\tau$ (from eq. 2). This can be easily visualized from the physical description of the mass balance model shown in Fig. 2. The particles move as a slug and in each transfer step a slice of slug of height eight "h" (containing "m" number of cells/particles) is extracted into the top chamber and in each transfer the slug of cells/particles move by the same distance and hence the same number of cells/particles is extracted in each transfer, until cells are exhausted.

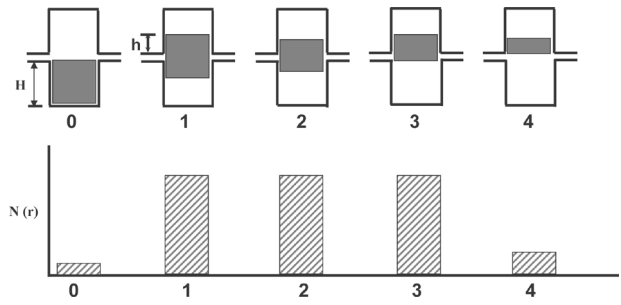

Figure 2. Conceptual description of multistage extraction of cells/particles.

The change in the number of particles in a stage "n" during step "r" will be equal to the number of bioparticles that migrated to the top chamber during step "r". So the general equation can be written for this situation, by material balance, for number of particles extracted in each transfer step.

$$-(N)_{n,r} + (N)_{n,r-1} = (m)_{n,r} \tag{4}$$

where $(m)_{n,r}$ is number of particles with mobility μ_E that migrated from stage "n" during step "r" to the top chamber; N is the total number of bioparticles present in any of the "n" bottom chambers, at $t = 0$;

Now using eq. (3), the eq. (4) can be written as

$$-(N)_{n,r} + (N)_{n,r-1} = (\mu_E E\tau/H)(N)_{r-1} \tag{5}$$

where $(N)_{r-1}$ = Number of bioparticles of mobility μ_E in stage "n" at step $(r-1)$, i.e. $(c\pi R^2 H)$ where c is the concentration of cells. Alternatively

$$(N)_{n,r} = -(\mu_E E\tau/H)(N)_{n,r-1} + (N)_{n,r-1} \tag{6}$$

This is a general equation, which enables us to estimate the number of bioparticles having mobility μ_E in any stage n, provided their concentration is known in the previous stage. The model calculations are shown in Examples #1 and #2, in Appendix 1.

Assuming that the bioparticles are uniformly distributed in the chamber and in each transfer step, they move in a plug flow under the influence of an applied electric field and hence the same number of particles migrate to the top chamber (that are contained by the slug of height "h" of eq. 3), the following material balance equation can be written as

$$(N)_{n,r} = -(r)(\mu_E E_\tau/H)(N) + (N) \tag{7}$$

where "r" is the step number and N is the initial concentration of the bioparticles of mobility μ_E.

From this generalized equation, the number of the bioparticles in a given stage can be calculated directly from the number of transfers, r, instead of step by step sequentially, as shown in Example #3 in Appendix 1.

Heat Transfer

A major problem in scale-up of electrokinetic processes is known to be heating which in turn causes mixing. However, the major advantage of the multistage process is the speed at which it performs separations. Hence our aim is to determine design modifications (such as provision

for proper heat transfer/removal) in order to reduce the adverse effects of heating, while scaling up the process based on the basic laws of heat generation and transmission.

Electrical energy is dissipated as heat according to the equation

$$W = IE/A \tag{8}$$

where W is the power density (watts/cm^3), I is the current (amperes) and E is the electric field strength (V/cm) and A is the area (cm^2) over which the field is applied. For the system which obeys Ohm's law

$$W = I^2/A^2K_E \tag{9}$$

where K_E is the electrical conductivity of the medium (mho/cm or Siemen/cm). It may be noted that the heat generation increases as the square of the current passed. For this reason, nearly all electrokinetic applications are in the most resistant media compatible with the unit operation meaning that low-conductance solutions must be used to have low current in order to operate for longer periods of time.

In an adiabatic stagnant system the temperature gradually increases uniformly with time as

$$\Delta T = \frac{IE\tau}{C_p\rho} = \frac{I^2\tau}{K_E A^2 C_p \rho} \tag{10}$$

where "τ" is the time of application of electric field, C_p is the specific heat of the media carrying the current, ρ is the density of the system (gm/cm^3). The above equation represents the maximum (adiabatic) temperature increase in the electrophoretic system.

For typical conditions shown in Appendix 2, the temperature increase (ΔT) due to application of the given field for a time period of 60 s (which is typical for one extraction step) is 0.03°C. The bioparticles migrate over a distance of 3.0×10^{-2} cm during this transfer step (Appendix 2). In order to migrate to the top chamber, for the bioparticles, 20 such steps are required. The total temperature rise in 20 steps will be 0.60°C which appears to be a tolerable temperature increase.

MATERIALS AND METHODS

The latest version of the Electrophoretic Countercurrent Extractor (ECCE) used was developed by SHOT, INC. which was designed for multistage aqueous two-phase extractions. The unit consists of two plates with opposing right cylindrical cavities into which samples are loaded.

These plates can be rotated with respect to each other and thus the cavities can be aligned or separated as desired (18,19).

The extractor consists of a 22-cavity multi-stage thin layer extraction system of diameter 9 mm (19). Half-cavities oppose each other in disks that are sealed together and rotate with respect to each other. The half cavities are disk shaped, and top cavities have flat tops while bottom cavities have flat bottoms. Both contain palladium (Pd) metal electrodes that produce an electric field when the two cavities are in phase with each other. Each cavity is only a few mm in height so that the fluid within it remains isothermal during the application of an electric field that transfers the separand particles or molecules from the bottom to the top cavity. As each separand is transferred to a new cavity, it is either drawn into the upper cavity by the electric field or left in the lower cavity, depending on its electrophoretic mobility. The assembly of the plates containing the chambers is shown in Fig. 3a. Identically designed plates assure uniform loading and sealing when the plates are clamped together. The experimental samples are loaded and withdrawn through the fill ports located on the side of each plate as shown in Fig. 3b. ECCE was driven by an independent power supply (Lamda, Model #LP-532-FM) for rotating the plates to bring the chambers into interfacial contact with each other. The chambers were powered by a constant-voltage power supply (Hewlett & Packard; Model #6215 A).

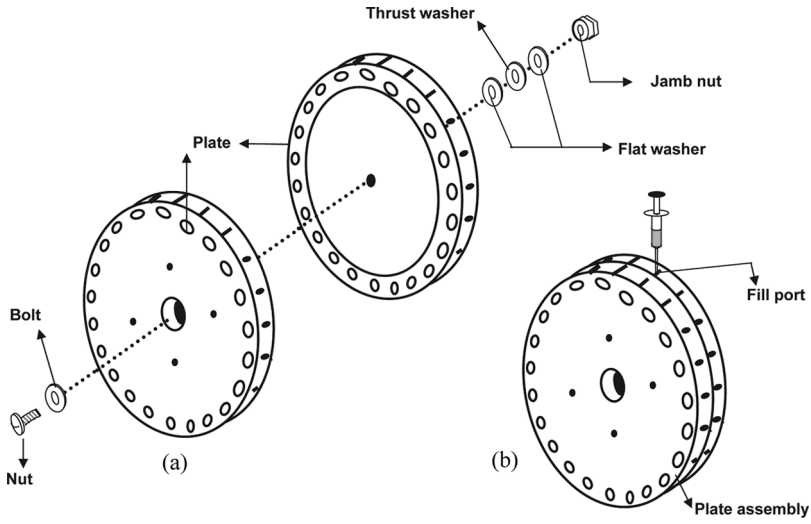

Figure 3. Assembly of multistage extraction plates containing the chambers: (a). Plates, (b). Filling port.

Table 1. Electrophoretic extraction of cells/particles at various field strengths

Sl. No	Initial cells	Transfer #1		Transfer #2		Transfer #3		Residual Cells		Expt Cont	
		Pred	Expt	Pred	Expt	Pred	Expt	Pred	Expt	E (V/cm)	τ (sec)
1	288	17	178	16	56	15	23	239	55	5	30
2	325	39	194	34	74	30	28	222	50	10	30
3	265	64	142	48	64	37	54	116	38	10	60

Two types of particles employed for the study were fixed Human Red Blood Cells (RBC) and Latex particles. The buffer used was 0.01 M phosphate buffer of pH 7.2. The cell/particle count was done using a haemocytometer. All the extraction experiments were repeated and average values (reading of minimum 3 counts) are reported in Tables 1 and 3.

RESULTS AND DISCUSSION

The ECCE unit with Pd metal electrodes was employed for the extraction experiments and used in tests of field generation, electrode performance, cell transfer, and heating. The unique applicability of the present equipment for the determination of electrophoretic mobility of the particles, in addition to cell separation was demonstrated.

Generation of Electric Fields

For initial experiments, the chambers were powered by a constant-voltage power supply, (Hewlett & Packard; Model #6215 A) set for 5 V or 10 V. With distilled water in the chambers the current density remained well below 1.0 mA; with 0.01 M phosphate buffer (pH 7.2) in the chambers, the current stabilized at 5 mA, corresponding to a conductivity of 1.33 mS/cm, which seemed high compared to the measured

Table 2. Counter current extraction without application of electric field

Sl. No	Initial cells	Transfer #1 Expt	Transfer #2 Expt	Transfer #3 Expt	Residual Cells
1	273	178	29.0	5.0	52.0
2	328	200	32.0	4.0	46.0

Table 3. Gravity settled cells/particles subjected to electrophoretic extraction of at various field strengths

S.No	Initial cells	Transfer #1		Transfer #2		Transfer #3		Transfer #4		Transfer #5		Transfer #6		Residual cells		Expt E (V/cm)	Cont τ (sec)
		Pred	Expt	Pred	Expt	Pred	Expt	Pred	Expt	Pred	Expt	Pred	Expt	Pred	Expt		
1	285	34	21	30	33	27	30	23	28	21	12	18	12	132	155	5	60
2	258	69	50	52	61	39	54	30	40	23	35	17	30	55	21	10	60
3	426	51	85	45	82	40	72	35	75	31	85	27	25	198	36	5	60
4	426	102	140	78	145	59	146	45	80	34	76	26	70	82	22	10	60
5	406	97	96	74	95	56	40	43	40	33	55	25	40	10	60	10	60

1,2 – Fixed blood cells : 3,4 – Latex particle 3.6 μm : 5 – hLatex particle 2.6 μm.

conductivity of 0.36 mS/cm. The applied electric fields were calculated using $E = I/k_E A$, which gives 1.85 V/cm.

Electrode Performance

Application of an approximately 5 V/cm electric field for up to 600 s did not produce any escaping electrolysis gases in case of distilled water. For 0.01 M phosphate buffer at >180 s and >600 s of 10 V/cm and 5 V/cm respectively showed the gassing near the electrode.

Electrophoretic Cell Transfer

Fixed human red blood cells, in the concentration range 90–245×10^4/ml, were placed in suspension in 0.01 M phosphate buffer (pH 7.2) in the lower cavity of stage-1 in a total volume of about 0.4 ml. A field of known intensity (5 or 10 V/cm) was applied for a specified time period (30 or 60 s). Then a fresh top chamber with buffer only was aligned with bottom chamber of stage 1. This process was repeated until several transfers had been completed. Cells were then removed from the top cavities, and the fraction of the original population transferred at each step was obtained by counting the suspended cells with a haemocytometer. In the preliminary experiments, the numbers of cells extracted into the top cavities (experimental values) were much higher than the predicted ones as shown in Table 1. Swapping of the liquids occur at liquid surfaces of respective chambers in a time period, when the chambers approach each other for extraction and depart after the transfer. The schematic diagram indicating the swapping of the liquid and flow pattern during the alignment and separation of the chamber is shown in Fig. 4. The swapping of liquid and enhanced mass transfer associated to such hydrodynamic flow for similar equipment was reported by Pollmann (20). In order to assess the magnitude of cell migration due to this phenomenon, a few control runs were performed without the application of an electrical field. The results are shown in Table 2. A considerable number of cells were found to migrate due to the hydrodynamic flow during the transfer steps, especially in the initial ones. This problem was alleviated by giving sufficient settling time for the cells in the lower cavity and by considerably reducing the speed at which the cavities align to each other during the transfer steps. Now when an electric field was applied, the cells move as a slug as described by the physical model of electrophoretic extraction in the earlier sections and also as shown in Fig 2. The electrophoretic extraction runs of fixed RBC and the latex particles are shown in Table 3. From this table, it can be noted that when the field

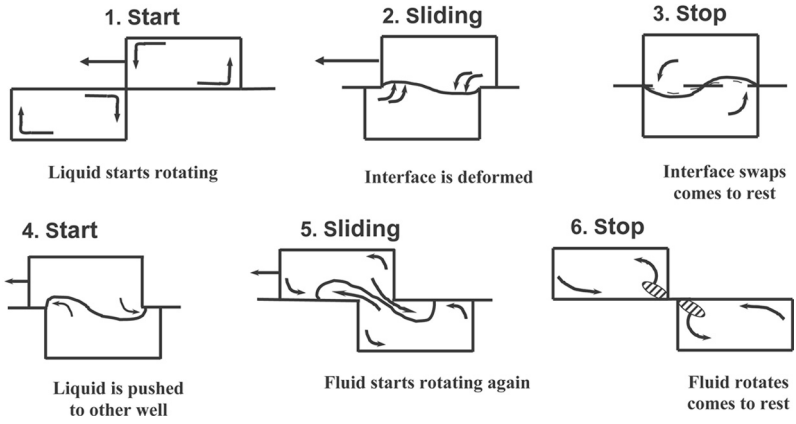

Figure 4. Schematic diagram indicating the swapping of liquid and flow pattern during alignment and separation of chambers.

strength was doubled the cell extraction was completed in much less number of transfers. For instance in the case of latex particles (size 3.6 μm) at 5 V/cm field strength about 450 particles were extracted in 5 transfers and when field strength was increased to 10 V/cm the similar number was extracted in 3 transfers only (Table 3). This can be appreciated from the fact that in eq. (3), the cell transport velocity increases proportional to the applied electric field strength under otherwise similar conditions.

The parity plot of the predicted and experimental values of the electrophoretically extracted cells/particles is shown in Fig. 5. The agreement can be seen to be good for fixed blood cells than for the latex particles, especially those of higher size range. The lower agreement for the latex particles over RBC could be attributed to higher interaction of RBC with buffer over the latex particles. Similarly, the higher surface energies of the latex particles coupled with mild gassing perhaps is contributing to the higher deviation in case of latex particles than RBC. The electrophoretic mobility values (cm^2/V-s) of RBC is matching with literature values more closely than latex particles may be for the same reasons.

Heating

Heating of the buffer causes convection current, which would have more effect on the swapping of smaller particles to the top chambers during transfers. Heating might also lower the effective field strength due to the variation in its conductivity with temperature. In order to overcome this problem, the

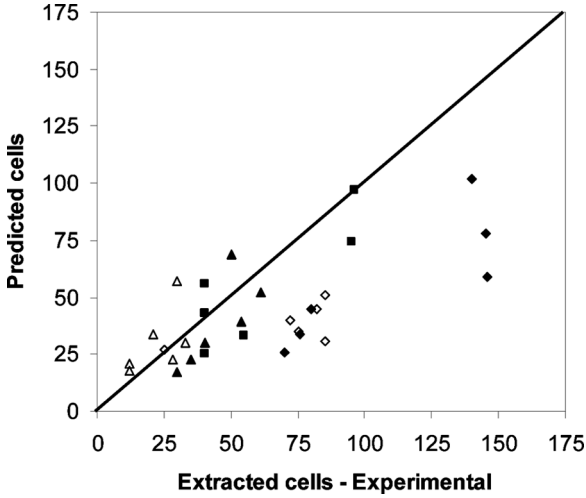

Figure 5. Parity plot for the electrophoretic extraction of cells and particles. ◆ Latex particles (3.6 μm); ■ latex particles (2.6 μm); ▲ fixed blood cells (4.6 μm).

strength of the phosphate buffer was reduced from 0.01 M to 0.002 M. It should be noted that the electrophoretic mobility of any given species increases with a decrease in ionic strength. This enhancement at lower concentration enables the separation of cells/particles whose mobilities are only slightly different from each other. Theoretical values of temperature increase (predicted values) were calculated using buffers of known conductivity and compared with exact measurements. In 0.01 M phosphate buffer, $K = 3.6\,mS/cm$, which is considered a high conductivity buffer for use in an electrophoretic instrument, a field of approximately 5 V/cm was applied. The Joule-heating calculation

$$\Delta T = (IE/C_p\rho)\tau = 17.24 \times 10^{-3}\tau \tag{11}$$

The above relationship was counterchecked by measuring the temperature with a thermistor probe over a 2-minute period (twice the typical time period of one electrophoretic transfer) to observe a rise from 23 to 24.2°C in 120 s and obtaining the following linear relationship

$$\Delta T = (1.2^\circ C/120\,s) = 10.0 \times 10^{-3}\tau \tag{12}$$

Interestingly the observed temperature rise was less than the expected rise.

This relationship scales linearly with conductivity and applied field. The actual experimental profile consisted of a rise from 23.0 to 24.2°C

Table 4. Electrophoretic mobility of cells/particles

Type	Size(μm)	ECCE Value (cm^2/V-s)	Literature Value (cm^2/V-s)
Fixed RBC	4.6	2.2×10^{-4}	2.0×10^{-4}
Latex particles	3.6	2.45×10^{-4}	2.2×10^{-4}
Latex particles	2.6	2.71×10^{-4}	1.0×10^{-4}

in 120 s. In this extreme case a temperature rise of 0.6°C per transfer (60 s of field application) could be expected; in 20 transfers (a typical experiment) the total temperature rise would be 12°C typically from 23 to 35°C. Thus when low-conductivity buffers are used there is no obvious reason to resort to thermoregulation since this temperature rise will be much lower.

One significant application of the present ECCE is direct measurement of electrophoretic mobilities as demonstrated in the present work. Capillary zone electrophoresis (CZE) is normally used to measure the electrophoretic mobility of solutes in free solution. Several undesirable features of CZE are absent in the present version of ECCE, namely

(a) Limitation to solutes since particles can not be evaluated,
(b) Need of a calibration standard due to electroosmotic back flow,
(c) Non-absolute mobility values as all measurements are relative,
(d) Small sample volume and hence can not provide recoverable amounts of separands, and
(e) No recovery of fractions is possible as both the ends of the capillary are submerged in buffer. In the present case the cells/particles that move electrophoretically to the top chamber are collected and counted. Using the eq. (3) the electrophoretic mobility (μ_E) can be estimated, as "m" is experimentally determined and other parameters except μ_E, are known.

In order to confirm the validity of this approach, the electrophoretic mobilities of fixed RBCs, which were well confirmed in literature, are estimated by this method. The values of the estimated mobilities of different cells/particles are shown in Table 4.

CONCLUSIONS

A combination of free electrophoresis and multistage extraction called ECCE was performed for the extraction of fixed RBC/Latex particles.

It offers several advantages, for instance, being a multistage process, it enables the fractionation of cells and easy collection of samples. Due to a short migration distance involved, the time of the electric field application in each transfer is less and hence the rise in temperature in turn would be less. From the experiments it is inferred that two versions of electrokinetic ECCE could be considered to address the problem of gas release at the electrodes

1. Low cost metal-electrode version (platinum and palladium electrodes) or more expensive version with membrane electrodes and
2. Electrode buffer recalculation.

The proposed mathematical model agrees satisfactorily with the experimental results of the extraction using fixed red blood cells and latex particles. The ECCE extractor could be used to measure the electrophoretic mobility of cells.

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APPENDIX I

Model Mass Transfer Calculations:

Consider the following values of the different parameters
Mobility of the bioparticles $\mu_E = 2.0 \times 10^{-4}\,cm^2/V\text{-}s$
Field, $E = 5\,V/cm$
Height of each chamber, $H = 0.5\,cm$
Time duration of application of electric field, $\tau = 1s$ and $5s$ per transfer (variable depending on the requirement)
Number of particles, $N = 200/ml$
Example #1: $\tau = 30s$

Step 1 (i.e. r = 1)
Using equation (6)

$$(N)_{n,r} = -(\mu_{1E} E\tau/H)(N)_{n,r-1} + (N)_{n,r-1} \quad (6)$$

$$(N)_{n,1} = -(2.0 \times 10^{-4} \times 5 \times 30/0.5)(200) + 200$$
$$= -12 + 200 = 188$$

Step 2 (i.e. r = 2)

$$(N)_{n,2} = -12 + 188 = 176$$

And this can continue as long as we do not change the operating conditions (i.e., either τ or E), and 12 particles (or 6%) of bioparticles will be electrophoretically extracted into the top chamber during each transfer step.

Example #2: $\tau = 30$s

Other conditions remaining the same Step 1 (i.e. r = 1)
Using equation (6)

$$(N)_{n,r} = -(\mu_{1E} E\tau/H)(N)_{n,r-1} + (N)_{n,r-1} \quad (6)$$

$$(N)_{n,1} = -(2.0 \times 10^{-4} \times 5 \times 30/0.5)(200) + 200$$
$$= -24 + 200 = 176$$

Step 2 (i.e. r = 2)

$$(N)_{n,2} = -24 + 176 = 152$$

so that 24 (12% of total) particles are electrophoretically extracted at each transfer.

Example #3: All conditions are same as example #2

$$(N)_{n,r} = -(\mu_{1E} E\tau/H)(N) + (N) \quad (7)$$

Directly calculating for Step #3 (r = 3)

$$(N)_{n,.3} = -(3)(2.0 \times 10^{-4} \times 5 \times 60/0.5)(200) + 200$$
$$= -3 \times 24 + 200 = 128$$

So 128 particles remained after 3 transfers in the 'n' th stage.

APPENDIX II

Model Heat Transfer Calculation:

Low conductivity buffer

$E = 5\,V/cm$

$k = 100\,\mu S/cm = 0.1\,mS/cm$

That is $I = 0.5\,m\ A/cm^2$

or $I = 0.5 \times 0.785 = 0.393\,mA$

$C_p = 0.99\,cal/gm^{\circ}C$

$\rho = 1\,gm/cm^3$

From the equation (10)

$$\Delta T = [0.393 \times 10^{-3} \times 5](t)/[0.99 \times 4.14 \times 1]$$
$$= [0.48 \times 10^{-3}](t)$$

For $t = 60s$

$$\Delta T = 0.03\,^{\circ}C$$

Referring to Mass transfer model (Appendix I)

$$h = \mu_E E \tau$$
$$= 10^{-4} \times 5 \times 60 = 3 \times 10^{-2}\,cm \quad (2)$$

High conductivity buffer and same other conditions as above

$k = 3600\,\mu S/cm = 3.6\,mS/cm$

That is $I = 18\,m\ A/cm^2$

or $I = 18 \times 0.785 = 14.13\,mA$

$$\Delta T = [14.13 \times 10^{-3} \times 5](t)/[0.99 \times 4.14 \times 1]$$
$$= [17.238 \times 10^{-3}](t)$$

For $t = 60\,s$

$$\Delta T = 1.03\,^{\circ}C$$

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