PREPARATIVE FREE-FLOW ELECTROPHORESIS OF PROTEINS, PEPTIDES AND RELATED COMPOUNDS

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For preparative electrophoresis particularly continuous methods are of interest, as they guarantee a high throughput. One of these methods is free-flow electrophoresis.

Free-flow electrophoresis is a continuous carrier-free electrophoretic method. The separation medium is either a uniform buffer or a discontinuous electrolyte system flowing through the separation cell as a thin fluidal film. Under the force of the electrical field applied rectangular to the flow direction, the substances to be separated are arranged into separated zones according to their mobilities or their different values of pI. The separated zones are continuously collected at the end of the separation cell by a fraction collector.

The applications of free-flow electrophoresis include the separation and concentration of inorganic ions, organic ions, peptides, proteins and enzymes as well as the separation of cells.

Memo:

SCALE-UP OF THE FREE FLOW ELECTROPHORESIS DEVICE

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There are two important obstacles which inhibit scale-up of the CPE: Thermal (buoyant) destabilization and inefficient use of the applied electric field. However, the magnitude these problems can be reduced by carefully examining their origins and then removing them from the device.

For instance, the former problem is overcome by redesigning the device to eliminate the unfavorable thermal gradients which drive the buoyancy instability. This is accomplished by modifying the thermal profiles in the CPE in such a way that they satisfy a sufficient condition for stability in every part of the chamber.

The latter problem is reduced by internally recycling the solute through the CPE. Recycling solute N times through the CPE chamber enables reduction of the power input by N^2. This second modification also reduces the dispersion due to crescent formation and solute density gradients found during operation of the device.

When these two ideas are combined into one device, our model predicts that scale factors of Order (1000+) over the General Electric CPE (Saville and Ostrach) are possible.

Memo:

AN EVALUATION OF A VIDEO IMAGE CORRELATION TECHNIQUE FOR THE ESTIMATION OF ELECTROPHORETIC MOBILITIES OF HUMAN BLOOD CELLS

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An apparatus is described for the automated measurement of electrophoretic mobility. This apparatus is attached to standard cell electrophoresis equipment (Mk.3, Rank Brothers, Cambridge, UK.). The composite video signal is analysed by a cell detector and correlator unit (Malvern Instruments, UK.) which is interfaced to a microcomputer providing control and data processing facilities.

We have demonstrated the accuracy and reproducibility of the apparatus in the determination of the velocity of monodisperse cell suspensions, e.g. the mobility of fresh, washed, human erythrocytes is routinely measured as -10.91 +/- 0.16 TU, in excellent agreement with previously published values. The wider application of this electrophoresis apparatus, ie. the estimation of velocity distributions of polydisperse cell suspensions, has been investigated using human peripheral blood lymphocytes as a model system. The apparatus has been shown to detect two populations of cells in the lymphocyte sample, one of about 30% with a mobility of -8.5 TU, and another of about 70% with a mobility of -11.5 TU, agreeing with the normal ratio of T and B lymphocyte populations. Measurements on clinical specimens show a good correlation between fast and slow velocity populations and the proportions of T and B lymphocytes estimated by rosetting or monoclonal antibody techniques.

Memo:

APPLICATIONS OF AN AUTOMATED CELL ELECTROPHORESIS EQUIPMENT WITH HIGH RESOLUTION - AN OVERVIEW

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More widespread consideration of cell electrophoresis as a clinical diagnostic tool and as an aid to basic biomedical research depends on the availability of equipments for performing measurements accurately and quickly. The automatic single cell electrophoresis (ASCE) realized by the PARMOQUANT equipment is used by several working groups for investigations in different fields of biomedical research. An overview about recent results with PARMOQUANT equipments is given.

Characterization of lymphoid cells, detection of in vivo and in vitro influence of different substances which was also used for characterization of anti-lymphocytic sera and immune drugs proved suitable applications. The throughput of the PARMOQUANT makes it suitable for routine investigations of lymphoid cells from patients with different diseases as well as in research programs which need many repeated measurements. By means of a computer program for analysis of cytopherograms we have the possibility for detection of small changes in the electrophoretic behaviour of subpopulations of lymphoid cells.

It can be concluded that the resolution of cell electrophoretic measurements by means of the presented ASCE equipment is now limited by the Brownian movement of the single cell.

The usefulness and limitation of simultaneous measurement of electrophoretic mobility, sedimentation coefficient and area of each single cell is discussed.

Memo:

ANALYSIS OF LYMPHOCYTE MOBILITY IN TUMOR BEARER BY A FULLY AUTOMATED ANALYTICAL INSTRUMENT

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Conventional instruments used for cell electrophoresis were operated so manually that it was difficult to obtain reproducible result. More recently, analytical instrument has been fully automated, from which the mobility histogram as well as the mean mobility was obtained by counting objectively through the opto-electronic image analytical system and rendered capable of measuring quantitative analysis.

With this automated instrument (Parmoquant), it was observed that the mobility histogram of peripheral lymphocyte shifted to the lower mobility zone in tumor-bearing (X5563-C3H/He) mouse. These phenomena are thought to be common as it was observed in other tumor-bearing mice or cancer patients. The mobility histogram of splenocyte in C3H/He mouse exhibited two main peaks. Nylon wool-passed cell corresponding to T cell showed one peak with the high mobility. Adherent cell showed two peaks, the high and low mobility peaks corresponding to T and B cells respectively. However, adherent T cell has lower mobility than passed T cell. The mobility histogram of KLH-specific suppressor T cell line originated from C3H/He mouse was located in the peak between normal T and B cells. From the results, it is postulated that nylon-passed T cell has higher mobility than a kind of suppressor T cell. Aberration of the mobility histogram pattern in the tumor bearer is thought to be mainly due to an increase in the low mobility T cell. The nature and function of T cell subset with the low mobility will be discussed.

Memo: