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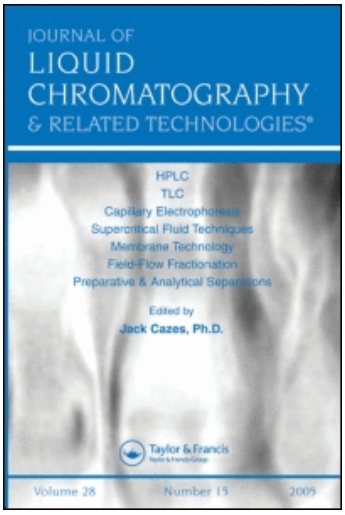

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PERFORMANCE AND ECONOMICS IN MICROPREPARATIVE CAPILLARY ELECTROPHORESIS OF OLIGOSACCHARIDES

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ABSTRACT

The advantages of micropreparative capillary gel electrophoresis include very high resolution, high speed and good recovery, with full automation. Nanomolar quantities of biopolymers, such as complex carbohydrates, can be collected for use in subsequent microsequencing or mass spectrometry. As in other preparative separation processes, the goal in preparative capillary gel electrophoresis is to maximize the production of a product with a given purity in the shortest time, i.e., to achieve the highest throughput. Another important factor is the economics of the operation. The cost of production in preparative capillary electrophoresis is a function of the cost of the automated system, the loading capacity of the capillary column and the system cycle time. Here we suggest a simple economic model for analyzing the economics of preparative capillary electrophoresis.

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INTRODUCTION

Capillary electrophoresis is predominantly an analytical tool[1] but also can be used for micropreparative purposes.[2-15] The feasibility of micropreparation was demonstrated in the early work of Hjerten et al.[2] They used open tubular capillaries, employing sweep liquid at the outlet end of the capillary, to move the sample components into a conventional HPLC detector and, subsequently, into a fraction collector. By taking advantage of the electroosmotic flow, the liquid containing the sample components could be easily moved from the capillary into the fraction collector.[3] Others used porous glass junction with no flow interruption during fraction collection.[9] Zare and co-workers employed an on-column frit which enabled them to collect fractions without interrupting the flow and the electrophoretic process.[4] Karger and coworkers[10] suggested the use of sheath flow assisted design that is able to collect fractions with uninterrupted applied electric field. The preparative capability is enhanced by employing wider bore capillaries, e.g., 0.2 mm i.d. or larger. Using gel-filled capillaries, with no electroosmotic bulk flow, the end of the capillary is dipped into a small collection vial containing 1-5 μL of water or buffer and acts as collection device.[7-12] Field programming can also be very useful for precise preparation of a single peak from the closely migrating other components in a complex mixture.[12,13] Just recently, Rush and coworkers[14] reported on multiple sequential fraction collection of peptides and glycopeptides by CE under applied voltage. Biologically active species, peptides, proteins or DNA fragments, could be easily collected with high recoveries for microsequencing as well as other microcharacterization methods, using capillary electrophoresis in either open or gel-filled capillaries.[8,12,13,15] Some of the commercially available capillary electrophoresis units, however, are equipped with automated fraction collectors and, thus, feature micropreparative capability.[16]

In analytical work, the objective is to obtain information on the composition of the sample and to separate a large number of components in a short period of time. In preparative work, however, the goal is often the purification of only one or several components; therefore, the separation of the other components in the sample may be unimportant.[17,18] Consequently, in preparative applications, the electrophoretic parameters must be optimized differently than in analytical work.

One of the most important factors in preparative capillary electrophoresis, besides the technical parameters and variables, is the economics of the system. It is important to note that, in preparative capillary electrophoresis, the amount of the collected material is small (nano- or micromolar range), but its value may be high enough to consider microfraction collection. Therefore, cost should also be taken into account in the optimization of the parameters of preparative capillary electrophoresis. Capillary electrophoresis processing costs are

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functions of the expense of the automated system, the loading capacity of the capillary column and the system cycle time. In this paper, we suggest an economic model for evaluating the efficiency of preparative capillary electrophoresis, based on loading capacity, product purity, yield, throughput and operating costs.

MATERIALS AND METHODS

Apparatus

In all these studies, the P/ACE™ system 5500 capillary electrophoresis apparatus (Beckman Instruments, Inc., Fullerton, CA, U.S.A.) was used in reversed (cathode on the injection side) polarity mode. The separations were monitored on-column by laser induced fluorescent (LIF) detection, employing a 4 mW Argon-ion laser with excitation wavelength of 488 nm/emission 520 nm. The temperature of the cartridge holding the gel filled capillary column was thermostated at 20°C ± 0.1°C by the liquid cooling system of the P/ACE instrument. The electropherograms were acquired and stored on an IBM 486/66 MHz computer and were evaluated with the System Gold™ software package (Beckman Instruments, Inc., Fullerton, CA, U.S.A.).

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Procedures

In the capillary gel electrophoresis experiments, the eCAP N-linked Oligosaccharide Profiling Kit (Beckman Instruments, Inc., Fullerton, CA) was used in conjunction of a PVA-I coated capillary column with a 20 cm effective length (27 cm total length, i.d. 50 μm /o.d. 375 μm). The samples were injected by pressure (0.5 psi, 5 sec) in the case of analytical or electrokinetically (10 kV for 10 sec) in the case of preparative load and reinjection of the collected fraction into the capillary. The fraction collection was accomplished by field programming, so the applied electric field strength was deceased from 1000 V/cm to 100 V/cm during collection in order to obtain higher precision.[8]

Chemicals

The maltooligosaccharide test mixture was labeled by 1-aminopyrene-3,6,8-trisulfonate (APTS) (both form Beckman Instruments, Inc., Fullerton, CA) via reductive amination as described elsewhere.[19] Fractions were collected into sample micro-vials containing 4 μL water.

The samples were stored at -20°C or freshly used. All the buffer solutions were filtered through a 0.20 μm pore size nylon filter and carefully vacuum degassed (100 mbar) before use.

THEORY

Maximum resolution in capillary gel electrophoresis for a given mixture requires optimization of the operational parameters such as effective column length, applied field strength, temperature, as well as composition of the buffer and the gel.[12,20,21] Resolution of any two components by capillary electrophoresis in a mixture is given by:[16]

$$R_S = 0.18\ \Delta\mu_e(E\ l\ /D\ \bar{\mu}_e)^{1/2} \qquad (1)$$

where $\Delta\bar{\mu}_e$ is the electrophoretic mobility difference between the two substances and μ_e is the arithmetic mean mobility for those species, E is the applied electric field, l is the effective length of the capillary and D is the diffusion coefficient of the solute in the gel-buffer system. Equation 1 demonstrates that, in capillary electrophoresis, higher potential gradients should lead to higher resolution. However, higher applied voltage increases Joule heating which, in turn, increases solute diffusion.

A concomitant increase in band spreading results in decreased separation efficiency. With longer effective column lengths, using the same applied electric field, the separation power can be increased in the price of longer migration time. Lower diffusion coefficients, due to lower temperature or higher media viscosity (higher polymer concentration and/or molecular weight), could also result in increased resolution. These separation variables must be optimized together with the preparative variables, such as sample load, yield, recovery, throughput and economics.

Similarly to preparative HPLC,[17,22] economical efficiency (E_f) in preparative capillary gel electrophoresis can be defined as the ratio of profit (P_r) and electrophoretic cycle time (t_c):

$$E_f = P_r/t_c \qquad (2)$$

The difference between produced value and production cost gives profit. With production cost, feed value and product value related to unit mass of feed, economical efficiency can be expressed as a function of throughput, T_p:

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$$E_f = T_p(v_p - c_E) - v_o m_o/t_c \quad (3)$$

where $T_p = m_p/t_c$ and m_p is the mass of product. v_p and c_E are the specific value of the product and the specific cost of the electrophoretic procedure; v_o and m_o are the specific value and mass of the feed with respect to the product. Although Equation 3 indicates that throughput may be increased by increasing m_p, this should not be at the expense of product purity, since lower purity reduces v_p and requires recycling of the overlapped fractions. Throughput can also be increased by decreasing the cycle time (t_c) of the process that strongly depends on the migration time of the solute, the recycle/re-equilibration time and the injection (feed) time. As long as the desired resolution between product and impurities is achieved, recycle/ re-equilibration time can be decreased in order to reduce cycle time. For example, the feed time can be decreased by increasing the injection voltage during electrokinetic injection.

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Equation 4 shows the throughput as a function of feed (m_o):

$$T_p = \frac{m_0}{t_c}\left[1 - (1 - p_0)r - p_0(1 - y_0)\right] \quad (4)$$

Key parameters in this equation are the cleanup ratio, r (mass of removed impurities/total mass of impurities in the feed) and yield, y_o, of the desired product. Using these terms, the mass of impurities removed and the loss of the desired component can be written as: $m_o(1 - p_o)r$ and $m_o p_o(1 - y_o)$, respectively. In these expressions $p_o = m_m/m_o$ is the purity of the initial feed and m_m is the mass of desired product in the feed.

RESULTS AND DISCUSSION

As a model, we have examined the capillary gel electrophoretic separation of APTS-labelled malto-oligosaccharide mixture in which the components differ only by one glucose unit between each other. Figure 1 shows the electropherograms of the malto-oligosaccharide test mixture in analytical (A) and preparative (B) injections as well as the reinjection of the collected glucose nonadekamer, (19-mer) (C) and octadekamer, (18-mer) (D). It is apparent that, in the analytical separation mode, all the components are well separated (Figure 1, trace A), but in the preparative mode (see overloading in Figure 1, trace B), they mostly overlap with each other. They even form doublets, suggesting that the local solute concentration is, probably, less than two orders of magnitude

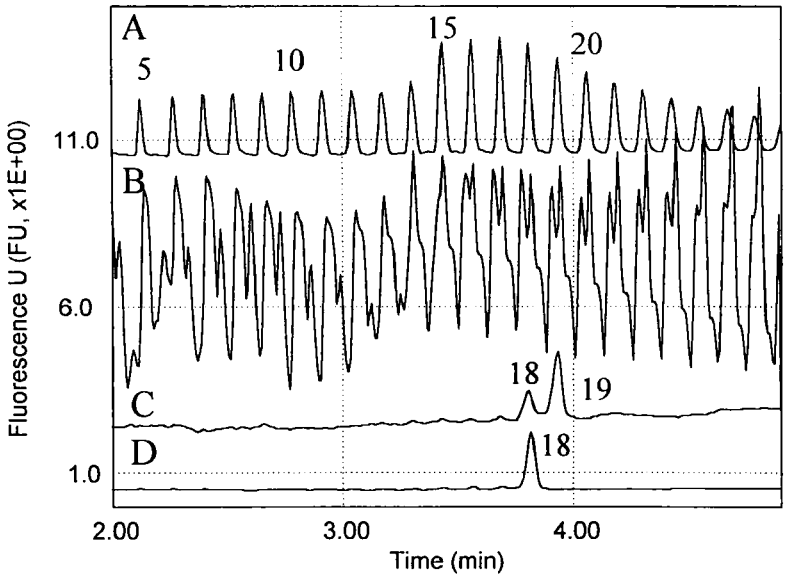

Figure 1. Capillary electrophoresis traces of the APTS-labelled malto-oligosaccharide test mixture in analytical (A) and preparative (B) separation modes and the re-injection of the collected nonadekamer, (19-mer) (C) and octadekamer, (18-mer) (D). Capillary: 270 x 0.05 mm (effective length, 200 mm); buffer, 25 mM acetate, pH 4.75; 0.4% polymeric additive; applied field, E = 1000 V/cm. The applied electric field during the fraction collection was 100 V/cm. Temperature: 20°C; LIF detection: excitation: 488 nm, emission: 520 nm. Injections: (A) 5 sec pressure, (B, C, and D): 10kV, 10 sec.

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different from the background electrolyte concentration.[23] Figure 1, trace C shows the reinjection of the collected 19-mer. Figure 2 shows that the collection of the glucose nonadekamer (19-mer) began at point t_{19} - w_A and ended at point t_{19} + w_B (w_A and w_B are distances on the graph). Here, a fraction, but not all of the very closely migrating (m_{r18}) of the total mass of the 18-mer, is eliminated as the 19-mer is collected. The removed mass of 18-mer (m_{r18}) can be calculated:[17]

$$m_{r18} = \frac{m_{18}}{A_{18}} \int_{t=0}^{t_{19}-wA} c_{18}\,dt \qquad (5)$$

where A_{18} is the total peak area of the 18-mer (concentration x time units) and c_{18} is the concentration at time t. Although, it is not the case here, the fraction of any closely migrating succeeding peak (e.g. 20-mer) could be calculated

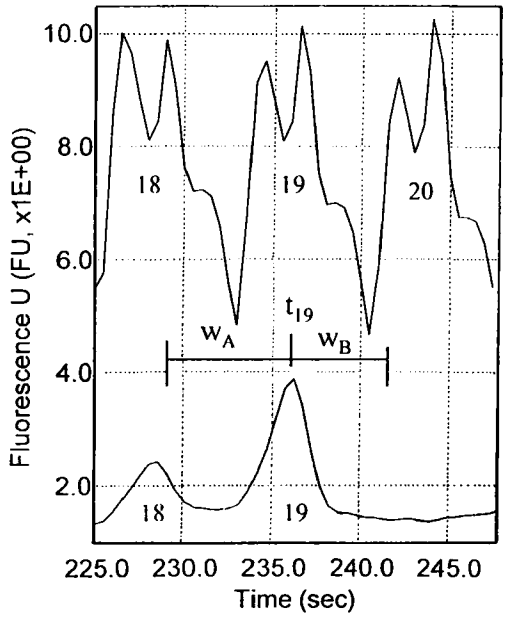

Figure 2. Preparative (upper trace) capillary electrophoretic separation of the APTS-labelled malto-oligosaccharide test mixture and the re-injection of the collected glucose nonadecamer (lower trace). The migration time of the 19-mer is t_{19}; w_A is the start and w_B is the end time of the fraction collection, respectively. Conditions are the same as in Figure 1.

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likewise. Thus, the cleanup ratio for the 19-mer, which is contaminated only by the components immediately preceding and following, can be expressed as:

$$r_{19} = \frac{m_{r18} + m_{r20}}{m_{18} + m_{20}} \qquad (6)$$

Figure 1, trace C and Figure 2, lower trace, show the analytical electropherogram of the fraction collected between points w_A and w_B. As expected from Fig.2 and Equations 5 and 6, the main peak is the 19-mer and the small preceding peak is the 18-mer.

As Equation 1 shows, in capillary gel electrophoresis, resolution can be increased by increasing the applied voltage and the effective column length,[16] or by decreasing the diffusion coefficient of the solute by using higher concentration and/or molecular weight polymer in the separation buffer. Also,

decreasing the time window of the fraction collection ($w_A + w_B$) will also increase the degree of purification at the expense of yield. Figure 1, trace D shows the very precise collection of the octadekamer (18-mer) with a decreased time window [$(w_A + w_B)_{18} < (w_A + w_B)_{19}$].

Electric field programming has been proven very useful in capillary electrophoresis fraction collection.[12,19] Then, a higher field, which is employed prior to and after the collection of the desired peak, can also be used to improve collection. The field can be decreased to a low value (usually 1/10 of the separation field strength) during collection without introducing significant band broadening.[12] Using this approach, high resolution can be accomplished and maintained during fraction collection in a simplified way due to the wider time window.

For the calculation of the throughput (Eq. 4), one needs to know the overall cleanup ratio of the purification process. Based on the example above, and considering that m_j is the mass of the j-th impurity, the overall cleanup ratio, the sum of all cleanup ratios, can be expressed as:

$$r = \sum_{i=1(\neq k)}^{n} r_i = \frac{\sum_{i=1}^{k=1} \frac{m_i}{A_i} \int_{t_0}^{t_k - w_A} c_i dt + \sum_{i=k+1}^{n} \frac{m_i}{A_i} \int_{t_k + w_B}^{t_T} c_i dt}{\sum_{j=1(\neq k)}^{n} m_j} \qquad (7)$$

where n is the number of components, k is the required component and t_T the total separation time.

Sometimes, the required amount of material cannot be collected in a single separation step, or the purity of the collected fraction is not satisfactory. In both cases, further purification/collection steps are required and the overall economical efficiency of the process will depend on the efficiency of all the steps. When the same separation is repeated over and over, the overall economical efficiency of preparative capillary electrophoresis, E_f, is the same as that of a single cycle and the overall economical efficiency is independent of the value of the intermediate products. When production of the desired component requires several different separation steps, Bellmann's principle of optimality can be applied to optimize the total efficiency of the overall purification process.[24]

The minimum cost of purification in multiple electrophoretic steps can also be expressed in terms of yield and cleanup ratio:

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$$\sum_{k=1}^{n} \frac{\sum_{i=1}^{n} T_{pi} c_i}{r_k} + \sum_{k=1}^{m} \frac{\sum_{i=1}^{n} T_{pi} c_i}{y_k} = 0 \qquad (8)$$

where n is the number of electrophoretic procedures. This equation shows that either the cleanup ratio (r) or the yield (y) can be adjusted (at the expense of the other) to achieve the optimum.

Throughput, specific cost and profit should be optimized as a function of all independent variables in order to achieve maximum economical efficiency. All variables discussed above will affect the viability of the purification process.

CONCLUSIONS

In this paper, an economic treatment is presented for the evaluation of preparative capillary electrophoretic procedures. Compared to conventional slab gel electrophoresis, micropreparative capillary electrophoresis offers the advantages of higher speed (minutes), recovery and resolution, combined with automation. If non-denaturing gel systems are used, even biologically active species can be collected without losing their activities. Rapid preparative separations with wider bore capillary columns are possible using an efficient cooling system. Multiple injections can also increase production. Using a gel-filled capillary column with optimal conditions, pure fractions can be conveniently collected up to the low microgram range. Commercially available instruments, equipped with fraction collectors that are computer controlled, are necessary for routine operation.

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