CONTINUOUS ELECTROEXTRACTION OF AMINO ACIDS USING POLY(ETHYLENE-GLYCOL)/CASEINATE AQUEOUS TWO PHASE SYSTEM

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ABSTRACT
In this paper we report on a method of continuous electroextraction of amino acids as model sample using caseinate/poly(ethylene-glycol) - PEG - two phase system in a microchip able to separate compounds based on their differences in electrophoretic mobility and solvent affinity. Fundamentally, the phase boundary replaces a physical membrane, suppressing the diffusion. When external potential is applied, the molecules cross this barrier selectively. The selectivity of the amino acids extraction is the result of their electrochemical properties and applied voltage.

KEYWORDS: Aqueous two-phase system, electroextraction

INTRODUCTION
This paper presents a membrane free microfluidic chip for continuous electroextraction of amino acids, performed using an aqueous two phase system. The first was caseinate solution, the acceptor phase of the extraction, while the second was poly(ethylene-glycol) (PEG) solution, the donor phase. Our chip was able to perform separation in short channels, using the phenomenon of partitioning of amino acids in aqueous two phases system [1] in conjunction with electrophoretic migration. Most of previously the published work on extraction [2] or dialysis were based on membranes, adding an additional step to the chip fabrication protocol. The absence of membranes in the method described in this contribution simplifies the preparation of the chip and its adaptation to different conditions and target compounds.

THEORY
In the absence of an external electric field, the molecules will diffuse between the donor and acceptor phase according to the affinities. In the presence of an external electric field, the charged compounds will migrate either along or against the field, depending on their charge. By setting the external electric field below the threshold value the molecules get stuck at the phase boundary due to the interfacial tension between the phases. They will remain in the phase that they have a higher affinity for. Once the field strength reaches the threshold, the molecules will be able to cross the boundary and migrate from the donor phase to the parallel acceptor phase. This threshold value is a function of the electrochemical properties of the compounds. Both the distribution constant (K_d) and the electrophoretic mobility influence the separation. The system then performs an electroextraction process with the phase interface replacing a physical membrane. The main difference of the design presented here in comparison to other chips presented in literature is the use of collector channels. This role is performed by the channels 1 and 5 (Figure 1a), placed perpendicularly to the flow. The external electric field is applied across these channels, and the extraction takes place in the region indicated by the dotted square in Figure 1a

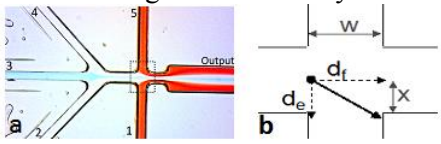


Figure 1. a) Photograph of the chip used in the extraction with the three streams in different colors. The region indicated by the dotted line is where the extraction takes place, detailed in schematic b.

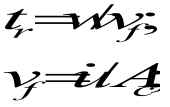
One way of influencing the mobility, and thus the separation efficiency, is the modification of the background electrolyte pH. We have calculated the mobilities as function of pH for lysine, tryptophan and glutamic acid. Effective separation can be obtained when the pH ranges between 4.5 and 11, but pH values lower than 7 are prohibitive due to the gelation of caseinate donor phase.

Another parameter that may affect the results is the flow rates used. The schematic of the theoretical movement of an ion in the extraction space is presented in Figure 1b. The variable d_f is the displacement caused by fluidic motion, d_e is the electrophoretic displacement, x is the minimum distance that the ion should be displaced to be trapped by the collector channel and w is the channel width.

The minimum value of residence time (t_r) to promote the second level of separation can be calculated according to equation 1, where v_i is the velocity of the ion perpendicular to the flow, μ its mobility and E is the applied electric field.

 (1)

The residence time can be used in the set of equations 2 to determinate the flow rates ($\dot{u}$), in which v_f is the flow linear velocity and A_c is the channel cross-section area.

 (2)

Substituting t_r in equations 2 and rearranging them results in equation 3. Solving the equation to the chip and field parameters presented in this paper and the mobility of glutamic acid in pH 10, it is possible to conclude that the flow rate should be between 0.2 to 0.3 μL min^{-1}. The EOF was not included in this calculation.

 (3)

EXPERIMENTAL

Fluorescein-labeled glutamic acid and lysine were added to the donor phase, a 6% solution of PEG 6000 in tetraborate buffer (pH 10). The acceptor phase was a 10 % solution of caseinate in the same buffer. The microfluidic chip (Figure 1) had 5 inputs and a single output. Each channel had both, width and depth, of 70 μm. External (red) and intermediate flow (white) carried the acceptor phase, both with a flow rate of 0.5 μL min^{-1}. The internal flow (blue) was the donor phase at 0.3 μL min^{-1}.

RESULTS AND DISCUSSION

The separation principle is demonstrated in the first line of Figure , with applied external field as a parameter. The phase boundary acted as a virtual membrane, promoting the selective transport of molecules according to the electric field strength. Practically no transport was observed across the phase boundary without an external electric field (Figure 2a). The application of an electric field of 7.4 kV m^{-1} between reservoirs 1 and 5, with all other inputs floating, caused movement of amino acids towards the positive potential. Nevertheless, the strength of the field was not high enough for amino acids to cross the phase boundary (Figure 2b). Once the applied electric field strength was 14.7 kV m^{-1}, the molecules with higher mobility were able to cross the phase boundary (Figure 2c) and, once in the acceptor phase, migrate according to the applied potential. This lead to the conclusion that there is a threshold in the electric field for the diffusion of molecules across the phase boundary.

The ability to cross the barrier is related to the physicochemical properties of the molecule, making the extraction selective. This phenomenon was confirmed by the mass spectrometry analysis of the output stream (third line in Figure 2). The recoveries of both amino acids were similar, without an external electric field and with the field at a strength of 7.4 kV.m^{-1}. Once the electric field strength was further increased, the recovery of higher-mobility lysine dropped much faster than the recovery of

glutamic acid. These results indicate that control of external electric fields could be used for selective extraction.

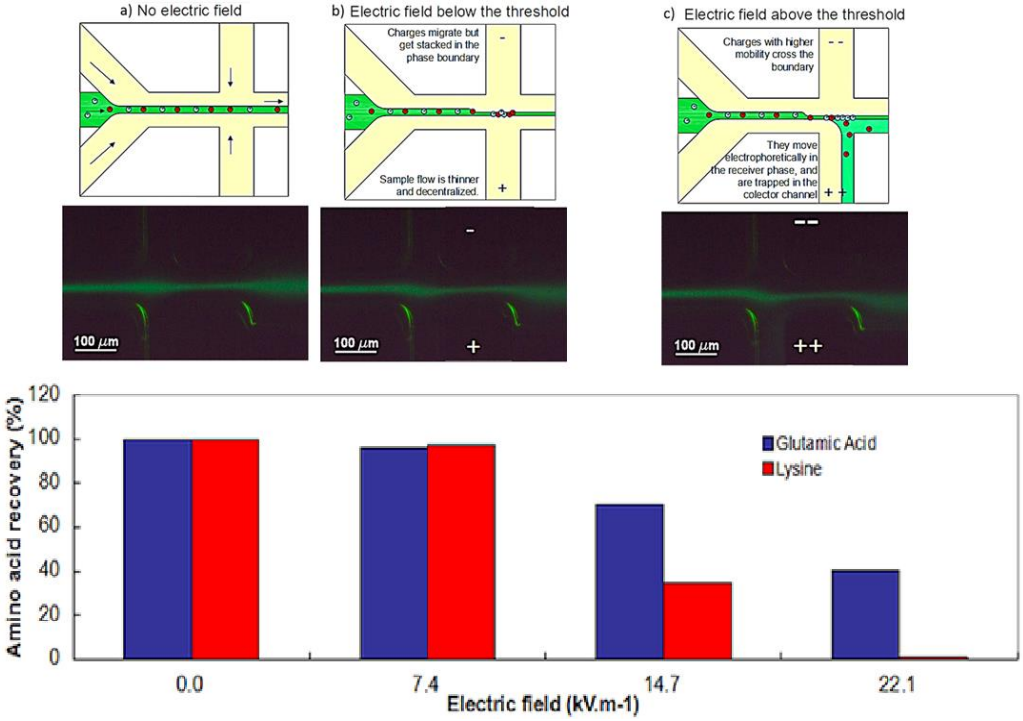


Figure 2. An illustration of the effect of the field in the flow, and fluorescence images of the experimental results when a) no electric field b) an electric field below the threshold (7.4 kV m^{-1}) and c) and electric field above (22.1 kV m^{-1}) the threshold is applied.

CONCLUSION

The use of two different phases as donors and acceptors results in membrane-like behavior of the phase boundary. Furthermore, there is a threshold in the electric field for the migration of molecules across it. Once the threshold is reached, the amount of molecules that migrate seems to be proportional to the applied potential. These results indicate that the use of a two-level separation process represents an option for parallel selection of multiple target compounds. However, changes in the extraction media properties such as pH, conductivity etc. can increase the differences in mobility between some of the compounds in the sample. The technique can also be used for sample clean-up by removing undesirable components from the complex matrix.

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