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Micellar Affinity Gradient Focusing: A New Method for Electrokinetic Focusing

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This report describes a novel method for the concentration and separation of neutral and ionic hydrophobic analytes based on a combination of the analytes' electrophoretic mobility, and affinity for partitioning into a micellar phase. This method of simultaneous separation and concentration of species, which we call micellar affinity gradient focusing (MAGF) is effectively a combination of micellar electrokinetic chromatography (MEKC)[1,2] and temperature gradient focusing (TGF).[3] MEKC separates neutral species on the basis of their partitioning into an ionic micelle. TGF is similar to related counter-flow gradient focusing methods such as field gradient focusing[4−6] and separates analytes based on differences in electrophoretic mobility. However TGF is much easier to implement, simply requiring the application of a temperature gradient along a separation channel. As described here, MAGF uses a temperature gradient to achieve what is essentially a focusing mode analog to MEKC.

There are several unique and advantageous features of MAGF over existing separation methods. First, because MAGF is a focusing method, it combines concentration and separation, giving increased sensitivity relative to separation techniques such as capillary electrophoresis (CE) and MEKC. In contrast to transient concentration methods such as stacking,[7,8] sweeping,[9] or capillary isotachophoresis,[10] the point at which the analyte is focused is a true equilibrium zero-velocity point so that there is no theoretical upper limit to the concentration enhancement. Second, like MEKC, MAGF separates analytes on the basis of a combination of electrophoretic mobility and partitioning with the micellar phase.[11] This allows for selectivity based on different chemical properties and hence facilitates the focusing and separation of classes of analytes (such as neutral analytes) that could not previously be focused. Third, the method is simple to implement in microfluidic or capillary formats and requires much shorter channel/capillary lengths than traditional methods such as CE or MEKC. The combination of these advantages makes MAGF a significant new tool for rapid, sensitive, and selective separations.

Specifically, MAGF is implemented through a spatial gradient of the analyte retention factor, k, for a pseudostationary micellar phase. The total analyte velocity u_T is given by:

$$u_T = (u_B + u_{EP})[1/(1 + k)] + u_{MC}[k/(1 + k)] \qquad (1)$$

where u_B and u_{EP} are the velocity of the buffer (mobile phase) and the electrophoretic velocity of the analyte respectively, u_{MC} is the velocity of the micelles, and k is the retention factor. For neutral analytes u_{EP} is zero. As eq 1 shows, a spatial gradient in the retention factor results in a spatial velocity gradient; with appropriately chosen conditions the velocity of the analyte will be zero at a unique point in the separation channel, thus focusing (or concentrating) and separation will occur.

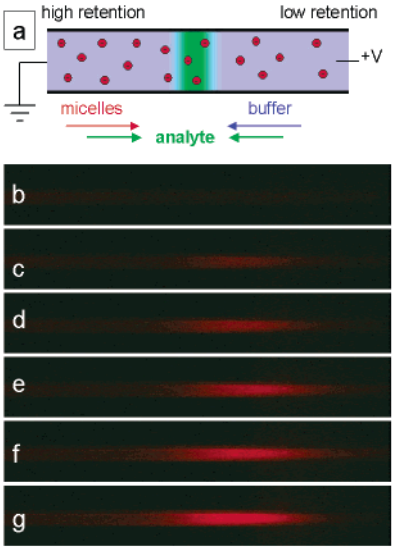

Figure 1. (a) Schematic representation of MAGF. (b−g) MAGF focusing of rhodamine B as a function of time. The fluorescence micrographs were collected 0, 10, 20, 30, 40, and 50 s after application of voltage. Focusing conditions: +63 V cm^{-1}, T_{hot} 80 °C, T_{cold} 10 °C in 5 mmol/L SDS, 5 mmol/L carbonate, 5% EtOH (wt/wt) adjusted to pH 9.4. The starting concentration of rhodamine B was 2.5 μmol/L. The final concentration was 32 μmol/L, an enhancement of 13-fold. Each image shows a 1.5-mm length of the separation channel.

The retention factor depends on the distribution coefficient, K, and the phase ratio, β according to eq 2:

$$k = K\beta \qquad (2)$$

The phase ratio is the ratio of the volume occupied by the pseudostationary (micelle) phase to the volume of the mobile phase. The distribution coefficient is a measure of the affinity of an analyte to partition into the hydrophobic interior of the micelle. Thus, the retention factor gradient can be created by modulating K, β, or both. For example, as the critical micelle concentration (CMC) of most surfactant solutions is temperature dependent, a gradient in the phase ratio can be established via a temperature gradient.

The illustration in Figure 1a shows a schematic of the focusing mechanism. The gradient in retention factor is created by application of a temperature gradient along the separation channel length. In this scheme, the CMC of the surfactant increases with temperature, and consequently, the phase ratio decreases with increasing temperature. A combination of voltage and hydrodynamic pressure is applied so that the mobile phase moves in one direction while the micelles move in the opposite direction. Thus, when the analyte partitions with the pseudostationary phase, it moves in one direction (left to right in this figure), and when it partitions into the mobile phase, it moves in the opposite direction. Given that there is a spatial

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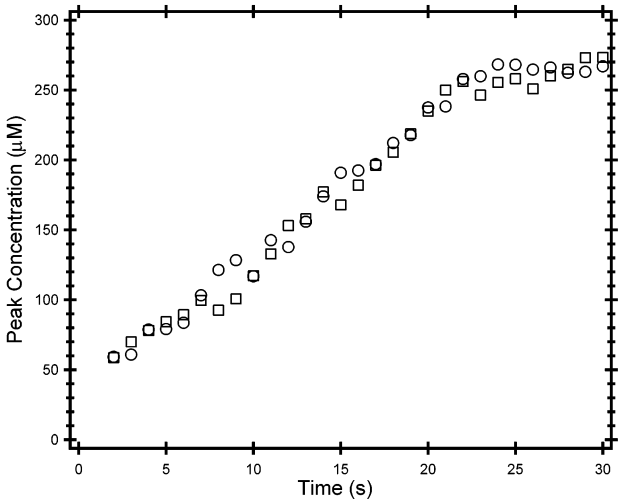

Figure 2. Plot of concentration as a function of MAGF time for anthracene. Focusing conditions: −833 V cm^{-1}, T_{hot} 80 °C, T_{cold} 20 °C. The initial concentration of anthracene was 10 μmol/L in 15 mmol/L SDS, 8 mmol/L carbonate, 25% EtOH (wt/wt) at pH 10.0. The circles and squares represent different trials.

gradient in the retention factor, the analyte will preferentially partition with one of the two phases on the basis of its spatial position in the channel. Therefore, the total analyte velocity will be positive on one side of the temperature gradient and negative on the other side, and it will be equal to zero at a unique point along the gradient where the analyte will be focused.

Although MAGF bears many similarities in its implementation to TGF, it focuses analytes via a different mechanism and is functionally unique from TGF. Further, it is important to note that, whereas TGF requires a buffer whose ionic strength is a function of temperature, MAGF can be accomplished in any buffer capable of supporting micelle formation. The principle of MAGF is demonstrated with sodium dodecyl sulfate (SDS), a typical surfactant used in MEKC, in carbonate buffer. In addition to surfactant, an organic solvent (ethanol) was added to the buffer in concentrations ranging from 5 to 25% (wt/wt) in an effort to influence the temperature-dependence of the CMC.[12] The purpose was to create a linear velocity gradient over the entire temperature range employed. The focusing experiments were performed in polycarbonate microchannels (50 μm wide, 30 μm deep) or fused-silica capillaries (30 μm inner diameter). The temperature gradient was generated with a previously described apparatus.[3] Briefly, the microchannel or capillary is mounted between two thermostated copper blocks, one of each high and low temperature. The space between these two blocks (2 mm) defines the spatial region for focusing and separation. Figure 1 shows the MAGF focusing of zwitterionic rhodamine B at a pH of 9.4, where it has a near-zero electrophoretic mobility. The polycarbonate microchannel was initially filled with a uniform concentration of dye in the buffer. The fluorescence micrographs in Figure 1b−g show the appearance of a concentrated band during the initial 50 s of MAGF, leading to an increase in concentration of roughly 13 times between b and g in Figure 1.

In addition to zwitterionic dyes, the focusing of uncharged anthracene was also performed. A plot of concentration of anthracene versus MAGF focusing time is shown in Figure 2. In these experiments, the focusing of anthracene was performed in a fused-silica capillary (30 μm i.d., 3 cm length). The plotted concentration was the average concentration in the 2-mm long gradient zone as determined from a calibration performed with 10, 50, 100, 200, and 500 μmol/L anthracene solutions under the same temperature conditions. After performing MAGF for 30 s, the concentration of anthracene increased 27-fold. It is anticipated that improvements in the concentration factor are possible by optimizing experimental parameters such as the applied electric field and the concentration and type of organic solvent used.

Figure 3. Fluorescence micrograph illustrating the separation of rhodamine B (red band) from rhodamine 110 (green bands). The faint green band to the left of the intense green band is an impurity in rhodamine 110. For scale, the image is 2 mm long. Focusing conditions: −300 V cm^{-1}, T_{hot} 80 °C, T_{cold} 20 °C in 20 mmol/L SDS, 5 mmol/L carbonate, 25% EtOH (wt/wt) adjusted to pH 9.4.

The distinct advantage of MAGF to simultaneously concentrate and separate analytes is shown in Figure 3. To demonstrate this capability, MAGF was used to separate and focus two similar dyes, rhodamine B and rhodamine 110. Initially, rhodamine B was introduced to the capillary and focused within the 2-mm gradient zone. Next, rhodamine 110 was added to the input reservoir, and after a few minutes of focusing, a baseline-resolved green band appeared to the right of the red band.

To our knowledge, MAGF is the first electrophoretic focusing method capable of simultaneous concentration and separation of species on the basis of properties other than electrophoretic mobility (charge-to-friction ratio) or isoelectric point. As with traditional CE approaches, the use of this new technique and others like it will require an examination of the tradeoffs between resolution or peak capacity on one hand and concentration or detection limits on the other. With CE, this is a simple one-to-one tradeoff: improvement in one comes at the cost of the other. With MAGF and other newly developed focusing techniques,[3−6,9,10] injection time is introduced into the tradeoff, so that an improvement in detection limit does not necessarily come at the cost of poorer resolution. For example, if greater concentration enhancement is needed, a MAGF separation could simply be run with a longer injection time, with (theoretically) no degradation of resolution. Further MAGF experiments to explore the limits of this three-way tradeoff and to increase the range of analytes that can be focused are already under investigation in our laboratory. Because MAGF combines the high selectivity provided by MEKC with the nearly unlimited concentrating ability of TGF, it has significant potential as a high throughput method for separations of neutral and ionic hydrophobic analytes.

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