

Stacking effect via solvent polarity differences in micellar electrokinetic chromatography with aqueous-organic background electrolyte

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Abstract

In this manuscript, a new stacking mode for micellar electrokinetic chromatography with anionic micelles and neutral analytes was investigated. The variation in the velocity of the analytes between the sample and the separation zones, needed for stacking effects, was obtained by using solvents with different polarities, the concentration of salts and micelles being identical between the two zones. Whereas a pure aqueous solvent was used in the injection zone, up to 30% ACN was used in the separation zone. In such conditions, higher interactions between the analyte and the micelles are obtained in the sample zone than in the separation zone, this due to the presence of the organic solvent. As the velocity of neutral analyte depends on its interactions with the moving micelles, its speed will drop when crossing two distinct zones where in the second zone its interactions with the micelles are lower than in the outgoing zone. This will induce a stacking effect. This approach was validated using a background electrolyte in the separation zone made with 10 mM disodium hydrogen phosphate, 15 mM sodium dodecyl sulphate and 15% ACN. The three triazines (simazine, atrazine, and terbuthylazine), used as test analytes, were dissolved in 10 mM disodium hydrogen phosphate, 15 mM sodium dodecyl sulphate and either 15 or 0% ACN. A stacking factor of around 2 was obtained injecting the sample in 0% ACN rather than 15% ACN. This was in accordance with the theoretical values predicted measuring the effective mobilities in both zones.

Keywords: MEKC; Triazines; Efficiency; Acetonitrile

1. Introduction

Micellar electrokinetic chromatography (MEKC) was proposed in 1984 by Terabe et al. [1] to separate neutral molecules via capillary zone electrophoresis (CZE). In this approach a charged surfactant, such as sodium dodecyl sulphate (SDS), is added in the background electrolyte (BGE) at a concentration higher than its critical micellar concentration (cmc). The separation of the neutral molecules is achieved because of differences in their partition coefficient to the hydrophobic component of the charged micelles acting as a pseudo stationary phase (PSP). The capacity factor, k' , is the ratio of the total number of the solute in the micelle to those in the aqueous phase and is related to the migration time of the electroosmotic flow (EOF), t_{EOF} , the migration time of the free micelle, t_{mc} , and the analyte migration time, t_m , by [2]

$$k' = \frac{t_m - t_{EOF}}{t_{EOF}(1 - t_m/t_{mc})} \quad (1)$$

Using the apparent electrophoretic mobility, eq (1) is equivalent to

$$\mu_s = \frac{\mu_{EOF}}{1+k'} + \mu_{mc} \left(\frac{k'}{1+k'} \right) \quad (2)$$

where μ_s , μ_{EOF} and μ_{mc} are the solute, EOF and micelle apparent electrophoretic mobility, respectively. With the effective mobility defined as $\mu_s^{eff} = \mu_s - \mu_{EOF}$ and $\mu_{mc}^{eff} = \mu_{mc} - \mu_{EOF}$, eq (2) can be rearranged to

$$\mu_s^{eff} = \mu_{mc}^{eff} \left(\frac{k'}{1+k'} \right) \quad (3)$$

Whereas, originally MEKC was used for the separation of neutral molecules, nowadays, micelles based separations are often used with ionic molecules that could be separated using capillary zone electrophoresis. This is because PSPs provide various ways to locally modify the velocities of solutes, allowing online sample pre-concentration approaches [2-6].

Pre-concentration approaches can be separated in two main types: field enhanced sample stacking and sweeping [4]. Field enhanced sample stacking is a generic pre-concentration mode where the sample is in a solution of lower conductivity than the one filling the rest of the capillary. Thus, the analyte velocity is higher in the sample zone inducing a stacking effect when entering the zone of high conductivity that will reduce the effective length of the sample plug. Sweeping approaches are limited to MEKC and related CE techniques that used charged micelles. The pre-concentration is obtained using a sample solution without micelles. During the electrophoretic run, charged micelles, present in the BGE,

will enter the sample zone, pick-up the analyte and concentrate it [7]. Most often a combination of both approaches is used and with control conditions an increase in concentration higher than 100 fold can be observed [8].

Recently, a new approach has been proposed, micelle to solvent stacking MSS [9, 10] in which cationic molecules are injected in an electrolyte containing SDS but without organic modifier, whereas the separation is performed in a BGE with high organic content. When entering the BGE, the micelles collapse and release their analyte in a very narrow zone.

The aim of this work is to validate a new stacking mode where the composition of the sample and separation zone only differs by their organic content (0 in the injection zone, 5 – 30% in the separation zone). Because the interactions between the solute and the micelles are dependent on the polarity of the solvent, the effective mobilities will be lower in the separation zone than in the sample zone (eq 3). This will induce a stacking effect as detailed below.

The dependence of the capacity factor with the partition coefficients between the solvent and the micelles, P_{mw} , is given by [11]

$$k' = P_{mw} \frac{v(C_{sf} - cmc)}{1 - v(C_{sf} - cmc)} \approx P_{mw}v(C_{sf} - cmc) = K_{mw}(C_{sf} - cmc) \quad (4)$$

where C_{sf} and cmc are the micelles and critical micelles concentration respectively, v is the molar volume of surfactant and K_{mw} is the solute-micelle binding constant.

The presence of organic solvent will not only decrease the value of K_{mw} (eq 4), as expected from the distribution of a hydrophobic analyte between the hydrophobic micelle and the hydrophilic solvent, but will also modify the equilibrium of formation of the micelle and thus the cmc . Organic modifier can even prevent the formation of the micelles [9, 10], such effect is usually observed at a concentration of organic solvent higher than 30% [12]. Below, in general, the capacity factor decreases as the organic content increases [13]. From eq (3), it can be seen that a decrease in k' will cause a decrease in the mobility of the solute. Thus a stacking effect should be observed if the sample zone does not contain organic modifier but the BGE does. Moreover, the stacking factor should be equal to the ratio of the analyte velocities in the sample zone to the BGE zone. This effect will be verified in the experimental part using three triazines (atrazine, terbuthylazine, and simazine) as test compounds.

2. Experimental

2.1. Chemicals

All chemicals used are of analytical grade: atrazine, terbuthylazine, and simazine (Riedel-den-Haën), sodium dodecyl sulphate (99%, for electrophoresis, Sigma Aldrich), disodium hydrogen phosphate (Fluka), sodium hydroxide (Fluka) and acetonitrile (HPLC gradient grade,

VWR, Prolabo). Ultra-pure water, used in the preparation of standard solutions, running BGE was obtained using a Milli-Q Millipore system (Milli-Q plus 185).

2.2. Capillary Electrophoresis

The Capillary Electrophoresis (CE) analyses were performed using a commercial instrument (Beckman P/ACE MDQ (Fullerton, CA, USA)), equipped with a photodiode array detector. Approximately 1 mm of the capillary external coating was removed by burning the extremities prior to conditioning; the capillary extremities were then polished to increase the efficiency of the method and decrease of baseline noise.

2.3. Capillary column conditioning

A fused-silica capillary with a total length of 50 cm (40 cm to detector) and 75 μ m of internal diameter was used. New capillaries were first conditioned with 1 M NaOH for 30 min followed by ultra-pure water for 15 min. The capillary was washed with NaOH 0.1 M for 10 min followed by ultra-pure water for 10 min at the beginning of each working day and with ultra-pure water for 5 min at the end of the day. Between runs the capillary was flushed with BGE for 3 min. All the capillary conditioning steps were performed at a pressure of 138 KPa (20 psi).

2.4. Standard solutions and running BGE

Triazines stock solutions were prepared in ACN with a final concentration of 500 ppm and were stored in dark, at 4°C, for no more than 1 month. Analysis of the stock solution by capillary electrophoresis shows no significant variations in the concentration of each triazine after a month [14]. The running BGEs were made up using various percentage of ACN and concentration of SDS. For every BGE the final concentration of disodium hydrogen phosphate was 10 mM. BGEs were not filtered but sonicated for 15 min.

2.5. Separation conditions

Electrophoretic separations were performed in direct polarity at 20 kV. The temperature was maintained at 25°C. Running BGEs were changed every 6 runs.

2.6. Data analysis

Electrophoretic figures of merits (migration time, peak area, plate number and skewness) were measured using Finnee (<http://finnee.yolasite.com/>) by fitting a Gaussian or an HVL function [15,16].

3. Results and Discussion

3.1. Effect of SDS concentration and percentage of acetonitrile in the separation of triazines

The method for the separation of triazines was modified from a previous published method in our group [14]. To study the effect of both the SDS concentration and percentage of acetonitrile in the separation various BGE were prepared with a constant final concentration of disodium hydrogen phosphate of 10 mM, a concentration

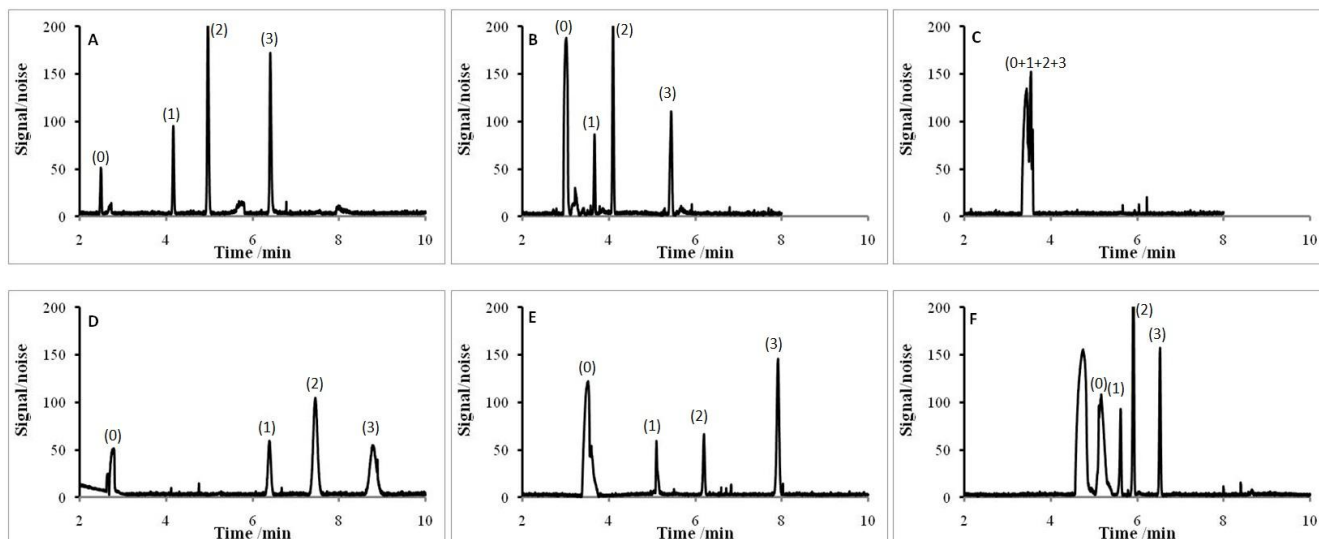


Figure 1. Selected electropherograms showing the separation of (1) simazine (2) atrazine and (3) terbuthylazine in a 10 mM Na_2HPO_4 with (A, B and C) 15 mM SDS or (D, E and F) 60 mM SDS and (A and D) 0% ACN, (B and E) 15% ACN or (C and F) 30% ACN. Each analyte was at a concentration of 2.5 ppm diluted in water and injected at 5 psi for 3 sec. The separation was performed in direct polarity at 20 kV in 50 cm long (40 cm to the detector) fused silica capillary with an ID of 75 μm .

of SDS ranging from 5 mM to 60 mM and the percentage of acetonitrile between 0 and 30%. The sample zone was made of terbuthylazine (1), atrazine (2), simazine (3) at a concentration of 2.5 ppm each, 0.5% of acetone (0) and 0.5% ACN used as the electroosmotic flow (EOF) marker in 10 mM Na_2HPO_4 aqueous solution.

Selected electropherograms are displayed in Figure 1 with BGEs made with 10 mM Na_2HPO_4 (final concentration) and (A) 0% ACN, 15 mM SDS; (B) 15% ACN, 15 mM SDS; (C) 30% ACN, 15 mM SDS; (D) 0% ACN, 60 mM SDS; (E) 15% ACN, 60 mM SDS; (F) 30% ACN, 60 mM SDS. As expected, comparing (A), (B) and (C) and (D), (E) and (F), the effective mobility decreases

as the organic content increases (-3.36 , -1.49 and $-0.15 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ in (A), (B) and (C) respectively for atrazine for example), this due to lower interactions between the solutes and the micelles as the organic content increases.

In Figure 2 is plotted the effective mobility of atrazine as a function of the SDS concentration where (A) is in a pure aqueous BGE, (B) with 15% ACN and (C) 30% ACN, each data point corresponding to the average of 3 replicates. The relative standard deviation of the measured effective mobility was always below 0.5%. The effective mobility of atrazine increases as the SDS concentration increases. This is in accordance with eq. 3

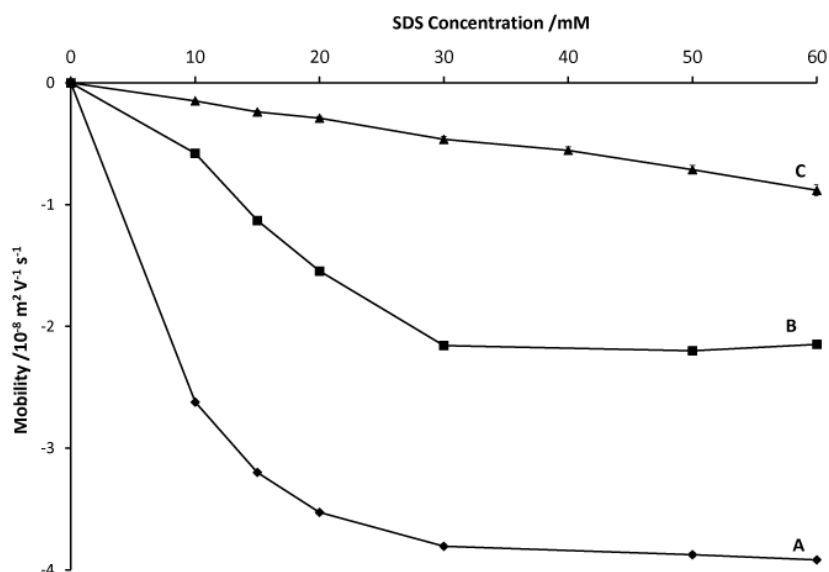


Figure 2. Effective mobility of atrazine measured at different concentrations of SDS and different percentages of ACN (A: 0%, B: 15% and C: 30%). The error bars correspond to the standard deviation calculated using three replicates.

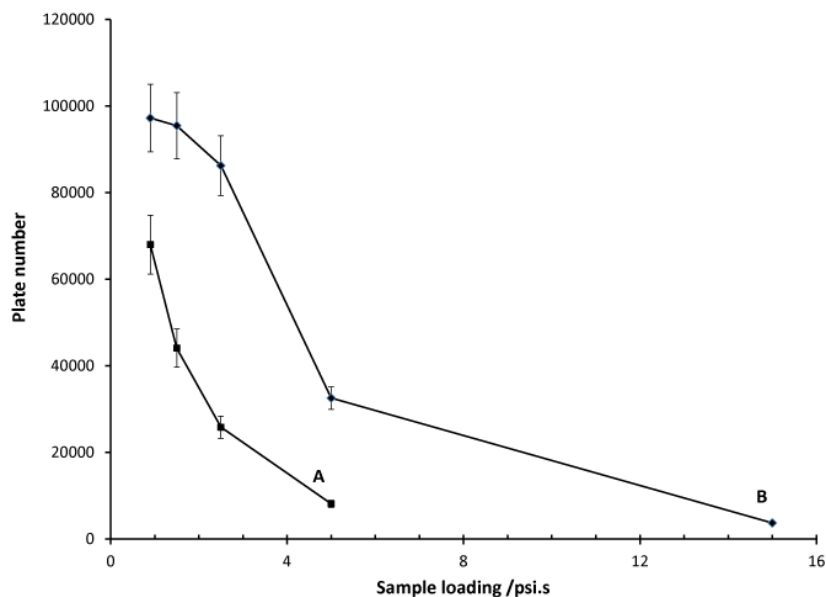


Figure 3. Plate number measured with the atrazine peak as a function of the sample loading (injection pressure time injection time) measure in a 10 mM Na_2HPO_4 15 mM SDS and 15% ACN BGE. The sample (2.5 ppm of each triazine) was either injected in (A) 10 mM Na_2HPO_4 15 mM SDS and 0% ACN or (B) 10 mM Na_2HPO_4 15 mM SDS and 15% ACN. The error bars correspond to the standard deviation calculated using three replicates.

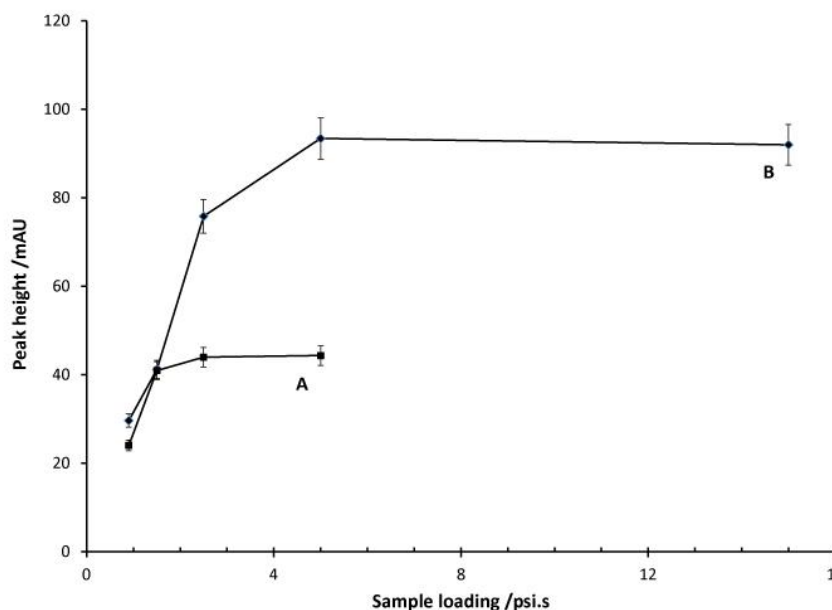


Figure 4. Peak height measured with the atrazine peak as a function of the sample loading. Other conditions as in Fig. 3. The error bars correspond to the standard deviation calculated using three replicates.

and 5. It is interesting to notice that the effective mobilities does not tend to the same values as it will be expected if the micelle mobility was the same in all solvents. As discussed previously, at equivalent SDS concentration, a lower mobility is observed at higher ACN content, this indicates a decrease in the binding constant as the ACN content increases. Figure 2 shows, that at identical EOF, the atrazine velocity will be higher in a BGE made by dissolving the salts in pure water than in a BGE containing the same ionic species and a

percentage of ACN. This not only can be used to tailor the separation selectivity and speed but only to pre-concentrate neutral analytes. This will be tested in the next part using 15 mM SDS 15% ACN in the BGEs as they provide a good compromise in terms of separation speed and resolution.

3.2. Stacking effect due to difference in the polarity of the solvent between two zones.

To study this effect, a separation was performed in a BGE made of 10 mM Na_2HPO_4 and 15 mM SDS with 15% ACN. The samples (atrazine, simazine, and terbuthylazine) were dissolved in solution with the same salt concentration (final) and either with 15% or 0% ACN. The loading capacity in each solvent used for the sample preparation was measured by injecting the sample at various pressures (0.3 to 0.5 psi) and for different injection times (3 to 10 sec). For each run the plate numbers were calculated and the peak height was measured.

For atrazine as analyte, the plate number and the peak height as a function of the loading are plotted in Figure 3 and Figure 4 respectively. When the sample is injected in BGE (Figure 4A), the peak height plateau at 1.5 psi.s (injection at 0.3 psi for 5 s) whereas when the sample is injected in a buffer with the same salt concentration as the BGE but without ACN, the peak height plateau at 5 psi.s (injection at 0.5 psi for 10 s) (Figure 4B). However it should be emphasized that at this loading the peak efficiency is also severely reduced. The plate number of atrazine at this sample loading is equal to 32500 whereas at lower loading, the plate number can reached 97200. It is interesting to notice that when there is 15% ACN in the injection zone, the maximum efficiency is equal to 68000, 30% lower than when the injection zone contains only water. This is because with a 75 μm ID capillary, even an injection of 3 s at 0.3 psi (5.5 mm injection length [17]) contributes significantly to the overall peak width, the stacking obtained using pure water allows to reduce this contribution.

Selected electropherograms are shown in Figure 5. The electropherograms were obtained using an

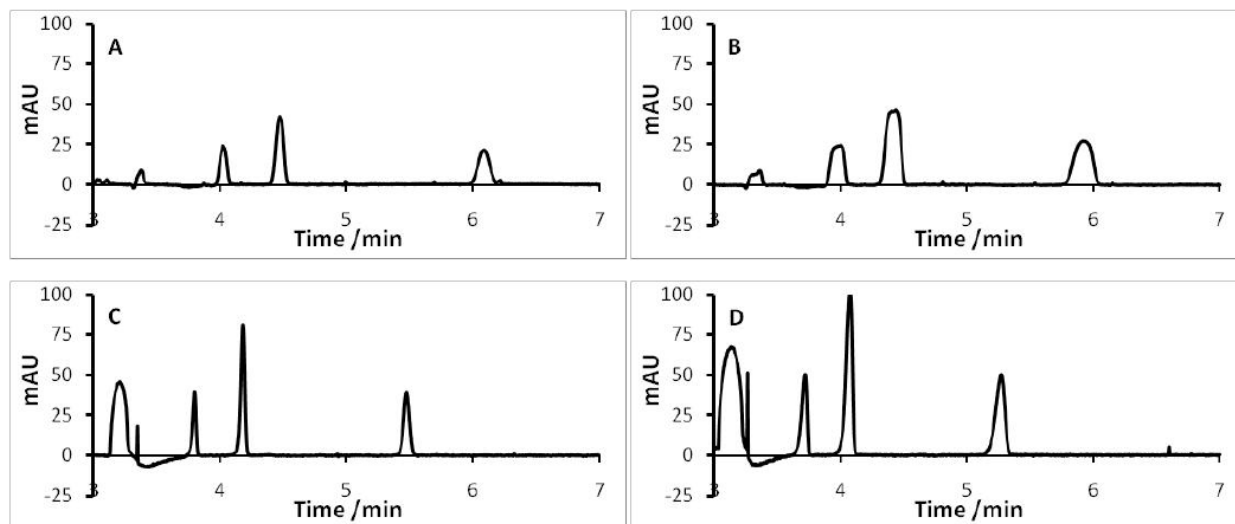


Figure 5. Selected electropherograms obtained with a 10 mM Na_2HPO_4 15 mM SDS and 15% ACN BGE. The sample was either injected in (A and B) 10 mM Na_2HPO_4 15 mM SDS and 15% ACN or (C and D) 10 mM Na_2HPO_4 15 mM SDS and 0% ACN at a pressure of 0.5 psi and 5 s for (A and C) or 10 s for (B and D).

Table 1. Theoretical and experimental stacking factors.

| | Mobility / $10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ | | Stacking factor | |
|---------------|--|--------------------|-----------------|-----------------|
| | 0% ACN | 15 % ACN | Theoretical | Measured |
| Simazine | -2.54 ± 0.01^a | -0.84 ± 0.02^a | 1.67 ± 0.15 | 1.66 ± 0.06 |
| Atrazine | -3.26 ± 0.02^a | -1.27 ± 0.04^a | 2.09 ± 0.14 | 1.95 ± 0.10 |
| Terbutylazine | -4.18 ± 0.03^a | -2.25 ± 0.06^a | 3.12 ± 0.09 | 1.87 ± 0.05 |
| EOF | 7.14 ± 0.07^b | 5.09 ± 0.02^b | NA | |

^a Effective mobility

^b Apparent mobility

injection at 0.5 psi for 5 s (A and C) or at 0.5 psi for 10 s (B and D). The samples were either dissolved in 10 mM Na_2HPO_4 , 15 SDS with 15% ACN (A and B) or 0% ACN (C and D).

The stacking factors for all three analytes have been measured using the ratio of the peak height obtained when the samples are dissolved in a matrix without ACN to the peak height obtained when the samples are dissolved in BGE. A sample loading of 2.5 psi.s was used for this task (Figure 5A and Figure 5C). Results are summarized in Table 1, with the theoretical stacking factors, estimated using the effective mobilities obtained in a BGE without ACN and a BGE with 15% ACN.

A stacking factor of 1.6 and 2 was obtained for simazine and atrazine respectively, in accordance with the predicted values using the speed ratio between the sample and BGE zones. However, for terbutylazine, significant differences were obtained between the predicted (3.1) and experimental (1.9) values. This might be due to other dispersive effects that limit potential gain. However the stacking effect due to the difference in the polarity between the sample and separation zones has clearly been observed.

4. Conclusions

In this work, it was demonstrated that a difference of polarity between the sample and separation zones will induce a stacking effect when working in MEKC with charged micelles and neutral analytes. With triazine as test compounds a stacking factor of 2 was obtained injecting the samples in 0% ACN and separating the sample in 15% ACN. This stacking mode can be used in conjunction with other online preconcentration mode, such as field enhance stacking, allowing to significantly improve the limit of detection.

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