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Short Communication

Effect of acetonitrile in the sampling solution on the analyte peak shape in micellar electrokinetic capillary chromatography

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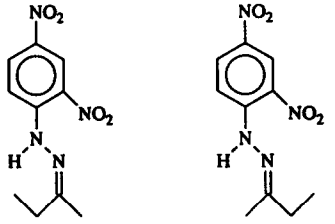
Abstract

When acetone-2,4-dinitropnenylhydrazone is dissolved in pure acetonitrile and analyzed by micellar electrokinetic capillary chromatography, peak splitting is observed. As the amount of acetonitrile is reduced in the sample solution, the peaks converge and a single, narrow peak is observed.

Peak splitting due to geometric isomerism has previously been observed in gas and liquid chromatographic analyses of dinitrophenylhydrazine derivatives of aldehydes and asymmetrical ketones, but the acetone derivative has no such isomerism. Peak splitting is thought to be a result of the interaction of acetonitrile with the micelles in the separation buffer. This artifact may occur whenever analytes are prepared in a buffer containing a large concentration of organic phase; decreasing the concentration of organic phase should produce simpler electropherograms.

1. Introduction

In the separation of 2,4-dinitrophenylhydrazine derivatives of aldehydes and ketones by gas and liquid chromatography, numerous authors have observed peak splitting for single derivatives [1–6]. The presence of the two peaks is attributed to the existence of *syn* and *anti* isomers of the derivatives of aldehydes and ketones that are not symmetrical about the carbonyl group. The solvent-generated phenomenon observed in this work was initially seen with 2-butanone-2,4-dinitrophenylhydrazone, which has geometrical isomerism about the sp^2 hybridized nitrogen:



2-butanone-2,4-dinitrophenylhydrazone

To bar isomerism as the possible cause of the double peak, the data presented are for the acetone derivative, which has no such isomers.

In chromatography, operating in the linear portion of a sample's isotherm will generate a Gaussian concentration distribution for a single compound, barring extra-column band broadening. The situation becomes more complex in electrophoresis, where Mikkers *et al.* [7] have

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shown that the electrophoretic mobility of the analyte ion relative to that of the carrier ion will strongly influence the analyte concentration distribution and hence peak shape in an electropherogram.

Terabe *et al.* [8] created a chromatography–electrophoresis hybrid technique, micellar electrokinetic capillary chromatography (MECC), by adding micelles to the aqueous buffer. The micelles act as a pseudo-stationary phase and allow neutral species to be separated by means of their partitioning between the micellar and aqueous phases.

In this study, acetone-2,4-dinitrophenylhydrazone, separated by MECC, produced a double peak when the sample was dissolved in pure acetonitrile, and the two peaks coalesced as the ratio of acetonitrile to aqueous buffer decreased. These peak shape anomalies cannot be due to differences in ionic strength of the sample and running buffers as described by Mikkers *et al.* [7]. Peak shape anomalies of neutral species (such as acetone-2,4-dinitrophenylhydrazone) separated in MECC must be described by a different mechanism.

2. Experimental

2.1. Reagents

2,4-Dinitrophenylhydrazine and acetone were from Aldrich, sodium monohydrogenphosphate was from Fisher Scientific, acetonitrile, sodium dodecyl sulfate (SDS) and sodium dihydrogenphosphate monohydrate were from BDH, and potassium hydroxide was from J.T. Baker. Water used was from a Nanopure water purifier from Barnstead. All chemicals were reagent grade or better.

Acetone-2,4-dinitrophenylhydrazone crystals were prepared by a standard method [9–11], and were recrystallized from ethanol and dried.

The aqueous electrophoresis buffer was 20 m*M* in both SDS and phosphates, buffered at pH 7, and was filtered twice through 0.22-μm Millipore GS filters. The 0.50 *M* potassium hydroxide purging solution was filtered once with a 0.2-μm Nalgene disposable filter.

2.2. Procedure

The MECC separations were performed on an automated Waters Quanta 4000 capillary electrophoresis (CE) system with UV absorbance detection at 254 nm. The capillary was 60.0 cm × 74 μm I.D. × 363 μm O.D. On-column detection was 52.4 cm from the sampling end, on a portion of the capillary that has had its coating gently burned off. A Fisher Recordall Series 5000 strip chart recorder was used to record the 10 mV full scale signal (spanning 0.005 absorbance units) from the CE instrument.

Injection was by means of electromigration at 1.00 kV for 10 s, ramped over the first 0.5 s. The separation was performed at 15.0 kV, ramped over the first 5 s of the run. Both ends of the capillary as well as the platinum electrodes were immersed in the aqueous buffer. The samples consisted of 0.2 m*M* acetone-2,4-dinitrophenylhydrazone dissolved in mixtures of acetonitrile and aqueous buffer having 100, 80, 60, 40 and 20% (v/v) of acetonitrile.

The capillary was purged by vacuum each day first with 0.50 *M* potassium hydroxide, then with the aqueous buffer (5 min each). A buffer purge was also used if the buffer was changed or if an error occurred during a run.

3. Results and discussion

Fig. 1 presents electropherogram peaks from the acetone-2,4-dinitrophenylhydrazone sample eluting after approximately 16 min. When the sample is dissolved in pure acetonitrile, there are two distinct peaks, but as the acetonitrile is replaced with aqueous buffer, the splitting is reduced. The peaks are much closer together for 80% acetonitrile, while the leading peak is merely a shoulder on the trailing peak for 60% acetonitrile; a single peak is observed for both 40 and 20% acetonitrile, but the peak sharpens going from 40 to 20% acetonitrile. Table 1 shows the migration times for the acetone-2,4-dinitro-

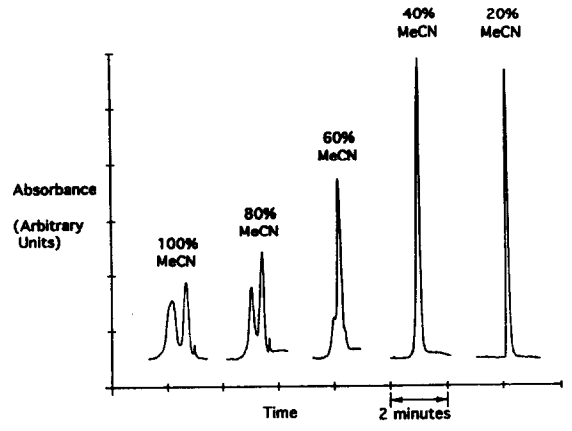

Fig. 1. Demonstration of peak splitting for acetone-DNPH when dissolved in solvents containing a varying fraction of acetonitrile. The volume fraction of acetonitrile is noted above each data set. The data from five runs are shown in the single figure; absolute migration time data are given in Table 1.

phenylhydrazone peaks, as well as the acetonitrile peaks in the same run.

The appearance of several peaks for a single compound without isomers would suggest the possibility of impurities. To establish the identity and purity of the compound, nuclear magnetic resonance and mass spectra, as well as an elemental analysis were performed. The 300 MHz ^1H NMR spectrum in C^2HCl_3 was free of extraneous peaks, and the peak shifts, splittings and integrated areas were consistent with the hydrogen found in the structure. The peaks observed, described as [shift (splitting, coupling constant, number of hydrogen)] with units of ppm and Hz for the shifts and coupling constants, are as follows: d_H (C^2HCl_3; 300.133 MHz) 2.10 (s, 3H), 2.20 (s, 3H), 7.95 (d, J 9.5, 1H), 8.28 (ddd, J 9.5, 2.7, 1.0, 1H), 9.11 (d, J 2.5, 1H), 11.0 (s, 1H). A high-resolution electron impact mass spectrum yielded the parent-ion peak at 238.0701 u; the closest match possible using the principle isotopes ^{12}C, ^1H, ^{14}N and ^{16}O is 238.0702 u for $C_9H_{10}O_4N_4$, which is the empirical formula for acetone-2,4-dinitrophenylhydrazone. The carbon, hydrogen and nitrogen elemental analysis yielded results in agreement with calculated values (in brackets): C, 45.11%, 45.08% (45.38%); H, 4.05%, 4.11% (4.23%); N, 23.10%, 23.18% (23.52%).

Table 1
Migration time data for acetone-2,4-dinitrophenylhydrazone as a function of acetonitrile concentration in analyte dissolution solution

Sampling solution	Migration time (min)	
	Acetonitrile	Acetone-2,4-dinitrophenylhydrazone
100% Acetonitrile	6.3	16.5, 17.0
80% Acetonitrile	6.0	15.2, 15.6
60% Acetonitrile	6.0	15.4, 15.6
40% Acetonitrile	6.0	15.5
20% Acetonitrile	5.9	15.4

Migration time is given for the baseline disturbance associated with the acetonitrile content of the sample solution. The migration time for the two major peaks associated with acetone-2,4-dinitrohydrazone is given for the 100, 80 and 60% acetonitrile data. A single peak was observed for the 40 and 20% acetonitrile data.

We interpret the splitting phenomenon by the combination of two processes: (1) the dissolution of micelles into individual surfactant ions at high concentrations of acetonitrile and (2) the favorable partitioning of the solute in the micellar pseudo-phase *versus* acetonitrile. If this is the case, then when the sample plug is drawn into the capillary, the analyte will immediately begin partitioning into micelles in the aqueous buffer in contact with both ends of the plug. Two regions of high analyte concentration will develop at the ends of this acetonitrile plug. The acetonitrile progresses much more rapidly than the analyte or the micelles along the capillary, so the acetonitrile plug will overtake the micelles on its leading edge. As these micelles are overtaken, they will be dissolved in the acetonitrile and will release any analyte molecules they contain. The analyte will partition again back into the micelles at the leading edge of the acetonitrile plug. This process will continue until longitudinal diffusion of the acetonitrile plug reduces the acetonitrile concentration to the point where the plug can no longer dissolve the micelles it is overtaking. Then the acetonitrile will pass over the leading region of analyte, having separated it from the trailing region.

As described, this mechanism would be expected to produce less peak separation as the initial concentration of acetonitrile decreases; when the initial concentration of acetonitrile is insufficient to dissolve the micelles, then analyte should not be depleted in the acetonitrile region. Hence a decrease in the initial acetonitrile concentration means that less time will be required to dilute the solvent enough to lower the critical micelle concentration (the concentration above which individual surfactant ions come together to form micelle aggregates) beneath that of the total SDS concentration. This prediction is in agreement with what is observed; when the acetonitrile component of the sample solution drops from 100 to 80% (v/v), the two peaks are seen to be sharper and closer together; the leading peak is also smaller.

As an alternative hypothesis, peak splitting might be caused by precipitation of the analyte in the acetonitrile plug; redissolution in the micelles could lead to peak splitting. A series of samples with 100, 80, 60, 40 and 20% (v/v) of acetonitrile but with the acetone-2,4-dinitrophenylhydrazone concentration diluted by a factor of 4 to 0.05 m*M* were prepared. If precipitation were a problem, a decrease in analyte concentration should decrease the fraction of analyte that is precipitated, which should decrease the peak splitting. However, the splitting did not disappear but was instead more pronounced, with the trailing peak equal in size to the leading peak. The effect of reducing the acetonitrile in the sampling solution was similar to that seen with the undiluted derivative, with the two major peaks getting progressively closer together and coalescing into a single peak for the 40% acetonitrile solution.

4. Conclusions

This phenomenon is not general; while we observed the peak splitting with acetone-2,4-dinitrophenylhydrazone and 2-butanone-2,4-dinitrophenylhydrazone, the peak splitting was not observed with phenylthioyhdantoin derivatives of aspartic and glutamic acids. Our interpretation of the phenomenon relies on high solubility of analyte in micelles and low solubility of analyte in acetonitrile. In those cases where analyte is soluble in acetonitrile, peak splitting is not expected.

When anomalous peak shapes are observed for analyte injected from a buffer containing relatively high concentrations of organic phase, the possibility of peak splitting must be considered. As a simple diagnostic, the sample should be prepared in a buffer that more nearly matches the separation buffer. If the peak splitting is reduced, then this partitioning mechanism may be in operation.

5. Acknowledgements

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6. Note added

A reviewer for this manuscript has observed a similar phenomenon for samples dissolved in methanol.

7. References

[1] H. Kallio, R. Linko and J. Kaitaranta, *J. Chromatogr.*, 65 (1972) 355.
[2] H. Kallio and R. Linko, *J. Chromatogr.*, 76 (1973) 229.
[3] Y. Hoshika and Y. Takata, *J. Chromatogr.*, 120 (1976) 379.
[4] R. Smith and I. Drummond, *Analyst*, 104 (1979) 875.
[5] V. Uralets, J. Rijks and P. Leclercq, *J. Chromatogr.*, 194 (1980) 135.
[6] M. Bicking, W. Cooke, F. Kawahara and J. Longbottom, *J. Chromatogr.*, 455 (1988) 310.

[7] F. Mikkers, F. Everaerts and T. Verheggen, *J. Chromatogr.*, 169 (1979) 1.
[8] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
[9] T. Nolan, D. Bornhop and N.J. Dovichi, *J. Chromatogr.*, 384 (1987) 189.
[10] R. Shriner, R. Fuson and D. Curtin, *The Systematic Identification of Organic Compounds*, Wiley, New York, 4th ed., 1956, p. 219.
[11] L. Papa and L. Turner, *J. Chromatogr. Sci.*, 10 (1972) 747.