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Short communication

Mixed capillary electrophoretic and reversed-phase electrochromatographic separation of 4-*n*-alkylbenzoic acids

Andrew G. McKillop[a], Roger M. Smith[a,*], Raymond C. Rowe[b], Stephen A.C. Wren[b]

[a]*Department of Chemistry, Loughborough University, Loughborough, Leics. LE11 3TU, UK*
[b]*Zeneca Pharmaceuticals, Hurdsfield Industrial Estate, Macclesfield, Cheshire SK10 2NA, UK*

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Abstract

The apparent electrophoretic mobilities of 4-alkylbenzoic acids with side chain lengths of greater than seven carbon atoms in bonded phase capillaries were anomalously high compared to shorter homologues. This was identified as resulting from their slower migration rates caused by interactions with the wall of the column. The effect could be reduced by the addition of acetonitrile to the buffer solution suggesting a reversed-phase partitioning process. These compounds thus demonstrated a mixed electromigration and open-tubular reversed-phase electrochromatographic separation. However, because a relatively wide bore column was used, the efficiencies were poorer than previously reported for open-tubular electrochromatographic separations. © 1998 Elsevier Science B.V.

Keywords: Retention mechanism; Alkylbenzoic acids

As part of a study of the separation of homologous 4-*n*-alkylbenzoic acids in capillary electrophoresis, we have observed a mixed electrophoretic and reversed-phase chromatographic mode of separation when a bonded phase column was used to reduce the electroosmotic flow and increase resolution. Initially, the 4-*n*-alkylbenzoic acids from 4-methylbenzoic to 4-decylbenzoic acids were examined on a 50-μm silica capillary at pH 8.5. They showed the expected systematic decrease in migration rates from $-2.738 \cdot 10^{-4}$ to $-1.880 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ with increasing chain length. We have previously reported a similar change in migration rates for the homologous alkylpyridines [1,2]. The migration of the benzoic acids was against the electroosmotic flow and all the acids were eluted in a short separation window of about 3 min between thiourea as neutral marker and benzoic acid. In order to reduce the electroosmotic flow and broaden this window, the separation was examined on a C_1 alkyl-bonded capillary. However, under these conditions the octyl, nonyl and decyl alkylbenzoic acids were eluted relatively much later than on the silica column. This made their apparent mobilities anomalously similar to those of the much shorter chain acids. For example, *n*-decylbenzoic acid ($-2.631 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$) was eluted about the same time as 4-ethylbenzoic acid ($-2.575 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$). When the same mixture was examined on a C_{18}-bonded capillary similar effects were again observed. In both cases the peak shapes of these longer chain analytes were less symmetrical

*Corresponding author.

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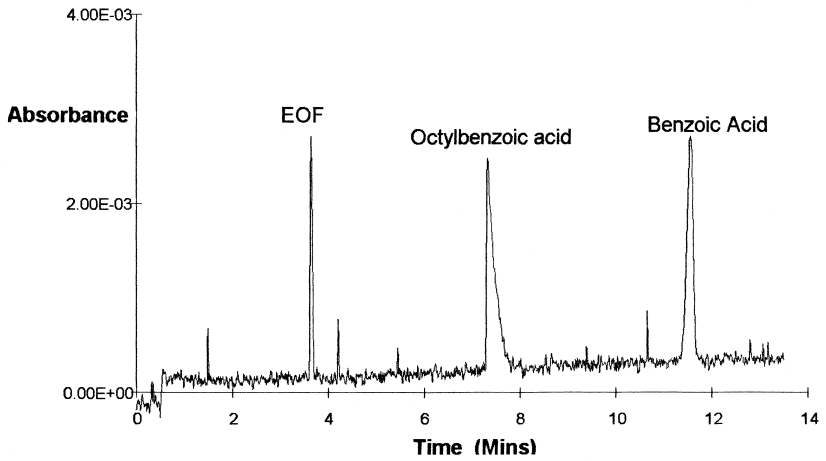

Fig. 1. Electropherograms of the separation of octylbenzoic acid on a C_1-bonded phase capillary. Conditions: 50 m*M* lithium borate buffer, 30 kV applied voltage.

and broader that those of the shorter chain acids (Fig. 1).

It therefore appeared that the longer chain acids were being retained by a reversed-phase partitioning into the alkyl-bonded phase and were being separated by a combination of electrophoresis and open tubular electrochromatography (Fig. 2). To confirm this explanation the separations were repeated using acetonitrile as a modifier in the buffer solution. As the level of acetonitrile increased up to 15% the separations reverted to those on the uncoated silica capillary. In addition if a sample of the alkylbenzoic acids was pumped through the column in an aqueous buffer using hydrostatic pressure the longer chain

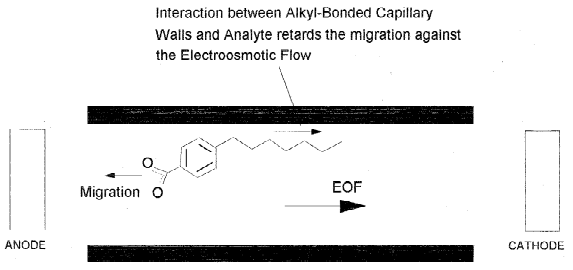

Fig. 2. The forces acting on the longer chain benzoic acids in bonded phase capillaries.

acids were again retained compared to the other short acids. Similar chromatographic retention factors were observed on the C_1 coated capillary for the nonylbenzoic acid in both pressure driven ($k=0.37$) and electrophoretic migrations ($k=0.35$). Under electroosmotic flow, neutral analytes, like hexanophenone, were also retained demonstrating that the bonded capillary wall was acting as an open-tubular reversed-phase column. Similar separations were reported in 1983 by Tsuda et al. [3] using much narrower capillaries but very few studies have re-examined or extended this work.

There have been numerous examples of adsorption and wall effects in electrophoresis and of the efforts made to eliminate the interactions [4]. In most cases these effects have been attributed to polar interactions or the hydrophobic interaction of proteins and peptides from aqueous solutions. However, in the present case the interaction appears to be that of a reversed-phase partitioning into the alkyl bonded phase and although the peak shapes are poorer than the non-interacting acids they are still reasonable.

We consider that this reversed-phase retention may also be present but be unrecognised in other separations on alkyl-bonded capillaries. If only a single compound was being examined the user would not be aware of the interaction with the wall of the

capillary, which in the present study were only recognised because of the change in the relative elution order of a series of homologous analytes.

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