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Wall adsorption of small anions in capillary zone electrophoresis induced by cationic trace constituents of the buffer

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Abstract

Fe(III), present as a trace constituent in the buffer, induces wall adsorption of small anions in coated and uncoated fused-silica capillaries even in the ppb concentration range. For di- and tribasic benzoates, drastic adsorption effects were found when the carboxylate groups were in an *ortho* position, leading to severely tailing peaks and a lower reproducibility of migration times. Benzoates with carboxylate groups in 1,3- or 1,4-positions were less affected than the *ortho* derivatives. The adsorption of the anions is related to Fe(III) attached to the capillary wall.

1. Introduction

In capillary zone electrophoresis (CZE), highly reproducible migration times and peak shapes can be obtained with the presumption that the measurements are carried out under controlled conditions. Many effects may influence the electrophoretic behaviour of the separands, but the reproducibility can be affected most unpredictably by adsorption effects at the capillary wall. This is caused by the fact that the inner surface often changes its state not only from run to run, but even during one run. Although advanced theories on adsorption phenomena in CZE have already been formulated [1–5], the behaviour of the system is difficult to predict, because the values of the adsorption–desorption isotherms or rate constants, parameters which are necessary

for accurate calculation, are unknown in nearly all instances.

Although adsorption is the usual principle for HPLC separations, it is not desirable in CZE. However, it is often observed with biopolymers such as proteins, which can exhibit different interactions, both hydrophobic and Coulombic attraction. The latter takes place between positively charged analytes and the negatively charged sites on the fused-silica surface (see, e.g., ref. 6). In contrast, such behaviour is uncommon for small and negatively charged ions such as aromatic carboxylates, the separands on which this paper is focused. These anions are normally not adsorbed under the pH conditions where they are formed, because the silanol groups of the fused-silica surface (also weak acids) are in that case also negatively charged. Hence, a high reproducibility of the migration times can be obtained for these solutes, especially when the electroosmotic flow is suppressed. In

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this instance the precision of the migration times, expressed by the resulting relative standard deviation, is in the range of a few tenths of a percent.

This was in fact the case during our investigations concerning the prediction of the optimum pH of the buffer as described in previous papers [7,8]. Strong deviations occurred, however, in some instances, which were unexpected and unusual for such systems. They could be related to adsorption of the separands induced by higher charged cationic constituents present at trace concentrations in the buffer.

This paper reports on a systematic investigation of the source and the magnitude of these adsorption effects in CZE. Such effects limit the applicability of particular ions to adjust the electrophoretic selectivity by secondary equilibria in the solution, *e.g.*, due to complexation reactions.

2. Experimental

2.1. Reagents

Chemicals used for the preparation of the buffer (sodium acetate, 0.01 mol/l) were acetic acid and sodium hydroxide; FeCl₃ and CaCl₂ were added to the buffer (all of analytical-reagent grade from Merck, Darmstadt, Germany). The following compounds were used as test substances, each at a concentration of 0.02 mg/ml in water: 1,2-benzenedicarboxylic acid, 1,3-benzenedicarboxylic acid and 1,2,4-benzenetricarboxylic acid (all 99%; Aldrich, Steinheim, Germany), 1,4-benzenedicarboxylic acid and 1,3,5-benzenetricarboxylic acid (both 98%; Aldrich), 1,2,3-benzenetricarboxylic acid dihydrate (98%; Fluka, Buchs, Switzerland).

For the coating procedure, methylcellulose (Methocel MC, 3000–5000 mPa s; Fluka) was cross-linked using formic acid and formaldehyde (both of analytical-reagent grade; Merck). Water was distilled twice from a quartz apparatus.

2.2. Apparatus

The instrument used for the measurements was a P/ACE System 2100 (using System Gold

6.01 software) (Beckman, Palo Alto, CA, USA) equipped with a UV absorbance detector. The absorbance was measured at 214 nm at the anode side of the capillary. The separation capillary was made from 75 μm I.D. fused silica (Scientific Glass Engineering, Ringwood, Australia). The total length was 0.269 m and the effective length (the distance from the injector to the detector) was 0.202 m. The capillary was thermostated at 25.0°C. Electrophoresis was carried out either with or without electroosmotic flow at a total voltage of 5000 V (field strength 18 600 V/m), leading to an effective potential drop of 3750 V along the migration distance from the injector to the detector.

Injection of the sample was carried out by pressure for 1 s from pure aqueous solution. Between the runs the capillary was rinsed with buffer for 5 min. The coating procedure described by Hjertén [9] was applied.

3. Results and discussion

Electropherograms, which were measured for the experimental evaluation of the theoretical prediction of the resolution as a function of the pH of the buffer, described in a previous paper [8], are presented in Fig. 1 for the solutes given in Table 1. It was found that they were highly reproducible under controlled conditions also in the long term, namely over several months, even with different capillaries (see Fig. 1a). Migration times were obtained within a deviation of less than 1%, and the peak shapes remained nearly identical.

During this investigation the reproducibility was, however, found to be drastically reduced in one series, leading to large variations in migration times and changes of peak shapes. One example of this effect is shown in Fig. 1b. The substantial peak tailing indicated the presence of adsorption effects.

Based on the large number of deviating electropherograms, a certain tendency could be derived with respect to the extent of peak tailing and shift of migration times: those components with carboxylic functions in an *ortho* position seemed to exhibit the strongest effects (see, *e.g.*,

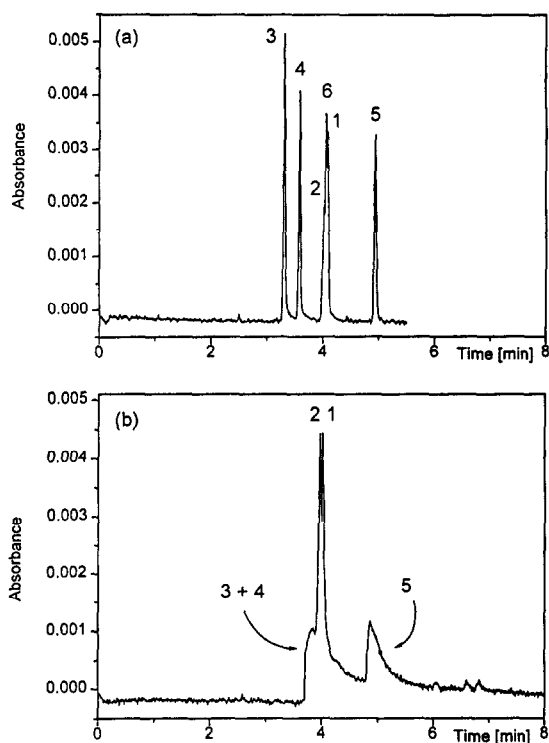


Fig. 1. Typical electropherograms of the test benzenecarboxylates in coated capillaries at pH 5.03: (a) representative of the long-term reproducibility; (b) with deviations of migration times and peak shapes obtained with a buffer having enhanced Fe^{3+} and Ca^{2+} contents. Numbering of the separands according to Table 1. Conditions: sodium acetate buffer, 0.01 mol/l; coated fused-silica capillary ($75 \mu\text{m}$ I.D., total length 0.269 m, effective length 0.202 m); total voltage, 5000 V; effective potential drop, 3750 V; detector located on the anode side of the capillary.

Table 1
Separands investigated

No.	Separand (benzene- carboxylic acid)	No. of <i>ortho</i> positions
1	1,3-Dicarboxylic	0
2	1,4-Dicarboxylic	0
3	1,3,5-Tricarboxylic	0
4	1,2,4-Tricarboxylic	1
5	1,2-Dicarboxylic	1
6	1,2,3-Tricarboxylic	2

Fig. 1b). This led us to the presumption that the interaction with some cationic species attached to the wall is possibly operative, probably favoured by the formation of a ring that stabilizes the adduct. In the following investigations, the test solutes were therefore subdivided into two groups: (i) with substituted carboxylic groups in non-*ortho* positions and (ii) with one or two *ortho*-substituted carboxylic functions (see Table 1).

To clarify the origin of these effects, a number of instrumental and chemical parameters were varied. As a result of the systematic investigation of all the sources possibly involved, it was found that the replacement of the sodium hydroxide stock solution used hitherto with a freshly prepared solution led again to the highly reproducible electropherograms that we were used to obtaining.

Based on this result, an analysis of a number of inorganic components in the sodium hydroxide solution used so far was carried out by atomic emission spectrometry with an inductively coupled plasma (ICP), which gave an enhanced content of iron and calcium, namely about 800 ppb of Fe and 1 ppm of Ca, which is at least three orders of magnitude higher than that specified by the manufacturer. The origin of those traces is unknown to us. Possibly the solution was contaminated from the glassware used during the long-term manipulations.

The effect of these two cations as buffer trace constituents on the electrophoretic behaviour of the analytes was therefore investigated in more detail. All these effects were originally observed in coated capillaries, but both types of capillary surfaces, coated and uncoated, were subjected to the following investigations.

3.1. Coated capillaries

Electropherograms obtained in coated capillaries with suppressed electroosmotic flow are shown in Figs. 2 and 3 for different concentration levels of Fe^{3+} and Ca^{2+} . In pure buffer (indicated by zero iron and calcium, respectively) all separands show fairly symmetrical peaks. Whereas the peak shapes remain almost symmetrical on addition of Ca^{2+} to the buffer

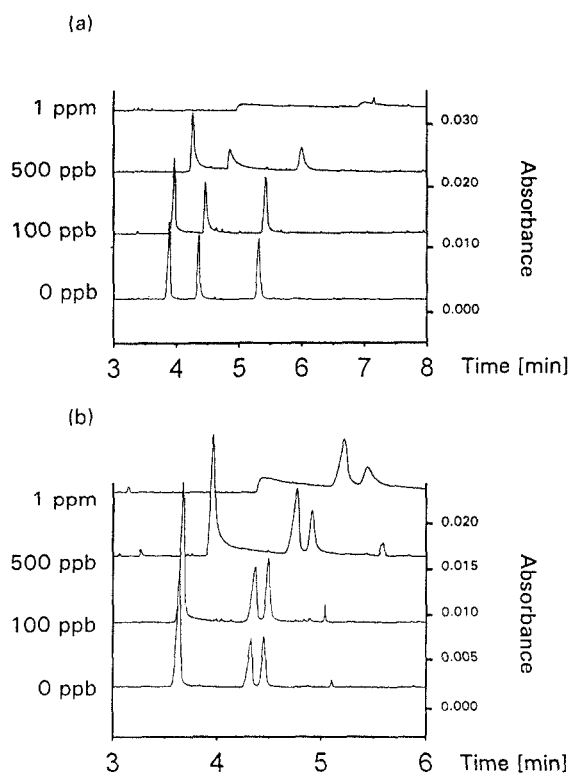


Fig. 2. Electropherogram of (a) *ortho*-substituted and (b) non-*ortho*-substituted benzenecarboxylic acids in a coated fused-silica capillary at different concentrations of Fe^{3+} in the buffer electrolyte solution at pH 4.50. Migration sequence of separands: (a) 4, 6, 5; (b) 3, 2, 1 (numbering according to Table 1). Conditions as in Fig. 1.

even up to 10 ppm (w/w) (with the occurrence of only a slight tailing for components 4 and 6), a very different situation arises when Fe^{3+} is added: even at 100 ppb (w/w) the peaks of the two first migrating *ortho*-substituted separands (4 and 6, Fig. 2a) tail significantly, and at 1 ppm of Fe^{3+} the peaks are so distorted that only two of them can be recognized in the electropherogram. The increase in tailing is accompanied by the retardation of the separands, leading to an increase in retention times.

It follows from Fig. 2b that the non-*ortho* derivatives undergo this adsorptive effect to a much smaller extent. At least the peaks of components 2 and 1 remain nearly symmetrical even up to 1 ppm of Fe^{3+} . As Ca^{2+} seems to

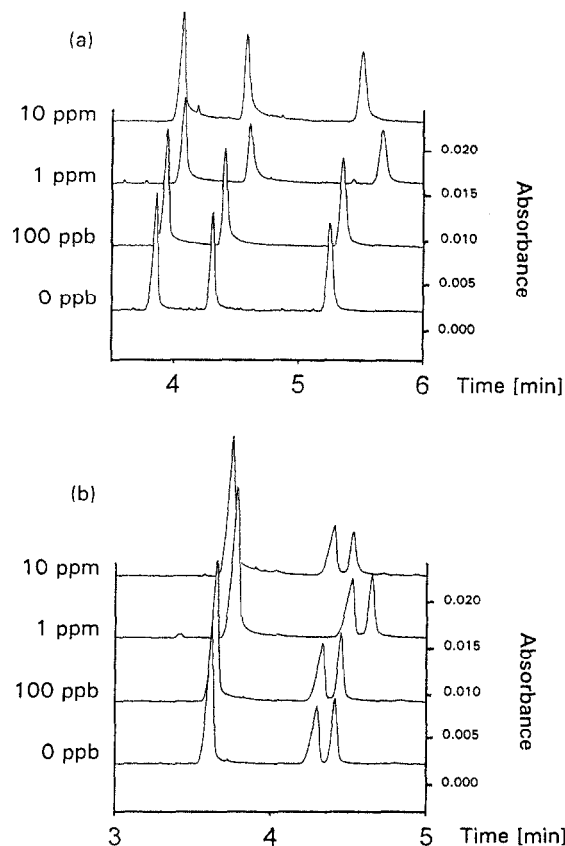


Fig. 3. Electropherogram of (a) *ortho*-substituted and (b) non-*ortho*-substituted benzenecarboxylic acids in a coated fused-silica capillary at different concentrations of Ca^{2+} in the buffer electrolyte solution at pH 4.50. Migration sequence of separands as in Fig. 2. Conditions as in Fig. 1.

have only a minute effect on solute adsorption, the further discussion is focussed on Fe^{3+} .

In Fig. 4 the electropherograms of three subsequent injections of the *ortho*-substituted solutes is shown for two different Fe^{3+} concentrations. From the similar shape of the electropherograms it can be assumed that steady conditions concerning the attachment of the Fe species on the solid capillary surface are established for a given concentration of Fe in the buffer.

It is known from the literature that doping of (bare) silica particles with ions in HPLC [10–19], applied to change the selectivity of the chromatographic system, can influence the retention of certain solutes. For the doping with Fe^{3+} [17]

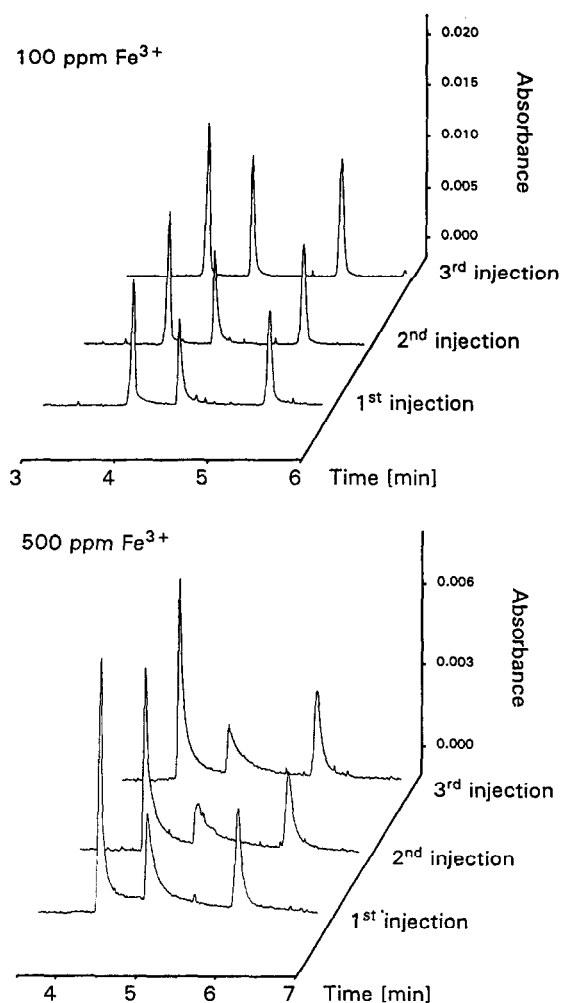


Fig. 4. Effect of three repetitive injections obtained at two different concentration levels of Fe^{3+} , 100 and 500 ppb, of the buffer solution at pH 4.50 for *ortho*-substituted benzenecarboxylic acids. Migration sequence of separands as in Fig. 2a. Conditions as in Fig. 1.

it was found that for compounds possessing hydroxyl functions (phenols), adsorptive retardation leads to an increase in the capacity factors by up to a factor of two, whereas components without such functional groups were not found to be retarded. An analogous mechanism seems to be operative in our system, although not a bare, but a coated silica surface is under consideration. This assumption is supported, however, by experimental findings of peak broadening in CZE in the presence of triply charged lanthanoids due

to wall adsorption for C-1 modified fused-silica capillaries [20].

3.2. Uncoated capillaries

The electropherograms obtained with uncoated capillaries, presented in Fig. 5, show symmetrical peaks in the absence of Fe^{3+} . In contrast to coated capillaries, the migration times are longer, because the (relatively low) electroosmotic flow occurring in bare fused silica at pH 4.50 is directed against the electrophoretic migration of the anionic solutes. Those anions with low ionic mobilities are stronger influenced by this counterflow. In that event, a higher separation selectivity can be obtained. This ef-

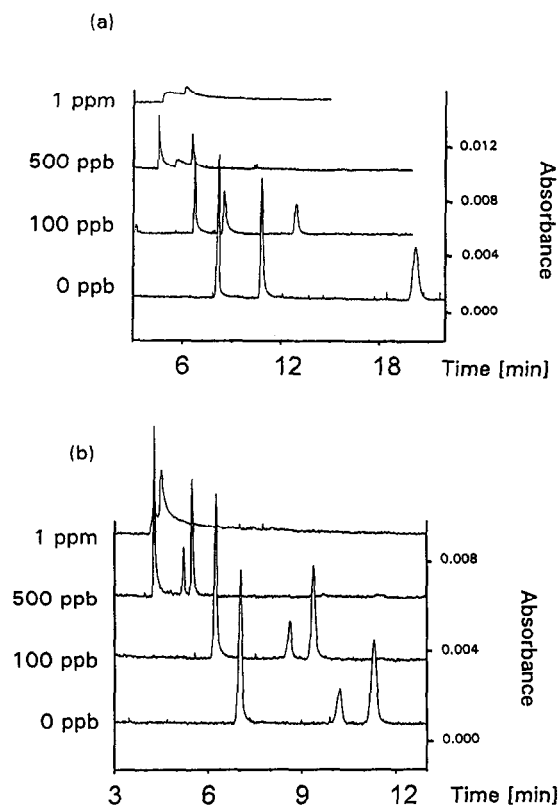


Fig. 5. Effect of the Fe^{3+} concentration of the buffer electrolyte solution at pH 4.50 on peak shapes and migration times of (a) *ortho*-substituted and (b) non-*ortho*-substituted benzenecarboxylic acids in uncoated fused-silica capillaries. Migration sequence of separands as in Fig. 2. Conditions as in Fig. 1.

fect can be observed, *e.g.*, for the pair of separands 1 and 2. This pair is baseline separated in the coated capillary, but exhibits a much higher resolution in the uncoated capillary.

When Fe^{3+} ions are added to the buffer, two effects can be observed: first, the migration times decrease, and second, the peaks show increasing tailing. The former effect is caused by the decrease in the charge density and thus the zeta potential due to the adsorption of the Fe species on the silica wall. This effect is well known. It is used to modify the electroosmotic flow by addition of, *e.g.*, alkylamines to the buffer (see, *e.g.*, refs. 6 and 21). It is also in agreement with results observed in the presence of other higher charged cations in the buffer [22,23].

In the case under consideration, the decrease in the electroosmotic flow leads, *e.g.*, at about 500 ppb of Fe^{3+} to electroosmotic conditions as for the coated capillaries shown above. Owing to the attachment of the Fe species on the wall, adsorptive sites for the separands seem to be formed, reflected by severe peak tailing, yet again, especially of the *ortho*-substituted anions. From the comparison with Fig. 2, it can be concluded that this extent of adsorption is of the same order for coated as for uncoated capillaries. The coating does not prevent or even reduce the adsorption effect.

With respect to the nature of the Fe species attached on the wall only speculations are possible. Given the value of $2.64 \cdot 10^{-39}$ (at 25°C) for the solubility product of iron(III) hydroxide [24], at pH 4.5 the solubility of $\text{Fe}(\text{OH})_3$ is considerably exceeded for the concentration range under investigation. On the other hand, it is well known that such hydroxides form colloids before precipitation. These colloidal particles can be attached on the wall in addition to the free Fe^{3+} ions. If the colloid is formed in fact [and it is not a metastable condition which is established, namely a supersaturated solution of $\text{Fe}(\text{OH})_3$], it must consist of particles of very small size, because these particles were not visible, *e.g.*, as spikes, in the detector.

The assumption that the resulting phenomena are in fact caused by adsorption on the capillary

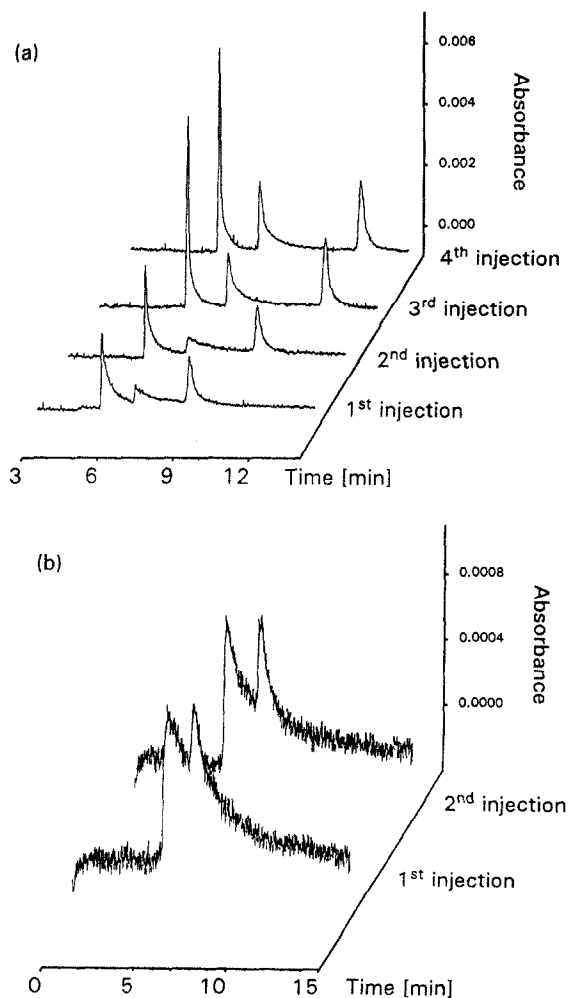


Fig. 6. "Memory effect" on the peak shapes of *ortho*-substituted benzenecarboxylic acids due to attachment of Fe(III) on the wall of the uncoated fused-silica capillary after replacing the buffer containing (a) 500 ppb and (b) 1 ppm of Fe^{3+} by an Fe^{3+} -free running buffer at pH 4.50. Prior to each run the capillary was rinsed with 20 column volumes of Fe-free buffer. Migration sequence of separands as in Fig. 2a. Conditions as in Fig. 1.

surface rather than by secondary equilibria in the bulk liquid phase is supported by the observed "memory effect" when the buffer containing Fe^{3+} is replaced with an Fe^{3+} -free electrolyte, as shown in Fig. 6: the tailing of the peaks still remains.

Acknowledgements

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