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Short communication

Stacking of weakly cationic compounds by acetonitrile for capillary electrophoresis

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Abstract

The inclusion of acetonitrile in the sample causes stacking especially in presence of sodium chloride. It is shown here that the type of the separation buffer and the conditions for the stacking of cationic versus anionic compounds are quite different. Anionic compounds stacked better in high ionic strength inorganic buffers such as borate and phosphate, while the cationic drugs and endogenous substances stacked better in amine and zwitterionic buffers. About 10–20% of the capillary volume can be loaded with sample. Shielding against the negative charges of the silica is a critical factor in stacking of the cationic compounds by the acetonitrile in the sample. The ionic strength of the separation buffer and the addition of organic modifiers affected the stacking to a limited extent.

Keywords: Stacking; Buffer composition; Acetonitrile

1. Introduction

Stacking methods are very important for improving the detection limits in capillary electrophoresis (CE). Usually, a large volume of sample is injected on the capillary and under appropriate conditions the analyte is concentrated into a sharp band. Several methods are used for stacking [1]. In particular, acetonitrile stacking, as we described earlier, offers several practical advantages for sample concentration such as the removal of proteins and toleration of salts [2–4]. It is useful for concentrating many acidic compounds in samples of biological origin such as drugs present in serum. Our attempts for stacking the cationic compounds with the common buffers such as borate and phosphate demonstrated a limited stacking could be obtained [5].

Several factors affect, directly or indirectly, the stacking. Some of these factors are related to the sample itself e.g., ion concentration and pH [6]; while others are related to the separation buffer, e.g., the ratio of the separation buffer conductivity to that of the sample. Cationic compounds and hydrophobic compounds tend to bind to the negatively charged surface of the capillaries and indirectly degrade the stacking as we illustrate in this work. We show here that the type of buffer is important in the stacking of cationic compounds. Many basic endogenous and exogenous compounds can be successfully stacked in zwitterions and amine-containing buffers. These buffers tend to decrease the interaction of the cationic compounds with the silica surface and thus favor stacking.

2. Experimental

2.1. Chemicals

CHES [2(*N*-cyclohexylamino)ethanesulfonic acid], tricine [*N*-tris(hydroxymethyl)methylglycine], anhydrous betaine, ethylenediamine and triethanol-

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amine were obtained from Sigma (St. Louis, MO, USA).

2.2. Electrophoresis buffer

Most of the work was done with a buffer composed of 160 μl triethanolamine, 50 m*M* tricine and 10% acetonitrile. The apparent pH is adjusted to 8.6 or as described.

2.3. Stock drug solutions

The different drugs were prepared at 1 mg/ml in acetonitrile and diluted in 1% sodium chloride to reflect the ionic contents of the serum.

2.4. Acetonitrile stacking

An aliquot of 50 μl of the different drugs diluted in 1% NaCl was mixed with 100 μl acetonitrile; vortex mixed for 15 s and centrifuged at 14 000 *g* for 20 s.

2.5. CE instrument

A Model 2000 CE (Beckman Instruments, Fullerton, CA, USA) was set at 12 kV and 254 nm. The sample was introduced hydrodynamically at low pressure on a 40 cm×50 μm I.D. untreated silica capillary for 70 s (or as specified) filling 12% of the capillary volume (to the detector) and electrophoresed for 10 min at 12 kV (~35 μA).

3. Results and discussion

Most biological samples such as serum contain sodium chloride at about 1% which causes band spreading in CE due to the low field strength; especially when the sample volume exceeds 1% of the capillary volume. Thus, it is important to explore simple methods which can concentrate a large sample volume directly on the capillary ''stack'' without the adverse effects of the salts. The addition of acetonitrile to the sample eliminates proteins and enables tolerating a high concentration of salts with good stacking [2]. Many of the weakly anionic compounds stack well in acetonitrile in the presence of salts; however, they require separation buffers with high ionic strength [2]. Fig. 1 (top) illustrates that 210 m*M* borate buffer, pH 8.6 caused good stacking for the two anionic compounds iothalamic acid and theophylline. About 10% of the capillary was filled with sample which contained one volume of 1% saline and two volumes of acetonitrile. The electropherogram shows good peak shape and high plate number. Unfortunately, the cationic compounds quinine, *N*-acetylprocainamide and doxepin, in presence of 1% sodium chloride and with large sample loading, could not be detected in this buffer. On the other hand, these cationic compounds stacked well in a triethanolamine buffer containing tricine. For example, the theoretical plate number (*N*) for *N*-acetylprocainamide is close to 400 000, Fig. 1 (bottom). However, the anionic compounds did not stack that well in triethanolamine buffer, e.g., *N* for theophylline is 60 000 vs. 104 000 in the borate.

An increase in the electrophoresis buffer concentration enhances the stacking and the separation for the anions [2]. The cationic compounds separated better but the stacking did not improve with an increase in buffer concentration as illustrated in Table 1.

Since the cationic compounds migrate rapidly, the addition of acetonitrile decreased the migration and at the same time greatly improved the resolution of these compounds (Fig. 2). Also the selectivity changed by the addition of acetonitrile as one of the contamination peaks (c2) (Fig. 2) changed its relative migration. However, the effect on the stacking was variable for each compound (Fig. 2). For example, the *N* value for doxepin increased two-fold with addition of acetonitrile; while that for quinine improved slightly, about only 23%, Table 1. The unpredictable effect of both the buffer concentration and the organic modifiers on the stacking of the cations probably is due to the differences in the interaction of these compounds with the capillary wall.

Usually, under non-stacking conditions, the sample volume is kept well below 1% of the capillary volume to avoid band spread. Fig. 3 illustrates that the peak height increased almost linearly with an increase in the sample volume; however, the plate number decreased partially because of the decrease in the capillary length. About 20% of the capillary

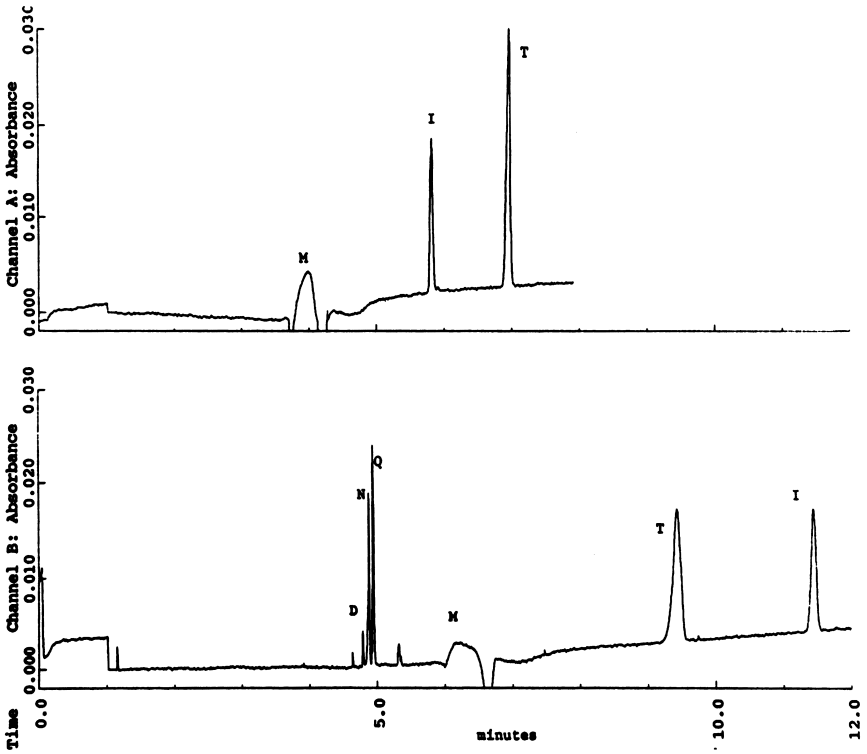

Fig. 1. Effect of the separation buffer type on stacking at sample loading of 12% of the capillary volume: (top) 210 m*M* borate buffer, pH 8.6; and (bottom) 160 m*M* triethanolamine, 50 m*M* tricine, pH 8.6 containing 10% acetonitrile. Separation of a mixture of weakly cationic and anionic compounds in the same run: doxepin (D, 50 mg/l), *N*-acetylprocainamide (N, 50 mg/l), quinine (Q, 20 mg/l), theophylline (T, 50 mg/l) and iothalamic acid (I, 20 mg/l) at 14 kV, 254 nm; (M=electroosmotic flow).

volume can be filled with sample with a theoretical plate number close to 200 000 which is good for many practical separations.

The tricyclic drugs are notorious in binding to the unmodified silica surfaces. For example, in high-performance liquid chromatography (HPLC) deactivated columns are necessary for the analysis of these compounds. Two tricyclic drugs (impramine and amitriptyline) in addition to procainamide and quinine are used here as examples for stacking. A borate buffer (180 m*M*, pH 8.0), which is suitable for the acidic compounds, was not useful for the stacking of these basics (Fig. 4). The addition of some zwitterions such as CHES to the borate buffer improved the analysis slightly. On the other hand, triethanolamine, a buffer known to shield against the negative charge on the surface of the capillary, was much more effective in improving the separation and stacking of these basic drugs. The addition of some zwitterions such as CHES (Fig. 4D) to this buffer improved the separation further so the procainamide becomes detectable as a small peak. Other zwitterions such as 3-cyclohexylamino-1-propanesulfonic acid (CAPS) (Fig. 4E), tricine and betaine affected, to different degrees, the stacking and the separation. For example, tricine offered much better separation

Table 1
Effect of the buffer molarity on stacking, as measured as peak height (mA) and as theoretical plate number (*N*), for doxepin and quinine

Triethanolamine (m*M*)	Doxepin		Quinine	
	N	mA	*N*	mA
320	170	12	150	27
160	250	13	210	32
80	230	14	190	33
160+50% acetonitrile	410	21	230	35

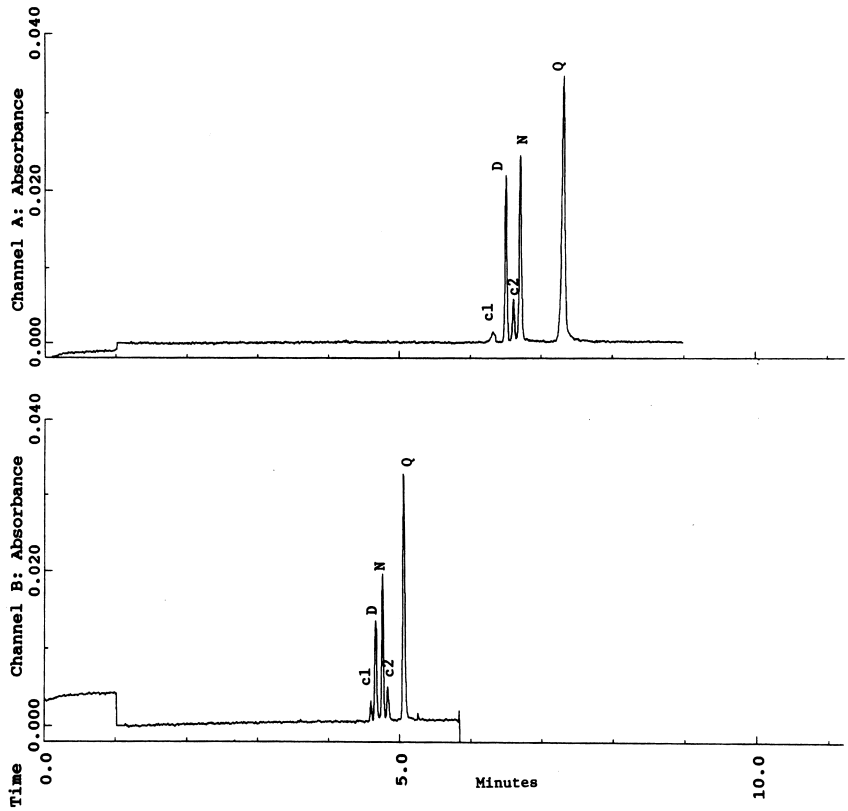

Fig. 2. Effect of acetonitrile on stacking at sample loading is 12% of the capillary volume: doxepin (D), *N*-acetylprocainamide (P) and quinine (Q): (top) in the presence, and (bottom) absence of 50% acetonitrile added to the separation buffer (60 m*M* triethanolamine, and 50 m*M* tricine, pH 8.5, at 254 nm, 11 kV. c1, c2=Contamination peaks. Time in min.

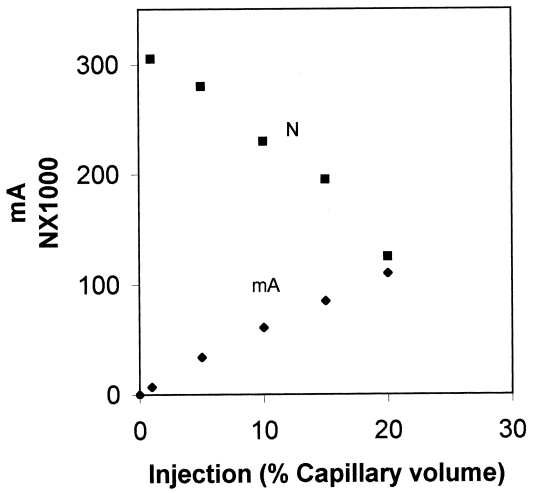

Fig. 3. Sample size as % of the capillary volume vs. theoretical plate number (*N*) and peak height as mA units for quinine at 11 kV, 254 nm. The buffer is 320 m*M* triethanolamine containing 150 m*M* CHES, pH 8.2, and 25% acetonitrile.

than betaine in this respect; however, it was not successful as a buffer (210 m*M*, pH 8.6) by itself without the triethanolamine. The effect of the zwitterions on the separation and the plate number for proteins is well documented [7,8]. Polyethylene glycol (PEG), which simply shields against the silica surface and increases the viscosity, also improved slightly the stacking indicating that adsorption of the cationic compounds to the surface of the capillary is a major problem.

We checked also the stacking of some endogenous substances such as dopamine, metanephrine and tyramine (Fig. 5). When the sample contained 1% sodium chloride, these compounds did not stack in a low ionic strength phosphate buffer (50 m*M*, pH 6.7). However, when triethanolamine is used in place of the phosphate as an electrophoresis buffer, a better separation and a better stacking is obtained. The separation is improved further by the addition of

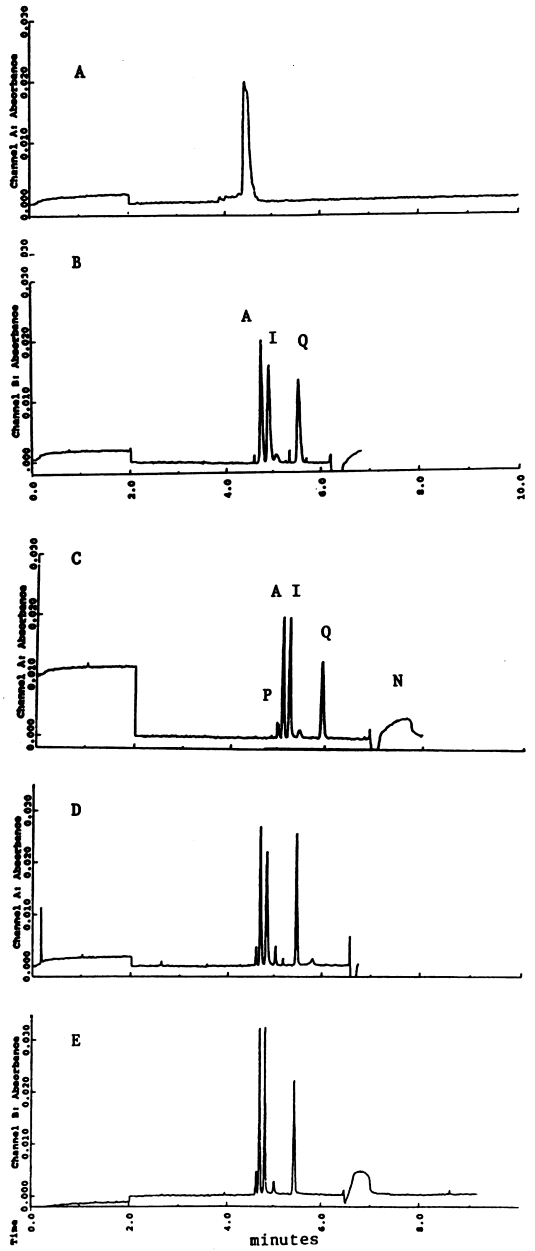

Fig. 4. Separation of procainamide, 20 mg/l (P), amitriptyline, 100 mg/l, (A), impramine 100 mg/l (I) and quinine 80 mg/l (Q) at 254 nm, 12 kV. (Sample loading is 6% of the capillary volume, N=electroosmotic flow): (A) 180 m*M* borate buffer, pH 8.2; (B) 180 m*M* triethanolamine, pH 8.2; 10% acetonitrile and 10% isopropanol; (C) buffer B+1% PEG; (D) buffer B+100 m*M* CHES; and (E) buffer B+100 m*M* CAPS.

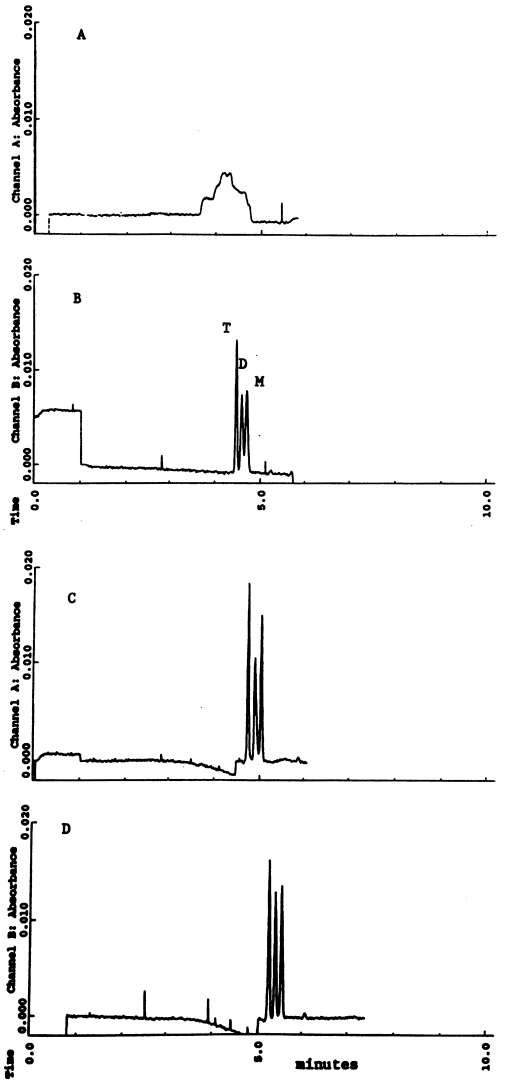

Fig. 5. Separation of 50 mg/l of each: tyramine (T), dopamine (D), and metanephrine (M) at 214 nm, 10 kV. Sample loading is 10% of the capillary volume: (A) 60 m*M* phosphate buffer, pH 7.2; (B) 80 m*M* triethanolamine, pH 7.2 containing 20% acetonitrile; (C) buffer B+100 m*M* tricine, pH 7.2; and (D) buffer B+100 m*M* CHES, pH 7.2.

CHES (Fig. 5D) or PEG to the buffer such that the sample volume can be increased above 10% of the capillary volume. We tried diethylamine as another buffer. It gave better stacking than the phosphate but it was inferior to that of the triethanolamine buffer. The addition of 20% acetonitrile to the buffer improved the plate number for metanephrine alone.

Several factors usually contribute to the overall band broadening or to the height equivalent of theoretical plate in CE such as diffusion, Joule heating, electrodispersion, injection plug and adsorption to the capillary walls. The latter two are considered the major contributing factors [9]. Their effect on stacking is well demonstrated here in this work especially the wall effect which seems to be a very critical factor for the cationic compounds. The variable effects of the organic modifier and the buffer ionic strength on the stacking indicate that there are several factors playing different roles in the stacking of these compounds as expected.

The triethanolamine as a buffer was used earlier for the stacking. It enhanced the stacking and offered a transient isotachophoresis step for insulin analysis [8,10]. This work illustrates that cationic compounds can be stacked for capillary zone electrophoresis using acetonitrile. About one fifth of the capillary can be loaded with sample; however, these compounds require different types of buffers compared to the anionic compounds. It is important to point out that this stacking is not only applicable to pure standards but occurs also for compounds present in serum samples deproteinized with acetonitrile. We have added several of these compounds such as procainamide to the serum; deproteinized the samples with acetonitrile and obtained the same separations as demonstrated for pure saline solutions.

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