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Field amplified injection in the presence of salts for capillary electrophoresis

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Abstract

Salts in the sample are detrimental to the stacking by the field-amplified injection. However, physiological samples often contain salts at levels of about 1% which can diminish the peak height or cause band spreading instead of stacking. Using different analytes which contain salts, we demonstrate that the presence of acetonitrile at 66% in the sample reverses the deleterious effect of salts and favors the stacking by the electrokinetic injection. The advantage of this type of stacking is that it favors certain analytes over others and it can give, in some instances, better theoretical plate numbers.

Keywords: Field amplified injection; Injection methods; Sample stacking; Hydrodynamic injection; Hippuric acid

1. Introduction

Samples from physiological origin normally contain salts at about 1% concentration. Unless they are removed, salts restrict or diminish the amount of sample which can be loaded on the capillary and can cause peak broadening especially in the case of field amplified injection [1,2]. Desalting is not suited for routine analysis especially on small sample volumes. Previously, we have shown that stacking in the hydrodynamic injection mode can be achieved in the presence of physiological amounts of salts in the sample, provided acetonitrile is added to the sample itself [1,3–5]. About half of the capillary volume can be filled with sample [3] yielding a concentration factor of 10–20 times obtained by such a simple maneuver and thus improving the detection signal by such factor. In addition to that, proteins, which can adsorb onto the capillary wall and can ruin the separation, are eliminated too in this process decreasing the need for a lengthy capillary wash with sodium hydroxide after each sample [6]. However, as the sample size is increased the effective capillary length decreases too, leading to a drop in the theoretical plate number [7]. The mechanism behind this stacking is not clear but is thought to involve the low conductivity of the acetonitrile with a transient-isotacophoretic-like effect brought about by the salts [5].

The transfer of the analytes in the sample by electromigration to the capillary is greatly affected by the salts which in turn affect the field strength. If the salt concentration is high enough in the sample no peaks are detected. Here it is demonstrated that the addition of acetonitrile to the sample can produce a stacking effect in the electrokinetic injection similar to that by the hydrodynamic injection, in

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spite of the presence of salts in the sample. A low concentration of salt in the presence of acetonitrile improves the stacking and increases the theoretical plate number. This effect is dependent greatly on the ionic strength of the separation buffer. The ease of the electro-injection, better reproducibility of the long injection time, and the elimination of the lengthy capillary wash between samples render this technique of injection quite suitable especially for the laboratory-made instruments.

2. Material and method

2.1. CE instrument

Most of the work was performed on a Model 2000 CE (Beckman Instruments, Fullerton, CA, USA) set at 12 kV and 254 nm. The sample was introduced on a short capillary 27.5 cm (effective length 21 cm)× 50 μm I.D. of untreated silica capillary for 50 s filling about 33% of the capillary volume (pressure mode) and electrophoresed for 3 min. The capillary was washed for 0.5 min with 0.2 *M* NaOH and filled with the electrophoresis buffer for 1.0 min.

2.2. Electrophoresis buffer

Boric acid 150 mmol/l adjusted to pH 8.5 with 2 *M* NaOH.

2.3. Field amplified injection

The sample, containing hippuric acid, was injected electrokinetically for 99 s (or as specified) at 8 kV.

2.4. Hippuric acid stock standard

(200 mg/l) dissolved in electrophoresis buffer. This standard was diluted 10 fold in 1% saline or water as specified.

3. Results and discussion

We used in these experiments hippuric acid dissolved in different diluents as an example to investigate the effect of salts on the stacking by the electrokinetic injection. When hippuric acid in the sample is injected electrokinetically under non-stacking conditions, i.e. dissolved in the same electrophoresis buffer (or in 1% NaCl) the salts or the high ionic strength in the sample deteriorates the separation and affects greatly the peak height in CE as expected, Fig. 1. Short injection time about 2–5 s can be tolerated to a certain extent but the detection signal for the hippuric acid is very poor, Fig. 1A. Increasing the injection time to improve the detection

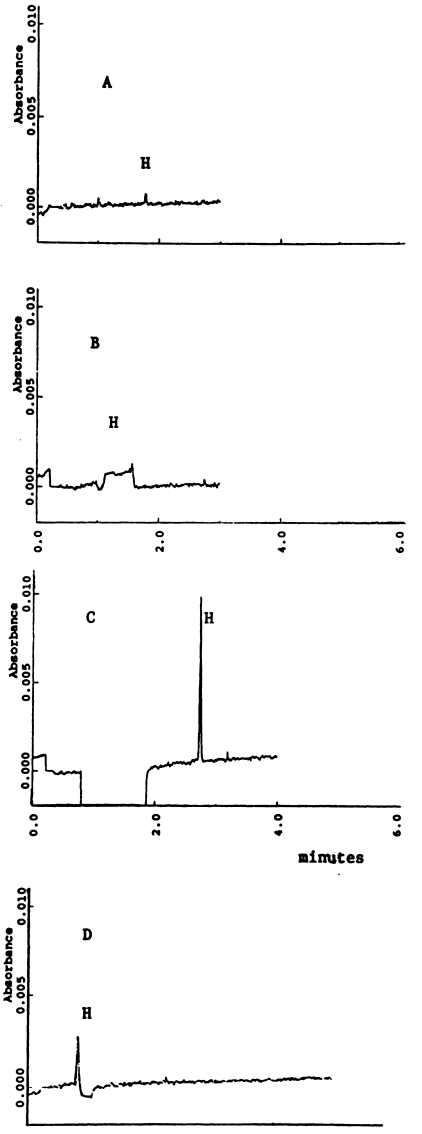

Fig. 1. Effect of the sample diluent on the peak height for hippuric acid by electrokinetic injection (8 kV) with separation at 10 kV. The sample contained hippuric acid (H) 20 mg/l, and in addition to that it contained in A, B, and C 1% NaCl: (A) Hippuric acid in 1% NaCl (100 μl)+200 μl water. Injection, 3 s. (B) As in A, but the injection is 60 s. (C) Hippuric acid in 1% NaCl (100 μl)+200 μl acetonitrile. Injection, 60 s. (D) Hippuric acid in water (100 μl)+200 μl acetonitrile. Injection, 60 s.

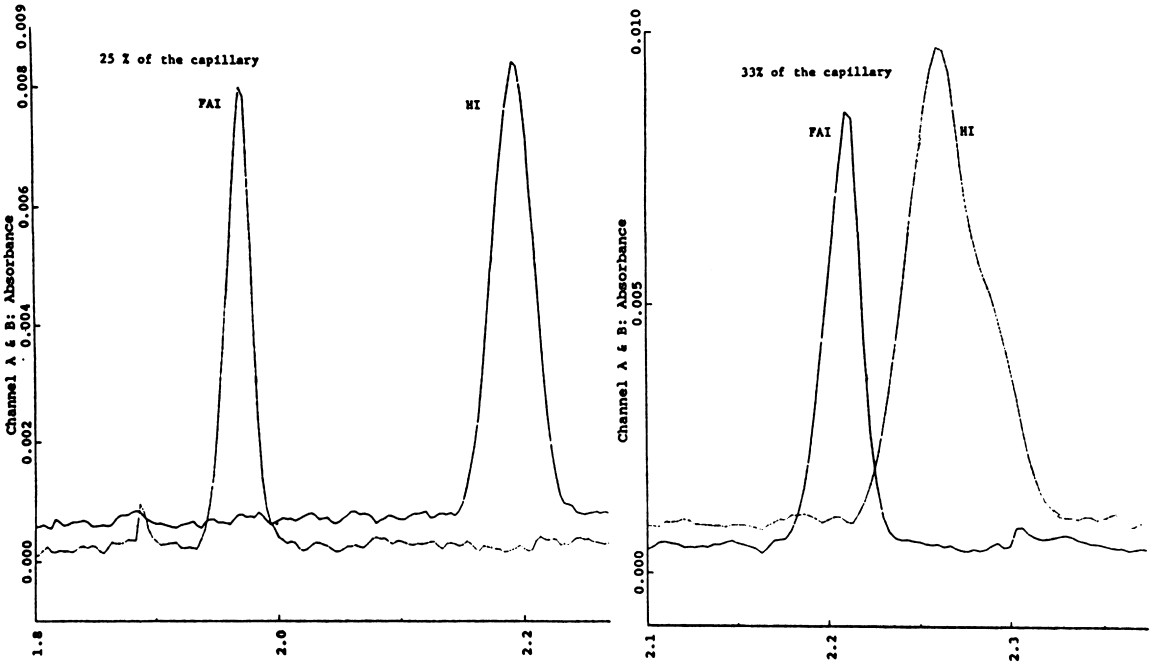

Fig. 2. The peak shape of hippuric acid by the hydrodynamic injection (HI) at 25% and 33% of the capillary volume vs. that of the electrokinetic injection (FAI) (15 s at 9 kV). The sample (100 μl) contained hippuric acid in 1% NaCl in addition to 200 μl acetonitrile with separation at 10 kV.

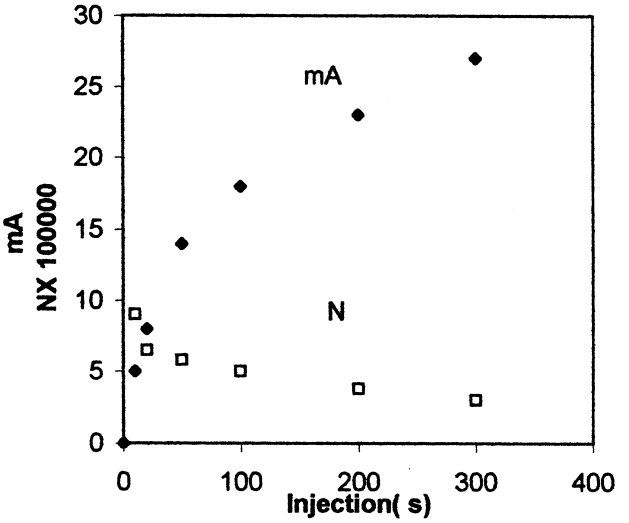

Fig. 3. Effect of sample size by electrokinetic injection on peak height and theoretical plate number for hippuric acid. Other conditions as in Fig. 2.

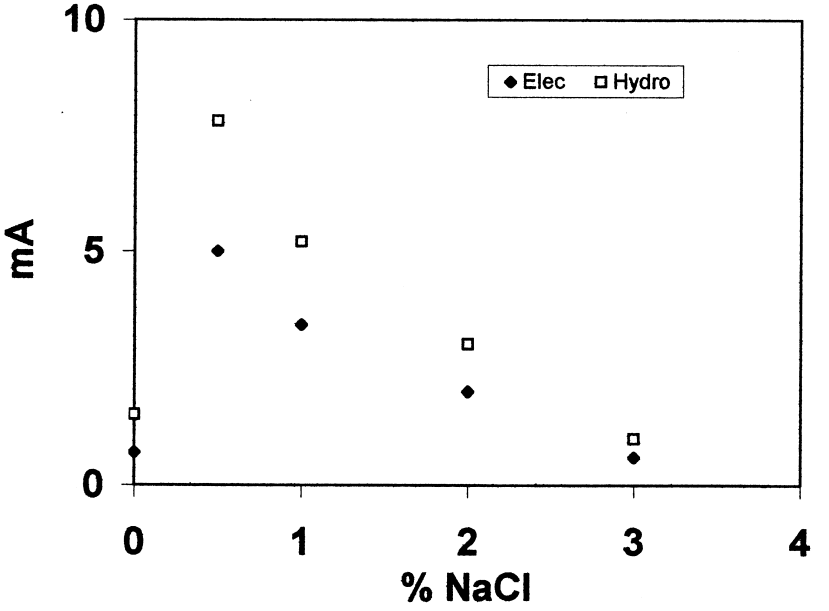

Fig. 4. Effect of the salt content in the sample on the peak height by both electrokinetic and hydrodynamic injections for hippuric acid (H). Analysis conditions as is in Fig. 1C.

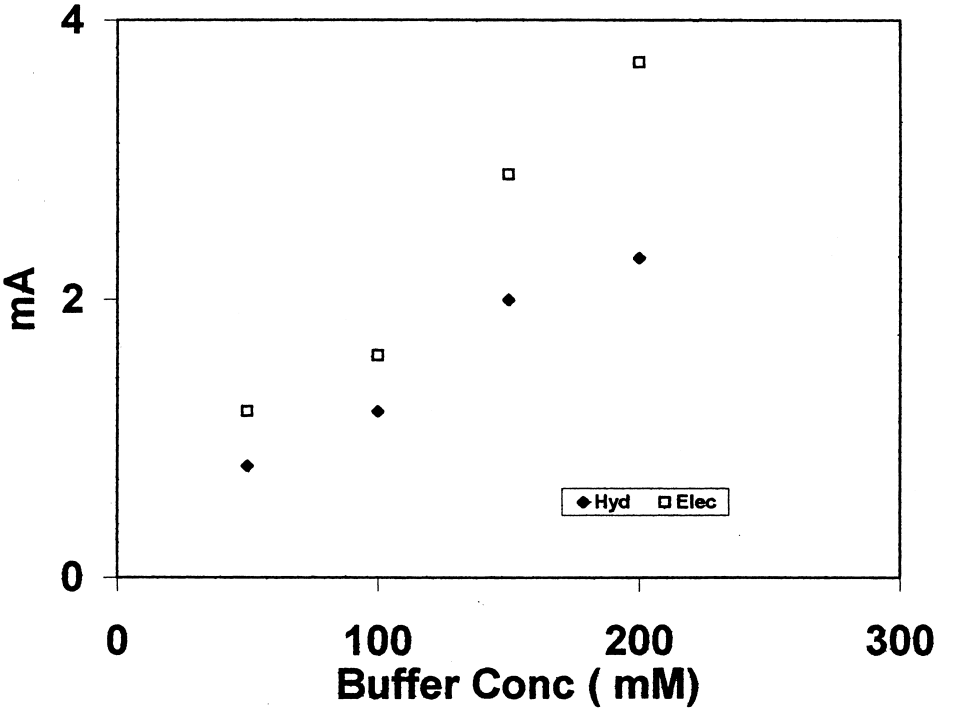

Fig. 5. Effect of the separation buffer concentration on the stacking by both hydrodynamic and electrokinetic injections for hippuric acid; analysis conditions as in Fig. 1C.

through stacking leads to a broad peak (Fig. 1B) or no peaks at all. On the other hand, when acetonitrile is added to the previous samples, at a concentration of 66%, the harmful effect of salts are reversed, and stacking for the hippuric acid peak, H occurs (Fig. 1C) similar to what we have described previously with the hydrodynamic injection [3]. The effect of this stacking is dependent on the presence of acetonitrile and salts. In absence of salts the acetonitrile gives a limited stacking for the hippuric acid, Fig. 1D, due to the low conductivity.

The electrokinetic injection has some advantages and some drawbacks especially for quantification of multiple components. The components enter the capillary at different rates based on their mobility and the field strength and thus give peaks with different heights/areas. However, this can be an advantage in some situations when a rapidly migrating analyte, is sought for analysis. The majority of the commercial instruments can be used for sample introduction using either hydrodynamic or electrokinetic injections. Fig. 2 illustrates that for the same peak height the electrokinetic injections give narrower peak (better plate number) than the hydrodynamic. For example, the hydrodynamic injection gave a good peak shape up to 25% of the capillary

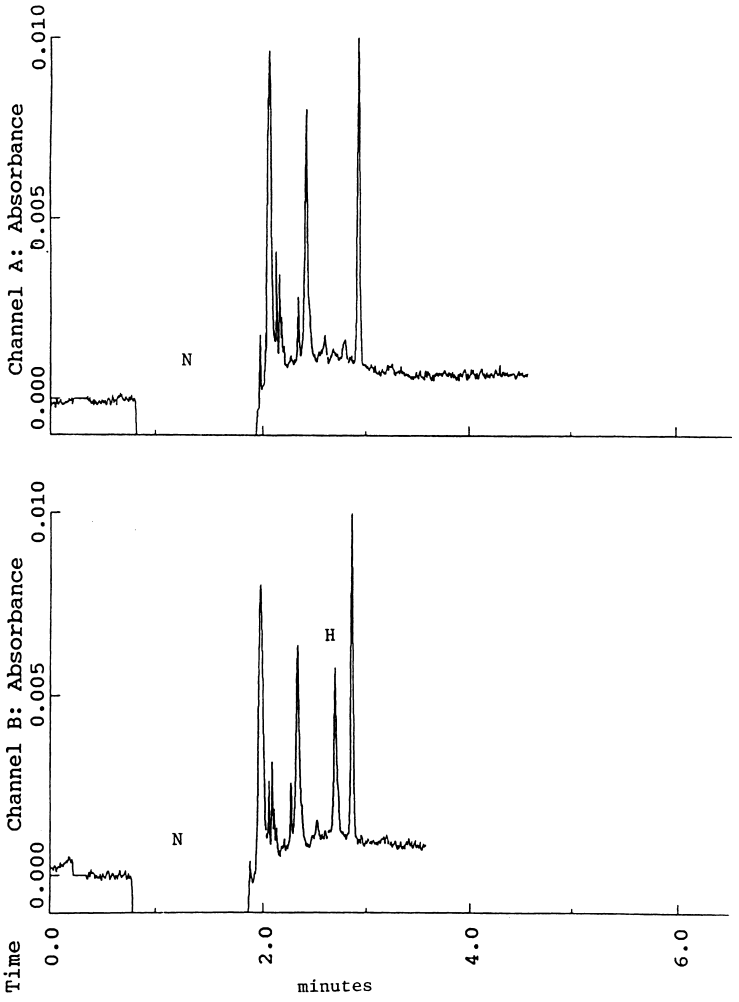

Fig. 6. (A) The supernatant of a serum sample (100 μl) deproteinized with (200 μl) acetonitrile; (B) as in (A) but with hippuric acid (H), 10 mg/l added to the supernatant, 60 s at 8 kV injection.

volume. At a sample loading of 33% the peak starts to split which is more evident as the sample loading is increased to 41% giving rise to two separate peaks. In general, the electrokinetic injection gave 3-times increase in peak height over that for the hydrodynamic injection. This effect is probably due to the fact that in the hydrodynamic injection the effective capillary length decreases, as the sample size is increased [3]. As with the hydrodynamic injection [7], an increase in the injection time, the peak height increases non-linearly; while the theoretical plate number decreases, Fig. 3.

Serum contains sodium chloride at about 1% which causes band broadening or diminished peak height. In addition to that the serum contains a high concentration of proteins which adhere to the capil-

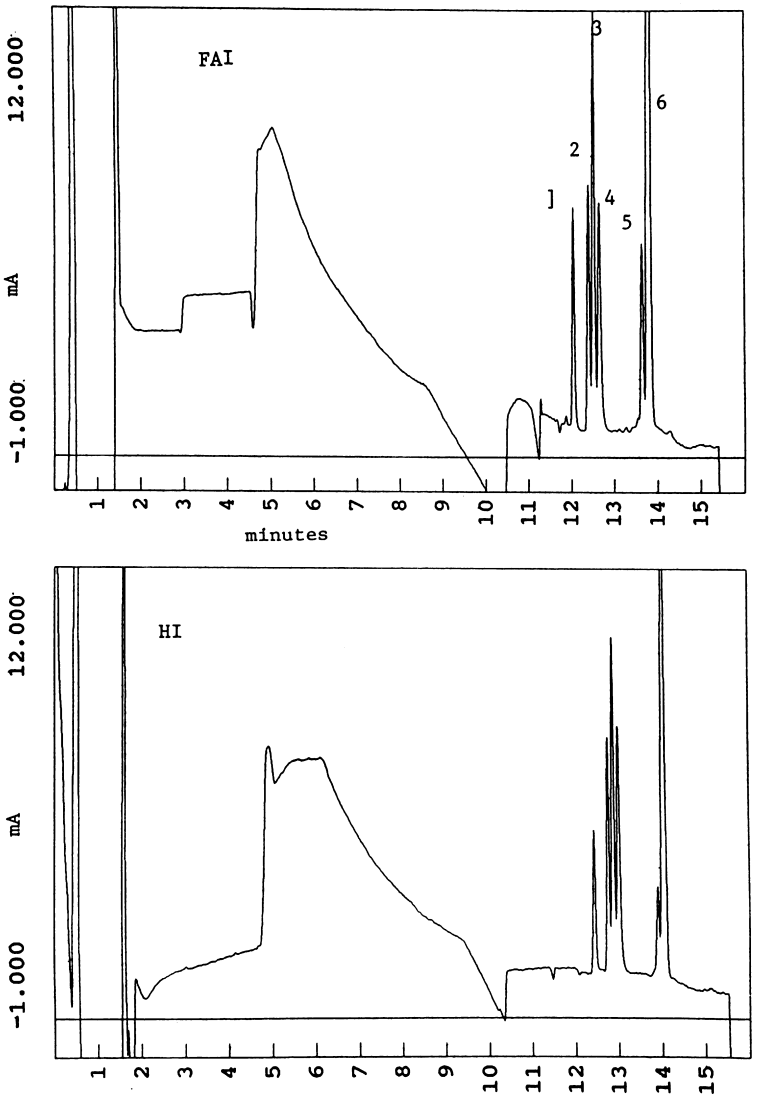

Fig. 7. Effect of the type of injection: (A) hydrodynamic vs. (B) electrokinetic for several catecholamines (50 mg/l) in 1% NaCl (100 μl) and acetonitrile (200 μl): 1-dopamine, 2-normetanephrine, 3-metanephrine and epinephrine, 4-norepinephrine, 5-impurity, and 6-quinine (10 mg/l). Conditions (Waters CE, capillary 50 cm×75 μm, 15 kV 100 s injection 229 nm; separation buffer 10 g tricine, 3 ml triethanolamine/100 ml at pH 7.8.

lary wall and ruining further the separation. Serum deproteinization with two volumes of acetonitrile eliminates the proteins but the salts remain in the supernatant. The effect of different salt concentrations in the presence of acetonitrile on the peak height is illustrated in Fig. 4. Low concentrations of salts can be tolerated; however as the concentration is increased there is a continuous decrease in the peak height, Fig. 4. This effect becomes important in quantification of samples with different salt content such as urine samples. In this case, for accurate quantification, standards have to be added to the sample directly.

Fig. 5 illustrates that the separation buffer concentration affects the peak height for both the electrokinetic as well as the hydrodynamic injection. As the molarity of the buffer increases relative to the sample buffer the stacking force also increases producing higher theoretical plate number and peak height [8]. High concentration of the separation buffer enables tolerating more salt in the sample and decreases the interaction of the analytes with capillary wall too.

In practice, it is important to be able to extend this data to the analysis of various compounds present in physiological fluids such as serum. Fig. 6 illustrates that the addition of hippuric acid to the supernatant of the serum gives a sharp peak. In fact most of the unknown compounds in the chromatogram have also sharp peaks regardless of the long injection time (60 s) and the short capillary length. Since the different peaks were present in the wide injection zone N (Figs. 6, 1C) the width of the neutral zone to that of the different peaks indicates visually the degree of the stacking obtained for each peak. It is important to point out that the supernatant of serum has a pH of ~7.4. Thus some cationic compounds may not be ionized at this pH. A decrease in the pH of the supernatant might be necessary for detecting such compounds [9]. Fig. 7 illustrates not only hippuric acid can be stacked by the electro-injection but other compounds such as the different catecholamine metabolites can be stacked too using different set of conditions of separation.

The acetonitrile does not only lead to stacking of the samples containing salts but indirectly improves precision of quantification. In CE precision is dependent on two important factors; the concentration of the analyte and injection time [6]. Both of these parameters are improved in this type of stacking leading to a better overall precision.

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