Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1066 (2005) 205–210
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Organic solvent high-field amplified stacking for basic compounds in capillary electrophoresis

Zak K. Shihabi*

Department of Pathology, Wake Forest University School of Medicine, Baptist Medical Center, Winston-Salem, NC 27157, USA

Received 31 August 2004; received in revised form 12 December 2004; accepted 14 January 2005
Available online 30 January 2005

Abstract

Many water-miscible organic solvents, especially acetonitrile and acetone, bring along significant degrees (~30 times) of stacking by electroinjection through high-field amplified injection for the basic compounds compared to that for aqueous buffers or water. The relative stacking of different compounds in acetonitrile or acetone is different compared to that for water. Stacking by electroinjection in organic solvents is less stringent and easier to accomplish in practice. Acids and salts, in aqueous solutions, can ruin the stacking for both organic and aqueous solvents; however, this effect can be better tolerated by diluting the sample in acetonitrile. Thus, this stacking is termed "organic solvent high-field amplified injection". This stacking by electroinjection is enhanced by increasing the electrophoresis buffer concentration and can be better than that by pressure injection. From the practical aspects, some cationic drugs present in serum such as amiodarone can be detected at the therapeutic levels by electroinjection on the capillary after protein precipitation by acetonitrile.

Keywords: Stacking; Basic drugs; High-field; Electromigration; Electroinjection; Pressure injection; Amiodarone; Quinidine

1. Introduction

It remains to be a common practice in CE to dissolve the sample in aqueous buffers, especially in a dilution of the same separation buffer, or just in plain distilled water [1,2] and inject it hydrodynamically on the capillary. This approach is simple and gives satisfactory separation in the majority of analyses. However, for compounds present at low concentration, it may not be a good choice for a sensitive detection. Previously, we have shown that anionic compounds dissolved in acetonitrile and salts and injected hydrodynamically concentrate 10–30 folds on the capillary (stacking) leading to improved sensitivity [3]. The acetonitrile has the added advantage of removing the excess of proteins present in the sample [4,5]. The basic mechanism behind this type of stacking is pseudo-transient isotachophoresis [5]. Stacking of basic compounds is more difficult than that for anionic ones [6]. Many of these compounds are hardly water-soluble and also they tend to adsorb to the capillary walls distorting their stacking. Many of the pharmacologically active drugs are basic compounds such as; amiodarone, morphine, codeine, oxycodone, tricyclics and catecholamines. These compounds are present in low concentration. Practical methods for measuring these compounds from biological tissues by CE are greatly needed.

Electroinjection (electromigration) is not used as commonly as the hydrodynamic injection for sample introduction in CE because it is subject to many variables, which can lead ultimately to concentration bias. On the other hand, Palmer et al. [7] as well as we [8] have shown that the electroinjection has the ability to concentrate the sample on the capillary far more than can be achieved by the hydrodynamic injection [7]. In theory, the capillary can be loaded very rapidly with sample at the inlet, beyond its full size; yet it remains to provide a good separation and good theoretical plate number [7,8]. This concentration is greatly needed to improve the poor sensitivity of the CE. Unfortunately, the electroinjection is greatly affected by salts and acids in the sample. Often

* Tel.: +1 336 716 2639; fax: +1 336 716 9944.
E-mail address: zshihabi@wfubmc.edu.

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.021

cationic compounds are dissolved in dilute acids in order to solubilize them or to convey the positive charge. Excess of acids can arise also during protein precipitation especially in biological fluids such as plasma. Neutralization of acids can be difficult since it results in an excess of salts, which again ruins the separation.

In order to improve the detection limits of the basic compounds in CE, the combination of electroinjection and organic solvent field amplified stacking is investigated in this work. Here, we investigate the effect of diluent (aqueous versus organic) on the stacking by the electromigration, i.e. under field-amplified injection on the analysis. We demonstrate that electroinjection, in conjunction with dissolving the sample in acetonitrile, yields far better stacking than that obtained by pressure injection in conjunction with aqueous solvents. We study the effects of acids and salts on both types of injections. Furthermore, we attempt to extend these studies to the analysis of drugs in serum. Since stacking under electroinjection from acetonitrile or organic solvents is different from that of aqueous solutions it is suggested to be termed as "organic solvent high-field amplified injection".

2. Materials and method

2.1. Chemicals

Tyramine HCl, propranolol HCl, quinidine sulfate dihydrate, quinidine anhydrous and amiodarone HCl were obtained from Sigma Chemicals (Saint Louis, MO, USA); Quinine dihydrate from Aldrich Chemicals, Milwaukee, WI, USA); and amitriptyline HCl from USP Inc, Rockville, MD, USA.

2.2. Stock solution

Stock solution (300 mg/l in 20% methanol in water) was prepared of the following compounds: tyramine, amitriptyline, propranolol, and quinine sulfate. This stock solution was diluted 20 fold either in water, or acetonitrile. Amiodarone 100 mg/l was dissolved in 50% methanol. Quinidine anhydrous and quinidine sulfate (500 mg/l) were prepared in water.

2.3. Instrument

A Model 2000 CE instrument (Beckman, Fullerton, CA, USA) with a short capillary 30 cm × 50 μm (I.D.) (Polymicro Technologies, Scottsdale, AZ, USA) was set at 8 kV, 214 nm with hydrodynamic sample injection at 10 s or by electroinjection at 2 kV for 3 s.

2.4. Separation buffer

The separation buffer was phosphate, 60 mmol/l, pH 6.2.

3. Results and discussion

Electroinjection from water or low ionic aqueous buffers leads to a high degree of sample concentration on the capillary due to the high-field strength [2,9,10]. Here, two model basic drugs propranolol and quinine were dissolved in several diluents and the sample was introduced by electroinjection. The peak height of these two compounds in acetonitrile was about 40 times higher compared to that in distilled water or for that prepared in the diluted electrophoresis buffer. This indicates that a better stacking is taking place in many of these organic solvents relative to that for water. The mechanism for stacking in organic solvents, to some extent, is similar to that for water, i.e. high-field strength injection. However, it is also different since it is occurring in organic solvents which can affect the field strength, ionization and solubility of the analytes and also of the co-ions differently from aqueous solvents. Other organic solvents such as ethanol, isopropanol and acetone brought also similar stacking (Fig. 1). However, the degree of stacking and the relative ratio of the peaks heights are different. For example the ratio of propranolol to quinine peaks are 10, 3, and 2 in acetone, acetonitrile and water, respectively. Organic solvents also have the advantage that small ions and salts which can ruin the separation [10], have limited solubility in these solvents. For this reason, this type of stacking can be specifically termed "organic solvent high-field amplified injection".

In the second experiment, we studied the stacking efficiency (ES) [11] of quinidine as a sulfate and as anhydrous, where

$$\mathrm{ES} = \frac{\text{concentration in the sample zone}}{\text{concentration of the concentrated band}}.$$

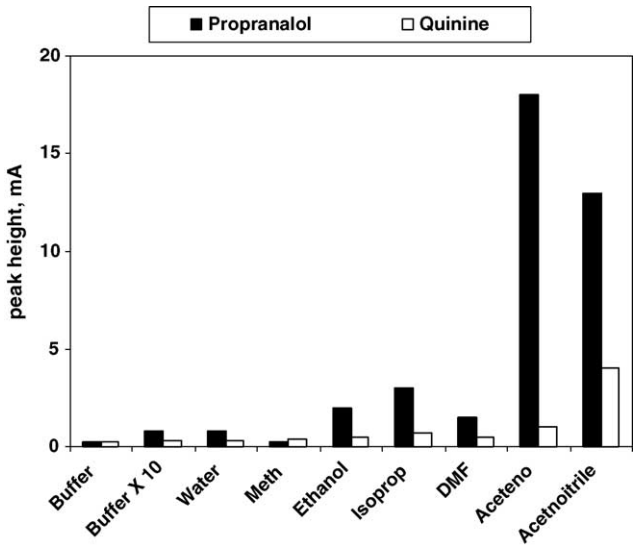

Fig. 1. Effect of different solvents on sample stacking of propranolol and quinine by electroinjection, 3 s at 3 kV: electrophoresis buffer, electrophoresis buffer diluted 10 times, water, methanol, ethanol, isopropanol, dimethylformamide, acetone and acetonitrile, respectively.