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Original Article

Effect of Capillary Rinsing Protocol on the Reproducibility of Separation in Capillary Electrophoresis with Indirect UV Detection

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Abstract

Capillary electrophoresis (CE) with indirect UV detection is an interesting analytical method for the analysis of drugs and pharmaceuticals. Good and reproducible capillary quality is needed to develop robust methods and to facilitate method transfer in CE. It is widely accepted that preconditioning procedures are indispensable in capillary electrophoresis in order to achieve reproducibility of migration times and peak areas.

In order to explore different aspects of this technique, a set of experiments were performed using vigabatrin as a model drug. The effects of capillary rinsing between each run was investigated using basic (NaOH 0.1 M) and acidic (phosphoric acid 0.1 M)-wash cycles. The results of 10 consecutive injection of the model drug after each of the two wash cycles, reveal that more reproducible results obtained when acid-wash cycle was performed as a capillary conditioning protocol. The higher pH changes during basic-wash cycle and its effects on the characteristics of the capillary inner surface were suggested as a source of greater variation between consecutive runs.

Keywords: Capillary electrophoresis; Indirect UV detection; Capillary rinsing protocol.

Introduction

In the research and industrial pharmaceutical settings, the measurement of chemical properties such as purity, chiral purity, inorganic ion content, and identity confirmation is routinely performed by HPLC and other chromatographic techniques. In recent years, there have been rapid developments in capillary electrophoresis (CE) as an analytical technique. The employment of CE for the analysis of drugs and pharmaceuticals has been demonstrated in excellent reviews (1, 2). The popularity of CE in the pharmaceutical fields has been accelerated by its simplicity, high efficiency and selectivity, and large separation capacity (3).

Indirect UV detection is often used in CE for the analysis of chemical compounds that lack an intrinsic chromophore or fluorophore. In indirect detection, an absorbing or fluorescing ion, typically called the probe, is added to the buffer. The probe ions are displaced by analyte ions of the same charge and similar mobilities. Displacement of the probe by the analyte produces a decrease in signal (4, 5). The main advantage of indirect photometric detection is that it offers universal detection.

There are only a few reports discussing the use of CE with indirect detection for drugs and pharmaceuticals (6). In order to explore different aspects of indirect UV detection in CE applied

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for drug and pharmaceutical analysis, a set of experiments were performed using vigabatrin, an anti-epileptic drug (7,8) with poor UV absorptivity, as a model drug (9). The aim of the present work is to investigate the effects of rinsing of the capillary before the main analysis, on the efficiency and reproducibility of the CE with indirect UV detection method.

Experimental

Materials

All solutions were prepared with double distilled water. Vigabatrin and 8-aminocaprylic acid (internal standard, I.S.) were purchased from Sigma (St. Louis, MO, USA), benzyl triethyl ammonium hydroxide (BTEA) was obtained from Lancaster (Winham, USA) as a 40% solution in methanol. All of the chemicals used were reagent grade or better, and were used without further purification.

Buffers were prepared from hydrated sodium salts of dihydrogen orthophosphate (Merck, Darmstadt, D-6100, Germany) after the required amount of BTEA had been added. The pH was adjusted using 0.1 M phosphoric acid or 0.1 M NaOH (Merck).

CE Instrument and conditions

All experiments were performed on a Biofocus 3000 (BioRad, Hercules, CA, USA) instrument equipped with an on-column diode array UV absorbance detector. Data acquisition and control were preformed using Biofocus 3000 Operating software version 6.0 (BioRad Laboratories, Hercules, CA, USA) for Windows 95 on a Pentium II personal computer. Untreated fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 µm, an outer diameter of 365 µm, and a total length of 40 cm (32 cm to the detector) were used. In all experiments, the capillary was thermostatted at 25°C. Samples were introduced by hydrodynamic injection (50 mBar pressure injection) for 5 sec at the anodic end of the capillary and detected at 214 nm. Separations were performed at 25 kV, which was experimentally determined to be within the linear portion of the Ohm's plot.

Results and discussion

Correctly choosing an indirect detection probe and providing suitable buffering of the background electrolyte (BGE) are very important factors in the development of a robust analytical method (10-12). Vigabatrin and I.S are both amphoteric compounds that bear a positive charge in low pH buffers. Thus, to begin method development a 10 mM sodium phosphate buffer at pH 2.5 was prepared and used as BGE. Further modifications were performed on the buffer composition and pH. The final buffer composition was then 5 mM sodium dihydrogen phosphate and 5 mM BTEA at pH 2.2.

Capillary Rinsing Protocol

A CE method may consist of several steps: pre-rinse wash cycle with, for example dilute sodium hydroxide solution or water, pre-rinse wash cycle with the running buffer, sample injection, separation process and post-rinse wash cycle with water or sodium hydroxide solution. In CE it is important to rinse the capillary between runs to regenerate the silanol functionality on the capillary surface. This ensures a reproducible electroosmotic flow, and thus reproducible migration times. This is especially important when indirect UV detection is applied. Adsorption of probe molecules can cause baseline noise and artifacts in indirect detection (13).

Traditionally, in CE methods, alkaline rinses are used to wash the capillary between runs (14). In our study, acidic buffer conditions had been established for the indirect UV detection of vigabatrin and I.S. Thus, rinsing the capillary with alkaline solutions between each run, may cause dramatic changes on the capillary wall. This may affect reproducibility of the method.

Figure 1 shows the reproducibility observed for ten consecutive injections of a standard solution of 100 µg/ml vigabatrin and I.S. using

Table 1. Reproducibility of the method under two protocols for rinsing the capillary between runs.

Wash Cycle	RSD (%) for migration time	RSD (%) for peak-height ratio
Basic	1.1	3.4
Acidic	0.2	1.2

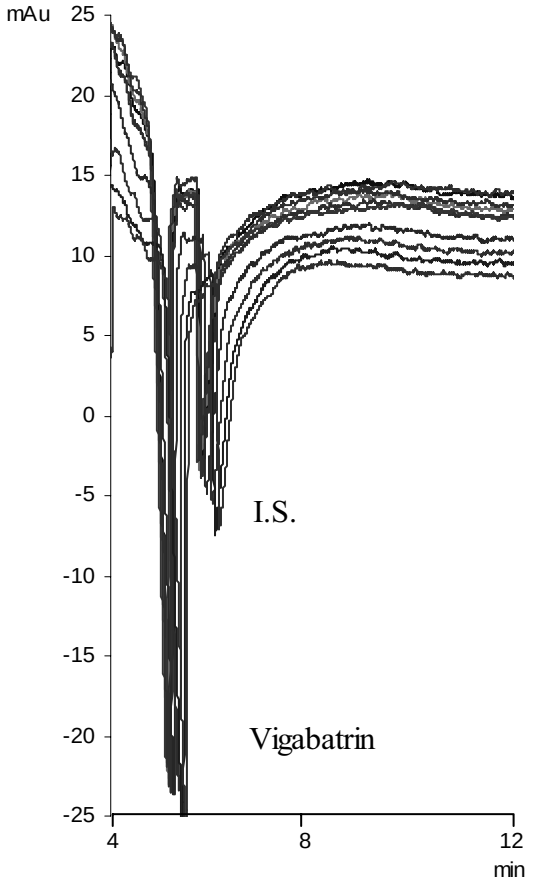

Figure 1. Electropherogram showing 10 consecutive injection of vigabatrin and 8- aminocaprylic acid (I.S.) using basic wash cycle (NaOH 0.1 M) for between run capillary rinsing. CE and indirect UV detection conditions are described in Experimental.

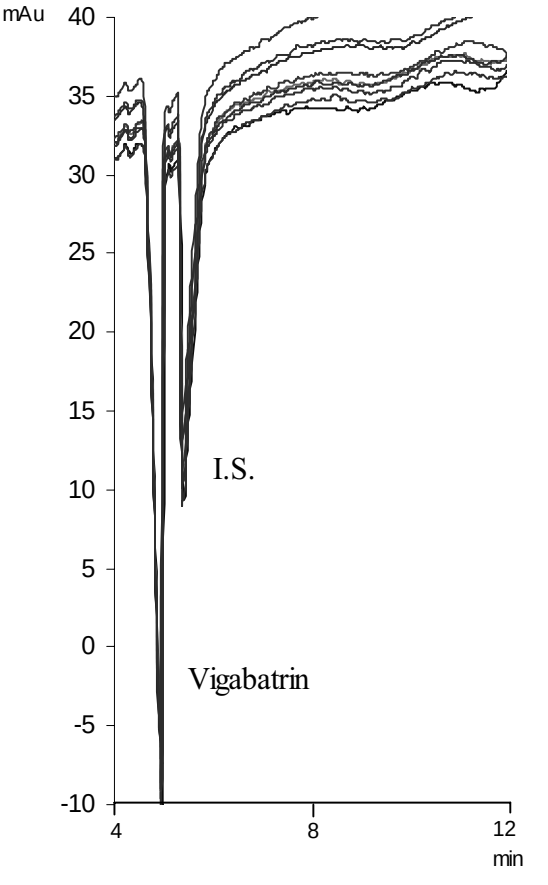

Figure 2. Electropherogram showing 10 consecutive injection of vigabatrin and 8- aminocaprylic acid (I.S.) using acidic wash cycle (phosphoric acid 0.1 M) for between run capillary rinsing. CE and indirect UV detection conditions are described in Experimental.

between-run rinses of 0.1 M NaOH for 1 min, followed by water for 1 min, and then the running buffer for 2 min. The same repeatability experiment was performed except that acidic between-run rinses by means of 0.1 M phosphoric acid rinse for 1 min followed by the buffer for 2 min was used. The results are shown in Figure 2. Better reproducibility was obtained using the acid wash cycle (Table 1). It is seen that between run variations under acid-wash cycle is lower than the basic-wash cycle. This is mainly because dramatic pH changes during basic-wash cycle, definitely affected capillary inner wall characteristics, which in turn, would affect any possible interaction between the capillary surface and the probe and analytes. This is especially important when a positively charged compound (like BTEA) are used as a probe for indirect UV detection, where interaction of the probe ions and negatively charged silanol groups on the fused silica capillary inner wall should affect the reproducibility of the indirect detection method. In case of acidic-wash cycle, the capillary inner surface experienced little changes during wash cycle, which provide more robust indirect detection method.

Capillary conditioning, before and between each runs is very important in obtaining reproducible results. This is especially important in CE with UV indirect detection. In experiments using acidic buffers, it is recommended to use acid-wash cycle to maintain characteristics of capillary inner surface close to the conditions encountered during run cycle. This will ensure robustness of the method.

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