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Evaluation of a monolithic silica column operated in the hydrophilic interaction chromatography mode with evaporative light scattering detection for the separation and detection of counter-ions

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Abstract

In this work a monolithic silica column operated in the hydrophilic interaction chromatography (HILIC) mode in conjunction with an evaporative light scattering detector (ELSD) was investigated. Lithium, sodium and potassium were used as the test counter-ions for this evaluation. Chromatographic properties of this column operated in the HILIC mode were determined by varying key mobile phase parameters, such as pH, flow rate, buffer strength, acid and organic modifier. As organic content was increased from 60 to 90% acetonitrile, retention time increased on average by a factor of seven for the test cations listed above. Buffer concentration and pH were also observed to have an effect, although not as significant as the HILIC effect that was observed by changing organic content. Flow rates up to 5 mL/min were utilized to perform counter-ion separations in less than 3 min. After examining the changes in retention, resolution, and peak shape an optimized method was established and then further evaluated for linearity, reproducibility, and limit of detection (LOD) for sodium. Linearity was acceptable with an R^2 value of 0.999 across the working-standard range and a LOD of 0.1 μg/mL was calculated. The reproducibility on the counter-ion determination from pharmaceutical sodium salts was 1.6% R.S.D. on average, and the accuracy of the counter-ion prediction was approximately 3% from theory when salt content was corrected for potency.
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Keywords: Monolithic columns; Hydrophilic interaction chromatography; Evaporative light scattering detection; Counter-ion; Silica column

1. Introduction

Monolithic silica columns consist of macropores and mesopores that form a network of interconnected flow paths. Due to the porous nature of this packing material which is nearly 80% porosity as compared to 65% with packed columns, higher flow rates are possible without an excessive increase in back pressure. The macropores are approximately 2 μm in size and offer channels for the analytes to be transported rapidly to the active sites within the pores. Due to the high surface area within the column, a reduction in diffusion path length and flow resistance, as well as, an increase in mass transport is achieved; thus, a faster high efficiency separation is possible. The main advantage of reduced run time is realized when higher flow rates can be utilized with monolithic columns.

Tanaka et al. published reviews on the capabilities and possible limitations afforded by monolithic column technology [1,2]. The concept of monolithic (or single piece) columns is not new, as Kubin et al. introduced a size exclusion monolith in 1967, but the performance was not good based upon permeability considerations [3]. In the mid nineties a more robust manufacturing process was introduced offering the full advantages of monolithic columns that could now be realized [3].

Recently, monolithic octadecylsilyl-silica gel columns were utilized for the high-speed ion determination of rainwater acidity using conductimetric detection [4]. In this study, H^+ was separated from Na^+, K^+, Mg^{2+}, and Ca^{2+}. To accomplish this separation, the silica gel column was first

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pre-treated with lithium dodecylsulfate. For the separation of anions, a reversed-phase C18 (RP-18e 4.6 mm × 50 mm, Merck KGaA) column was permanently coated with didodecyldimethylammonium bromide, and used in conjunction with conductivity detection [5]. The separation of seven anions in less than 60 s was accomplished with this methodology. The main draw back in this work was indicated to be the limited lifetime of the column, which was approximately 12 h. The use of monolithic columns in the pharmaceutical industry was examined in relation to performance in a good manufacturing practices (GMP) environment [6]. This evaluation utilized a C18 reversed-phase monolithic column with a phosphate buffer mobile phase. When compared to a conventional method with a particle packed column (RP18, 5 μm, 250 mm × 4 mm), the monolithic column method reduced the analysis time by a factor of 6 while achieving equivalent resolution. However, there was a dramatic loss of theoretical plates as the flow rate was increased from 1 mL/min to 5 mL/min. Although, the work suggested that for smaller molecules higher optimum flow rates could be achieved. Additional findings of their work indicated that the monolithic column offers comparable repeatability, reproducibility, and stability when compared to a packed column.

The approach presented here uses a monolithic silica column operated in the hydrophilic interaction chromatography (HILIC) mode with evaporative light scattering detection (ELSD). Alpert coined the term hydrophilic interaction chromatography for the separation of proteins, peptides, and polar molecules [7], although this mechanism had been previously established for the separation of carbohydrates [8,9]. The HILIC mode employs polar stationary phases with mixed aqueous/organic mobile phases creating a stagnant enriched water layer around the polar stationary phase. This enriched layer allows analytes to partition between the two phases based on their polarity. In contrast to reversed-phase (RP) chromatography, where a non-polar stationary phase is employed and analyte elution is facilitated by the organic strength of the mobile phase, analyte elution is facilitated by the aqueous component of the mobile phase in HILIC mode. The separation mechanism and retention order in the HILIC mode is therefore opposite to that of the RP mode. Although the HILIC mode is more similar to the normal phase (NP) and polar organic modes, it is different in that the HILIC mobile phases contain a relatively high amount of water (5–40%), which can provide a significant solubility advantage for very hydrophilic samples. The HILIC mode can be generated by a variety of polar stationary phases, for example, the counterion piperazine has been determined utilizing the HILIC mode on a cyano column [10] and polar pharmaceutical analytes have been separated using both amino and silica columns [11]. The HILIC mode has also been employed for chiral separations using cyclodextrin and macrocyclic antibiotic based packings [12,13].

In HPLC, the evaporative light scattering detector has an extensive application base, but it is especially important when ultraviolet (UV) detection is not feasible. The concept and operation of commercially available evaporative light-scattering detectors as sensitive universal detectors has been thoroughly discussed in the literature [14]. The ELSD has been shown to successfully detect many substances, such as, phospholipids [15], triglycerides, fats and fatty acid esters [16], carbohydrates [17], synthetic polymers [18], steroids [19], and amino acids [20]. The HPLC–ELSD system has also been extremely useful for the determination of pharmaceutical impurities, raw materials, cleaning verification and small organic compounds [21–24]. A more recent niche for the ELSD in the pharmaceutical industry is for the detection and quantitation of counter-ions from pharmaceutical salt forms. Our laboratory first introduced the applicability of HPLC–ELSD for the detection and quantitation of inorganic ions, such as chloride and sodium [25,26]. A comparison of the HPLC–ELSD technique to ion chromatography, capillary electrophoresis, and titration for the determination of Cl^- in pharmaceutical drug substances has been statistically compared and it was determined that the four techniques were equivalent [26]. However, the ELSD is a cost effective detector that can be utilized with many other HPLC applications in addition to the analysis of counter-ions (e.g. assay and impurity determinations for compounds lacking a strong chromophore) which gives it a unique advantage over other techniques. The aim of this paper is to show the application of a new monolithic silica column operated in the HILIC mode for the detection and rapid quantitation of lithium, sodium and potassium using ELSD.

2. Experimental

2.1. Chemicals

Ammonium acetate, sodium chloride, lithium chloride, and potassium chloride were obtained from Fisher Scientific (Fairlawn, NJ). Acetonitrile was purchased from Burdick and Jackson (Muskegon, MI). Acetic acid, naproxen sodium, and warfarin sodium were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO). A sodium standard solution was acquired from Fluka Chemika (Buchs, Switzerland). The pH 2.00 and 7.00 buffers were from Red Bird Service (Osgood, IN). Deionized water and nitrogen were from an inhouse system. The new chemical entity sodium salt was synthesized at Eli Lilly and Company (Indianapolis, IN).

2.2. Equipment

The HPLC system consisted of a Hewlett-Packard 1050 pump and auto sampler (Wilmington, DE) integrated with an Alltech 800 evaporative light scattering detector from Alltech Associates (Deerfield, IL). The detector was operated at 50 °C, 3.8 bar nitrogen and a gain setting of eight throughout the experiments. A Chromolith Performance SI (100 mm × 4.6 mm) silica column from Merck KgaA was

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