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Electrospun Nafion-Polyacrylonitrile nanofibers as an ion exchange ultrathin layer chromatographic stationary phase

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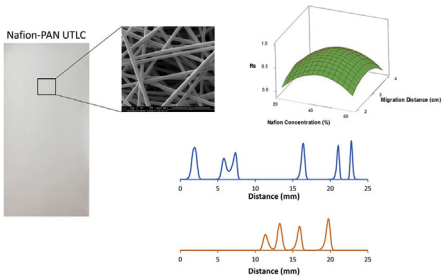
HIGHLIGHTS

- Nafion-Polyacrylonitrile (PAN) electrospun nanofibers as ion exchange stationary phase in UTLC.
- Optimization of separation resolution by fractional factorial design and Box-Behnken design.
- Nafion-PAN UTLC exhibits excellent solvent compatibility and separation efficiency.
- Nafion-PAN UTLC is suitable for separation of both small and large biomolecules.

GRAPHICAL ABSTRACT



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ABSTRACT

An ion-exchange method to separate charged biomolecules on ultrathin layer chromatographic (UTLC) plates using electrospun Nafion-Polyacrylonitrile (PAN) nanofibers as the stationary phase is described. Sulfonate groups on Nafion provide the ion-exchange sites. The addition of PAN (a higher molecular weight polymer than Nafion) was used to facilitate the nanofiber formation process using electrospinning. Electrospinning parameters and separation conditions were optimized using fractional factorial design and response surface methodology. Nafion-PAN nanofibers containing 45% (w/w) Nafion with 0.407 mmol/g of SO_3H group and 16.0 mmol/g of fluorine as an ion exchange stationary phase for UTLC were evaluated using the separations of amino acids and proteins, followed by visualizations using ninhydrin and fluorescamine, respectively. The electrospun Nafion-PAN plates showed high chemical stability under various mobile phase conditions. Mobile phase velocity decreased with the addition of Nafion into the electrospinning solutions. The sources of band broadening of analyte spots were investigated. The separation of amino acids showed high selectivity and separation efficiency. The separation of four proteins demonstrated the feasibility of Nafion-PAN UTLC for separating large biomolecules.

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1. Introduction

Thin layer chromatography (TLC) is a simple chromatographic method that involves the separation of multiple samples and standards on an open layer developed by a mobile phase [1]. It is used in many areas including synthetic chemistry [2], food science [3], pharmaceutical industry [4] as well as clinical research [5] due to its simplicity, rapidness and low cost. High performance thin layer chromatography (HPTLC), an extension of TLC, was developed in 1970s using smaller diameter particles (5–20 μm) [6]. HPTLC offers better chromatographic efficiency, faster separation, lower analyte and mobile phase consumption and lower detection limits [7]. In order to further improve the chromatographic performance and reduce analysis time and the amount of consumables, ultrathin

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layer chromatography (UTLC) was introduced in 2001 using monolithic silica as the stationary phase. Compared to classic TLC with thicknesses of 100–400 μm, UTLC utilizes sorbent layers as thin as 5–25 μm, approximately [8]. There are many materials that have been developed as stationary phases for TLC, including the most commonly used silica gel and modified silica gel, and less frequently used aluminum oxide, cellulose, polyamides and ion exchange resins [9]. Recently, our group has reported electrospun nanofibers as the stationary phases for UTLC. Various polymers can be electrospun to produce nanofibers. Polyacrylonitrile (PAN) [10], glassy carbon [11], polyvinyl alcohol (PVA) [12], silica [13], carbon nanorod-filled polyacrylonitrile [14] were successfully fabricated as stationary phases for UTLC using the electrospinning method. All of these electrospun UTLC plates showed enhanced separation efficiency, decreased use of solvents and increased speed of analysis compared to commercial HPTLC plates.

Nafion is a synthetic perfluorinated cationic polymer developed by Dupont in the 1970s [15]. As shown in Fig. S-1, a hydrophobic tetrafluoroethylene (Teflon) backbone makes Nafion highly chemical resistant, while sulfonate groups on the side chains provide proton conductivity. Scaling down Nafion into the nanometer scale via electrospinning can further improve its proton conductivity [16]. During the electrospinning process, the shear force elongates fibers and orients ionic domains along the fiber axis direction, and the aligned ionic structures result in higher conductivity [16,17].

Unfortunately, the pure Nafion polymers or polymer solutions have a low shear viscosity that does not allow electrospinning of the polymer [18]. Therefore, another polymer is needed to blend with Nafion for electrospinning to take place. Several researchers have successfully blended Nafion with carrier polymers, such as poly (acrylic acid) (PAA) [17,19], poly (vinyl) alcohol (PVA) [20,21], poly (ethylene oxide) (PEO) [15,22–24] and polyacrylonitrile (PAN) [25,26]. In our group, PAN and multi-walled carbon nanotubes (MWNT) filled PAN have been previously electrospun as UTLC stationary phases and showed substantially improved chromatographic performance [10,14]. Thus, in this work, PAN was chosen as the carrier polymer to electrospin with Nafion to create ion exchange UTLC stationary phases.

Separations of amino acids and proteins are essential in food science [27], agricultural science [28] and pharmaceutical industry [29]. Ion exchange chromatography (IEX) is one of the most popular chromatographic techniques for separating amino acids and proteins owing to its high capacity, high resolving power and easy controllability of the separation process [30]. In practice, IEX for amino acids and proteins is more often conducted in column chromatography, which exhibits disadvantages in costly equipment, time consuming experiments and difficulties in detection [31]. Employing the ion exchange technique on UTLC eliminates these requirements for time and expensive instruments. Also, the detection in UTLC can be realized by a simple spray reaction, by absorption of ultraviolet light, by using fluorescent labeling, or even direct observation in the case of colored analytes [32]. Electrospun Nafion-PAN nanofibers have particularly attractive properties as the ion exchange UTLC stationary phase, including excellent chemical stability and easy accessibility of ion exchange sites which allow sufficient interaction between analytes and the stationary phase.

In this paper, we report the first cation exchange electrospun UTLC method and an evaluation of its chromatographic performance by separating amino acids and proteins. Fractional factorial design and response surface methodology were used to optimize the Nafion-PAN stationary phase and separation conditions. The chemical stability of the electrospoun nanofiber mat was also investigated.

2. Materials and methods

2.1. Materials

Nafion containing solution LIQUION 1115 (15% by weight NAFION®, 1100 equivalent weight) was purchased from Ion Power Inc. (New Castle, DE). Polyacrylonitrile (PAN), average M_w 150,000 g mol^{-1}, was purchased from Sigma-Aldrich (St. Louis, MO). *N*, *N*-Dimethylformamide (DMF) (99.8%), HPLC grade methanol (99.9%), acetonitrile (99.9%), 2-propanol (99.9%) and methylene chloride (99.9%) were acquired from Fisher Scientific (Fair Lawn, NJ). Ethanol (91%) was purchased from Decon Labs Inc. (King of Prussia, PA). Buffer reagents MES hydrate (minimum 99.5%) and ammonium formate (≥99%) were purchased from Sigma-Aldrich; sodium chloride (100%) and ammonium hydroxide (certified ACS plus) were purchased from Fisher Scientific. Water was purified to 18.1 MΩ by a Barnstead Nanopure Infinity System from Thermal Scientific Inc. (Odessa, TX).

The amino acids including arginine (Arg), lysine (Lys), histidine (His), valine (Val), phenylalanine (Phe) and alanine (Ala); Arg, Lys and Ala were purchased from Sigma-Aldrich, Val was purchased from Amresco (Solon, OH), His was purchased from Matheson Coleman & Bell (Gardena, CA), and Phe was purchased from Eastman Chemical Company (Kingsport, TN). Proteins included lysozyme (LYZ) from chicken egg, bovine serum albumin (BSA), α-chymotrypsin (CHY) from bovine pancreas and myoglobin (MGB) from equine skeletal muscle. LYZ, BSA and MGB were obtained from Sigma-Aldrich, and CHY was purchased from Worthington Biochemical Corporation (Lakewood, NJ). Visualization spray reagents ninhydrin (≥98%), fluorescamine (≥98%) and triethylamine (≥99.5%) were purchased from Sigma-Aldrich.

2.2. Instrumentation

The morphology of electrospun nanofiber mats was characterized using a FEI Nova NanoSEM 400 (FEI Corporate, North America NanoPort, Hillsboro, OR) scanning electron microscope (SEM). Each sample was sputter coated with AuPd for 90 s at 17 mA using a Cressington sputter coater (Cressington Scientific Instruments, Watford, UK) to create a conductive surface before SEM analysis. Fiber diameters were measured on SEM images using ImageJ software (Available from the National Institute of Health at http://www.rsbweb.nih.gov/ij/index.html). The statistical experimental designs and the optimization calculations were performed using Minitab 17 (Minitab, Inc., State College, PA).

2.3. Preparation of Nafion-PAN solution

Pure Nafion was obtained following the drying/curing procedure provided by Ion Power Inc. Dried Nafion and PAN were dissolved in DMF and stirred for at least 24 h using a magnetic stirrer under gentle heating.

2.4. Electrospinning

A syringe pump (Harvard Apparatus, Holliston, MA), a high voltage power supply (Spellman, Hauppauge, NY), a stainless steel collector covered with aluminum foil (Reynold Super Strength) and a Plexiglas enclosure were used as the electrospinning apparatus. A nitrogen purge and a VWR Traceable® humidity sensor were used to control and monitor the relative humidity in the closed chamber while electrospinning. Nanofibers were electrospun at room temperature with relative humidity below 20%. The distance between the collector and the tip of the needle was kept at 15 cm. The voltage was kept at 16 kV, and the flow rate was kept at 0.3 mL/h.

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