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Uniaxially aligned electrospun cellulose acetate nanofibers for thin layer chromatographic screening of hydroquinone and retinoic acid adulterated in cosmetics

Siripran Tidjarat, Weerapath Winotapun, Praneet Opanasopit, Tanasait Ngawhirunpat, Theerasak Rojanarata *

Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand

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ABSTRACT

Uniaxially aligned cellulose acetate (CA) nanofibers were successfully fabricated by electrospinning and applied to use as stationary phase for thin layer chromatography. The control of alignment was achieved by using a drum collector rotating at a high speed of 6000 rpm. Spin time of 6 h was used to produce the fiber thickness of about 10 μm which was adequate for good separation. Without any chemical modification after the electrospinning process, CA nanofibers could be readily devised for screening hydroquinone (HQ) and retinoic acid (RA) adulterated in cosmetics using the mobile phase consisting of 65:35:2.5 methanol/water/acetic acid. It was found that the separation run on the aligned nanofibers over a distance of 5 cm took less than 15 min which was two to three times faster than that on the non-aligned ones. On the aligned nanofibers, the masses of HQ and RA which could be visualized were 10 and 25 ng, respectively, which were two times lower than those on the non-aligned CA fibers and five times lower than those on conventional silica plates due to the appearance of darker and sharper of spots on the aligned nanofibers. Furthermore, the proposed method efficiently resolved HQ from RA and ingredients commonly found in cosmetic creams. Due to the satisfactory analytical performance, facile and inexpensive production process, uniaxially aligned electrospun CA nanofibers are promising alternative media for planar chromatography.

1. Introduction

Electrospinning is a facile, robust and versatile technique for the fabrication of nanofibers for laboratory and industrial use. In this process, polymer liquid, i.e. solution or melt, loaded in a syringe is charged by a high voltage. Once the electrostatic force overcomes the surface tension on the polymer droplet, the liquid is ejected from the nozzle tip towards the grounded collector while solvent evaporates or melt solidifies. This phenomenon results in the formation of extremely small fibers with high surface area, high porosity and controllable compositions and size. Taking advantages of these features, electrospun nanofibers are attractive for a wide array of applications ranging from filtration, medical wound dressings, tissue engineering, drug delivery and so on [1]. So far, nanofibers electrospun by conventional methods are collected as nonwoven layers with randomly arranged structures. Later, aligned nanofibers can be produced through different means e.g. by using high speed rotating mandrel, electrostatic metallic staple and a pair of permanent magnets [2]. It is known that, for some applications, an ordered structure such as uniaxially or radially aligned nanofiber is more desirable. For instance, uniaxially aligned nanofibers with anisotropic properties has been shown to satisfy electrical, optical and mechanical purposes [3–5]. In addition, the aligned nanofiber scaffolds used in cell culture and tissue engineering not only mimic the parallel structure of fibrous tissues e.g. collagen in tendon or nerve, but also promote the migration and extension of cells [6,7].

In analytical chemistry, electrospun nanofibers have been currently applied to electrochemical and optical based detection systems, solid phase extraction and membrane separation [8]. Besides, electrospinning has recently become a new route to produce stationary phase for thin layer chromatography (TLC). There have been the reports about the fabrication and use of electrospun nanofibers as TLC stationary phase for the separation of amino

* Corresponding author at: Department of Pharmaceutical Chemistry and Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand. Tel.: +66 34 255800; fax: +66 34 255801.
E-mail addresses: rtheerasak@yahoo.com, teerasak@su.ac.th (T. Rojanarata).

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acids, laser dyes, steroidal compounds and food preservatives. However, the polymers used for the fabrication were limited to glassy carbon [9], polyacrylonitrile (PAN) [10–12], polyvinyl alcohol (PVA) [13] and cellulose acetate (CA) [14] In other aspects, electrospun nanofiber layers were improved for the detection of ultraviolet-active compounds by incorporation of photoluminescence indicator and were hyphenated with electrospray-ionization mass spectrometry [12]. Concerning the aligned electrospun nanofibers, Olesik et al. reported the use of aligned PAN nanofibers to separate a mixture of β-blockers and steroidal compounds. Compared to those with random orientation, the aligned nanofibers improved reproducibility and separation efficiency as well as shortened run time [11]. The alignment of PAN nanofibers was also observed in the work done by Kampalanonwat et al. However, in that study the delicate investigation aimed to control the aligment such as the optimization of rotating speed of drum collector has not yet been conducted [12]. These reported performance enhancements motivate further pursuit of new electrospun TLC materials.

CA is an environmentally degradable material made from the most abundant biopolymer on earth i.e. cellulose [15]. It is much cheaper than some synthetic polymers such as PAN. In addition, nanofibers made from CA can be readily used after electrospinning process for TLC without additional steps such as pyrolysis or chemical crosslinking which are usually required for the fabrication of glassy carbon and PVA nanofibers, respectively. Recently, TLC plates made from non-aligned CA nanofibers have been prepared and used with eco-friendlier hydro-alcoholic mobile phases for the analysis of prohibited steroids in traditional medicines [14]. However, no reports related to the fabrication of aligned CA nanofibers used for TLC have been available.

The present study extends this work by optimizing drum collector rotation speed and spin time to produce new reversed-phase TLC plates with uniaxially aligned CA nanofibers. TLC performance of the aligned nanofibers was compared to that of non-aligned ones as well as commercially available silica based plates. Finally, their application was demonstrated by using them for screening hydroquinone (HQ) and retinoic acid (RA) adulterated in cosmetic creams since both of them are prohibited substances for use in cosmetics in many countries due to the side effects including scaling of skin, stinging, flushing and ochronosis [16,17]. From the study, the aligned CA nanofibers were proven to be the efficient TLC media for resolving the analytes within a shorter run time. Furthermore, this work reports for the first time about the advantage of aligned nanofibers over the non-aligned ones in the term of the lower mass of the analyte which could be visualized on the plates.

2. Materials and methods

2.1. Materials

Cellulose acetate (CA; M_w = 30 kDa; degree of acetylation ≈ 2.4), hydroquinone (HQ; purity ≥ 99.0%), retinoic acid (RA; purity ≥ 98.0%), vitamin C (VC; purity ≥ 99.0%), vitamin E (purity ≥ 96.0%), resorcinol (RS; purity ≥ 99.0%), α-arbutin (AR; purity ≥ 98.0%), sodium metabisulfite (purity ≥ 98.0%) and phosphomolybdic acid were purchased from Sigma–Aldrich, St. Louis, MO. Solvents used in this study i.e. *N*,*N*-dimethylacetamide (Labscan, Thailand; purity ≥ 99.5%), acetone (Carlo, Italy; purity ≥ 99.5%), glacial acetic acid (Merck, Germany; purity ≥ 99.8%), hexane (Merck, Germany; purity ≥ 99.0%), methanol and ethanol (Merck, Germany; purity ≥ 99.9%) were of analytical grade. Distilled water was used throughout the experiments. Commercial TLC plates i.e. silica gel 60 and silica gel 60 RP-18 plates (particle size of 10–12 μm, layer thickness of 200 μm on aluminum backing) were purchased from Merck. Cosmetic cream samples were collected from local markets in Nakhon Pathom province, Thailand, during January–March 2014.

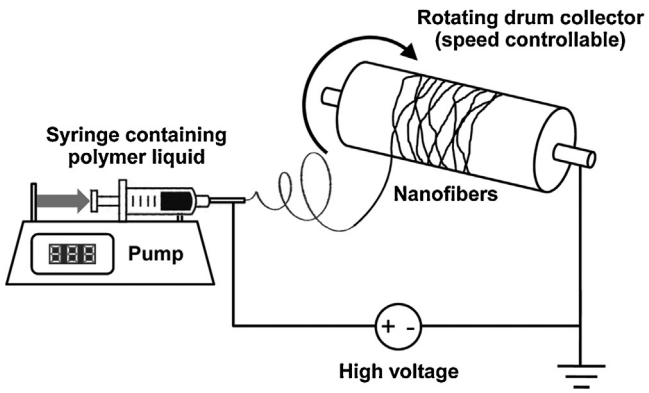

Fig. 1. Setup of electrospinning instrumentation for fabrication of nanofibers.

2.2. Fabrication and characterization of electrospun CA nanofibers

2.2.1. Electrospinning of CA nanofibers

Initially, 17% (w/v) CA solution was prepared by dissolving 1.02 g of CA powder in 6 mL of 2:1 (v/v) acetone/*N*,*N*-dimethylacetamide at room temperature (30 °C). Then, 5 mL of the solution was loaded into a glass syringe equipped with a blunted stainless steel needle (diameter ≈ 0.9 mm) attached to a pump (Fig. 1). Electrospinning was performed by ejecting CA solution from the needle tip at the feeding rate of 0.4 mL h^{-1} towards the rotating drum which functioned as a grounded collector under the electric field using electrical potential of 17.5 kV from a high voltage supply. The distance between the needle tip and the aluminum foil (standard household quality with the thickness of about 15 μm) that was wrapped around the drum (circumference ≈ 19 cm) was 15 cm. To fabricate vertically aligned nanofibers, the drum was set to rotate at different high speeds (4500, 6000 and 7500 rpm) and the nanofibers were collected at different spin times (1, 2, 4, 6, and 8 h). Non-aligned nanofibers were prepared in a similar way except that the drum rotation speed was 350 rpm. After electrospinning, the aluminum foil containing CA nanofiber layer was removed from the collector and cut into rectangular shape (7.5 × 2.5 cm^2) by selecting the area with the uniform thickness. The cut foil was then fixed onto the glass backing using double sided adhesive tape to produce CA nanofiber plate which was ready to use for TLC separation. It should be noted that this fixation is limited with regard to solvent resistance; thereby the use of thicker foils can be an option to avoid the tape.

2.2.2. Characterization of CA nanofibers

To observe the effect of drum rotation speed and spin time on the morphology and alignment, electrospun CA nanofibers fabricated under different conditions were subjected to scanning electron microscopy (SEM) (Camscan, MX-2000). The specimens for SEM were prepared by coating nanofibers with thin layer of gold (thickness ≈ 150 Å) by using sputtering device. The diameters of fibers and the thickness of layers were measured directly from SEM images (n = 50), using the image analysis software (JMicroVision V.1.2.7, University of Geneva, Geneva, Switzerland). The alignment of nanofibers was assessed as the number of fibers oriented in a determined direction with respect to a vertical reference line (0° angles). The angles from which individual fibers were deviated from this line were determined (n = 50) and then plotted as a function of frequency in a histogram. The narrow distribution of nanofiber angles indicated more alignment.