Talanta 115 (2013) 208–213

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Electrospun cellulose acetate nanofibers as thin layer chromatographic media for eco-friendly screening of steroids adulterated in traditional medicine and nutraceutical products

Theerasak Rojanarata*, Samarwadee Plianwong, Kosit Su-uta, Praneet Opanasopit, Tanasait Ngawhirunpat

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

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form
28 April 2013
Accepted 29 April 2013
Available online 3 May 2013

Keywords:
Cellulose acetate
Nanofiber
Electrospin
Thin layer chromatography
Steroid
Adulteration

ABSTRACT

Nanofibers fabricated from cheap, naturally derived biopolymer, namely cellulose acetate via facile electrospinning technique were successfully applied for the first time to use as separation media for thin layer chromatography (TLC). From the optimization studies, uniform, bead-free nanofibers with good adherence to the backing plates were obtained by electrospinning 17% (w/v) cellulose acetate solution prepared in acetone/*N*,*N*-dimethylacetamide (2:1, v-v), using a feed rate of 0.6 mL/h and an electrostatic field strength of 17.5 kV/15 cm for 4 h. The nanofibers exhibited reversed phase characteristics, thereby offering the possibility to use simple, polar and more environmental friendly mixtures of water and alcohols as mobile phase. In this work, the application of the fabricated fibers was illustrated by using them combined with the optimal mobile phase e.g. ethanol/water (40:60, v-v) for the screening of steroids adulterated in traditional medicine and nutraceutical products. Due to the satisfactory separation performance, electrospun cellulose acetate nanofibers were shown to be an efficient alternative for TLC media and could be potentially used for the development of green and facile analytical methods.

1. Introduction

Thin layer chromatography (TLC) is the simplest of all widely used chromatographic methods. It is quick, cost-effective and practical for the analytical tasks in many fields including pharmaceutical and food sciences [1]. Typically, conventional TLC plates are made by spreading the slurry of stationary phase which is dispersed in a suitable solvent, sometimes in the presence of binder such as gypsum or polymethacrylate, onto a plate. For better performance, smaller and more even spherical particles are used in high-performance TLC (HPTLC) and more recently the stationary phases with monolithic structures have been developed through specialized manufacturing processes for ultra-thin layer chromatography (UTLC) [2]. Despite the continuous improvements of the efficiency of stationary phases, it is obvious that the layer materials practically used for TLC are polar compounds including the predominant silica gel and less frequently used aluminum oxide and cellulose. This indicates that for TLC, normal phase adsorption chromatography is by far the more important mode of separation and there are no significant problems in using this system [3]. On the contrary, the separation of analytes on less polar stationary phase is rather limited. Until now, few choices of reversed phase TLC plates have been commercially available and most of them rely on the chemically bonded, silica-based phases which are generally expensive. For these reasons, the facile fabrication of cheap alternative media for such purpose is still in demand, including the use of low cost polymers from natural sources.

In the material sciences, electrospinning is well recognized as a straightforward and fascinating process used to fabricate non-woven micro- or nanofibers from the polymer solutions or melts [4]. This technique relies on the application of electrical field between the tip of a nozzle and a collector. Once the electrostatic forces overcome the surface tension of the polymer solution at the nozzle tip, a jet stream is drawn from the tip while the solvent is evaporating and the produced fibers are deposited on the collector. Since electrospun nanofibers have superior characteristics such as a high specific surface area, high porosity, high adsorption capacity and wide possibilities for surface functionalization, they are of interest and used for a broad range of applications including filtration, biosensors, enzyme immobilization, drug delivery and tissue engineering [5]. Surprisingly, while the number of publications pertaining to the versatility of electrospun nanofibers is rapidly growing and their applications in analytical chemistry have emerged as clearly

* Corresponding author. Tel.: +66 34 255800; fax: +66 34 255801.
E-mail addresses: teerasak@su.ac.th, rtheerasak@yahoo.com (T. Rojanarata).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.04.078

reviewed by Chigome and Torto [6], little research has been done on the use of electrospun nanofibers as TLC media. During the past few years, only Olesik's group at Ohio State University have successfully fabricated electrospun nanofibers from polyacrylonitrile, glassy carbon and crosslinked polyvinyl alcohol and described their potential uses as UTLC stationary phases [7–10].

Cellulose acetate (CA) is an acetate ester of cellulose, the world's most abundant naturally occurring biopolymer. By acetylation at hydroxyl groups on cellulose to an average degree of substitution of 2–4 acetyl groups per glucose units, the reversed phase characteristic is created [11]. Although pre-coated TLC plates of CA are not commercially obtainable to date, CA was formerly used to prepare TLC plates by conventional slurry spreading method for the separation of polyaromatic hydrocarbons and some enantiomers [11,12]. At present, there are several reports about the electrospinning preparation of CA nanofibers [13,14] and their applications e.g. for the build-up of extracellular matrix mimicking scaffolds [15] and for the delivery of therapeutic agents [16,17]. Nevertheless, none of them involves with the utilization of CA nanofibers for TLC. In this work, electrospinning was used as an alternative technique to fabricate the layers of CA fibers onto the plates and the parameters affecting the characteristics of fibers were studied and optimized. Furthermore, the practical application of the nanofibers from this naturally derived polymer to TLC was demonstrated for the first time by using them as efficient stationary phases for the screening of steroidal adulterants in traditional medicine and nutraceutical products. Due to the reversed phase nature of CA, the separation required only a simple polar mobile phase consisting of water and alcohol, thereby additionally offering the environmental friendliness feature.

2. Materials and method

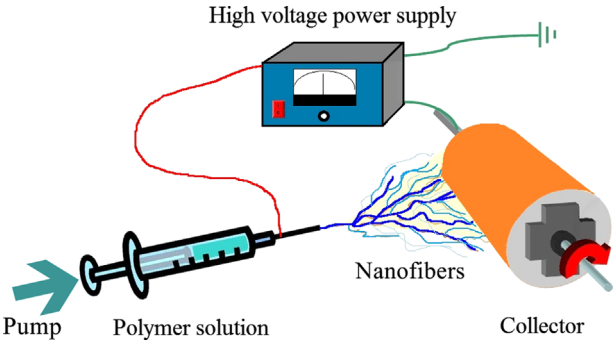
2.1. Materials

Cellulose acetate (M_v=30 kDa, degree of acetyl substitution ≈2.4) was purchased from Sigma-Aldrich (St. Louis, MO). The solvents in this work including acetone (Carlo, Italy), *N*,*N*-dimethylacetamide [DMAc, Labscan (Asia), Thailand], methanol (Merck, Germany) and ethanol (Merck) were of analytical reagent grade and used without prior purification. Dexamethasone (99.7% purity) and prednisolone (99.8 % purity) reference standards were from Department of Medical Sciences, Ministry of Public Health, Thailand. The commercial unmodified silica plates were purchased from Merck. The samples of traditional medicine and nutraceutical products were collected from local markets in Thailand.

2.2. Electrospinning fabrication of CA nanofibers

CA solutions were prepared at a concentration of 17% w/v by dissolving a weighed amount of CA powder in the mixed solvents containing acetone and DMAc (2:1, 1:1 or 1:2, v-v). The mixtures were stirred at room temperature for 1 hour to obtain the clear solutions and subsequently subjected to electrospinning.

Electrospinning (Scheme 1) was carried out at 25 °C by loading 5 mL of the polymer solution into a glass syringe attached to a pump to deliver the polymer solution to a blunt gauge-20 stainless steel needle which was attached to the open end of the syringe as the nozzle. The feed rate of the solution was controlled at 0.2, 0.6 or 1.0 mL/h. The pieces of aluminum sheets (9 × 15 cm) were attached onto a rotating metal drum (diameter of 6 cm) which functioned as the fiber collector and the grounding electrode. A fixed electrical potential of 17.5 kV was applied across the distance of 15 cm between the tip of the nozzle and the outer surface of the drum



Scheme 1. Schematic illustration of electrospinning setup.

(viz. the electrostatic field strength of 17.5 kV/15 cm). The rotational speed of the drum was 360 rpm. The electrospin times used were 2, 4 and 6 h.

After the CA fibers were formed on the aluminum sheets, the plates were cut by selecting the portions with a uniform thickness of fiber mats to the size of roughly 8.5 × 4 cm. Cutting was done with care to prevent the nanofiber mats from the damage and detachment from the plate.

2.3. Characterization of electrospun CA nanofibers

The morphology of CA nanofibers was observed under a scanning electron microscope (SEM; Camscan Mx2000, Obducat Camscan Ltd, Cambridge, UK) The specimens were prepared by cutting a small section of the fiber mat and coating with thin layer of gold using a sputtering device. The thickness of gold layer was approximately 150 Å. The diameter of electrospun fibers and the thickness of mats were directly measured from SEM top-view and side-view images, respectively using the image analysis software (JMicroVision V.1.2.7, University of Geneva, Geneva, Switzerland). The measurements were done at 10 individual locations taken from 3 SEM images. The chemical functionality of fibers was examined by using Fourier transform infrared (FTIR) spectroscopy by crushing and pressing the samples into KBr pellets. The FTIR spectra were recorded over the range 400–4000 cm^{-1} by using Nicolet Magma-IR system 750.

2.4. TLC procedures for screening of steroidal adulterants in real samples

2.4.1. Preparation of standard and sample solutions for the screening of steroidal adulterants

The standard solutions containing reference standards of dexamethasone or prednisolone at 1 mg/mL were prepared in absolute ethanol. For the preparation of sample solutions, about 50 mg of pulverized sample was extracted with 1 mL of ethanol in a microcentrifuge tube. The mixture was then mixed and sonicated for 10 min to improve the dissolution and the release of the drugs from the sample matrix. Subsequently, it was centrifuged and the clear supernatant was collected for TLC analysis.

2.4.2. TLC on electrospun CA nanofiber plates

The separation of dexamethasone and prednisolone was performed on 8.5 × 4 cm electrospun CA nanofiber plates by spotting 1 μL of sample or reference standard solutions, 1 cm from the bottom edge. Binary mixtures composed of methanol/water or ethanol/water at different ratios were used as mobile phases. The separation was run in a closed chamber until the front of mobile phase had moved 7 cm from the origin. The spot detection was done by spraying the plate with a mixture of 0.2% w/v tetrazolium

blue and 2% w/v sodium hydroxide in methanol. Then it was heated for 2 min to visualize the violet–blue spots of steroids under visible light.

The migration of the steroids on TLC plate was characterized by hR_f value, defined as $100 \times R_f$ value, the ratio of the distance moved by the solute to the distance moved by the mobile phase front. The quality of chromatographic separation between the two steroids was estimated by the resolution (R_s) parameter which was defined as the distance between two zone centers (d) divided by the average widths (W) of the zones

$$R_s = \frac{d}{(W_1 + W_2)/2}$$

2.4.3. TLC on CA plates prepared by slurry spreading and on commercial silica gel plates

Since CA plates are not commercially obtainable, they were prepared in the laboratory by spreading the slurry of finely ground CA powder and gypsum in a mixture of methanol and water onto the glass plates, following the method previously described [12]. Then the plates were used to separate the steroids by using ethanol/water (40:60, v-v) mobile phase and the separation performance was compared with that of the electrospun CA nanofibers. In addition, the separation on the commercial silica gel plates was conducted in order to confirm the reversed phase characteristics of electrospun CA nanofibers by observing the inversed migration sequences of the analytes between these two systems. The TLC procedures run on the commercial silica gel plates were similar to that using electrospun fiber plates, except that dichloromethane/methanol (90:10, v-v) was used as mobile phase.

3. Results and discussion

3.1. Optimal conditions for electrospinning process

It is known that the parameters related to the polymer solution as well as the production process affect the spinnability of polymers and the morphology of fibers. Although some literatures have previously described the conditions for electrospinning CA [13–17], their specific purposes of fabrication and the desired properties of resultant fibers might be different from that suited for use as TLC media. Thus, in this study three important parameters i.e. type of solvent, feed rate and spin time were varied and optimized while keeping the concentration of CA and the applied electric field constant at 17 % (w/v) and 17.5 kV/15 cm, respectively. As shown in Fig. 1(a)–(c), the morphology of fibers was noticeably varied, depending on the composition of solvents. Among the solvents investigated, only CA in acetone/DMAc (2:1, v-v) produced uniform bead-free fibers. Since the formation of beads is likely to occur when low-volatility solvent is used [18], CA solution prepared in the solvent with higher proportion of acetone (boiling point of 56 °C) evaporated faster than that of DMAc (boiling point of 166 °C) and gave rise to the formation of smooth fibers without beads. Consequently, acetone/DMAc (2:1, v-v) was used in this study as the solvent for CA.

With regard to the feed rate, this parameter did not have a pronounced effect on the variation of morphology since the fibers obtained from the electrospinning of CA in acetone/DMAc (2:1, v-v) at the different feed rates for 4 h were entirely bead-free (Fig. 1(d)–(f)). However, the diameters of fibers became slightly larger from 0.40 ± 0.15 to 0.59 ± 0.24 and 0.66 ± 0.14 μm when the feed rates were 0.2, 0.6 and 1.0 mL/h, respectively. This finding was in agreement with the previous work which concluded that the faster feed rate increased the input of the polymer content into the jet, but lowered the volume charge density at the nozzle, eventually giving rise to the formation of fibers with larger diameters [19]. Since smaller fibers were desired to achieve the high specific surface area and faster rate of spinning was preferred in order to shorten the fabrication time, the feed rate of 0.6 mL/h was regarded as optimal. Ultimately, when the electrospinning processes conducted at this feed rate were compared in the aspect of spin time, it was obvious that the fiber layers were thicker with the increasing time (Fig. 1(g)–(i)). The average thickness values were 18.84 ± 0.53, 21.39 ± 0.61 and 46.13 ± 0.84 μm for the fiber layers collected at 2, 4 and 6 h, respectively. Noteworthily, the use of too short spin time (i.e. 2 h) resulted in fluffy fibers that could be seen at microscopic level. In addition, the layers formed were not thick enough to effectively resolve the mixture of steroids (data not shown). On the contrary, too long a spin time (i.e. 6 h) caused partial detachment of the fibers from the backing sheets, leading to the uneven moving front of mobile phase when plates were used for the separation. Based on these results, the spin time suited for our case was considered to be 4 h.

3.2. Characteristics of electrospun CA nanofibers

The morphology of CA nanofibers prepared under the optimized electrospinning conditions as previously described was illustrated in Fig. 1(a) or (e), and (h). The average diameter of the fibers was 0.59 ± 0.24 μm. The thickness of the layers deposited on the plates was 21.39 ± 0.61 μm, much thinner than that of commercial classical silica gel plates which was about 200–250 μm. The fibers were found to be resistant to several polar protic solvents e.g. water, methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol and some non-polar solvents e.g. benzene, toluene, diethyl ether and hexane. However, they were soluble in some polar aprotic solvents such as dimethylformamide, dimethylsulfoxide, pyridine, acetone, dichloromethane and tetrahydrofuran. Therefore, the use of these pure solvents as mobile phase should be avoided or at least.

To examine the chemical functionality, CA nanofibers were analyzed by FTIR in comparison with the original CA and their spectra are shown in Fig. 2. It was found that both exhibited the identical characteristic peaks at 3500 cm^{-1} for –OH, 2960 cm^{-1} for –C–H, 1750 cm^{-1} for $>C=O$ and 1250 and 1040 cm^{-1} for –C–O–. From this information, it is evident that the electrospinning process did not cause the chemical alteration to the functional groups present in CA molecule, thereby preserving the reversed phase property of the polymer.

3.3. Opimization of TLC mobile phase and application of CA nanofibers to the analysis of real samples

To demonstrate the usability of the electrospun CA nanofibers for TLC, the fabricated plates were employed to separate two synthetic steroids, namely dexamethasone and prednisolone, the adulterants commonly found in traditional medicine and nutraceutical products. Chemically, both steroids are very similar, except that dexamethasone possesses a fluorine atom in the B ring and a methyl group in the ring D. For consumer protection programs, a facile but efficient screening method which can be performed in field is preferable. In this case, TLC is undoubtedly a powerful means since the analysis can be done in remote areas away from the laboratory without the requirement of sophisticated techniques or high-cost instruments, suited for developing countries. In addition, many samples can be screened on a single plate with low usage of solvents. For this purpose, the optimal mobile phase was investigated. Different from cellulose or silica gel which possesses numerous hydroxyl groups, CA has a lower polarity due to the presence of acetyl moiety. The separation taking place on this polymer was therefore equivalent to the reversed phase mode in

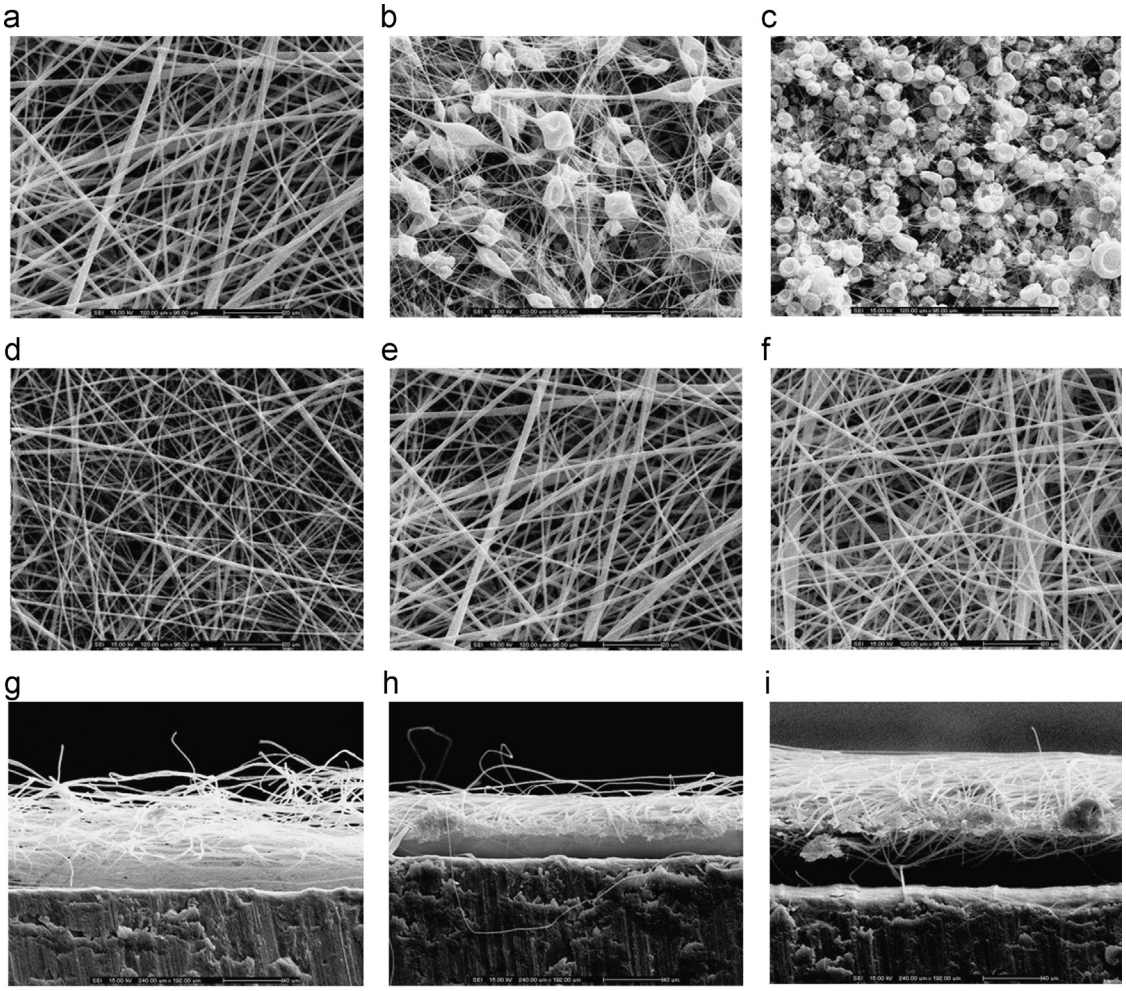


Fig. 1. (a–c) Effect of acetone:DMAc ratio (v:v) used in the solvents: (a) 2:1, (b) 1:1, (c) 1:2, on the nanofibers electrospun by using 17% (w/v) at the feed rate of 0.6 mL/h for 4 h. (d–f) Effect of the feed rate: (d) 0.2, (e) 0.6, (f) 1.0 mL/h, on the nanofibers electrospun by using 17% (w/v) CA in acetone:DMAc (2:1, v-v) for 4 h. (g–i) Effect of the spin time: (g) 2, (h) 4, (i) 6 h, on the fiber layers electrospun by using 17% (w/v) CA in acetone:DMAc (2:1, v-v) at the feed rate of 0.6 mL/h.

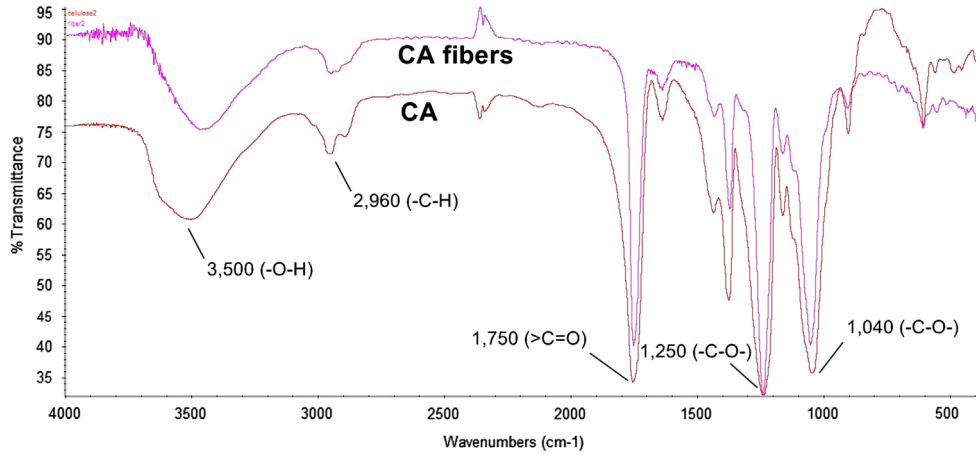


Fig. 2. FTIR spectra of CA as pure compound and electrospun CA nanofibers.

partition chromatography, as confirmed by the inversed migration sequences of dexamethasone and prednisolone when they moved on CA nanofiber plate versus the silica gel plate (data not shown). In this study, the polar solvents consisting of water and the organic modifier i.e. methanol or ethanol were chosen. In the methanol/water system, the solvent ratio of 60:40 (v:v) was the most proper because the ratio of 50:50 (v:v) produced tailing spots whereas the ratio of 70:30 (v:v) worsened the resolution (Table 1). For all ratios of methanol/water tested, the developing times that mobile phase moved from the start to the end point of the plate were generally in the range of about 30–50 min. For ethanol/water mobile phase, the effective solvent ratios were found at the lower proportion of alcohol than that in the methanol/water system, probably due to the higher elution strength of ethanol. The ethanol/water ratio of

40:60 (v:v) and 50:50 (v:v) were both effective to separate the two steroids, although better resolution and more acceptable separation time of about 30 min were obtained when the ethanol/water (40:60, v-v) was employed. The use of a higher content of ethanol in the mobile phase not only impeded their migration on the plate, but also consumed a greater amount of organic solvent.

When electrospun CA nanofiber plates were applied to the analysis of real samples, it was proven that they were capable of resolving dexamethasone from prednisolone as well as from the matrix when either methanol/water (60:40, v-v) or ethanol/water (40:60, v-v) was used as mobile phase. The results obtained from the separation on CA nanofiber plates are exemplified and shown in Fig. 3(a) and (b). In contrast, TLC plates consisting of the layers of fine CA powder failed to resolve these two analytes (Fig. 3(c)), indicating the importance of the preparation of the stationary phase in nano size to achieve the successful separation on this polymer. By using electrospun CA nanofiber plates, the proposed method allowed the detection of the steroids at the minimum level of 0.1 μg (Fig. 4). In the aspect of the stability of the fabricated nanofiber plates, it was found that the plates which were kept in airtight condition for 6 months at room temperature remained satisfactory visual appearance (color and the adherence to the backing), microscopic morphology (shape and size of fibers) and chromatographic performance as their initial stage.

Concerning the environmental compatibility, ethanol is accepted as a green solvent because of its biomass origin e.g. from agricultural feedstock and its biodegradability. Furthermore, it is safer to human and ecosystems and requires simpler handling of waste, compared to some other organic solvents commonly used in TLC such as chloroform, dichloromethane and acetonitrile. Therefore, besides the ease and the efficiency, the proposed TLC separation using the reversed phase electrospun CA nanofibers combined with the ethanol/water mobile phase is considerably benign to users and friendly to the environment.

Table 1
The developing time used, hR_f and R_s values obtained from the separation of dexamethasone and prednisolone on the electrospun CA nanofiber plates using different mobile phases.

Mobile phase	Developing time*	hR_f		R_s
(% v/v)	(min)	D**	P**	
Methanol:water				
50:50	28 ± 7	39.39	59.85	1.47
60:40	45 ± 8	59.09	74.24	1.44
70:30	47 ± 5	84.09	93.94	1.20
Ethanol:water				
40:60	33 ± 4	47.01	67.91	2.32
50:50	60 ± 8	55.22	72.39	1.35
60:40	89 ± 5	69.40	78.36	1.00

* Defined as the time that the solvents spent on moving from the start to the end of the plate for the distance of 7 cm.

** D is dexamethasone and P is prednisolone.

4. Conclusion

CA nanofibers were fabricated with ease by electrospinning technique onto the aluminium sheets and applied as the separation media for TLC for the first time. From the optimization experiments, uniform, bead-free nanofibers with good adherence to the backing were obtained by electrospinning 17 % (w/v) CA solution prepared in acetone/DMAc (2:1, v-v) using a feed rate of 0.6 mL/h and an electrostatic field strength of 17.5 kV/15 cm for 4 h. Unlike most commercial plates, the resultant fibers exhibited reversed phase behavior owing to the acetyl functionality substituted on the hydroxy groups. This feature provided the opportunity to use simple polar solvents such as a mixture of water and alcohols as the mobile phase. By using the fabricated nanofibers with the optimal mobile phase such as ethanol/water (40:60, v-v) for the screening of steroids adulterated in traditional medicine and nutraceutical products, it was proven that the electrospun

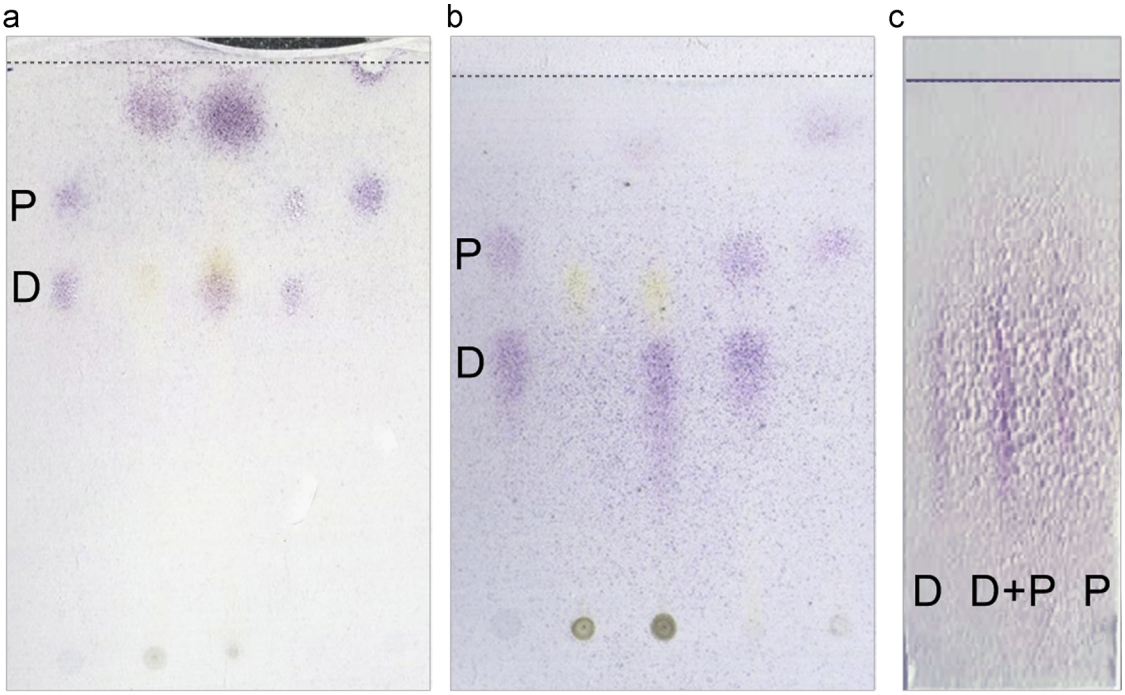


Fig. 3. The separation of dexamethasone and prednisolone in the selected samples of traditional medicine and nutraceutical products on the electrospun CA nanofiber plates (layer thickness of 21 μm) using (a) methanol/water (60:40, v-v) and (b) ethanol/water (40:60, v-v) as mobile phase in comparison with (c) CA plate conventionally prepared by using CA powder (layer thickness of 500 μm). For (a) and (b), lane 1 to 5 (from left to right) represent the standard mixture of dexamethasone and prednisolone, sample without steroids, sample adulterated with only dexamethasone, sample adulterated with both steroids and sample adulterated with only prednisolone, respectively.

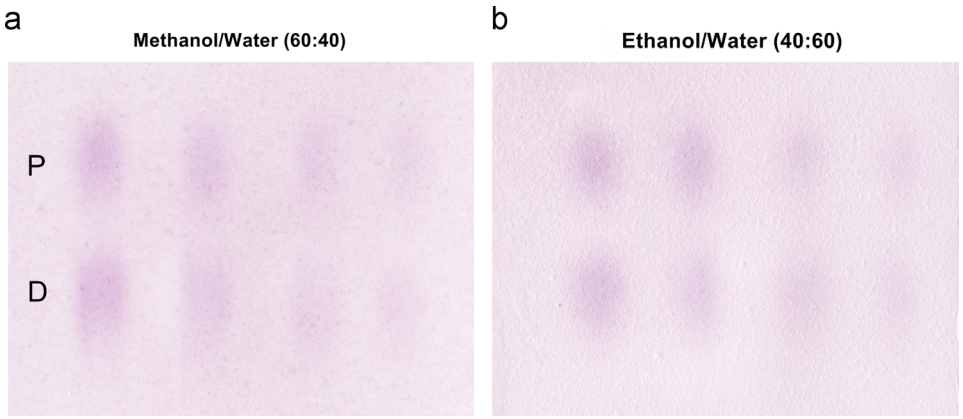


Fig. 4. The level of steroids that can be detected on the electrospun CA nanofiber plates using (a) methanol/water (60:40, v-v) and (b) ethanol/water (40:60, v-v) as mobile phase. The quantities of each steroid in lane 1 to 4 (from left to right) are 1, 0.5, 0.25 and 0.1 μg, respectively.

nanofibers produced from this naturally derived biopolymer were the efficient alternative for TLC media and could be devised for the development of green and facile analytical methods.

Acknowledgments

The authors gratefully acknowledge the financial support for this work from Silpakorn University Research and Development Institute (SURDI 55/01/19) and Thailand Toray Science Foundation (2011 Science and Technology Research Grants). Also, we would like to thank Eugene Kilayco for his valuable help in editing and proofreading the manuscript.

References

[1] J. Sherma, B. Fried, Handbook of Thin-layer Chromatography, third ed., Marcel Dekker, New York, 2003.
[2] M.J. Brett, L.W. Bezuidenhout, J. Chromatogr. A 1183 (2008) 179–185.
[3] B. Spangenberg, C. Poole, C. Weins, Quantitative Thin-layer Chromatography, Springer, Berlin Heidelberg53.
[4] J. Doshi, D.H. Reneker, J. Electrostat. 35 (1995) 151–160.
[5] N. Bhardwaj, S.C. Kundu, Biotechnol. Adv. 28 (2010) 325–347.
[6] S. Chigome, N. Torto, Anal. Chim. Acta 706 (2011) 25–36.
[7] J.E. Clark, S.V. Olesik, Anal. Chem. 81 (10) (2009) 4121–4129.
[8] J.E. Clark, S.V. Olesik, J. Chromatogr. A 1217 (2010) 4655–4662.
[9] T. Lu, S.V. Olesik, J. Chromatogr. B 912 (2013) 98–104.
[10] M.C. Beilke, J.W. Zewe, J.E. Clark, S.V. Olesik, Anal. Chim. Acta 761 (2013) 201–208.
[11] P.E. Wall, Thin-layer Chromatography: A Modern Practical Approach, Royal Society of Chemistry, Cambridge, 2005.
[12] T. Kowalska, J. Sherma, Thin Layer Chromatography in Chiral Separations and Analysis, CRC Press, Taylor & Francis, Florida, 2007.
[13] M.W. Frey, Polym. Rev. 48 (2) (2008) 378–391.
[14] S. Tungprapa, T. Puangparn, M. Weerasombut, I. Jangchud, P. Fakum, S. Semongkhol, et al., Cellulose 14 (2007) 563–575.
[15] D. Han, P. Gouma, Nanomedicine 2 (1) (2006) 37–41.
[16] P. Taepaiboon, U. Rungsardthong, P. Supaphol, Eur. J. Pharm. Biopharm. 67 (2007) 387–397.
[17] O. Suwantong, P. Opanasopit, U. Ruktanonchai, P. Supaphol, Polymer 48 (2007) 7546–7557.
[18] S.A. Theron, E. Zussman, A.L. Yarin, Polymer 45 (2004) 2017–2030.
[19] K. Sawicka, P. Gouma, S. Simon, Sensor Actuat. B: Chem. 108 (2005) 585–588.