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Original Article

Preparation and characterization of gac loaded cellulose acetate nanofiber patch

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Abstract

The aims of this study were to fabricate cellulose acetate (CA) nanofiber patch containing gac extract using electrospinning process, and to characterize and investigate release and stability of the patch. The patch was successfully prepared using electrospinning process from 17% w/v CA and 3.40% w/v gac extract dissolved in 2:1 v/v acetone/ dimethylacetamide. Diameter of the obtained nanofibers of the patch was 341±63 nm and the gac extract was entrapped well in the nanofibers. Burst release was observed in the release profile of beta-carotene in the extract from the patch and approximately 62 % of beta-carotene was released in an hour. The beta-carotene content in the nanofiber patch remained 50% of the initial amount after 6 months at 40°C and 75%RH, while the physical properties of nanofiber patch were unchanged. This study may enhance the use of gac-loaded CA nanofiber patch and the applications of nanofibers for cosmetic purpose.

Keywords: gac extract, beta-carotene, nanofiber, patch, characterization

1. Introduction

Gac (*Momordica cochinchinensis*), a tropical plant found in southeast Asia, is used as food and traditional medicine. It is rich of beta-carotene, lycopene, vitamin A and vitamin E. These compounds in gac provide strong antioxidant activity which can be used in cosmetic products (Ishida, Turner, Chapman, & McKeon, 2004; Kubola & Siriamornpun, 2011).

Applications of the nanofibers include filters, composite reinforcement, tissue engineered scaffolds, wound-dressing materials and transdermal drug delivery (Kumar *et al.*, 2012). Electrospinning process has been widely used to generate nanofibers where the diameters in micrometer down to nanometer range can be fabricated. The apparatus consists of an electric high voltage power supply, a liquid container connected with a small diameter needle and a metal collector. High voltage electric field is used to induce charge of a polymer solution in the container resulting the polymer jet flying from the needle tip to the metal collector. While the solution jet flies to the collector, the solvent is dried out and ultra-fine fibers accumulate as a flat sheet on the collector. The collected sheet could be cut to be patches at desired sizes. Nanofibers containing active ingredients have been developed for cosmetic skin care benefit and medical purpose (Jatoi, Kim, & Ni, 2019; Sharaf & El-Naggar, 2018). In this study, gac loaded nanofibers were expected to improve stability of the active compounds in gac extract. Moreover, the nanofiber patch would facilitate deposition of the active compounds on skin due to the occlusion effect of the patch.

Polymer concentration, viscosity, conductivity, voltage, flow rate and distance between tip to metal collector are parameters involved in the electrospinning process. These parameters impact the morphology and mechanical properties of nanofibers (Kumar *et al.*, 2012). Viscosity of the polymer solution can modify the dimensions of nanofibers. If the

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viscosity is increased, the nanofibers diameter may increase. If the viscosity is too low, the formation of beads on the nanofibers would be obtained. The high voltage charge may result in long and thin fibers. Proper flow rate is required for perfectly dried nanofibers when they reach the collector (Megelski, Stephens, Chase, & Rabolt, 2002). The nanofiber diameter is decreased when the distance between tip and collector is increased (Jaeger, Bergshoef, Batlle, Schonherr, & Vancso, 1998). Cellulose acetate (CA) is a common biopolymer widely used in medical applications including electrospinning nanofiber patches (Khoshnevisan *et al.*, 2018; Konwarh, Karak, & Misra, 2013).

The objectives of this work were to prepare patch of gac-loaded CA nanofibers using electrospinning process, to characterize the nanofiber patch and to investigate release and stability of the nanofiber patch.

2. Materials and Methods

2.1 Materials

Gac fruit was purchased from Nakornpathom province, Thailand in September, 2017. Cellulose acetate (CA; acetyl content = 39.8%; Mw = 30,000) and beta-carotene standard (97.6%) were purchased from Sigma, Aldrich, Switzerland. N, N-dimethylacetamide (DMAc), absolute ethanol, acetone, hexane, methanol (HPLC grade), acetonitrile (HPLC grade), triethanolamine (TEA) and sodium acetate were purchased from RCI Labscan, Thailand. Ethanol (95% v/v), sodium carbonate (Na_2CO_3) and acetic acid were purchased from The Liquor Distillery Organisation (Thailand), Ajax Finechem (Australia) and Merck (Germany), respectively.

2.2 Methods

2.2.1 Preparation of gac extract

Preparation of gac extract was modified from previous study (Barba, Hurtado, Mata, Ruiz, & de Tejada, 2006). Two grams of dried fruit was macerated 30 minutes in 100 ml of mixed solvent (hexane: acetone: ethanol, 50:25:25) and 15 ml of water. Then it was filtrated and the obtained solution was evaporated using a rotary evaporator (Buchi, Switzerland) at 40 ºC until dried. Gac extract was protected from light and kept at 4 ºC until further use. Percent yield was calculated regarding Equation 1.

$$\%\ \text{Yield} = \frac{\text{Weight of crude extract}}{\text{Initial weight of fruit}} \times 100 \qquad (1)$$

2.2.2 HPLC determination of beta-carotene

An Agilent 1260 Infinity II Liquid Chromatography System (HPLC, USA) was used to quantify the amount of beta-carotene (Barba *et al.*, 2006). Chromatograhic separation of the beta-carotene was achieved by the use of a Zorbax SB-C18 C18 column (particle size = 5µm; column dimension = 4.6 x 150 mm) with a Zorbax SB guard column (particle size = 5 µm; column dimension = 4.0 x 10 mm). The mobile phase for beta-carotene separation was 90:10 v/v of methanol/acetonitrile: 9 µM triethanolamine and the flow rate of 0.9 mLmin^{-1}. The injection volume was 100 µl. An UV detector for beta-carotene was set at the wavelength of 475 nm. All of the sample solutions were filtered through a nylon filter 0.45 µm prior to injection. A calibration curve prepared from beta-carotene standard solution in 90:10 v/v of methanol/acetonitrile: 9 µM triethanolamine (the mobile phase) was carried out over the concentration range of 15-80 µg/ml. The gac extract was properly dissolved and diluted using the mobile phase and injected to the HPLC system to determine the beta-carotene content.

2.2.3 Preparation of CA nanofibers without and with gac extract

The CA solution in the concentration range of 13-20 % w/v was prepared in 2:1 v/v acetone:DMAc (Liu & Hsieh, 2002). Prior to electrospinning process, viscosity and conductivity of the solution was determined using a Brookfield DV-III programmable viscometer (USA) and a SUNTEX conductivity meter (Taiwan), respectively. Then, the CA solution was loaded into a 10 mL plastic syringe (diameter 14.5 mm) with an attached gauge-20 stainless steel needle (outer diameter = 0.91 mm). The process was carried out at 25°C. The solution was electrospun under the electrical charge of 17.5 kV and a tip-to-collector distance of 15 cm. The feeding rate of the solution was controlled at ~1 ml hour^{-1} by means of a KD Scientific syringe pump. Nanofibers were collected on a metal collector covered by a piece of aluminium foil and, consequently, a fabricated patch of nanofibers was obtained. The patch was left in an air ventilation hood until the patch weight did not change and then kept in a desiccator. This process was modified from previous work (Suwantong, Opanasopit, Ruktanonchal, & Supaphol, 2007). Morphology of the obtained nanofibers were evaluated using SEM images as presented in 2.2.4.1. The concentration of CA providing smooth surface, no bead and small diameter of the nanofibers was selected to incorporate with gac extract as follows. The selected CA solution was mixed with various concentrations of gac extract (5 - 25 wt.% based on the weight of CA powder). These mixtures were processed in the same manner to prepare patches of CA nanofibers containing gac extract at various concentrations. SEM images of these CA nanofibers containing gac extract were determined and the highest gac loaded CA nanofiber patch presenting smooth surface and small average diameter was selected. The selected nanofiber patch was investigated for mechanical properties (2.2.4.2), beta-carotene content (2.2.4.3), release profile (2.2.5) and stability (2.2.6).

2.2.4 Characterization of the nanofibers

2.2.4.1 Morphology

Morphology of the CA nanofibers was monitored using a scanning electron microscope (SEM, QUANTA 250, USA). A fabricated nanofiber patch (0.5 cm x 0.5 cm) was coated with a thin layer of gold using a JEOL JFC-1100E sputtering device prior to observation under SEM (10000X). Diameters of the electrospinning nanofibers were measured directly from SEM images using a SemAfore 4.0 software.

2.2.4.2 Mechanical properties

The mechanical properties consisting of stress, strain and elongation at break were tested on a Narin universal testing machine (gauge length of 40 mm and crosshead speed of 10 mm min^{-1}, Thailand). These measurements were modified from Suwantong *et al.* (2007). The thickness of the patch was measured using a Mitutoyo digital micrometer with a 0.001 mm accuracy and the average thickness was calculated from eight-point measurement. This experiment was performed on the fabricated nanofiber patch of 120 ± 10 µm in thickness which was cut into a rectangular shape (10 mm x 100 mm). The rectangular patch was placed on the machine and force was applied. Stress, strain and elongation at break of the patch were recorded. The experiment was done in triplicate.

2.2.4.3 Determination of beta-carotene in gac-loaded CA nanofibers

The amount of beta-carotene in the nanofibers was analyzed. A 30 mg-patch, which was cut from the nanofiber sheet, was accurately weighed and completely dissolved in 10 ml of 2:1 v/v acetone/DMAc and 8 ml of the acetate buffer solution and then adjusted to 25 ml using the HPLC mobile phase. The diluted solution was filtered and injected to HPLC system as previously mentioned and the content of beta-carotene in the 30 mg-patch was calculated. Loading efficiency of the nanofiber patch was calculated using Equation 2.

$$\text{Loading efficiency} = \frac{\text{Beta carotene entrapped in nanofiber patch}}{\text{Beta carotene loaded}} \times 1 \qquad (2)$$

2.2.5. Release of gac-loaded CA nanofiber patch

The release study was performed in a buffer solution (pH 5.5) at room temperature and beta-carotene was used as an active marker. One liter of the buffer solution contained 3.676 g of sodium acetate and 0.31 g of acetic acid and 5 g of Tween 80 dissolved in water. A 30 mg-patch of gac-loaded CA nanofibers was placed on a glass dish with a diameter of 100 mm. The buffer solution (25 ml) was gently poured into the glass dish. The patch was totally immersed in the buffer solution and the dish was gently sharked at 50 rpm. At a specified period ranging between 0 and 8 hours, 15 ml of the release medium was taken out from the dish and filtered through a nylon filter. Then, amount of beta- carotene in the release medium was analyzed using HPLC. The fresh buffer solution (15 ml) was added into the dish right after each sampling. A beta-carotene released vs time profile was plotted. The experiment was done in triplicate.

2.2.6 Stability test

Thirty milligram-patches of nanofibers loaded gac extract were accurately weighed, separately wrapped in aluminium foil and placed in sealed plastic bags. All stability samples were kept in an incubator (40 ± 2 ºC and 75 ± 5 %RH). The patches were taken to determine their morphology and beta-carotene content at specified times during 0-6 months. Percent of beta-carotene in remaining the patches was calculated using Equation 3. The experiment was done in triplicate.

$$\%\ \text{Remaining} = \frac{\text{Beta - carotene content (day t)}}{\text{Beta - carotene content (day 0)}} \times 100 \qquad (3)$$

3. Results and Discussion

3.1 Preparation of gac extract

The percent yield of gac extract from the dried fruit was 24.57 ± 0.07 % w/w. The extract presented a sticky dark brown paste. Beta-carotene is one of major active compounds in gac fruit and could be analyzed using the HPLC method. The standard curve of beta-carotene is shown in Figure 1. Content of beta-carotene in the gac extract was 0.21 ± 0.01 g/g. The beta-carotene content was reported in the range of 3.00 – 52.02 mg/g of gac extract (Kubola & Siriamornpun, 2011). In the current study, beta-carotene content in the gac extract was much more than that in the previous report. This deviation could be due to harvesting stage, storage time and source of the fruit (Bhumsaidon & Chamchong, 2016).

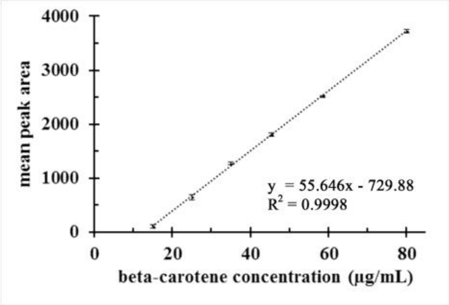


Figure 1. Typical standard curve of beta- carotene

3.2 Preparation of CA nanofibers without and with gac extract

Prior to electrospinning process, viscosity and electrical conductivity of the CA solution were measured and are summarized in Table 1. The increment of CA concentration enhanced the viscosity of the solution while it slightly affected the conductivity. The addition of gac extract slightly increased the viscosity of the CA solution, while the electrical conductivity was slightly decreased. However, the concentration of CA was still the major influence on viscosity of the solution. The decrease of conductivity could be due to the presence of non-ionizable compounds in the extract such as beta-carotene and lycopene.

3.3. Characterization of the nanofiber patches

3.3.1. Morphology

SEM images and diameters of the nanofibers are presented in Figure 2. Beads were noticeable on the nanofibers produced from 13% w/v CA solution where the

Table 1. Shear viscosity and electrical conductivity of CA solution and CA solution containing gac extract

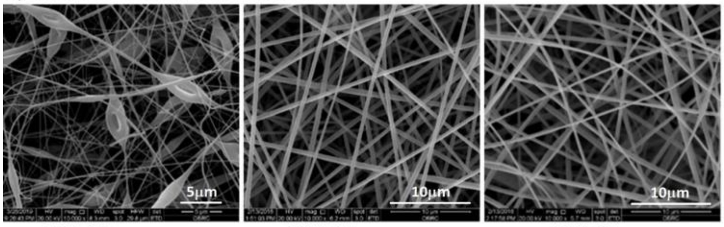
Condition	Shear viscosity (mPa s)	Electrical conductivity (μS cm^{-1})
13 % CA	328.15 ± 1.48	7.38 ± 0.12
17 % CA	418.33 ± 2.08	8.27 ± 0.06
20% CA	589.15 ± 1.76	8.72 ± 0.10
17% CA+5% gac	410.21 ± 1.74	7.98 ± 0.20
17% CA+10 % gac	430.53 ± 2.01	7.24 ± 0.14
17% CA+15% gac	446.04 ± 1.64	6.64 ± 0.08
17% CA+20% gac	457.67 ± 5.51	6.68 ± 0.10

viscosity was low (Table 1). Smooth and evenly uniform nanofibers without bump or bead were obtained from 17 and 20% w/v CA solutions. Diameters of the electrospinning nanofibers were measured from SEM images. Fiber diameter from 20% w/v CA solution was larger than that from 17% w/v CA solution. This result is consistent with previous studies (Fong, Chun, & Reneker, 1999; Nezarati, Eifert, & Cosgriff-Hernandez, 2013). They explained that the surface tension in the solution jet is sufficient to induce droplet/bead formation on the fibers in order to reduce surface area during the electrospinning process. This bead formation was opposed by the increase of viscosity. High concentration of polymer provided high viscosity and high viscoelastic force that reduced bead formation and also impedes the axial stretching during the electronspinning process leading to a larger fiber diameter.

A higher surface area to volume or mass ratio was obtained from the smaller diameter of nanofibers. The high surface area to volume or mass ratio would facilitate the release of active compounds from the nanofibers. Thus, 17% w/v CA solution was chosen because the nanofibers from 17% w/v CA solution would provide a higher area to mass ratio than 20% w/v CA solution. The various concentrations of gac extract was dissolved in 17% w/v CA solution and nanofibers containing gac extract were prepared from these solutions. The SEM images of the nanofibers loaded gac extract are presented in Figure 2. The maximum amount of gac extract that could be loaded in the nanofibers was 20 wt% based on CA powder or 3.40% w/v in the solution. The gac-loaded CA nanofibers from this selected concentration were smooth, evenly uniform fibers without beads and aggregation of gac extract. It implied that gac extract was incorporated well in the fibers. No nanofibers could be fabricated at 25 wt% gac loaded.

The average diameter of CA nanofibers was 306 ± 68 nm, while that of the gac-loaded CA nanofibers was 341 ± 63 nm. The addition of gac extract in the CA solution caused are increase of shear viscosity and a decrease of electric conductivity of the solution leading to the increase of fiber diameter. As mentioned, the morphology of electrospinning nanofibers depends on shear viscosity and electrical

a) various CA concentrations

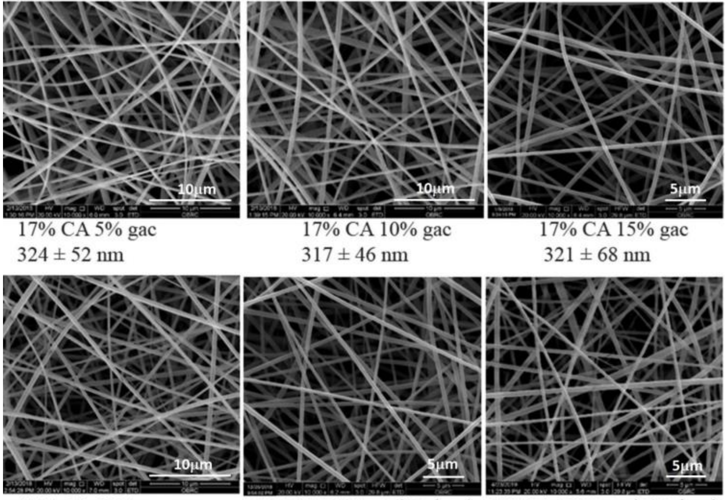


13% CA
488 ± 458 nm

17% CA
306 ± 68 nm

20% CA
495±44 nm

b) various gac concentrations in 17% CA solution



17% CA 5% gac
324 ± 52 nm

17% CA 10% gac
317 ± 46 nm

17% CA 15% gac
321 ± 68 nm

17% CA 20% gac
341 ± 63 nm

17%CA 20%gac (90 days)
395 ± 53 nm

17%CA 20%gac (180 days)
391 ± 98 nm

Figure 2. SEM images and diameters of CA nanofibers; a) various CA concentrations, b) various gac concentrations in 17% CA solution

conductivity of the polymer solution. When the viscosity is proper, the solvent is distributed throughout the polymer molecules leading to long and smooth fibers without beads (Kumar *et al.*, 2012). After that, the increase of viscosity leads to a larger average diameter of the fibers. The jet of high electrical conductivity solution is subjected to a great tensile strength force where a decrease of fiber diameter is obtained. The opposite result is observed in low conductivity solution (Son, Youk, Lee, & Park, 2004).

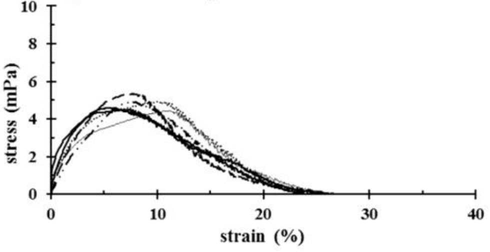
3.3.2. Mechanical properties

The mechanical properties of the patches of CA nanofibers prepared from 17% CA solution and gac-loaded CA nanofibers prepared from 17% CA and 3.40% gac extract solution were investigated in terms of stress at maximum load, strain at maximum load and elongation at break as presented in Table 2. Stress-strain curves of the nanofiber patches are shown in Figure 3. This characterization represented deformation and elasticity of the patches according is the force applied. The patches should be flexible and could attach on skin. The gac loaded CA nanofiber patch expressed slightly low of these mechanical parameters and wide deviation of the stress-strain curves. It could be due to the gac extract partially intervening and interacting with the CA polymer entanglement.

Table 2. Mechanical properties of CA nanofiber patch and gac-loaded nanofiber patch (n=6)

Type of electrospinning CA nanofiber patches	Stress at maximum load (MPa)	Strain at maximum load (%)	Elongation at break (%)
CA nanofiber patch	4.75 ± 0.34	8.45 ± 0.34	27.19 ± 0.34
Gac-loaded nanofiber patch	4.70 ± 1.12	7.85 ± 2.20	26.82 ± 3.59

a) CA nanofiber patch



b) gac-loaded CA nanofiber patch

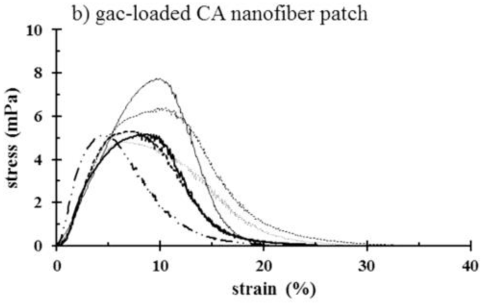


Figure 3. The stress-strain curves of a) CA nanofiber patch and b) gac-loaded CA nanofiber patch

3.3.3. Determination of beta-carotene in gac-loaded CA nanofibers

Beta-carotene content in gac-loaded CA nanofibers was determined using HPLC method. Actual amount of beta-carotene was 1.035 mg in a 30 mg-patch of the nanofibers. Thus, the content of beta-carotene in the nanofibers was 3.45 ± 0.11% by weight. Based on calculation of loaded amount of the extract, the beta-carotene content in the patch should be 3.50% by weight. This deviation of the actual content from the calculated value was probably due to variation of beta-carotene content in the extract and loss of beta-carotene during the nanofiber preparation process. It was reported that beta-carotene in toluene solution was rapidly degraded at approximately 50 percent in 8 hours and in 24 hours when it was exposed to UV light and day light, respectively (Scita, 1992). The loading efficiency of nanofiber patch was 98.76±3.08%.

In previous studies, the deposition of beta-carotene on stratum corneum was observed after the 0.2% beta-carotene cream was applied on skin and beta-carotene concentration of 20 mg/mL in emulsion was used to protect skin from sun damage (Darvin, Fluhr, Meinke, *et al.*, 2011; Darvin, Fluhr, Schanzer, *et al.*, 2011). In the current study, the nanofiber patch contained beta-carotene at 3.45±0.11% or 34.5±1.1 mg/g. The loading dose of beta-carotene in the patch was more than that of others and suitable for cosmetic propose. The nanofibers could act as reservoirs which slowly released beta-carotene.

3.4. Release of gac-loaded CA nanofiber patch

The cumulative released amount of beta-carotene from gac-loaded CA nanofiber patch was presented as the percentage of total amount of beta-carotene in the patch as shown in Figure 4. The gac-loaded CA nanofiber patch showed burst release of beta-carotene in first 60 minutes where the accumulative amount was 61.71 ± 2.37%. The release reached plateau in 3 hours. The result agreed with previous study and it was explained that fabricated nanofiber patches containing active ingredients presented burst release because the nanofiber patches provided large surface area and small interfibrous pores to facilitate diffusion of active ingredients (Suwantong *et al.*, 2007). At 8 hours, the cumulative beta-carotene released from the nanofiber patch was 77.76 ± 1.04 %. Beta-carotene was not completely released from the patch because beta-carotene might be dissolved and trapped in the polymer matrix. The trapped amount depended on solubility of the compound in the medium and in the matrix (Tungprapa, Jangchud, & Supaphol, 2007).

3.5. Stability test

Morphology of the nanofibers did not change over 6 months as shown in the SEM images (Figure 2). The nanofibers were still smooth without any aggregation of gac extract on their surface. There was no significant difference of the nanofiber diameters between before and after storage. The % remaining of beta-carotene in the patch vs time profile is shown in Figure 5. Regarding the profile, the degradation of beta-carotene was likely a first order kinetic reaction

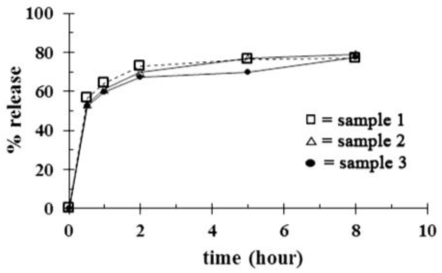


Figure 4. The percentage of cumulative release profile of beta-carotene from gac-loaded electrospinning CA nanofiber patch (n=3)

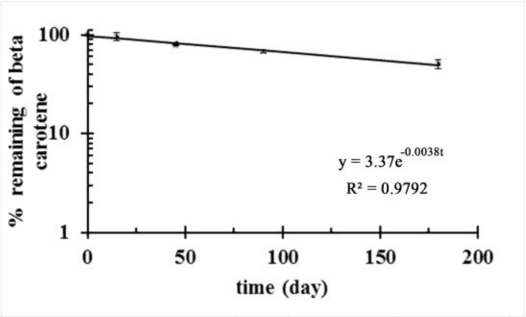


Figure 5. Concentration time profile of beta-carotene in gac-loaded CA nanofiber patch kept at 40 ºC

(correlation coefficient, r^2 = 0.9792) which agreed with the previous study (Peinado, Mason, Romano, Biasioli, & Scampicchio, 2016). Approximately fifty percent of beta-carotene in this current study disappeared in 6 months at 40 ºC (k = 0.0266 week^{-1}) while half-life of spray dried beta-carotene was only 2.4 days at 21ºC (k = 0.0122 hr^{-1}) and that of beta-carotene in gac oil was 97 days at 45 ºC (k = 0.050 week^{-1}). Not only could beta-carotene be stabilized by incorporating in nanofibers but it was also stabilized by encapsulating in hydrolyzed starch (Wagner & Warthesen, 1995). Beta-carotene has been widely used in cosmetic products to improve skin hydration balance and elasticity (Igielska-Kalwat & Mahmood, 2018). However, beta-carotene is decomposed easily especially by light, oxygen and high temperature (Woodall, Britton, & Jackson, 1997). The gac loaded CA nanofiber patch was physically stable and the nanofibers could stabilize beta-carotene but the degradation was still observed. Thus, the gac loaded CA nanofibers should be protected from heat and light.

4. Conclusions

The gac-loaded electrospinning nanofiber patch had potential for cosmetic use. In this study, CA nanofibers loaded with gac extract were successfully prepared by electrospinning technique from solution of 17% w/v CA and 3.40% gac extract dissolved in 2:1 v/v acetone/ dimethylacetamide. The gac extract was incorporated well in the nanofibers. The obtained nanofiber patch contained 3.45 ± 0.11% by weight beta-carotene. The average diameter and the mechanical properties of the gac-loaded CA nanofibers were slightly different from that of the CA nanofibers. The release profile of beta-carotene from the patch presented both burst release and sustained release. Although beta-carotene in the nanofibers was probably protected from the environment, degradation of the compound was still noticed without significant change of the nanofiber morphology.

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