International Journal of Biological Macromolecules 80 (2015) 139–148

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

# Improving agar electrospinnability with choline-based deep eutectic solvents

Ana M.M. Sousa[a,b], Hiléia K.S. Souza[a], Joseph Uknalis[b], Shih-Chuan Liu[b,c], Maria P. Gonçalves[a], LinShu Liu[b,*]

[a] REQUIMTE/LAQV, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[b] Dairy Functional Food Research Unit, United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA
[c] School of Health Diet and Industry Management, Chung Shan Medical University; Hospital Department of Nutrition, Chung Shan Medical University. 110, Sec. 1, Jian-Guo North Rd., Taichung, 402, Taiwan, ROC

ARTICLE INFO

*Article history:*
Received 18 March 2015
Received in revised form 12 June 2015
Accepted 18 June 2015
Available online 23 June 2015

*Keywords:*
Agar
Electrospinning
Microfibers
Nanofibers
Deep eutectic solvents
PVA

ABSTRACT

Very recently our group has produced novel agar-based fibers by an electrospinning technique using water as solvent and polyvinyl alcohol (PVA) as co-blending polymer. Here, we tested the deep eutectic solvent (DES), (2-hydroxyethyl)trimethylammonium chloride/urea prepared at 1:2 molar ratio, as an alternative solvent medium for agar electrospinning. The electrospun materials were collected with an ethanol bath adapted to a previous electrospinning set-up. One weight percent agar-in-DES showed improved viscoelasticity and hence, spinnability, when compared to 1 wt% agar-in-water and pure agar nanofibers were successfully electrospun if working above the temperature of sol–gel transition (~80 °C). By changing the solvent medium we decreased the PVA concentration (5 wt% starting solution) and successfully produced composite fibers with high agar contents (50/50 agar/PVA). Best composite fibers were formed with the 50/50 and 30/70 agar/PVA solutions. These fibers were mechanically resistant, showed tailorable surface roughness and diverse size distributions, with most of the diameters falling in the sub-micron range. Both nano and micro forms of agar fibers (used separately or combined) may have potential for the design of new and highly functional agar-based materials.

Published by Elsevier B.V.

## 1. Introduction

In recent years, the electrospinning technique has become the preferred method for the fabrication of polymer nano and microfibers with high surface-to-mass ratios, enhanced mechanical performance and high porosity [1–4]. Briefly, a high voltage is applied to the polymer solution or melts held at a tip of a capillary by its surface tension. When the applied electric field is such that overcomes the surface tension of the droplet, a jet forms and stretches towards the grounded collector (or counter electrode). If the solvent is volatile, it will evaporate as the jet travels towards the counter electrode [5,6]. However, if non-volatile solvents are used (*e.g.* ionic liquids, ILs), the fibers should be recovered through the use of a coagulating bath [7,8]. Despite this apparent simplicity, an intricate conjugation of factors is involved in electrospinning. Parameters that govern the spinnability include the ambient temperature and relative humidity, the feed rate of the solution, the applied electric field, and the distance from the capillary tip-to-collector [2]. Other aspects related to the properties of the polymer solution (*e.g.* concentration, viscosity, viscoelasticity, surface tension) as well as the polymer and solvent intrinsic properties are also crucial for the electrospinning process.

Micro and nano forms of polymers can be equally advantageous in the design of new products [1,2,9]. Several cutting-edge materials have also been developed from the combination of nanofibers with micro-scale structures. Collagen nanofibers and starch-based microfibers have been combined to design tissue scaffolds benefiting from the great biochemical functionality of the former and the superior mechanical strength and greater porosity of the latter [9]. Edwards et al. developed a composite scaffold formed when electrospinning poly(lactic-co-glycolic acid) nanofibers onto a micro-scale multiwalled carbon nanotube [10]. Lee and Kim combined electrospun fibers and microsized structures of polycaprolactone with cell-embedded alginate-based hydrogels to develop hierarchical scaffolds for hard tissue engineering [11].

* Corresponding author. Tel.: +1 215 233 6486.
*E-mail address:* LinShu.liu@ars.usda.gov (L. Liu).

http://dx.doi.org/10.1016/j.ijbiomac.2015.06.034
0141-8130/Published by Elsevier B.V.

Agar is a natural polymer extracted from selected red seaweeds mainly used as gel in food and biotechnological applications [12,13]. Properties such as biocompatibility, biodegradability and non-toxicity, make agar also ideal for more sensitive fields such as the biomedical [14–16]. Knowing that nano and microfibers may afford performances and properties not seen in bulk materials [2,9,17], processing agar down to these scales could be a major breakthrough in the development of cutting-edge and highly functional biomaterials. Very recently, our group has taken the first steps to fabricate agar fibers by an electrospinning technique using water as solvent and polyvinyl alcohol (PVA) as co-blending polymer [18]. Agar containing PVA nanofibers were successfully produced using a tubeless spinneret attached inside the electrospinning chamber, set to operate at 50 °C. In this first approach however, the pure agar solution (*i.e.* 1 wt% agar-in-water) showed inadequate spinnability and only blends with high PVA contents (30/70 and 20/80 agar/PVA mass ratios prepared from a 10 wt% PVA starting solution) yielded continuous fibrous mats.

The choice of solvent is a decisive element in electrospinning [2]. In many cases, ILs have shown to be good alternatives to volatile solvents (*e.g.* water) [7,8,19]. These solvents are highly versatile and extremely efficient at dissolving natural polymers and this has led to a growing interest in natural fibers made from ILs in fields such as the biomedical [20].

Deep eutectic solvents (DESs), also known as IL analogues, are much cheaper and easier to prepare than ILs and for that reason, are currently being focus of a lot of attention [21,22]. The DES concept arises from the adequate mixture of halide salts with hydrogen bond donors to form eutectics that can be operated at room temperature as fluids. Particular emphasis has been given to DESs derived from the quaternary ammonium salt (2-hydroxyethyl)trimethylammonium chloride (choline chloride, ChCl), due to its very low cost, biodegradability, non-toxicity as well as renewable nature [21,22]. Very recently, Mukesh et al. produced chitin nanofibers by sonication using a DES based on ChCl and thiourea [23]. A eutectic mixture with a freezing point of 12 °C is also formed when mixing ChCl (melting point, m.p. = 302 °C) and urea (m.p. = 133 °C) at 1:2 molar ratio [21].

In the present study, we tested for the first time the DES ChCl/urea prepared at 1:2 molar ratio, as an alternative solvent medium to process agar by electrospinning. To the best of our knowledge, this is the first report on production of electrospun agar nanofibers without the use of a co-blending polymer as well as the first time this DES is used as solvent medium for electrospinning. Due to the non-volatile nature of the DES a coagulating ethanol bath was used to collect the fibers. The solvent bath was adapted to an electrospinning set-up used in a previous study where we had used a rotating cylindrical drum to collect agar/PVA nanofibers from aqueous solutions [18]. PVA was added to agar [24,25] to further improve agar-in-DES spinnability and produce composite fibers with tailorable morphology. The morphology of the fibers was examined by scanning electron microscopy (SEM) and interpreted in light of the rheological properties of the spinning solutions.

## 2. Materials and methods

### 2.1. Materials

The PVA (average Mw = 89,000–98,000 Da, 99+% hydrolyzed) and commercial agar (A-7002, $(C_{12}H_{18}O_9)n$) were both purchased from Sigma–Aldrich Co. (St. Louis, MO) and were the same as used in our electrospinning study focusing agar aqueous solutions [18]. This commercial agar sample was previously characterized in our lab [26] following standard procedures: viscosity-average molecular mass, $M_v$ ~138 kDa; 3,6-anhydro-α-L-galactose, 3,6-AG (%) ~44; sulfate esters, $SO_3^-$ (%) ~1.6. Details concerning the followed experimental protocols used for agar characterization can be found elsewhere [26]. The choline chloride (>98%; $C_5H_{14}ClNO$) and urea (>99%; $CH_4N_2O$) were also purchased from Sigma–Aldrich.

### 2.2. Methods

#### 2.2.1. Preparation of the DES

The choline-based DES was prepared according to a previous procedure [27]. Briefly, the dried ChCl (70 °C overnight in an oven) was mixed with the appropriate amount of the H-bond donor, *i.e.*, urea at 1:2 molar ratio. The mixture was heated at 70 °C under stirring until formation of a homogeneous liquid. Subsequently, it was left to cool down to 25 °C and kept at that temperature (above the freezing point of the solvent).

#### 2.2.2. Preparation of agar/PVA spinning solutions

Agar and PVA powders were pre-dried overnight at 40 °C in a vacuum oven, prior to use. The following concentrations were considered for the starting solutions: 1 wt% (agar) and 5 wt% (PVA). Both concentrations were defined according to preliminary tests. Each starting solution was prepared separately considering a solution volume of 15 mL and taking into consideration the density of the solvent (1.25 g/cm$^3$ at 25 °C; [22]). The appropriate amount of agar (0.15 g) or PVA (0.75 g) was mixed with the DES and heated in an oil bath, in closed cap vials, with temperatures ranging between 120 and 130 °C and under vigorous stirring. In each case, the solution was initially heated to 120 °C, kept at this temperature for 20 min after which, the temperature of the oil bath was increased to 130 °C until a homogeneous solution was obtained. This typically occurred after 20–30 min of total dissolution time. Blends with different mass ratios (100/0, 50/50, 30/70, 20/80, 0/100 agar/PVA) were prepared by weighing the appropriate amounts of each starting solution into closed cap vials. Finally, the mixtures were heated again at 120 °C, under vigorous stirring, until a homogeneous solution was obtained (~20 min).

#### 2.2.3. Rheological measurements

The rheological characterization of the spinning solutions was performed in a stress-controlled rheometer (ARG2, TA Instruments, USA) as described elsewhere [18]. Each sample was degassed in a vacuum oven for 5 min at 100 °C before being placed on the hot Peltier (90 °C). A cone-and-plate (4 cm diameter, 2° angle and a 54 μm gap) was the chosen geometry to carry out the experiments. Frequency scans were recorded after cooling the solutions from 90 to 50 °C at a cooling rate of 1 °C/min and a fixed angular frequency, $\omega$, of 6.28 rad/s, followed by an equilibration period at 50 °C. Mechanical spectra were recorded at 50 °C, over the range 0.1–75 rad/s.

The relationship between the magnitude of the complex viscosity, $|\eta^*|$, and $\omega$, was determined using the power law equation (Eq. (1)) [28],

$$|\eta*| = K\omega^n \quad (1)$$

where $K$ is the dynamic consistency index and $n$ the dynamic power-law factor. In the limit, $n$ can assume the value −1 (completely elastic system) or 0 (completely viscous system). The strain conditions were chosen according to the linear viscoelastic region defined during strain sweep tests. A common strain of 2% was selected for all samples. Steady shear measurements were carried out at 50 °C, in the range of shear rates 1–200 s$^{-1}$. Three replicates were performed in each case.