Chemical Engineering Journal 331 (2018) 177–184

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Strength enhanced hydrogels constructed from agarose in alkali/urea aqueous solution and their application

Sen Wang[a], Rongrong Zhang[a], Yiwen Yang[a], Shuangquan Wu[a], Yan Cao[a], Ang Lu[a,*], Lina Zhang[a,b,*]

[a] College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China
[b] School of Chemistry and Chemical Engineering, Guangxi University, Nanning 530004, China

ARTICLE INFO

Keywords:
Agarose
Dissolution
Nanofibers
Sustainable development
Bioconversion

ABSTRACT

As a breakthrough to the traditional method of dissolving with heat, agarose aqueous solution was successfully obtained in alkali/urea system via freezing/thawing for the first time. The agarose dissolution was a physical process, proved by ^{13}C NMR. The results of dynamic light scattering and atomic force microscopy demonstrated that agarose existed as extended chains in the solution and easily aggregated in parallel to form nanofibers. The viscosity and rheology measurements indicated that the agarose solution exhibited higher stability at room temperature than that traditionally dissolved in hot water, favoring the processing of agarose to fabricate various materials. Moreover, the new agarose hydrogels were fabricated directly from the agarose solution, showing more homogenous structure, enhanced thermal stability and mechanical properties than that prepared by hot water. The compression fracture stress of the agarose hydrogels was 3.7 times of that by hot water, as a result of the reinforcement by the nanofibers, leading to greater applicability. Furthermore, the agarose hydrogels displayed excellent biocompatibility, showing potential applications in wider fields. Especially, the agarose hydrogel exhibited effective germination and growth for the use as soilless culture medium, superior to the hydrogel prepared by hot water. This work provided not only new insights of dissolving agarose with cooling temperature and fabricating strength enhanced hydrogels, but also significance for the dissolution and green conversion of biomass which fits sustainable strategy about utilizing the products from agriculture and ocean.

1. Introduction

Facing the threats of the depletion of non-renewable resources and environmental pollution, the world requires another industrial revolution in which our sources of energy are affordable, accessible and sustainable [1]. The resolution "Transforming our world: the 2030 Agenda for Sustainable Development" adopted by the General Assembly of the United Nations, as well as the Article 14 of the United Nations' Sustainable Development Goals (SDGs) is dedicated to conserving and using the oceans and their resources for sustainable development. The seas provide us with food, materials, livelihoods and recreation, and managing these ecosystem services effectively can help us to eradicate poverty, develop sustainable economies and adapt to global environmental changes [2]. The marine organisms have developed unique properties which are unparalleled by their terrestrial counterparts in some cases. The agarose extracted from red algae of seaweeds is a linear polymer made up of 3-linked β-D-galactopyranose (G) and 3,6-anhydro-α-L-galactopyranose (A), and is playing important roles in various areas, such as food and pharmaceutical industry, electrophoresis, tissue engineering as well as energy materials [3–7]. The large number of hydroxyl groups makes agarose insoluble in cold water and many common organic solvents. It is commonly dissolved in hot water and forms brittle hydrogel as temperature decreases [8]. Moreover, the agarose solution dissolved in water at high temperature is unstable at room temperature. The single traditional method of processing the agarose with hot water gives weak mechanical properties of the hydrogels and limits the scope of the researcher and the functionalization and applications of the marine resource [9]. In previous reports, agarose could be also dissolved in ionic liquids to expand its utilization [9]. A worthwhile endeavor would be to first study the agarose dissolution, which can be less expensive and more facile, and then to fabricate materials with better properties.

In our laboratory, a series of aqueous solutions as solvents have been successfully developed to dissolve stubborn natural polysaccharides such as cellulose, chitin and chitosan at low temperature, and various regenerated materials have been constructed [10–13].

* Corresponding authors at: School of Chemistry and Chemical Engineering, Guangxi University, Nanning 530004, China (L. Zhang).
E-mail addresses: anglu@whu.edu.cn (A. Lu), zhangln@whu.edu.cn (L. Zhang).

http://dx.doi.org/10.1016/j.cej.2017.08.118
Received 18 April 2017; Received in revised form 2 August 2017; Accepted 24 August 2017
Available online 24 August 2017
1385-8947/

However, these solvents have never been used to dissolve agarose. In the present work, breaking through the limitation of traditional dissolving method with heat, 4 wt% LiOH/16 wt% urea aqueous solution was chosen to dissolve agarose via a freezing-thawing process, supplying a new option for the dissolution and processing of agarose with enhanced properties, to help further expand the comprehensive utilization of the marine resources. The agarose solution was investigated by nuclear magnetic resonance spectroscopy (NMR), dynamic light scattering (DLS), atomic force microscopy (AFM), viscosity and rheology tests to clarify the dissolution process and mechanism. A significant enhancement of the agarose solution stability in the alkaline solvent was observed, compared with hot water, and agarose hydrogels were fabricated by regeneration in anti-solvents, showing good mechanical properties and excellent biocompatibility. This work enriched the possible applications for agarose, and furthermore proved the universality of the alkali/urea aqueous solvent in dissolving biopolymers at low temperature, which was different from the traditional heating methods. It is important for the research and development of renewable resources, and the conversion from the algae to agarose hydrogels by a green process (Fig. S1).

2. Experimental section

2.1. Materials

Agarose samples (M_w of 5.5×10^4 by viscosity [14]), dimethyl sulfoxide (DMSO)-d4 and deuterium oxide were purchased from Sigma-Aldrich (St. Louis, USA). Commercially available lithium hydroxide monohydrate ($LiOH{\cdot}H_2O$), urea and other reagents were of analytical grade (Shanghai Chemical Reagent Co., China), and were used without further purification.

2.2. Preparation of agarose hydrogels

The aqueous solution containing 4 wt% LiOH and 16 wt% urea was prepared as the solvent. A certain amount of agarose powders (3 wt%) was dispersed into the solvent, stirred for 5 min and then stored in a refrigerator (−20 °C) until completely frozen. The solid was then thawed while stirred extensively at room temperature to form agarose solution. Transparent agarose solution was obtained by centrifugation at 6000 rpm for 5 min to remove air bubbles. The alkaline solution was cast on a glass mold and then immersed into coagulating baths at room temperature until full regeneration to obtain agarose hydrogels. The coagulating baths were aqueous solution of 5 wt% boric acid or 5 wt% phytic acid to neutralize the alkali, and their resulting hydrogels were coded as Aga-1 and Aga-2, respectively. Traditional agarose hydrogel as the control prepared by dissolving in hot water and cooling was made and coded as Aga-h. It should be noted that, the viscosity of agarose solution in LiOH/urea with concentration below 2 wt% was very low and hydrogel did not occur in good shape in the anti-solvents. On the other hand, concentration above 5 wt% would lead to the insolubility of agarose in the solution. Therefore, a proper concentration of 3 wt% agarose in the solution was chosen for the fabrication of hydrogels.

2.3. Characterization

^{13}C NMR spectra of agarose dissolved in LiOH/urea and DMSO solution were recorded on a Bruker AVANCE III 600 NMR spectrometer at 298 K. The concentration of agarose solution is 2 wt%. The morphology of agarose in dillute alkaline solution (~0.001 mg/ml) and surface of hydrogels were observed in situ by atomic force microscopy (AFM) under AC-air mode (Cypher S, Asylum Research, Oxford Instruments). In detail, the dillute agarose solution was deposited on a freshly cleaved mica for 10 min, followed by thorough rinse with DI water to remove the alkali/urea and then dried. The surface of hydrogels was fastened and observed on mica directly. The morphology of the cross-section of the agarose hydrogel was characterized by breaking off the sample in liquid nitrogen and observing after lyophilization by scanning electron microscopy (ZEISS SIGMA FE-SEM). FT-IR spectra of the native and regenerated agarose were recorded on a Bruker TENSOR 27 spectrometer. The normal transmission mode was employed for IR measurement and spectra were recorded by overlaying 32 scans at a 4 cm^{-1} resolution. Thermogravimetric analyses were performed on TA Q500 under nitrogen atmosphere from 30 to 800 °C with a heating rate of 10 °C min^{-1}. X-ray diffraction (XRD) measurements were performed on an XRD diffractometer (D8-Advance, Bruker, Germany). The patterns of CuKα radiation (λ = 0.15418 nm) at 40 kV and 30 mA were recorded in the 2θ region from 5 to 76° at a scanning speed of 2°/min.

Dynamic light scattering (DLS) were used to characterize agarose in the dilute solution at 5 °C. A modified commercial light scattering spectrometer (ALV/SP-125, ALV, Germany) equipped with an ALV-5000/E multi-τ digital time correlator and a He-Ne laser (λ = 632.8 nm) was used at scattering angle θ of 30°. The agarose solutions were prepared at the concentration from 0.2 to 1.0×10^{-3} g/mL and made optically clean by filtration through 0.22 μm Millipore filters. The CONTIN program [15] was used to analyze the data. The hydrodynamic radius, R_h, was calculated by using the Stokes-Einstein relation as:

$$R_h = \frac{k_B T}{6\pi\eta_0 D} \quad (1)$$

where k_B was the Boltzmann constant, T the temperature in units of K, η_0 the viscosity of the solvent, and D the translational diffusion coefficient.

Relative viscosity (η_r) of agarose solution dissolved in LiOH/urea and hot water were measured for comparison with Ubbelohde viscometer in a water bath of 25 °C. The concentration of agarose solution is 0.1 wt%. TA Discovery Hybrid Rheometer (HR-2) was used for the rheology test of the 4 wt% alkaline agarose solution. Oscillation time sweep was conducted with a 40 mm parallel plate to measure the viscoelastic parameters of elastic storage modulus (G') and viscous loss modulus (G'') as a function of time at 5 °C, 25 °C and 45 °C. Constant frequency of 10.0 rad/s, and shear strains of 1% were employed which ensured the linearity of viscoelasticity.

The optical transmittance of the agarose hydrogels was observed with a UV−vis (UV-6, Shanghai Meipuda instrument Co., Ltd., Shanghai, China) spectrometer at a wavelength from 200 to 800 nm.

The mechanical properties of the agarose hydrogels were characterized by compression tests, which were performed on a universal testing machine (CMT6350, Shenzhen SANS, China) according to ISO527-3-1995 (E) at a speed of 1 mm/min.

2.4. Biological test

Human umbilical vein endothelial cells (HUVEC) suspension was plated into a 96-well microplate at 0.8×10^4 cells/well, and incubated with samples for 48 h, with the culture medium itself as the control. 20 μL MTT (5 mg/m L in PBS filtered for sterilization) was added into each well and incubated for another 4 h and then removed. 200 μL/well of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals and shook for 10 min. Subsequently, 150 μL of the supernatant was placed into a new 96-well micrometer plate to measure the absorbance at a test wavelength of 570 nm. Cell viability was calculated by the following equation:

$$\text{cell viability } (\%) = A_{test}/A_{control} \times 100\% \quad (2)$$

where A_{test} and $A_{control}$ were the absorbance values of the test and control groups, respectively.

To observe the cells on the hydrogels, the sterilized samples with diameter of 12 mm were placed in 24-well plates and then 1 mL fresh cells suspension (2×10^5 cells/mL) were seeded on the hydrogels, culturing at 37 °C for 24 h. The cell-seeded hydrogels were washed with