Carbohydrate Polymers 147 (2016) 409–414

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Development of chitosan/β-glycerophosphate/glycerol hydrogel as a thermosensitive coupling agent

Chih-Ling Huang[a,b], Yu-Bin Chen[a,*], Yu-Lung Lo[a,c], Yi-Hsiang Lin[a]

[a] Department of Mechanical Engineering, National Cheng Kung University, No. 1, University Road, Tainan 701, Taiwan
[b] Center for Fundamental Science, Kaohsiung Medical University, Kaohsiung 807, Taiwan
[c] Advanced Optoelectronic Technology Center, National Cheng Kung University, No. 1, University Road, Tainan 701, Taiwan

ARTICLE INFO

Article history:
Received 30 July 2015
Received in revised form 15 March 2016
Accepted 6 April 2016
Available online 8 April 2016

Chemical compounds studied in this article:
Acetic acid (PubChem CID: 176)
Chitosan (PubChem CID: 71853)
Glycerol (PubChem CID: 753)
β-glycerophosphate (PubChem CID: 6101544)

Keywords:
Chitosan
Coupling agent
Glycerol
Thermosensitive hydrogel
Ultrasonic
β-glycerophosphate

ABSTRACT

This work develops a dual-function thermosensitive hydrogel to prevent overheating, a side effect of focused ultrasound therapy. The proposed hydrogel has the components of chitosan, β-glycerophosphate, and glycerol. Its thermosensitive sol-to-gel transition gives an instant signal of overheating without the need of any awkward sensing device. Impacts of varying component concentrations on the sol-to-gel temperature, rate, and degree of transparency are also investigated. Chemical structures and ultrasonic coefficients after heating are obtained with a Fourier transform infrared spectroscopy and ultrasonic measurement, respectively. Optimized formula of the proposed hydrogel is 0.5% chitosan, 5% β-glycerophosphate, and 25% glycerol. This hydrogel has a high acoustic impedance (Z = 1.8 Mrayl) close to that of human skin, high ultrasonic transmission (T = 99%, which is normalized to water) from 25 to 55 °C, and low attenuation coefficient (α = 4.0 Np/m). These properties assure the success of dual functions of the hydrogel developed in this work.

1. Introduction

Focused ultrasound is a form of thermal therapy capable of destroying diseased tissues by protein denaturation at supra-physiological temperatures (Lin et al., 2012), with the trade-off being occasional cases of skin burns when tissues are overheated (ter Haar & Coussios, 2007). Fortunately, such a side effect can be prevented with an instant warning signal based on real-time temperature measurements. Common temperature sensors include a thermocouple, thermometer, and infrared thermometer. However, these are awkward additions to this form of therapy, and each has its own limitations. For example, a thermocouple and thermometer can only monitor temperature at one spot a time. Their sensing areas are limited and difficult to move during therapy. On the other hand, while an infrared thermometer can show the surface temperature for a wide area, it needs an additional and expensive read-out system (Medberry, Tottey, Jiang, Johnson & Badylak, 2012).

One potential method of warning on overheating is directly changing the transparency of the coupling agent employed during therapy. Coupling agents are often used to promote the conduction of ultrasound between air and human tissues. The simplest coupling agent is water due to its high transmission coefficient and low reflection coefficient. The operation time of water can be extended by adding hydrophilic compounds, such as glycerol, to slow down evaporation. Coupling agents can contain a wide range of components. For example, Vaseline is one kind of petrolatum with a high transmission like water (Casarotto, Adamowski, Fallopa & Bacanelli, 2004) and KY gel is a common and aqueous lubricant composed of water and glycerol (Poltawski & Watson, 2007). Finally, Biofreeze is an alcoholic hydrogel adding glycerol and menthol to provide the lubricant (Cage et al., 2012).

Since none of agents outlined above can warn overheating, this study proposes a thermosensitive hydrogel composed of chitosan (CS), β-glycerophosphate (β-GP), and glycerol. CS is biodegradable, biocompatible, and non-toxic. Its polymer chains have numerous

* Corresponding author.
E-mail address: ybchen@mail.ncku.edu.tw (Y.-B. Chen).

http://dx.doi.org/10.1016/j.carbpol.2016.04.028
0144-8617/

hydrophilic groups. β-GP is an organic and non-toxic compound approved by the Food and Drug Administration, while glycerol is a commonly used hydrophilic lubricant. CS/β-GP/glycerol is a transparent solution below the sol-to-gel temperature, but it becomes an opaque and turbid gel above this temperature. The transparency reduction is able to serve as an overheating signal.

In this work, we tune the compositions of the hydrogel such that the sol-to-gel transition temperature agrees with the supra-physiological temperature. The resulting hydrogel is able to give out a real-time overheating warning. The thermal and other physical properties of the proposed hydrogel are examined to assess its capabilities. First, the chemical structures of the CS/β-GP/glycerol hydrogel are analyzed using Fourier transform infrared spectroscopy. Second, the light transmission of the samples is measured to identify the sol-to-gel transition temperature of CS/β-GP/glycerol hydrogel with different compositions. Finally, the acoustic impedance (Z), attenuation coefficient (α), and ultrasound transmission of the optimized hydrogel are also evaluated.

2. Materials and methods

2.1. Materials

CS have positively charged amine group (NH_3^+). The protonation ($-NH_2 + H^+ \rightarrow NH_3^+$) generates when CS is dissolved in weak acid solution (Berger et al., 2004). CS is composed of glucosamine and derived by deacetylation of chitin. The positively charged NH_3^+ groups provide CS with many excellent properties, including bacteriostasis and biocompatibility, as well as promoting cell growth.

GP has α- and β-type, but β-GP has a more compact structure and shows more steric hindrance than α-type with its linear chains (Zhou, Jiang, Cao, Li & Chen, 2015). GP is an organic compound widely used in biomedical applications, and approved by the US Food and Drug Administration (Cho, Heuzey, Bégin & Carreau, 2006), while β-GP also plays an important role in thermo-reversible network hydrogel (Chenite et al., 2000; Zhou, Jiang, Cao, Li & Chen, 2015).

Glycerol has three hydroxyl groups (—OH) to provide excellent water-affinity, so that glycerol is normally used in surfactants, lubricants, plasticizer, food additives, cosmetics, and medical applications (Gholami, Abdullah & Lee, 2014). It is also a common component of commercial coupling agent products. The —OH groups of glycerol form hydrogen bonds with CS polymer chains and water (Lavorgna, Piscitelli, Mangiacapra & Buonocore, 2010).

2.2. Sample preparation

The three components of the CS/β-GP/glycerol thermosensitive hydrogel are prepared as follows. The 2% CS solution (W/V) is prepared by dissolving 2 g of CS powder (Aldrich) in 100 ml 0.5 vol% acetic acid solution. CS with medium molecular weight is used in this study. It is 75–85% deacetylated and its viscosity is 200–800 cP (1% CS in 1 vol% acetic acid solution). The solution is moderately stirred at room temperature until no phase separation or precipitation is observed. β-GP (Calbiochem) is prepared as a 30% (W/V) aqueous solution preserved at 4 °C for the later experiments. The glycerol (Katayama Chemical Co., Ltd.) is purchased from a vender. Once three components are ready, preparing CS-based hydrogel follows the way in a previous study (Zhou, Jiang, Cao, Li & Chen, 2015) with a little modification. The first step is to dilute 2% CS solution with deionized water to produce a homogenous CS solution. Glycerol is then added before the required amount of 30% (W/V) β-GP solution. In the last step, the CS/β-GP/glycerol solution is then well stirred to become homogenous.

In Test I, the CS concentration increases from 0 to 1.5% (W/V). If the CS concentration is above 1.5%, the CS/β-GP/glycerol solution forms a gel. In Test II, the β-GP concentration increases from 0 to 15% (W/V). When the concentration is more than 15%, the solution becomes a turbid solution at room temperature. In Test III, the glycerol concentration increases from 0 to 75 vol%. A high glycerol concentration makes the CS/β-GP/glycerol sample form a glycerol based solution, although the β-GP solution does not mix well. A benchmark used in all three tests is composed of 0.5% CS (W/V), 5% β-GP (W/V), and 25 vol% glycerol. This way, the impacts of each different composition can be easily compared.

2.3. Chemical analysis by FTIR

Chemical analysis of the hydrogels is performed using spectra from a Fourier transform infrared (FTIR) spectroscopy (Thermo Scientific™, Nicolet 6700). The FTIR spectra data are collected using commercial software (Thermo Scientific™, OMNIC™ Series) and recorded from 4000 to 950 cm^{-1}. The scanning resolution is 2 cm^{-1}, and each data is averaged over 128 scans. The FTIR spectra of samples are recorded at 25, 45, and 65 °C to analyze the chemical structures at these temperatures. A precise temperature controller based on a demountable liquid cell (PIKE Technologies, PN 111-40XX) is used in this study. The sample is placed in the demountable liquid cell with water-insoluble CaF_2 windows. The optical path through a sample is controlled by Teflon spacers of 0.05 mm thickness. This path is long enough for IR light within the solution to exhibit absorbance.

2.4. Sol-to-gel temperature measurement

We use the vial inverting method to roughly check the range of sol-to-gel temperature, while cloud point measurement is also used to accurately identify the sol-to-gel temperature. In the vial inverting method, each vial contains 1 ml sample and is immersed in a water bath for 1 min. The water is either at room temperature (25 °C) or one of the supra-physiological temperatures (45 and 65 °C). The vials are then inverted for 30 s to observe the flowing status of samples. In the cloud point measurement, a laser transmittance measurement system (Chen, Ho, Chiu & Chang, 2014) using 405-nm-wavelength incidence investigates the transmission (T) of samples. A sample is placed in the demountable liquid cell with CaF_2 windows during measurement. The heating rate is 0.5 °C/min, and the temperature is raised from 25 to 60 °C.

2.5. Ultrasound transmission measurement

The reflection mode is used to measure the acoustic impedance (Z), attenuation coefficient (α), and ultrasound transmission (T) of our samples. Information about the reflection coefficient is not shown because it can be obtained from 1–T, according to the energy balance. An ultrasonic transducer (Panametrics, model A314S) with a central frequency of 1 MHz is used. This transducer is excited by a function generator connected to a radio-frequency power amplifier (Huang, Shih, Liu & Lee, 2011). The transmission of water serves as the reference for normalization of other materials. The transducer is 25.4 mm away from a metal reflector in a container. The container is filled with a sample hydrogel. Z and α are the measured at temperatures ranging from 25 to 55 °C.

3. Results and discussion

3.1. Chemical analysis by FTIR

Fig. 1 shows the FTIR spectra of the benchmark (0.5% CS, 5% β-GP, and 25% glycerol) at 25, 45, and 65 °C in the range of