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# Preparation and properties of highly soluble chitosan–L-glutamic acid aerogel derivative

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ABSTRACT

The objective of this work is to improve the solubility of chitosan at neutral or basic pH using the supercritical carbon dioxide (sc.$CO_2$). A novel water-soluble chitosan–L-glutamic acid (CL-GA) aerogel derivative was synthesized by reaction of 85% deacetylated chitosan with L-glutamic acid (L-GA) in aq.AcOH subjected to solvent exchange prior to using sc.$CO_2$ as a nonsolvent for the polymer. The prepared aerogel derivative and molecular conformation of modified chitosan are characterized by using UV, FTIR, $^1$H NMR, and CD techniques. Some physical properties and surface morphology were analyzed by X-ray diffraction, differential scanning calorimetry (DSC), thermogravimetry (TG), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and porosimetry analysis. Overall, the sc.$CO_2$ assisted chitosan aerogel derivative opens new perspectives in biomedical applications.



## 1. Introduction

Chitosan is a partially *N*-deacetylated derivative of chitin, which is commonly found in shells of insects and crustaceans, as well as cell walls of some fungi, and is known as the second most abundant biopolymer in nature after cellulose. Chitosan has been well documented with some advantageous characteristics, including biocompatibility, biodegradability, hydrophilicity, non-toxicity, and nonantigenicity as well as bioadherence and cell affinity (Gong, Zhong, Zhao, & Zhang, 2000; Richardson, Kolbe, & Duncan, 1999). It is being used extensively in pharmaceutical and biomedical areas such as drug delivery vehicles, carriers of immobilized enzymes and cells, biosensors, ocular inserts, artificial organs, orthopedic materials, surgical devices and biodegradable packaging (Bernkop-Schnürch, Humenberger, & Valenta, 1998; Brito & Campana-Filho, 2004; Felse & Panda, 1999; Santos, Dockal, & Cavalheiro, 2005; Varna, Deshpande, & Kennedy, 2004), and in particular, temporary implants for fixations and supports in tissue regeneration, which are commonly known as tissue engineering scaffolds (Khor & Lim, 2003; Rinki, Dutta, & Dutta, 2007; Sasiprapha, Sei-ichi, & Suwabun, 2007).

The cationic nature of chitosan limits the versatility of aqueous solution and pH range because it only dissolves in some specific organic acids including formic, acetic, propionic, lactic, citric and succinic acid, as well as in a very few inorganic acids, such as hydrochloric, phosphoric, and nitric acid (Wang, Turhan, & Gunasekaran, 2004). The solubility of chitosan also depends on the p$K_a$ of these acids and their concentrations. Furthermore, chitosan solution is very viscous even at low concentrations, and its applicability in a commercial context is thus often restricted (Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998). Hence, improving the solubility of chitosan is crucial if this plentiful resource is to be utilized across a wide pH range. Strategies for improving chitosan solubility can be divided into three methods based on preparation principles. Firstly, homogeneous phase reaction (Sannan, Kurita, & Iwakura, 1976). Secondly, reducing the molecular weight of chitosan produces high solubility. The third and final method of improving solubility involves introducing a hydrophilic functional group to the chitosan, a technique also called the chemical modification method (Holme & Perlin, 1997). Many chitosan derivates including CM-chitosan (carboxymethyl chitosan), *N*-sulfofuryl chitosan, 5-methyl pyrrolidinone chitosan, and dicarboxymethyl and quaternized chitosan – have been developed, with a solubility range of 3–10 g/l obtained (Delben, Muzzarelli, & Terbojevich, 1989; Dung, Milas, Rinaudo, & Desbrieres, 1994; Muzzarelli, 1992; Watanabe, Saiki, Matsumoto, & Azuma, 1992; Heras, Rodriguez, Ramos, & Agullo, 2001; Gomes, Gomes, Batista, Pinto, & Silva, 2008). One of the reasons for the intractability of chitosan lies in the rigid crystalline structure and the acetamido

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or primary amino group residues that have an important role in the formation of conformational features through intra and/or intermolecular hydrogen bonding (Nishimura, Kohgo, Kurita, & Kuzuhara, 1991; Dutta & Singh, in press).

sc.$CO_2$ is a recent technique as a processing solvent in polymer applications such as polymer modification, formation of polymer composites, polymer blending, microcellular foaming, particle production and polymerization (Alsoy & Duda, 1999; Cooper, 2000; Kendall, Canelas, Young, & De Simone, 1999; Romain, Karine, Francoise, & Daniel 2003; Tomasko et al., 2003). Its gas-like diffusivity and liquid-like density in the supercritical phase allow replacing conventional, often noxious solvents with sc.$CO_2$. It has attracted particular attention as a supercritical fluid in the synthesis as well as processing areas for polymers owing to its attractive physical properties. It is non-toxic, non-flammable, chemically inert and inexpensive. Today the use of sc.$CO_2$ as a solvent can also be seen in the processing of various biodegradable/biocompatible polymers for pharmaceutical and medical applications in the forms of particles and microcellular foam (Reverchon & Cardea, 2004; Kho, Kalika, & Knutson, 2001; Matsuyama et al., 2001). The low thermal stability of biodegradable polymers and the lack of organic solvents in processing them are the main reasons for the emergence of sc.$CO_2$ as a replacement solvent.

There are only a few reports concerning chitosan/L-GA derivative available in literature. Poly(L-glutamic acid)-paclitaxel (PG-TXL) is a new water-soluble paclitaxel derivative that has shown remarkable antitumor activity against both ovarian and breast tumors (Chun et al., 1999). The polyL-GA derivatives are also used in chemotherapeutic and anticancer agents using the drug carrier (Yiyu et al., 2001). A chitosan formulation that can be easily administered, is less toxic, and has greater antitumor effect is needed. In this article the modification was done by the formation of amide linkage between $NH_2$ group of chitosan and COOH group of L-GA and dried using supercritical carbon dioxide (sc.$CO_2$) to form aerogel. When polypeptides are applied as polyelectrolytes, the polyelectrolyte chitosan derivatives obtained will be very useful in many biomedical applications. Polyelectrolyte chitosan derivatives consisting of chitosan and L-GA may possess advantages of both chitosan and L-GA, and have a broad range of uses in biomedical applications.

## 2. Experimental

### 2.1. Materials

The chitosan powder was a product of Sigma–Aldrich and a degree of deacetylation (DD) of 85% and L-glutamic acid (Sigma–Aldrich) were used as such. Carbon dioxide ($CO_2$) 99.9% purity was sourced from BOC Ltd. and used as such.

### 2.2. Measurements

The characterization of the chitosan derivative was carried out by using FTIR technique (Bruker ATR), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) with STA 600 thermal analyzer. The morphology was studied by using a JEOL JEM-1230 transmission electron microscope (TEM) and a JEOL JSM-5200 scanning electron microscope (SEM) at 80 and 5 kV, respectively. Electronic absorbance spectra (UV) were recorded on UV.1650PC spectrometer using 1.0 cm quartz cell at ambient temperature. CD spectra were recorded on a Jasco J-810 spectropolarimeter in DMSO. The porosity measurement was done by using the Micrometrics Porosimeter: ASAP2010 model. The $^1H$ NMR spectra for chitosan and CL-GA have been recorded on 500 MHz Jeol FX90Q FTNMR spectrometer using $D_2O$/DCl and X-ray diffraction (XRD) measurement on powder samples were performed with the Burker AXS General Area Detector Diffraction System (GADDS). Monochromatized Cu K$\alpha$ radiation ($\lambda = 0.154$ nm) was used. The powder samples were placed in 0.8 diameter Lindemann glass capillaries. The sample-detector distance was 10 cm. The intensity versus scattering-angle ($2\theta$) was plotted.

### 2.3. Preparation of chitosan derivative

CL-GA hydrogel derivatives were prepared by using chitosan powder (250 mg) dissolve into 1% aqueous acetic acid 37% (w/v). Water-soluble L-GA (50 mg) solution was added slowly to the chitosan gel and stirred for 6 h, until the chitosan solution turned into a more viscous gel and the magnet bar was stopped. The prepared hydrogel was then subjected to solvent-exchange into acetone and ethyl alcohol prior to sc.$CO_2$ treatment. The solvent-exchange product was placed inside a sealed chamber of the supercritical fluid (SCF) drying reactor (Thorr Co., USA). The temperature and pressure were raised to 40 °C and 100 bar, respectively. Further pressure was raised to 200 bar. The reaction was left for 90 min (Scheme 1) and a flow of $CO_2$ was then applied through the sample in order to replace all the organic solvent with $CO_2$. The pressure was then released slowly to the atmosphere. Finally the bluish powdered CL-GA aerogel was obtained.

## 3. Results and discussion

### 3.1. FTIR analysis of chitosan, L-GA and CL-GA derivative

Characteristic peaks assignment of chitosan (Fig. 1A) are: 3429 $cm^{-1}$ (O—H stretch overlapped with N—H stretch), 2921 and 2867 $cm^{-1}$ (C—H stretch), 1640 $cm^{-1}$ (amide II band, C—O stretch of acetyl group), 1592 $cm^{-1}$ (amide II band, N—H stretch) 1485–1380 $cm^{-1}$ (asymmetric C—H bending of $CH_2$ group) and 1035 $cm^{-1}$ (bridge O stretch) of glucosamine residue. The IR spectral band of L-GA (Fig. 1B): 2966 $cm^{-1}$ (O—H stretching), 2855 $cm^{-1}$ for (C—H stretching), 1690 $cm^{-1}$ (C=O group) and 1523 $cm^{-1}$ (N—H stretching of amino group). The (Fig. 1C) shows the significant peak of CL-GA derivative 3110 and 2966 $cm^{-1}$ (axial OH group of chitosan and glutamic acid), 1685 $cm^{-1}$ (amide linkage), 1556 $cm^{-1}$ (N—H bending stretching) and 1067 $cm^{-1}$ (bridge C—O—C stretch) of chitosan residue.

In the IR spectra of chitosan derivative, the strong peak at 1466 $cm^{-1}$ could be assigned to the asymmetry deformation of $CH_2$, and the C—O adsorption peak of secondary hydroxyl group becomes stronger and move to 1067 $cm^{-1}$ the intensity of primary alcohol 1035 $cm^{-1}$ due to C—O stretching vibration, becomes much smaller than in chitosan. The new peak appears at 2966 indicates the incorporation of the L-glutamic acid moieties. The FTIR results suggest that the COOH group of L-GA have been successfully bonded to the $NH_2$ group of chitosan main chain to form amide linkage.

The relative FTIR analysis of CL-GA derivative at different pH showed that a strong interaction will not occur at all pH ranges because the relative number of —$NH_3^+$ groups in chitosan is high and the number of —$COO^-$ groups in L-GA is low at low pH; an opposite trend will appear at high pH. As the number of one ionizing groups increases, the number of the other groups decreases. In CL-GA derivative, lower frequency shifts of amides I and II are found, and as the pH increases, the signal of the —OH group in —COOH of L-GA at 1442 $cm^{-1}$ weakens, and the characteristic absorption band of the C=O group at 1690 $cm^{-1}$ is combined with amide I. This may result from the strong electrostatic interaction between the —COOH of L-GA and the —$NH_2$ of chitosan. Furthermore, the formation of hydrogen bonds causes the C=O peak to shift to lower