



# Structural and rheological characterizations of nanoparticles of environment-sensitive hydrophobic alginate in aqueous solution



Kai Chen, Jiacheng Li\*, Yuhong Feng, Furui He, Qingfeng Zhou, Dunchao Xiao, Yiyuan Tang

College of Materials and Chemical Engineering, Hainan University, Haikou 570228, China

## ARTICLE INFO

### Article history:

Received 3 June 2016

Received in revised form 2 August 2016

Accepted 22 August 2016

Available online 24 August 2016

### Keywords:

Hydrophobic alginates

Electrostatic self-assembly

Nanoparticles

Steady-shear flow

Thixotropy

Dynamical viscoelasticity

## ABSTRACT

Amphiphilic polymers that form self-assembled structures in aqueous media have been investigated and used for the diagnosis and therapy of various diseases, including cancer. In our work, a series of environment-sensitive hydrophobic alginates (Ugi-Alg) with various weight-average molecular mass values ( $M_w \sim 6.7 \times 10^5$ – $6.7 \times 10^4$  g/mol) were synthesized via Ugi reaction. The structure of Ugi-Alg was characterized by  $^1\text{H}$ NMR spectrometer. The electrostatic self-assembly of different molecular weight ( $M_w$ ) and composition (M/G ratio) of Ugi-Alg chain under various  $\text{Na}^+$  concentrations, was investigated by dynamic light scattering, electron spin resonance experiments, and transmission electron microscopy. Result showed that by comparing to other Ugi-Alg, the mid- $M_w$  Ugi-Alg ( $M_w \sim 2.8 \times 10^5$  g/mol) could form stable and homogeneous nanoparticles in low  $\text{Na}^+$  concentration solution. However, G/M values exerted no obvious effect on nanoparticles structure. Additionally, steady-shear flow, thixotropy and dynamical viscoelasticity tests were performed to characterize the rheological behavior of Ugi-Alg aqueous solutions as influenced by  $M_w$  and M/G ratio. All of the samples exhibited a non-Newtonian shear-thinning behavior above a critical shear rate ( $\dot{\gamma}_{c2}$ ). The greater the  $M_w$ , the more sensitive the temperature-dependent behavior will be. The upward-downward rheograms showed that all of the systems evaluated in this study displayed a hysteresis loop, indicating a strong thixotropic behavior, and the thixotropic of mid- $M_w$  Ugi-Alg was the strongest. The dynamical viscoelastic properties were characterized by oscillatory frequency sweep, revealing the gel-like viscoelastic behavior of mid-low Ugi-Alg and the fluid-like viscoelastic behavior of high- $M_w$  Ugi-Alg.

© 2016 Published by Elsevier B.V.

## 1. Introduction

The design and fabrication of nano-scale biomaterials is important for applications in medicine. In particular, multifunctional organic nanoparticles have potential applications in medical diagnoses and therapies [1–3]. Self-assembly, phase separation, emulsification, and spray drying are typical methods for the fabrication of organic nanoparticles [4–6]. In the recent decades, self-assembled nanoparticles have been attracted much attention as potential delivery vehicles, because they enable the formation of nano-structures without the use of an excipient solvent or detergent. Moreover, their core-shell structures can readily encapsulate bioactive molecules, including anticancer drugs, into the core [7].

As materials science and technology develops, several types of amphiphilic polymers – such as star-, graft-, and block-type copolymers – have been designed and synthesized, leading to the production of self-assembled nanostructures with various sizes and shapes [8]. However,

While formation of self-assembled structures by various natural and synthetic polymers has been examined [5,18], few are promising for clinical application, mainly due to the toxicity of the polymers. Alginate, one of ubiquitous and relatively cheap goods, is biocompatible, biodegradable, lowly toxic, lowly immunogenic. Therefore, alginate has been extensively investigated and used in many biomedical applications [9–13]. Nevertheless, alginate does not form self-assembled structures unless hydrophobic moieties are introduced to the alginate backbone. Therefore, Ugi reaction, which was used given that the Ugi four-component condensation reaction is the most effective and unique method that can endow alginate with a specific property without the aid of a catalyst, and with inherent high atom economy and chemical yield [14]. The evaluation of alginate derivative synthesized via Ugi multicomponent reaction is still scarce.

Nano-scale drug delivery systems have undergone extensive development, and control of size and structure is critical for regulation of their biological responses and therapeutic efficacy. Self-assembled structures of alginate derivative, depends not only polymer concentration, temperature, and ionic strength, but also on the sequence and composition of the alginate chain and properties of hydrophobic chains. Lee et al., [15] and Soledad Lencina et al., [16] reported on the design and

\* Corresponding author.

E-mail addresses: 1479334044@qq.com (K. Chen), ljcfyh@263.net (J. Li), hn136.163@163.com (Y. Feng), 6583161@qq.com (F. He), 1203701089@qq.com (Q. Zhou), 1553137983@qq.com (D. Xiao), 1014601375@qq.com (Y. Tang).

fabrication of thermoresponsive polymeric (alginate–PNIPAAm) micelles from alginate conjugated with poly(*N*-isopropylacrylamide) (PNIPAAm); the self-aggregated structures of alginate–PNIPAAm were dependent on molecular weight (*M<sub>w</sub>*) of PNIPAAm, polymer concentration, temperature, and ionic strength. Yang et al., synthesized amphiphilic alginate esters (Alg–Cn) displaying DS and hydrophobic alkyl lengths, they illustrated that the value of sodium carboxymethyl cellulose (CMC) and the size of self-assembled micelle decreased with increasing hydrophobic alkyl chain length, provided that the DS of Alg–Cn is constant [17].

Being among the important parameters in alginate production, the rheological properties (including flow behavior, thixotropy and dynamical viscoelastic properties) of alginate have been studied extensively [18]. Nanoscale micelles or self-aggregates of amphiphilic molecule possess a series of specific rheological properties. Castrejón-González et al. [19] performed non-equilibrium molecular dynamics to determine the rheological behavior of micelles in two types of solvents. They found that data from flow curve were well fitted to a proposed model based on the Carreau–Yasuda equation and three regions in the flow curve were obtained: i) a Newtonian plateau, ii) a shear-thinning region and iii) a shear-thickening region. Nöbel et al. [20] validated the suitability of the application of Cole–Cole model in dairy products, including casein micelle suspensions, mixed casein micelle and glass microspheres suspensions and microgel suspensions (fresh cheese); this model was established for dairy products, and possible correlations with an altered microstructure were demonstrated. Zhang et al. [21] reported on the effect of shearing actions on the rheological properties and mesostructures on the aqueous solutions of polyvinyl pyrrolidone (PVP), carboxyl methyl cellulose (CMC), and CMC + PVP, which are simple water-based drilling fluids for gas hydrate drilling. The results show that three different types of network framework (namely thin films + thin rods, globular particles + thin rods, and thin films + thin slices) rendered various rheological behaviors to the aqueous solutions of polymers (CMC, PVP, and CMC + PVP).

In the present study, a series of hydrophobic alginates (Ugi–Alg) with various weight-average molecular mass (*M<sub>w</sub>* ~ 6.7 × 10<sup>5</sup>–6.7 × 10<sup>4</sup> g/mol) were synthesized via Ugi reaction. The structure of Ugi–Alg showing a degree of substitution were characterized by <sup>1</sup>HNMR spectrometer. Ugi–Alg nanoparticles were prepared via electrostatic self-assembly, and the M/G ratios (1.30–0.89), *M<sub>w</sub>* or addition of Na<sup>+</sup> (0.01–0.3 M), which all influence the size and structures of nanoparticles were further investigated via electron spin resonance (ESR) and dynamic light scattering (DLS). Morphology of the nanoparticles under different Na<sup>+</sup> concentrations were determined via transmission electron microscopy (TEM) experiments, respectively. To understand the rheological behavior of Ugi–Alg solutions, we tested the steady-shear flow, thixotropy and dynamical viscoelasticity by using a DHR-2 rheometer (TA Instruments). The results of the research could enrich and expand the applications of alginate derivatives in drug delivery (such as protein drug, anti-tumor drug and gene drug carriers) and drug controlled release.

## 2. Materials and methods

### 2.1. Materials

Samples of alginate, namely, a-Alg, b-Alg, and q-Alg, were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China), J & K Technology Co., Ltd. (Beijing, China), Qingdao Bright Moon Group Co., Ltd. (Shandong, China), respectively. J-Alg was degraded and purified from a-Alg. Their characteristics are summarized in Table 1. Formaldehyde, octylamine, hydrochloric acid, sodium hydroxide, ethanol, NaCl, and pyrene were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Cyclohexyl isocyanide was purchased from J & K Technology Co. Ltd. (Beijing, China). In addition, 5–doxyl stearic acid was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). These

**Table 1**

The G/M, DS and molecular weight of sodium alginates and their derivatives.

Sample	Mn(g/mol)	Mw(g/mol)	Mw/Mn	DS	G/M
a-Alg	513,428	752,943	1.47		1.74
a-Ugi-Alg	427,213	670,515	1.48	23.87%	0.92
b-Alg	181,642	273,645	1.51		1.32
b-Ugi-Alg	181,173	280,439	1.55	32.05%	1.30
q-Alg	197,322	307,236	1.56		1.02
q-Ugi-Alg	190,860	280,560	1.47	31.35%	0.89
J-Alg	62,838	72,557	1.15		3.73
J-Ugi-Alg	61,186	67,835	1.11	38.91%	3.56

chemicals were of analytical grade and used without further purification.

### 2.2. Synthesis and characterization of amphiphilic alginate derivatives

Four hydrophobic alginates (a-Ugi-Alg, b-Ugi-Alg, q-Ugi-Alg and J-Ugi-Alg) were synthesized based on previous studies [14,22–23]. First, an unmodified alginate was weighed (2.0 g; 2.5 wt%) and dissolved in 80 mL of water at room temperature; the solution was gently stirred overnight to achieve homogeneity. Second, the pH of the solution was adjusted to 3.6 with addition of 0.5 M HCl. Third, 0.05 g of formaldehyde, 0.17 g of octylamine, and 0.20 g of cyclohexyl isocyanide were successively added into the solution. The solution was subsequently stirred at room temperature for 24 h. Finally, the reaction mixture was diluted to 0.7 wt%, purified by dialyzing it against distilled water for 3 days, and then freeze-dried. The structure of the hydrophobic alginates was confirmed by <sup>1</sup>HNMR spectrometers. <sup>1</sup>HNMR was performed on a Bruker AV 400 nuclear magnetic resonance spectrometer. The samples were dissolved in D<sub>2</sub>O to a concentration of 10 mg/mL.

### 2.3. Gel permeation chromatography

Gel permeation chromatography was conducted by using a Waters 2695 high performance liquid chromatography system. The mobile phase used is pure water. The flow rate was controlled at 0.6 mL/min, and the temperature of the column was maintained at 45 °C.

### 2.4. Circular dichroism (CD) measurement

Solutions of NaAlg and its derivatives solutions (1.5 mg/mL) were prepared in deionized water. CD spectra were obtained at 25 °C by using a Jasco J810 spectropolarimeter. The wavelength-region between 180 and 270 nm was scanned [24].

$$\ln(P/T) = -0.0378 \times G\% + 1.1519 \quad (1)$$

where *P* is the peak height at 200 nm, *T* is the peak height at 212 nm, and *G*% represents β-D-guluronic acid percent content. Circular dichroism spectra of the alginate sample are seen in Fig. S1.

The values for the peak/trough ratio were calculated from the spectra; from these values the relative amounts of mannuronic and guluronic acid residues were calculated with reference to the calibration values [25].

### 2.5. Fluorescence measurement

Fluorescence was measured on a Hitachi F7000 fluorescence spectrophotometer by using pyrene as fluorescence probe. Such measurement is an efficient means to detect aggregation properties of the micelles via intra- or intermolecular associations of hydrophobically associating polymers [26]. The excitation wavelength was set to 335 nm, and the slit width was 2.5 nm. The fluorescence emission spectra were recorded at 335–600 nm. All tests were performed at 25 °C. Exactly 10 μL of 1.0 × 10<sup>−3</sup> M pyrene prepared in ethanol solvent was added

Download English Version:

<https://daneshyari.com/en/article/6481404>

Download Persian Version:

<https://daneshyari.com/article/6481404>

[Daneshyari.com](https://daneshyari.com)