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Insights into the rheological behaviors evolution of alginate dialdehyde crosslinked collagen solutions evaluated by numerical models

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ABSTRACT

The elaboration of the rheological behaviors of alginate dialdehyde (ADA) crosslinked collagen solutions, along with the quantitative analysis via numerical models contribute to the controllable design of ADA crosslinked solution system's fluid mechanics performance during manufacturing process for collagen biomaterials. In the present work, steady shear flow, dynamical viscoelasticity, creep-recovery, thixotropy tests were performed to characterize the rheological behaviors of the collagen solutions incorporating of ADA from the different aspects and fitted with corresponding numerical models. It was found that pseudoplastic properties of all samples enhanced with increasing amounts of ADA, which was confirmed by the parameters calculated from the Ostwald-de Waele model, Carreau and Cross model, for instance, the non-Newtonian index (n) decreased from 0.786 to 0.201 and a great increase by 280 times in value of viscosity index (K) was obtained from Ostwald-de Waele model. The forth-mode Leonov model was selected to fit all dynamic modulus-frequency curves due to its higher fitting precision ($R^2 > 0.99$). It could be found that the values of correlation shear viscosity (η_k) increased and the values of relaxation time (θ_k) decreased with increasing ADA at the fixed k value, suggesting that the incorporation of ADA accelerated the transformation of the collagen solutions from liquid-like to gel-like state due to more formation of C═N linkages between aldehyde groups and lysine residues. Also, the curves of creep and recovery phase of the native and crosslinked collagen solutions were simulated well using Burger model and a semi-empirical model, respectively. The ability to resist to deformation and elasticity strengthened for the samples with higher amounts of ADA, accompanied with the important fact that compliance value (J_{50}) decreased from 56.317 Pa^{-1} to 2.135 Pa^{-1} and the recovery percentage (R_{creep}) increased from 2.651% to 28.217%. Finally, it was found that the area of thixotropic loop increased from 8.942 Pa/s to 17.823 Pa/s with increasing introduction of ADA, suggesting that stronger thixotropic behavior was associated with higher amount of ADA. Furthermore, Herschel–Bulkley model was employed to describe the up and down curves of all samples and it was confirmed that all collagen solutions belonged to pseudoplastic fluid (the flow index < 1) without apparent yield stress and shear-thinning behaviors were more obvious with increasing additions of ADA according to the increasing consistency coefficient K values. Overall, this work contributed a new insight into the interactions between collagen and ADA based on quantitative rheological methods reflecting the different rheological properties and the results obtained should be of great utility in the extensive application of ADA crosslinked collagen solutions into diverse collagen-based materials.

1. Introduction

Collagen, the chief component of connective tissues, has been widely investigated in the fields of medicine, food, cosmetics and tissue engineering due to its high biocompatibility, controlled biodegradability and low antigenicity [1–3]. However, some demerits still cannot be neglected, especially, lower mechanical strength, thermo stability and vulnerability to enzymatic degradation [4]. Therefore, the use of collagen as biomaterials requires the introduction of exogenous cross-linking bonds to satisfy clinical requirements in profiles of both the desired physicochemical performance and the acceptable biocompatibility [5]. Generally, various synthetic cross-linkers, such as aldehydes (e.g., glutaraldehyde [GTA]), isocyanates (e.g., hexamethylene diisocyanate [HMDI]), have been applied to modify collagen-based biomaterials cautiously due to potential cytotoxic effects [6,7]. Recently, more emphasis is further to be placed upon naturally occurring cross-linkers out of consideration for their desired biocompatibility [8–10].

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Alginate (ALG), derived from various algae and bacterial sources, is a block copolymer composed of two different repeating units, (1, 4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers with varying proportions [11,12]. ALG has gained great attentions in a wide range of applications due to good gelation properties and structural resemblance with the extracellular matrices of tissues [13–15]. However, some disadvantages have limited its applications, for instance, poor protein adsorption ascribed to excessive hydrophilicity, overquick gelation leading to hard mixing of blended solutions, non-biodegradability corresponding to high-molecular weight and poor cellular attachment due to free of cell-adhesive groups [16]. Whereas, alginate dialdehyde (ADA) formed by selective oxidation of ALG can address many of the above mentioned issues, including delayed gelation [17], controlled biodegradability [18] and improved ability of protein adsorption compared to ALG. Additionally, injectable scaffolds derived from ADA with a high degree of oxidation demonstrate slower drug release rates. Specifically and importantly, C2-C3 positions of glucose residues of ALG are oxidized into two aldehyde groups, and resultant dialdehyde derivative can dissolve in aqueous solution and react with free amino groups of other polymers by formation of C═N linkages (Schiff's base), which provides the feasibility as a kind of naturally derived crosslinking agent [19]. Besides, ADA has been reported to be a viable and promising candidate because of its relatively facile availability and lower cost, compared with other biocompatible crosslinkers [20,21]. However, the investigations on ADA modification mainly focus on the improvements of physicochemical and biological performance of modified substrate [22–24], fewer works have paid attention to the rheological behaviors of collagen solutions cross-linked by ADA systematically and comprehensively. As well known, many information, such as design of flow processes, quality control and processing stability of collagen-based products could be obtained via rheological measurements. For example, steady shear test can imply that flow properties of samples in terms of their spreadability and firmness properties. Dynamic frequency sweeps are applied to investigate the entanglement network systems, a weak or strong gel property by analyzing data on the structure and energy reserve (G′) and consumption (G″). While, the ability of the resistance to deformation and the elastic response can be obtained by creep and recovery measurement, and thixotropy measurements can reveal the gradual breakdown and build-up of the microstructure with change of shear rate. Furthermore, a combined experimental and mathematical models framework can provide more detailed descriptions and effective guidelines for the practical industrial process. More importantly, most collagen-based manufactures are mainly based on liquid aqueous forms either used directly as medial injection or transferred into solid implants such as powders, sheets, fibers, sponges and hydrogels for tissue engineering, antibiotic wound dressings or drug deliver [25–27]. Hence, it is necessary to evaluate the rheological behaviors of ADA-crosslinked collagen solutions, which are of importance in their design and controlled quality in subsequent production process.

Hence, this study aims to evaluate the efficiency of ADA for crosslinking collagen solutions in profiles of rheological properties. Consequently, collagen solutions crosslinked by various amounts of ADA were prepared and comprehensive rheological behaviors (steady shear measurement, dynamical frequency sweep, creep-recovery measurement and thixotropy) were analyzed using rheometer in the present work. Furthermore, corresponding mathematical models were also employed to simulate and quantitatively analyze the experimental data, which could provide useful guidelines for better understanding the collagen gel formation mechanism induced by ADA and better directing the design of controlled-stable collagen-based products.

2. Materials and methods

2.1. Materials

The pepsin-soluble collagen used in this study was self-prepared from skin of the fresh grass carp (*Ctenopharyngodon idella*) according to our previous report [28]. The alginate dialdehyde (ADA) (the value of oxidation degree is 47.96 ± 2.67% approximately) was prepared according to the previous method [29]. Other reagents were used as received.

2.2. Preparation of the crosslinked collagen solutions

The crosslinked collagen solutions were prepared according to the previous method with slight modification [30]. Briefly, lyophilized collagen and ADA were dissolved in 0.5 mol/L acetic acid solution, respectively. And the pH values were adjusted to about 4.0 by 2 M NaOH to obtain the 5 mg/mL collagen solution and 1% ADA solution, respectively. Then, 1% ADA solution was added dropwise to collagen solutions, and the final ADA/collagen (w/w) ratios were 0:1, 0.01:1, 0.03:1, 0.06:1, 0.1:1 and 0.15:1, named as COL (pristine collagen), AC1, AC2, AC3, AC4 and AC5, respectively. All solutions were magnetically stirred for 24 h at 4 °C. The resultant collagen solutions were centrifuged at 8000 ×*g* for 10 min to remove entrapped air-bubbles, and then stored at 4 °C before the rheological characterization.

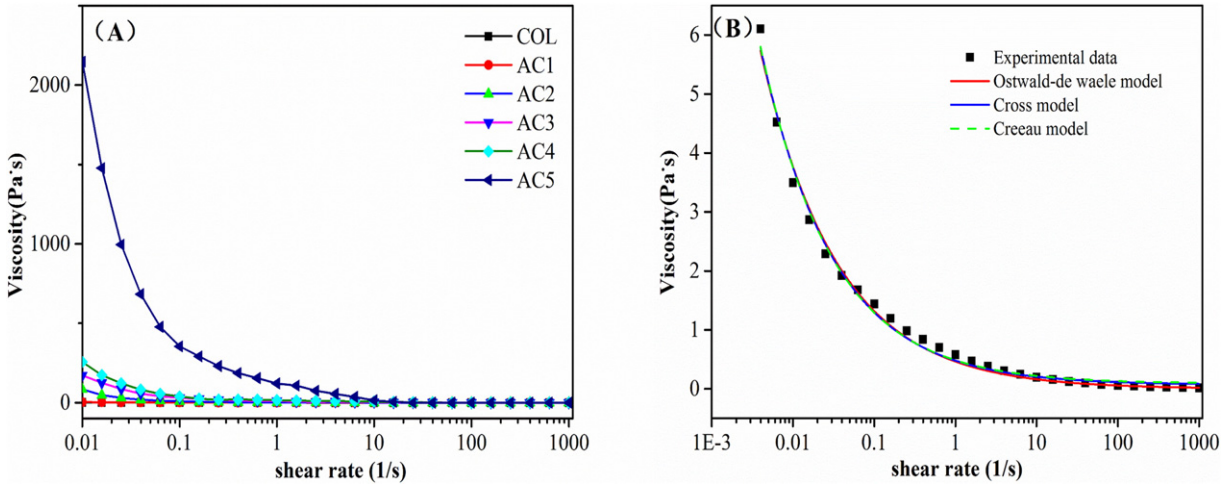

Fig. 1. (A) Flow curves of all samples with various ADA concentrations. (B) Experimental data for sample AC1 (ADA/COL = 1%) and corresponding fitted curves using Ostwald-de Waele, Carreau and Cross model, respectively.