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Glycerol-induced Modulation on the Hydration Dynamics and Rheological

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Properties of Collagen

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Abstract

Collagen is regarded as important biomaterial in biomedical and cosmetic applications due to its biocompatibility and biodegradability. Glycerol, a polar green solvent, enhances collagen stability by substituting water molecules in the hydration shell, maintaining its triple helical structure through hydrogen bonds. Understanding glycerol’s effects on collagen’s hydration dynamics is essential for optimizing concentrations for specific uses. NMR spectroscopy analyses show that adding glycerol increases T2 relaxation times in collagen, leading to a looser matrix. This research connects hydration dynamics to rheological properties, demonstrating that glycerol improves collagen’s pliability, lowers the elastic modulus, and enhances thermal stability, which is crucial for collagen-based cosmetics and biomedical applications.

Keywords: Collagen, Glycerol. T2 relaxation time. Rheology.

## 1. Introduction

Collagen is a vital protein that maintains the strength and integrity of connective tissues such as skin and cartilage. Its unique triple-helical structure, rich in glycine, proline, and hydroxyproline, is highly sensitive to hydration. Research by Bella et al. using X-ray crystallography

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revealed that water stabilizes collagen’s structure through hydrogen bonds and hydroxyproline-mediated bridges1. Glycerol, a well-known cosolvent, acts as a humectant and stabilizes collagen matrices by replacing water in the hydrogen-bonded network, as shown by Penkova et al2. It forms glycerol bridges between hydroxyproline residues and inhibits collagen type I fibrillogenesis, impacting collagen’s molecular aggregation. Despite glycerol’s influence on hydration dynamics in proteins, research on collagen hydration specifically is limited. Understanding collagen’s rheological characteristics is crucial for its cosmetic and biomedical applications. Glycerol alters the hydration dynamics in collagen matrices, affecting local hydrogen-bonding networks and mechanical properties. Our research focuses on the interplay between glycerol and collagen hydration dynamics and rheological performance. In this study, we use Nuclear Magnetic Resonance (NMR) spectroscopy to study the changes in hydration dynamics of collagen upon glycerol interaction. Later a rheometer was used to investigate the mechanical properties and elastic modulus of the collagen-glycerol system. This study helps to correlate changes in hydration dynamics which is a molecular level changes to macroscopic properties like rheology.

## 2. Methodology:

### 2.1. Preparation of Collagen-Glycerol Samples:

Collagen-glycerol composites were created by dissolving collagen in 100 mM acetic acid and adding glycerol at

concentrations of 1%, 5%, 10%, 15%, and 20%. The solution was stirred for 3 hours at 4 °C and incubated for another hour. Collagen concentration was set at 0.6 µM for solution studies.

### 2.2. NMR Measurements:

NMR studies of RTT fibers were performed using a Bruker Avance-III HD 400WB spectrometer to determine transverse relaxation times (T2) of collagen, assessing water mobility via the Carr–Purcell– Meiboom–Gill pulse sequence.

2.3. Rheological Evaluation:

Rheological properties of collagen-glycerol composites were tested using a Kinexus Pro+ Rotational Rheometer, maintaining constant temperature and applying various strain and frequency sweeps to determine viscoelastic behaviour.

3. Results and discussions

3.1. Analysis of Hydration Dynamics Using NMR

To investigate the molecular interactions and hydration dynamics of native RTT and RTT in glycerol, NMR relaxation measurements were performed. The transverse relaxation time (T2) reveals the mobility of water molecules within the collagen matrix, informing hydration behavior. T2 NMR relaxation times of water for native RTT and with varying glycerol percentages (1% - 20%) is given in Table 1. The T2 components correspond to bound, weakly bound, and free water, respectively3. The T2 short component, indicating bound water mobility, increased significantly with higher glycerol concentrations. This implies glycerol enhances water mobility, critical for collagen's structural integrity. However, structural integrity is only maintained up to 10% glycerol; beyond this, a gradual loss occurs.

Fig.1. Continuous fit of T2 relaxation time decays of

native RTT and glycerol-treated RTT's is given.

3.2. Analysis of Rheological Properties:

Rheological tests aimed to examine mechanical and viscoelastic changes in native collagen with addition of small molecule5. The viscometry test (0.1 to 1000 s-1) revealed shear-thinning behaviour, where viscosity decreases as shear rate increased (Fig.2). The frequency sweep test evaluated viscoelastic behaviour over 0.1 to 10 Hz at a constant strain of 0.5% and temperature of

Fig.2. Shear viscosity of native collagen (C) and collagen-

glycerol composites (CG1%, CG 5%, CG 10%, CG 15%

and CG 20%) at a shear rate of 1 s-1 is given.

Fig.3. Elastic modulus (G') of native collagen (C) and different collagen-glycerol composites (CG 1%, CG 5%, CG 10%, CG 15% and CG 20%) at a frequency of 1 Hz.

Fig.4. Denaturation temperature (Tdd) of native collagen (C) and different collagen-glycerol composites

(CG 1%, CG 5%, CG 10%, CG 15% and CG 20%)

from temperature sweep data.

25 °C (Fig.3). Results indicated that the elastic modulus (G') was consistently higher than the loss modulus (G") across all compositions, reflecting a predominantly elastic nature. CG 5% and CG 10% exhibited comparable storage and loss moduli to native collagen, indicating stability and flexibility for solution-based applications. A temperature ramp (25 °C to 50 °C) assessed thermal stability, with η* declining after 37 °C, suggesting collagen denaturation. The presence of glycerol raised the breakdown

temperature (Tdd) slightly compared to native collagen. The Tdd increases with higher glycerol percentages as given in Fig.4. obtained from tan δ versus temperature plot. These trends indicate that higher glycerol concentrations can weaken the composite's recovery of properties after breakdown, influenced by changes in the microenvironment.

4. Conclusion

This study demonstrates that glycerol significantly influences collagen's molecular stability, hydration dynamics, and mechanical properties. It stabilizes the tropocollagen structure and affects hydration at the microscopic level, which impacts macroscopic properties like rheology. Glycerol enhances flexibility by influencing bound water, as shown through NMR relaxation times. Additionally, glycerol preserves viscoelastic properties, softening collagen by reducing the elastic modulus and enhancing thermal stability due to hydrogen bonding. Optimizing collagen-glycerol composites can improve their applications in cosmetics and biomedicine.

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