Experimental Report

Proposal: **9-11-1654** **Council:** 10/2012

Title: Chitosan/gelatin enzymatically cross-linked hydrogels: Composition and temperature effects on the gel'smolecular structure.

This proposal is a new proposal

Researh Area: Materials

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Samples: Tilapia fish gelatin
Chitosan
microbial transglutaminase

Instrument	Req. Days	All. Days	From	To
D11	2	2	04/03/2013	06/03/2013

Abstract:

Hydrogels from biopolymers have been attracting increasing interest in biomedicine, but the lack of structural understanding hinders the development of new applications. Our work focuses on hydrogel scaffolds obtained from the blend of tilapia (fish) gelatin and chitosan. The hydrogels are cross-linked by the microbial enzyme transglutaminase. Two types of gelation will be studied: (a) Chemical gelation, enzymatic reaction (b)Physical-co-chemical gelation, enzymatic reaction done in presence of the gelatin physical network. As hydrogel is more than the added properties of its components and it also requires a particular set of mechanical properties, which are related to its structural organization at the nanoscopic level, we propose to use SANS to achieve an understanding of the structure of the networks at the nano-scale level, both after and during the gelation process. These experiments will give us precious insight into hydrogels architecture and properties allowing us to better correlate bulk and nano-properties in order to allow a better fine tuning of the final hydrogels.

Introduction

Hydrogels obtained from chemical or physical association of macromolecules are an important subject of materials science, due the ample array of design possibilities available and countless biomedical applications, for instance as skin substitutes, adhesives, drug delivery matrices. Biopolymers, inherently more suited for biomedical applications than their synthetic counterparts, have become a great focus of interest. Yet, due their natural complexity, their properties are still poorly understood. The applicability of these hydrogels for tissue engineering requires a specific set of properties. Amongst others, they should mimic the properties of the extracellular matrix they are supposed to replace, stimulating cell adhesion, growth and contact, and allowing diffusion of nutrients and metabolites. Previously in our group, extensive studies were conducted with gelatin,[1-3] which provides some of those desired properties, but not all. Blending gelatin with others biopolymers offers the possibility to explore synergistic effects and achieving the missing properties. Chitosan fulfils some of those requirements while adding other useful properties. For those reasons, mixtures of tilapia gelatin and chitosan were chosen as object of this study. As commonly used chemical crosslinkers are generally toxic, reactive and not easily quenched, we employ the microbial enzyme transglutaminase as cross-linker.[1-3]

However, the suitability of a hydrogel is more than the added properties of its components and it also requires a particular set of mechanical properties, which are related to its structural organization at the nanoscopic level. Thus, a fine control of the hydrogels nanostructure is required for its successful use.

Experiments and discussion

In this work, we investigated chitosan/tilapia fish gelatin gels cross-linked by the microbial enzyme transglutaminase (mTGase). Gelatin itself undergoes a thermally-reversible sol-gel transition. This phenomenon allows us to conduct the enzymatic cross-linking in two different micro-environments. At temperatures above gelatin melting temperature, both macromolecules are in the sol state. Instead, below gelatin melting temperature, gelatin is in the gel state while chitosan is likely to be dispersed within the physical network. The physical gelation is a faster process than chemical gelation, thus the cross-links will be formed in a constrained environment.[1-3]

The SANS study covered both structural and kinetics investigations. The effects of concentration of mTGase, gelation time and presence of a gelatin's physical network were evaluated.

The power law and Lorentzian equation[4,5] has been used to describe scattering from cross-linked gels in the dilute regime and provided good results for pure gelatin gels.[3]

$$I_l(q) = \frac{A}{q^m} + \frac{I_l(0)}{1+(q\xi)^n} + BKG \qquad \text{Equation 1}$$

The first term describes Porod scattering from clusters (exponent = *m*) in the low-*q* range and the second term characterizes the polymer chains behaviour detected in the high-*q* region. The exponent n relates to the chain thermodynamics. *A* and $I_l(0)$ are constants and ξ is the correlation length, which describe the size of the scattering centres. The analysis will be focused on n and ξ. For this analysis, the important values of n are for a highly swollen chain in a good solvent (chain with excluded volume): *n*=1.66 and for randomly branched Gaussian chains: *n*=2.28.

Both *n* and ξ values increase with time (Fig.1a) and enzyme concentration (Fig.1b) above 10 $U/g_{gelatin}$ of enzyme. At 40 U (highest concentration assayed), we obtained values ranging from *n* = 1.6 for short times (30 min) to 2.4 (24 hours) and ξ changes from 75 to 300 Å. This suggests a change from free swollen chains to restricted branched chains, as one could expect as the cross-linking progresses. After 24 hours, 20 and 30 U systems also reach ξ of 300 Å, but *n* is limited by enzyme concentration, reaching values of *n*=1.9 and 2.0 for 20 and 30 U, respectively, suggesting a different extension of cross-linking as a function of enzyme concentration. At the lowest concentration (10 U) *n* is rather time insensitive, ranging between 1.6 to 1.7. This suggests that at this concentration, the enzyme is not capable to produce extensive cross-linking. ξ ranges from 56 (30 min.) to 100 Å (24 hours) confirming the limited effect of the enzyme.

For gelation conducted below gelatin's gelation temperature, *n* showed little variation with enzyme concentration: $1.6 < n < 1.9$ after 24 hours (Fig.1c – 1st step). ξ also was relatively constant with values around 60 Å. This shows the ability of the gelatin physical network to constrict enzymatic cross-linking. Measuring the same samples at 37°C (Fig.1c – 2nd step), thus removing the physical network, no significant changes were observed for *n*, however, ξ showed large increase, reaching values of hundreds of angstroms. This could mean an agglutination of the scattering clusters, as they are no longer obstructed by gelatin's physical

network. Lowering the temperature to 21°C allows the regrowth of the physical network (Fig.1c – 3rd step). *n* values are still constant and the ξ shows a small decrease but still within 100s of angstroms. It is worth mentioning that SANS scattering patterns shows no measurable differences between chitosan/gelatin physical gels and solutions. Thus, the differences observed must come from changes in the chemical network.

When comparing chitosan/gelatin gels with pure gelatin gels across the different gels type (Fig.1d), it was observed that chitosan/gelatin chemical gels show much larger ξ while physical-co-chemical gels ξ values falls within similar range, reinforcing the pattern of the physical networking restricting the cross-linking process.

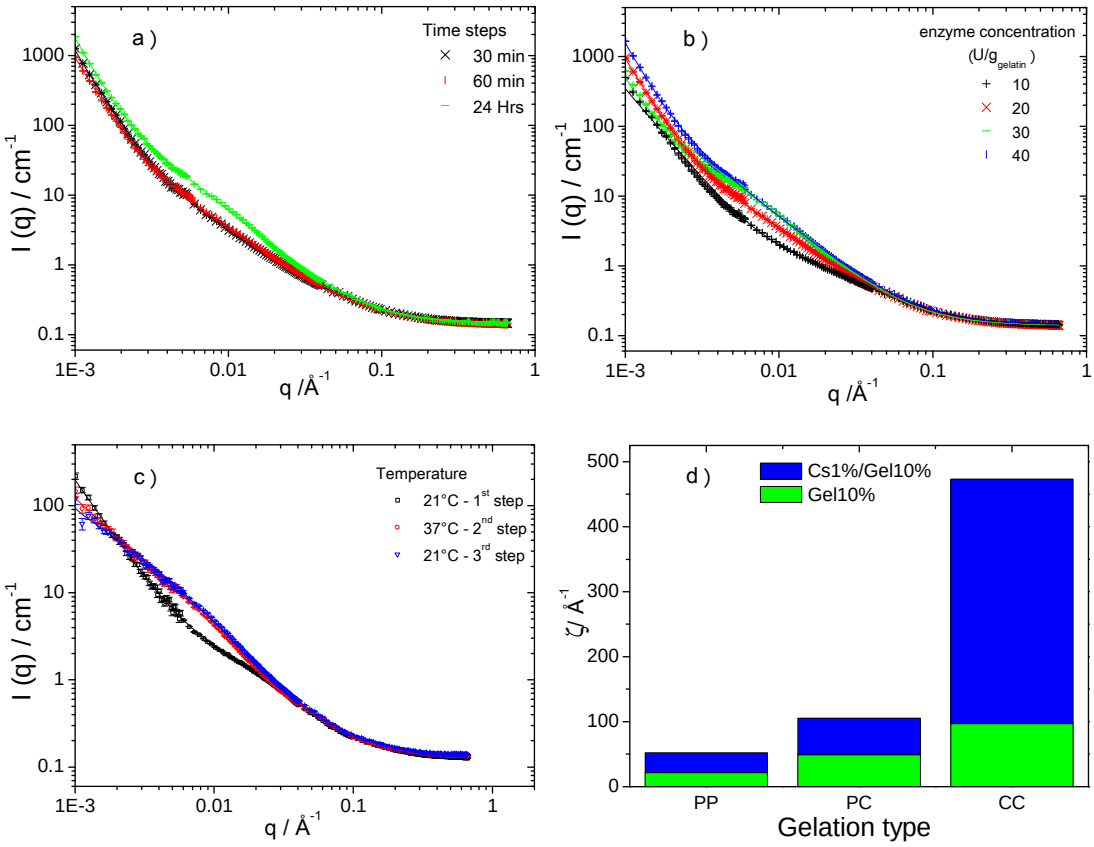

Fig. 1 – Neutron scattering patterns for enzymatic cross-linked chitosan/gelatin hydrogels : [a] as a function of time at 20 U mTGase/$g_{gelatin}$; [b] as a function of mTGase concentration after 24 hours of gelation; [c] in the presence and absence of gelatin physical network. [d] Correlation length for chitosan/gelatin and gelatin gels for different types of gels: PP=physical, PC=physical-co-chemical and CC chemical gels.

References

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