



Optimizing the formulation of oil-loaded thermosensitive Chitosan-based hydrogels by $^1\text{H-NMR}$ Relaxometry

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Introduction

Hydrogels represent an ideal class of polymeric material for various biomedical applications, including drug delivery, cell encapsulation and tissue engineering [1]. Thermosensitive hydrogels can be used as in situ forming implants. These biodegradable delivery systems are generally liquid formulations that form a semi-solid or solid depot after injection into the desired tissue or organ (Figure 1). General advantages are localized or systemic prolonged drug delivery periods, drug dosage reduction along with reduction of undesirable side effects and reduced frequency of application. Besides these advantages the application of in situ forming implants is less invasive and painful compared to common implants leading to an improved patient compliance and comfort. Chitosan is an amino-polysaccharide obtained by alkaline deacetylation of chitin (exoskeleton of shrimps or crabs), with various biomedical applications [2]. Chitosan is soluble in acidic solution and phase separates at $\text{pH} > 6$ to form a hydrogel. The addition of β -glycerol-phosphate (β -GP) to acidic chitosan solutions allows rising the pH to neutral without phase separation. These systems are thermosensitive and form hydrogels at temperature of 37°C and below [3]. The sol-gel transition of these chitosan- β -GP systems has been studied mainly by rheology [4]. To make these drug delivery systems accessible for poorly water soluble drugs, we developed an oil loaded chitosan hydrogel system. The aim of the present study was to optimize the composition of the gel to achieve sol-gel transition near body temperature and high oil content. To resolve the phase transition behaviour $^1\text{H-NMR}$ relaxometry was used as an alternative method. Rheology methods widely used to characterize hydrogels are often time consuming, risking dehydration of the gels and report only on macroviscosity. Phase transitions can be observed due to the dependence of the dynamic of molecular groups to temperature. The NMR T_2 relaxation time correlates to the dynamic viscosity as shown for Newtonian fluids [5] and water mixtures of microcrystalline cellulose [6].

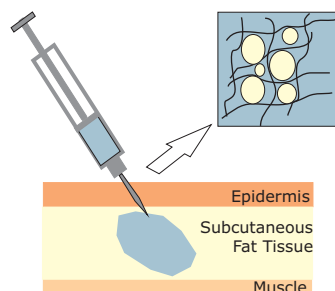


Figure 1: Concept of in situ gelling implants
Insert - theoretical structure of oil-loaded chitosan hydrogels

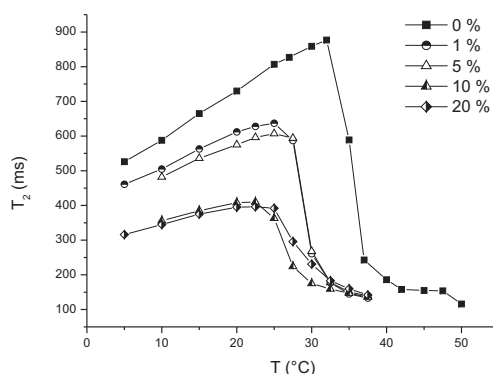


Figure 2: Temperature dependence of T_2 relaxation for chitosan gels with different oil content

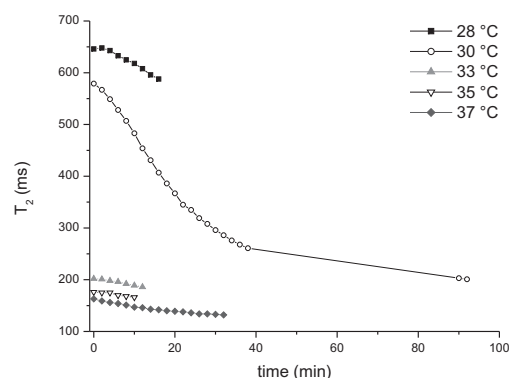


Figure 3: Time dependence of the relaxation time T_2 for chitosan gel loaded with 5% oil

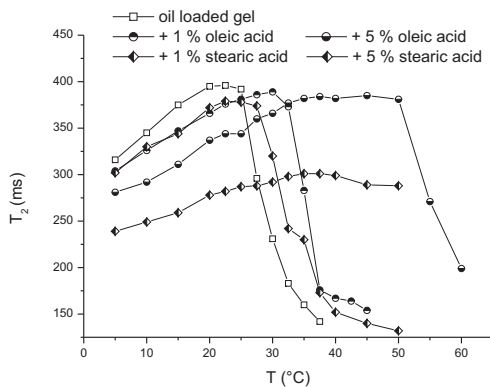


Figure 4: Influence of the addition of fatty acids on the temperature dependence of T_2 relaxation for chitosan gels (20% oil)

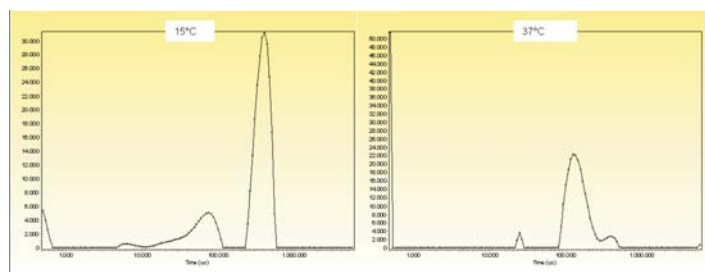


Figure 5: Distribution of the relaxation time T_2 before gelation at 15°C (left) and after gelation at 37°C (right) for chitosan gel loaded with 20% oil

Results

The sol-gel phase transition of the gels had a strong effect on T_2 , clearly visible by a sharp decrease in Figure 2. The NMR data showed that the sol-gel transition and the viscosity of the solutions were strongly influenced by the proportion of olive oil in the gel. Higher oil concentrations lead to onsets of gelation at lower temperatures and to higher initial dynamic viscosities (lower T_2 times). The NMR relaxometry also allowed the observation of gelation viscosity (Figure 3) and revealed that gelation is a time-consuming process. Once gelled they do not reliquify, even when cooled down and stored at 4°C . Further addition of fatty acids to the oil-loaded gels increased the sol-gel transition to higher temperatures (Figure 4). The compositions of the gels directly influenced their initial viscosities and the thermal behaviour. The water compartment of this type of gels behaves very uniform. After gelation the strong free water distribution peak is shifted from about 800 ms to about 100 ms (Figure 5). The mobility of the oil did not change during gelation with T_2 of about 100 ms. The wide distribution of T_2 is due to the non-conformous composition of olive oil. The inclusion of almost the whole water compartment at the gel-formation suggests that hydrogen bondings play a dominant role during sol-gel transition.

In conclusion NMR relaxometry is a very useful, fast and non-invasive method for determining the sol-gel phase transition of thermosensitive hydrogels and the gelation processes. Additionally it gives unique information of the status of water within the gels.

Experimental methods

Sample preparation

Chitosan (Chitoclear[®]FG95, Primex, Iceland) and olive oil were dissolved in 0.1 M hydrochloric acid. To the cooled chitosan-oil emulsion, chilled aqueous β -glycerolphosphate (β -GP) was added. The formulations contained 2.5% (m/V) chitosan and 16% (m/V) β -GP, loaded with 1 to 20% (m/V) olive oil. In further experiments 1 to 5% (m/V) stearic acid or oleic acid was added.

Viscosity determination by NMR-relaxometry

A 20 MHz NMR benchtop system Maran DRX2, Oxford Instruments Molecular Bio Tools UK, was used. The instrument is equipped with 18 mm sample access, variable temperature control and imaging unit. NMR relaxometry was performed in the temperature range of 5 to 50°C . The CPMG sequence was used for T_2 relaxation analysis. The recorded T_2 -NMR relaxation curves were fitted by a one-exponential decay.

References

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