RHEOLOGICAL ASPECTS OF THE FLOW OF THERMOSENSITIVE CHITOSAN SYSTEMS DURING INJECTION APPLICATION

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Abstract

The aim of this paper was to conduct preliminary instrumental tests to determine the possibility of injection applications for thermosensitive chitosan systems, including injection needles and application conditions. Among the many biomedical and pharmaceutical applications of chitosan, the minimally invasive thermosensitive scaffolds that form in vivo are an interesting solution. Despite many studies on colloidal chitosan systems undergoing sol-gel phase transition, almost no studies have examined their injectability. It has been stated that the use of acetic acid as a solvent reduces the forces needed for injection. Moreover, the key impact of injection temperature was determined. Storing the medium at room temperature before the injectability test led to a decrease in the value of forces needed for injection. The obtained results are discussed based on the change of the rheological properties of the chitosan hydrogels.

Keywords: *chitosan scaffolds, thermosensitive hydrogels, injectability*

Received: 15.03.2020
Accepted: 20.05.2020

1. Introduction

For many years, studies on the use of chitosan and its derivatives in biomedical and pharmaceutical applications have been described in the literature [1–5]. This is primarily due to its biological properties such as biocompatibility, biodegradability and nontoxicity [3, 5, 6]. Another very important aspect is the availability of this material at a much lower price compared with, and a similar structure to, hyaluronic acid. Potential applications of chitosan and its derivatives in medicine and regeneration include cellular scaffolds [1, 4, 7], controlled drug release systems [1, 2, 8, 9] and matrices produced by three-dimensional (3D) printing [10–13]. However, due to the growing interest in innovative treatment and regeneration methods, thermosensitive polymer systems [14–16] characterized by a lower critical solution temperature (LCST) [17] seem to be an interesting solution. Such systems exhibit the ability to undergo sol-gel phase transition due to a temperature increase. This phenomenon is most often used to design injectable cell scaffolds [18–22] in the form of a liquid, low-viscous sol, forming a spatial structure *in vivo*. The advantages of the injectable scaffolds are their reduced invasiveness compared with implantable ones and better filling of the existing irregularly shaped defects. Even though thermosensitive chitosan systems are often studied in many respects, their application potential in the form of low-invasive injections has been determined only based on LCST values. Detailed studies on this subject have not been conducted, and conclusions about their use as injectable cell scaffolds have been formulated only on their thermosensitivity and nonrepetitive, manual injection tests performed by the operator during preparation of the experimental medium. Instrumental tests have been rare and limited to a few papers discussing the effects of molecular weight [23] and hybrid chitosan/calcium phosphate ceramics [22, 24]. This small number of available studies compared with all studies conducted on thermosensitive chitosan hydrogels is very unexpected.

The injection application for many researchers is limited by the challenges associated with a patient's pain sensations and possible tissue damage at the injection site. Consequently, it is recommended to use the smallest needles. It should be noted that their use may cause difficult application or even prevent it. Properties such as pH, tonicity, osmolarity, drug concentration and excipients are considered when preparing the medicament for injection. The volume of the medium, viscosity, particle size and dosage form are also important [25, 26]. From a rheological point of view, injection application is the flow of medium through the capillary [27], which will be affected by factors such as syringe diameter and length, piston friction, injection speed, but above all needle diameter and length, as well as rheological properties of the injected medium. All these parameters will undoubtedly affect the application process and properties of the medium after injection. This issue is crucial in the case of polymer systems, which after injection must form a temporary scaffold.

The purpose of this paper was to conduct preliminary instrumental tests to determine the possibility of injection applications for thermosensitive chitosan systems, including injection needles and application conditions.

2. Materials and Methods

2.1. Materials

As an experimental material, chitosan from crab shells (degree of deacetylation: 81.8%; molecular weight: 680 kDa; CAS 9012-76-4) and disodium β-glycerophosphate (CAS 13408-09-8) were purchased from Sigma-Aldrich and used without further treatment. The solvents were 0. 1 M hydrochloric acid (CAS 7647-01-0) and 0.1 M acetic acid (CAS 64-19-7).

DOI: 10.15259/PCACD.25.016

Thermosensitive chitosan hydrogels, which underwent sol-gel phase transition under physiological conditions, were prepared in accordance with the methodology described in detail in the literature [28]. Four-hundred milligrams of crab-derived chitosan was clearly dissolved in 16 mL of 0.1 M hydrochloric acid or 20 mL of 0.1 M acetic acid. After 24-h storage at room temperature, the sample was cooled for 2 h at 4°C. Next, a suspension of disodium β-glycerophosphate (2 g of powder distributed in 2 mL of distilled water) was added dropwise to the colloidal chitosan solution.

2.2. Methods

Instrumental injectability tests were conducted using a Brookfield CT3-4500 texture analyser. In the measurements, the force needed to inject the test medium as a function of the distance travelled by the analyser cell was recorded. During the tests, 2-mL disposable syringes (Braun Injekt) filled with 0.5 mL of experimental medium were used. Moreover, injection needles (Zarys, dispoFINE) in several sizes – 16G (outer diameter 1.6 mm), 19G (1.1 mm) and 21G (0.8 mm) – with a 40 mm length were used. Control measurements were performed without the needle. The injectability process was carried out into the air. The measurements were carried out at 5°C (sample immediately measured out of the fridge) and 20°C (sample thermostated at the set temperature for about 2 h before measurement). For each sample, 3 to 5 replicates were performed, and the error did not exceed 4.5%.

Based on the obtained experimental curves, the characteristic parameters of the injection process such as initial glide force (IGF), i.e. force necessary to initiate the piston movement, dynamic glide force (DGF), i.e. force require to maintain the piston movement, and the maximum force value F_{max} recorded during the injection [29] were determined. The value of the total work done necessary to inject a specific volume of the experimental medium was also determined [30].

The influence of the solvent used and the application temperature on the change of rheological properties of research media was determined based on the oscillatory measurements in the frequency sweep test [31] using an Anton Paar Physica MCR 301 rotational rheometer equipped with a cone-plate measurement system (50 mm diameter, 1° cone angle and 48 μm cone truncation). The tests were performed at 5, 20 and 37°C.

3. Results and Discussion

Raw measurements data determining the forces as a function of distance traveled are presented in Fig. 1. The obtained experimental curves show a different course from those most frequently observed in the literature [30]. The experimental curves are similar to those obtained for high viscosity lipid systems [25]. In this case, the high viscosity of the systems determines the shape of the curve and the injection process. The characteristic first peak followed by a sharp decrease in recorded forces was not observed. This means that the value of the force necessary to initiate the piston movement is identical to the force necessary to maintain the piston movement during the injection process. In this case, it is crucial to overcome the resistance of the fluid flowing through the needle during injection. It is interesting that the use of the largest needle (16G) almost did not affect the increase in the value of forces needed for injection compared with the control measurement (without the needle).

Fig. 2 shows the determined values of dynamic force necessary to maintain the piston movement (DGF), which corresponds to the IGF. These data clearly revealed that the use of a smaller diameter needle requires a greater force for injection. Therefore, the selection of the optimal injection needle should not be limited to the smallest diameter needle to reduce patient pain because, as anticipated, its use may hinder application by the person giving the injection. In the case of control measurements at 5°C (Fig. 2A) and 20°C

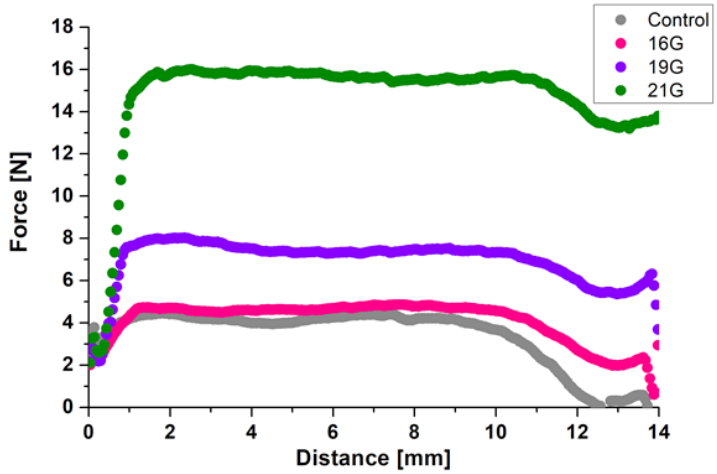

Figure 1. Force-distance curves for the chitosan/hydrochloric acid system at 20°C obtained for the ● control test (without needle) and ● 16G, ● 19G and ● 21G measurements.

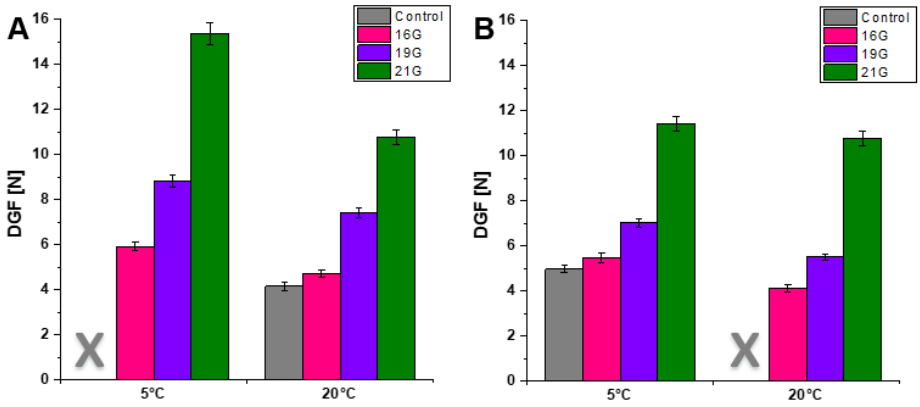

Figure 2. Dynamic glide force values determined at 5 and 20°C for chitosan hydrogels prepared with (A) hydrochloric acid or (B) acetic acid based on the ● control test (without needle) and ● 16G, ● 19G and ● 21G measurements.

(Fig. 2B), it was not possible to determine the DGF values (marked by 'x') due to the continuous decrease in the force value during the test.

The obtained experimental data also revealed the influence of the solvent used on the injectability process of thermosensitive chitosan hydrogels. Regardless of the needle, lower force values were observed for systems obtained by dissolving the polysaccharide in acetic acid. The smaller the diameter of the needle, the greater these differences were.

The measurements also determined the effect of temperature on the injection application. Regardless of the injection needle and solvent, heating the experimental medium for 2 h at room temperature led to a decrease in the DGF. Although these results may be questionable, due to the phenomenon of thermo-induced phase transition, they are fully correct. During the formation of the spatial structure under the influence of a temperature

DOI: 10.15259/PCACD.25.016

increase in the first stage, the value of dynamic viscosity, or complex viscosity in the case of viscoelastic fluid, decreases. This phenomenon has been repeatedly presented both in our earlier studies [20, 31–35] and from other groups [36, 37]. This finding indicates that it is preferable to inject the thermostated material that has been left at room temperature for several hours rather than inject material that has been stored at low temperature (e.g. in a refrigerator). It is worth noting that the sample storage time was not long enough to observe a rapid increase in the viscosity of the medium, which would indicate the development of a spatial network, and thus lead to difficult application.

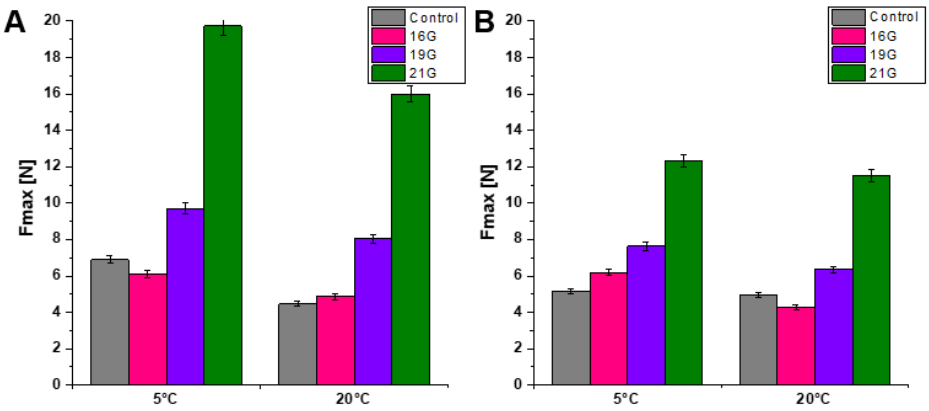

Figure 3. Maximum force values determined at 5°C and 20°C for chitosan hydrogels prepared with (A) hydrochloric acid or (B) acetic acid based on the ● control test (without needle) and ● 16G, ● 19G and ● 21G measurements.

Fig. 3 shows the values of maximum forces observed during the injection application. These data confirm the analyses based on DGF forces. For chitosan/hydrochloric acid samples thermostated at 5°C and chitosan/acetic acid samples thermostated at 20°C, higher maximum force values were observed with the control (without needle) than with the largest needle (16G).

From the application point of view, the value of the maximum force recorded during the measurement seems to be crucial. In all the analysed cases, the maximum force values (F_{max}) did not exceed the allowable value of 20 N [26]. However, in the case of hydrogels prepared with hydrochloric acid, the use of needles smaller than 21G and injection application at 5°C will most likely exceed the allowable force values. This risk is reduced by pre-heating the experimental medium to room temperature or by using acetic acid as a solvent.

The determined values of the total work done (W_T) necessary for injection are shown in Fig. 4. These data confirm the conclusions formulated above for the obtained values of dynamic and maximum forces. It is worth noting that the discussion of the results based on the total work done allows obtaining quantitative data regardless of the shape of the experimental curves. Thus, it is possible to determine the injection process in a quantitative manner for measurements in which dynamically changing force values as a function of distance were recorded. However, according to the available literature data, this parameter is not clear in terms of application, because so far no panel research has been conducted that would associate the values of total work done with the actual injection process.

DOI: 10.15259/PCACD.25.016

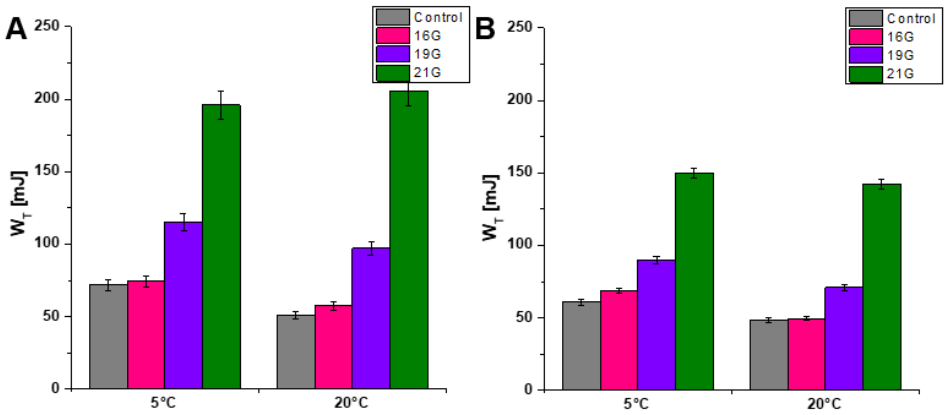

Figure 4. Total work done (W_T) values determined at 5°C and 20°C for chitosan hydrogels prepared with (A) hydrochloric acid or (B) acetic acid based on the ● control test (without needle) and ● 16G, ● 19G and ● 21G measurements.

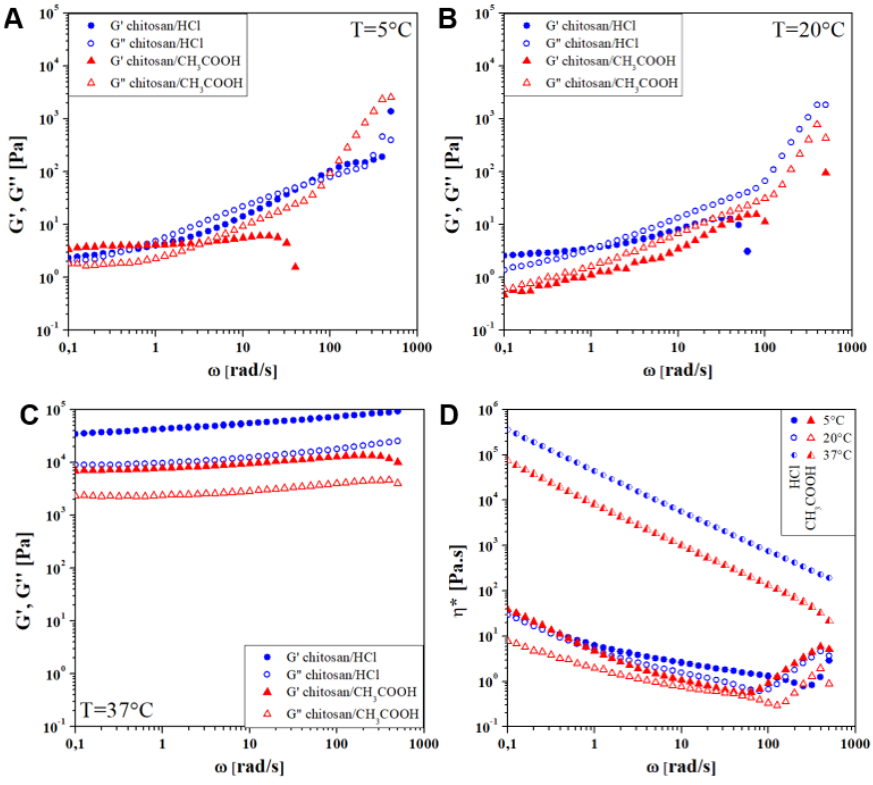

Figure 5. Changes in the storage modulus (G', closed markers) and loss modulus (G'', open markers) values of chitosan/hydrochloric acid hydrogels (blue markers) and chitosan/acetic acid hydrogels (red markers) at (A) 5, (B) 20 and (C) 37°C. (D) Changes in the complex viscosity values of chitosan/hydrochloric acid hydrogels (blue markers) and chitosan/acetic acid hydrogels (red markers) at 5, 20 and 37°C.

DOI: 10.15259/PCACD.25.016

In order to explain the above-described dependence of the forces and total work done values on the solvent used, measurements determining the rheological properties of the samples were carried out (Fig. 5). Based on the obtained mechanical spectra under isothermal conditions, the utilized solvent significantly affects the obtained values of dynamic moduli; namely, they are lower for chitosan/acetic acid systems. This phenomenon makes the application easier due to the less developed spatial structure and lower viscosity values. Based on the obtained rheometric data, the decrease in the value of dynamic modules, and thus the viscosity, was confirmed as the temperature increased (Fig. 5D). It is worth noting that, despite the lower polysaccharide concentration, in the case of chitosan/acetic acid systems, a spatial polymer network is formed at 37°C. This observation is based on the course of experimental curves of dynamic modules, whose value does not depend on the angular frequency (Fig. 5C).

4. Conclusions

Based on the preliminary injectability tests, thermosensitive chitosan hydrogels are promising materials in the context of injection application. The chitosan/glycerophosphate systems obtained by dissolving the polysaccharide in hydrochloric and acetic acid were injectable using 16–21G needles. As the diameter of the needle decreased, the values of the forces recorded during the injection increased. However, in all the examined cases, they did not exceed the admissible values determined based on panel tests. In practice, this means that the application should be successful without major difficulties for the person carrying out the injection. Hence, choosing the optimal injection needle should not be limited to the smallest diameter needle to reduce patient pain.

The viscosity of the experimental medium is a crucial parameter that affects the injectability process. The use of a less viscous hydrogel obtained as a result of dissolution of chitosan in acetic acid reduces the forces needed to inject the experimental medium.

Extremely interesting results – and not obvious in the context of application conditions – revealed that heating the sample for 2 h before the actual measurement facilitates application by reducing the forces necessary for injection. This is the result of a decrease in the viscosity of chitosan systems under the influence of temperature increase, this stage is preceded by the proper formation of the spatial matrix.

5. References

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