New instrumental criterion for evaluating the potential of injectable scaffolds for biomedical engineering applications

Anna Ryl* [ID], Piotr Owczarz [ID]

Lodz University of Technology, Department of Chemical Engineering, Wolczanska 213, 93-005 Lodz, Poland

* Corresponding author:
e-mail:
anna.ryl@p.lodz.pl

Abstract
When designing injectable scaffolds for biomedical applications, it is crucial to determine the conditions for the formation of unlimited structures, in particular the kinetics at constant temperature. Despite many studies, these conditions have not been characterized so far after injection, which is such an important application aspect. The aim of the research was to discuss the impact of the injection application on the polymer structure and to propose new criteria for assessing the potential of thermosensitive biopolymer sols, considering the flow under high shear rates during the administration. Based on the analysis of the obtained results of rheological tests, it was shown that flow through needles causes a significant change in the elastic properties that define the polymer structure, with almost unchanged viscous properties. As a consequence, the parameters characterizing the polymer coil change, which, combined with the quantitatively proven fragmentation, indicates that injection application may affect the size of the coils that will not reach the critical size of the aggregating nucleus. Finally, extended research procedures for the conscious design of injectable scaffolds are proposed as well as key rheological parameters to ensure thermoinduced aggregation preceded by shear during injection are provided.

Keywords
injectable scaffolds, hydrogels, sol-gel phase transition

1. INTRODUCTION

The development of science and proposing new technological solutions is a strong incentive for many researchers to improve the quality of life. One of the leading research areas is the development of modern methods of treatment and regeneration that would shorten the recovery time and at the same time be less invasive for the patient. One of the solutions are polymer scaffolds obtained by thermoinduced sol-gel phase transition, during which polymer aggregates form spatial matrices inside which medicinal substances and/or cells are trapped. Although for many researchers the term 'phase transition' is controversial, according to modern classifications based on Ehrenfest categories, this process should be classified as a second-order phase transition under non-equilibrium conditions (Rouwhorst et al., 2020) due to energy discontinuity at the critical point.

The dynamic development of research on polymer hydrogels, which are to serve as cellular scaffolds and smart drug carriers, most often concerns the determination of the architecture, biological and chemical properties of the formulated hydrogels. This is primarily due to the need to meet biosafety requirements, including the determination of broadly understood biocompatibility, as well as to ensure the transport of nutrients, e.g. oxygen and the trapping of medicinal substances inside the scaffold. Ultimately, the chemical and topographical properties together determine the morphology of the scaffolds, which can significantly influence cell/active substance adhesion (Reddy et al., 2021).

In addition to the above requirements for all hydrogels for biomedical applications, in the case of those forming a spatial polymer network under the influence of temperature changes, it is extremely important to determine the conditions of their thermoinduced sol-gel phase transition. This is due to the fact that the ability to inject the preparation, its composition and sterilization conditions depend on their precise determination. There are two main groups of measurement techniques used to determine gelation conditions. The first of them are measurements based on the analysis of the flow phenomenon and the determination of changes in rheological properties accompanying the phase transition. The second group includes optical measurements based on light scattering techniques. Much less frequently conducted research uses NMR spectroscopy techniques and differential scanning calorimetry.

The simplest methods, such as the inverted tube test and the falling steel ball test (Chung et al., 2002), are simple and do not require the use of advanced equipment. Unfortunately, apart from the one-point determination of gelation time/temperature, which is also burdened with a significant error, the methods make it impossible to determine the kinetics of the spatial structure formation. Moreover, it seems that the system remaining at higher temperatures for a longer time may lead to continuous structural changes, and thus lead to significant measurement errors; the structure at the beginning of the measurement will not correspond to that at the end of the measurement.

In order to determine the conditions of sol-gel phase transition, rheological tests are most often carried out in the oscillatory shear mode. For most of the research results presented in the literature, the conditions of the sol-gel phase transition are determined on the basis of non-isothermal kinetics (da Silva et al., 1995). In the simplest case, the gelation temperature is determined as the point of equalization of the values of storage G' and loss G'' moduli. Although this method is generally accepted and widely used due to its unambiguity, it should be emphasized that it only determines the location of the point where the change of the dominant characteristics of the medium is observed – from viscous to elastic, and this does not guarantee the exact determination of the formation point of the polymer network that does not show the flow phenomenon (Owczarz et al., 2018). Moreover, the obtained results depend on the applied deformation (angular frequency and amplitude). This disadvantage can be eliminated by using the measurement methodology proposed by Chambon and Winter (1987), based on the power-law dependence of dynamic modules on the angular frequency: $G' \sim G'' \sim \omega^n$. In the case of non-isothermal measurements, an alternative seems to be the use of the Fredrickson–Larson method, according to which energy discontinuity occurs at the spinodal decay temperature (Fredrickson and Larson, 1987). Its unquestionable advantage is the ability to describe systems characterized by a predominance of elastic properties from the beginning of the measurement (Owczarz et al., 2019c). Another method of determining the critical point of the phase transition is the method proposed by Owczarz et al. (2019a), based on the determination of the inflection point of the complex viscosity curve $\eta^* = f(t)$.

It is surprising that most researchers focus on measurements under non-isothermal conditions when characterizing thermosensitive hydrogels. The temperature value determined on their basis, at which the changes of the dominant features of the medium occur, is identified as the only condition ensuring the formation of a spatial structure at the designated temperature. Due to the use of thermosensitive systems as in-situ gelling scaffolds, it seems much more important to determine the kinetics of polymer network formation under isothermal conditions; the phase transition time at the temperature of the human body should be as short as possible to ensure the formation of the gel form before undesirable spillage of the polymer sol. Moreover, determining the kinetics of the spatial structure formation is extremely important because it affects the gel architecture and the release profile of the drug substance (Chung et al., 2002).

The interest in polymer hydrogels obtained as a result of thermoinduced sol-gel phase transition results from the assumed possibility of their minimally invasive injection application due to their liquid, relatively low-viscosity, sol form characterized by the flow phenomenon. The flow of these systems through the needles will be carried out under high shear rate conditions, which are always accompanied by an increase of shear stresses. These, according to multi-threaded research

conducted for colloids, can lead to reversible or irreversible deformations of the dispersed phase. It seems that such an intense shear field can lead to significant molecular changes in the colloidal polysaccharide suspension, e.g. coil fragmentation, and consequently affect the conditions of hydrogel formation induced by temperature increase.

So far, the only studies determining the effect of injection on the structure of polysaccharide systems and the conditions of thermoinduced sol-gel phase transition have been presented in the authors' previous work (Ryƚ and Owczarz, 2021a). For this reason, the aim of this work was to formulate criteria for assessing the application potential of injectable scaffolds for biomedical applications, which have not been considered so far, together with a discussion about changes observed under the influence of injection on the conformation of polymer coils constituting the starting material for hydrogel formation.

2. MATERIALS AND METHODOLOGY

Two derivatives of natural polysaccharides were used in the study: hydroxypropyl cellulose (Sigma Aldrich, CAS number 9004-64-2) and chitosan (Sigma Aldrich, CAS number 9012-76-4). The latter was obtained from the chitin derivative differed in origin: crab and shrimp, and consequently also in molecular weight, which was 680 kDa and 860 kDa, respectively. The hydroxypropyl cellulose (HPC) was characterized by a weight average molar mass of $Mw = 100$ kDa. Colloidal chitosan systems (2% w/w) were obtained in accordance with the preparation proposed by Chenite et al. (Chenite et al., 2000; Chenite et al., 2001). For this purpose, the powdered polymer was dissolved in 0.1M hydrochloric acid (Sigma-Aldrich, CAS number 7647-01-0), and the obtained acidic polysaccharide solution was stored for 24 hours at room temperature. In order to ensure gelation under physiological conditions, an aqueous suspension of disodium β-glycerophosphate salt (2 g of powder in 2 ml of water) was prepared and placed in the refrigerator for 2 hours before being mixed with the cooled polymer solution. Then, the suspension was added to the chitosan solution and, after thorough mixing, stored at 4 °C. HPC solution (10% w/w) was obtained by dissolving the powder in distilled water, and the resulting suspension was left for 48 h at room temperature until the polysaccharide was completely dissolved.

In order to determine the kinetics of phase transition, rheometric tests were carried out in the oscillatory shear mode at constant deformation. The determination of the mechanical spectra was accomplished by oscillation frequency sweep studies. The tests were carried out under isothermal conditions at 37 °C. Before the actual measurement, the test medium was injected with hypodermal needles in sizes 14G–25G, in which the sol flow was carried out under shear rate conditions in accordance with Table 1 (Ryƚ and Owczarz, 2020). During the research, the procedures proposed in the

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Table 1. Values of shear rates occurring during the flow in the needles used in the tests, assuming a plunger speed of 1 mm/s.

Needle	Outer Diameter (OD) [mm]	Inner Diameter (ID) [mm]	Shear Rate* [s^{-1}]	Adjusted shear rate [s^{-1}]	
				Chitosan ($n = 0.44$)	HPC ($n = 0.7$)
14G	2.108	1.6	186	245	206
15G	1.829	1.372	294	388	326
16G	1.651	1.194	447	589	495
18G	1.270	0.838	1292	1703	1430
19G	1.067	0.686	2356	3104	2608
20G	0.908	0.603	3469	4571	3841
21G	0.819	0.514	5600	7380	6200
22G	0.718	0.413	10,796	14,226	11,953
23G	0.641	0.337	19,871	26,185	22,000
25G	0.514	0.26	43,269	57,020	47,903

*the shear rate value is the nominal value for Newtonian fluid flow, which must be adjusted for non-Newtonian fluid

authors' previous work were followed (Ryl and Owczarz, 2021a). Control measurements were also carried out during which the tested sols were not subjected to injection or other initial shearing prior to the actual measurement.

3. RESULTS AND DISCUSSION

In previous author's studies (Ryl and Owczarz, 2021a), a significant effect of the short-term shear field observed during injection on the sol-gel phase transition conditions was demonstrated. However, regardless of the tested sol, the use of low (relative to injection) shear rates accelerated the phase transition of the studied polymer systems, and the dependence of the gel time on the shear rate was non-monotonic with a clear extreme, a direct comparison of the obtained results leading to interesting observations. The analysis of

the results obtained for the control measurements (i.e. not preceded by shear prior to gelation) shows that the hydroxypropyl cellulose sol underwent the fastest phase transition determined on the basis of equating the storage G' and loss G'' moduli (Fig. 1a). Subsequently, a change in the dominant properties from viscous to elastic was observed for crab-derived chitosan. After the longest measurement time, a characteristic point was observed in the case of chitosan of higher molecular weight of shrimp origin. Thus, based solely on the most frequently cited method of determining the phase transition time, it would be expected that the hydrogel from the cellulose derivative would be formulated the fastest. However, based on turbidimetric studies, it has been proved that thermostating the hydroxypropyl cellulose sol at 37 °C for 8 hours did not lead to the formation of a spatial polymer lattice (Ryl and Owczarz, 2021a). In addition, a direct comparison of the determined sol-gel phase tran-
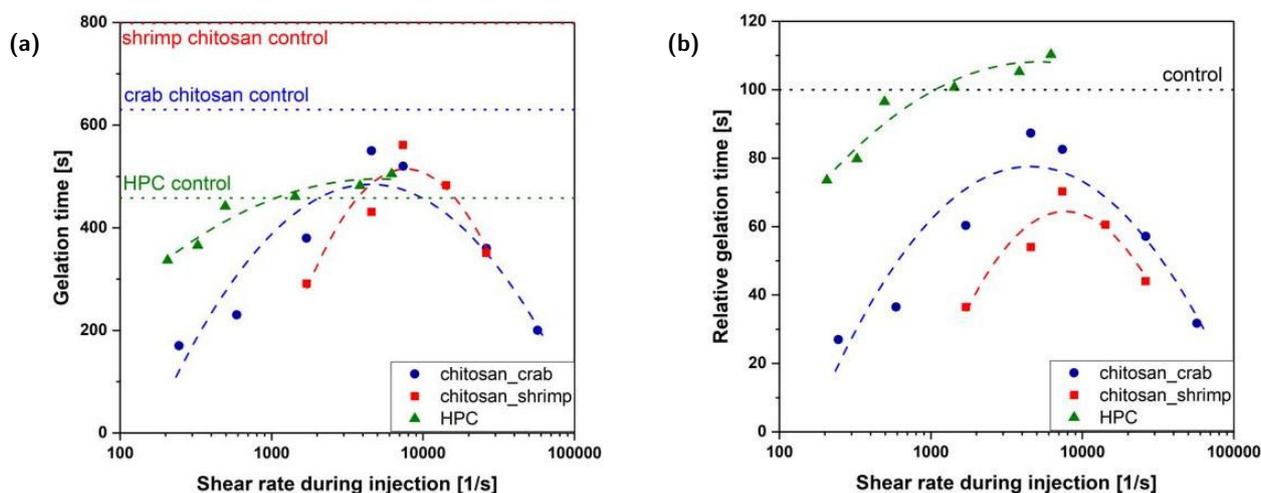
Figure 1. Dependence of gelation time (a) and relative gelation time (b) on shear rate during injection.

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sition times (Fig. 1a) allows to unambiguously confirm the much greater sensitivity of chitosan systems to the presence and intensity of the initial shearing interval observed during injection. It can be observed that despite much longer control gelation times determined for sols obtained with the use of chitosan, these systems after injection were characterized by a shorter time after which the dynamic modules were equalized than in the case of systems based on a cellulose derivative. Only in the case of using 20G and 21G needles for crab-derived chitosan and 21G needle for chitosan of shrimp origin (characterized by a higher molecular weight) a longer phase transition time was noted than for hydroxypropyl cellulose sol. In all other cases, the sol-gel phase transition time was shorter.

In order to take into account the results of control measurements and the actual impact of the injection on the gelation time t enabling comparison of the tested polymers, normalization was performed in relation to the control time $t_{control}$. For this purpose, the relative gelation time t_{rel} was determined according to Equation (1):

$$t_{rel} = \frac{t}{t_{control}} \cdot 100\% \qquad (1)$$

On this basis, it can be clearly stated that in the case of the hydroxypropyl cellulose sol, the maximum reduction of the phase transition time was 25% compared to the control measurements (without pre-shearing) and was observed during injection through a 14G needle – Fig. 1b. In the case of using an injection needle of the same size during the injection of crab-derived chitosan sol, the gelation time was shortened by 73%. The longest of the determined relative times was 83% and 70% for the crab-derived chitosan sol and the higher molecular weight chitin derivative ones, respectively. Particular attention should be paid to the data obtained after injecting sols with needles smaller than 21G, the use of which is recommended by global and European medical agencies for intramuscular and subcutaneous injections.

Based on the data obtained from injectability measurements presented in the authors' previous works, an even stronger potential of chitosan sols can be demonstrated compared to media obtained based on hydroxypropyl cellulose. Firstly, manual injection application was impossible due to exceeding the maximum allowable forces determined on the basis of panel tests. Moreover, using the 18G, a time similar to the control measurement (without pre-shearing) was reached, and the selection of 20–21G needles extended gelation time by 5 and 10%, respectively. In the case of chitosan sols, the determined dynamic forces reached a value of approx. 35 N, and the determined relative times for needles smaller than 21G amounted to a maximum of 60%; when the chitosan sol was injected with a 25G needle, the relative time was only 30%. In the case of injecting a sol with a higher molecular weight with the use of 25G and 14G − 16G needles, the predominance of elastic properties over viscous ones was observed from the beginning of the measurement or the gelation process occurred immediately.

Due to the non-obvious nature of the observed changes, it was decided to analyze the changes in viscoelastic properties caused by the injection – Fig. 2. Based on the dynamic modulus values, it can be seen that the injection causes much greater changes in the initial values of the storage modulus G' with almost unchanged values of the loss modulus G''. This clearly indicates that the flow through the needle under high shear rate conditions causes changes in elastic properties, which directly testifies to the changes taking place in the polymer network.

It can be observed that the flow through needles with large diameters (e.g. 14G), accompanied by a shear rate of approx. 250 1/s, caused an increase in the value of the storage modulus G' by approx. 30% compared to the control measurements. This indicates a greater amount of energy accumulated inside the system. This energy most likely comes directly from the pre-shear during injection. However, due to the moderate shear field, it does not lead to rapid structural
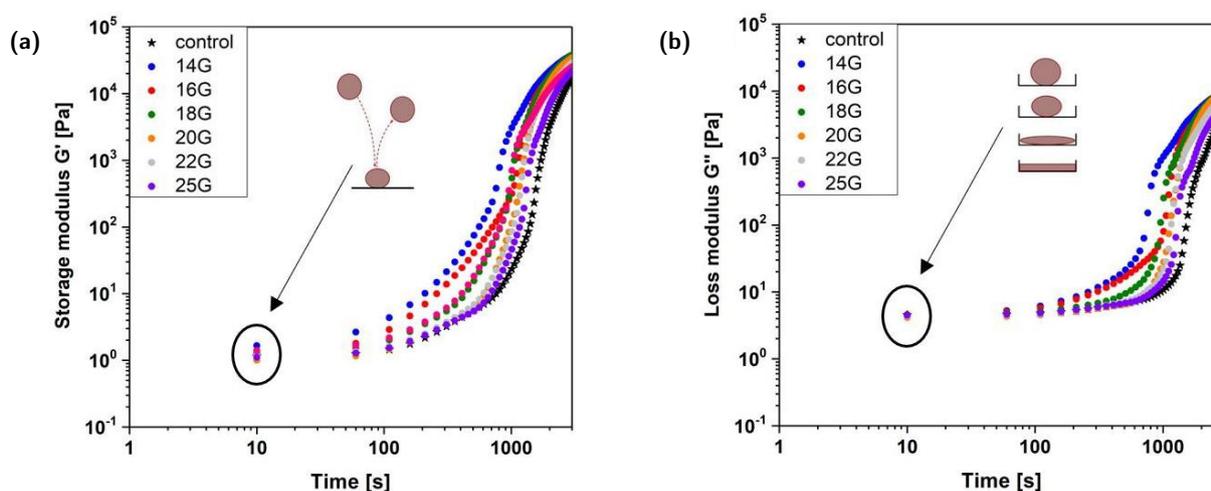
Figure 2. Kinetics of changes in storage modulus G' (a) and loss modulus G'' (b) during gelation after injection.

changes. The increase in the shear rate in the initial interval observed during the injection causes a decrease in the value of the storage modulus G'. Despite the fact that more and more energy is supplied to the system, the degree of its accumulation decreases compared to the largest of the needles used, which suggests significant changes in the system that dominate over the sole capacity of energy accumulation. It is worth noting that the storage modulus G' reaches a value close to the control measurements when using the 20G ($\dot{\gamma} = 4571$ s^{-1}) needle, the flow through which resulted in the maximum sol-gel phase transition time.

Based on the rubber elastic theory (RET) (Karvinen et al., 2019), the value of the storage modulus G' can be related to the mesh size ξ according to Equation (2).

$$\xi = \left(\frac{G' N_A}{RT} \right)^{-1/3} \tag{2}$$

Considering that the tests were carried out under isothermal conditions, it can be shown that the mesh size is inversely proportional to the value of the storage modulus G' according to Equation (3).

$$\xi \approx \sqrt[3]{\frac{1}{G'}} \tag{3}$$

This indicates that the flow through the needles with the smallest inside diameters results in a reduction of mesh size compared to the control measurement. Although in this case, due to the form of the sol, it seems more reasonable to refer to the average distance between the polymer chains inside the coil. This is due to the flow-induced change in conformation and elongation of the polymer coil in the direction of the velocity vector. Subsequently, an increase of the average distance between the polymer chains inside the coil associated with the progressive deformation of the dispersed phase is observed.

Due to the observed changes in the value of the storage modulus G' recorded immediately after injection at the beginning of gelation, it was decided to determine the mechanical spectra after flow through the needles determined in frequency sweep tests. It is worth noting that in the transition region between the glassy state and the plateau state, the course of the storage modulus G' curves as a function of angular frequency is independent of the needle size through which the injection was made. According to the Kasapis theory (Groot and Agterof, 1995; Kasapis et al., 2004), in this area a gradual immobilization of elastic chains and adjacent structural nodes of the resulting network is observed. The effect of the injection is visible in the plateau zone, the range of which also depends on the shear rate occurring during the flow through the needles. In this region, biopolymers form elastic networks, as a result of which elastic deformations occur and flow is not observed. As shown in Figure 3, the plateau region was recorded in the widest range of oscillation frequency for the largest diameter needle (14G), which narrowed with the increase of shear rate during injection caused by the selection of needles with smaller diameters. As in the case of measurements carried out at constant deformation, the injection of chitosan sol through needles with large diameters (14G-16G) caused an increase in the value of the storage modulus G', compared to the control measurements. According to the rubber elastic theory, this is related to the initial reduction of the average molecular weight of the polymer chain between neighboring crosslinks and the increase in crosslinking density, expressed by Equations (4) and (6), respectively:

$$M_C = \frac{c\rho RT}{G_e} \tag{4}$$

Due to the constant concentration and density as well as the unchanging conditions of the measurements, it can be unequivocally determined that the average molecular weight of the polymer chain between neighboring crosslinks is inversely
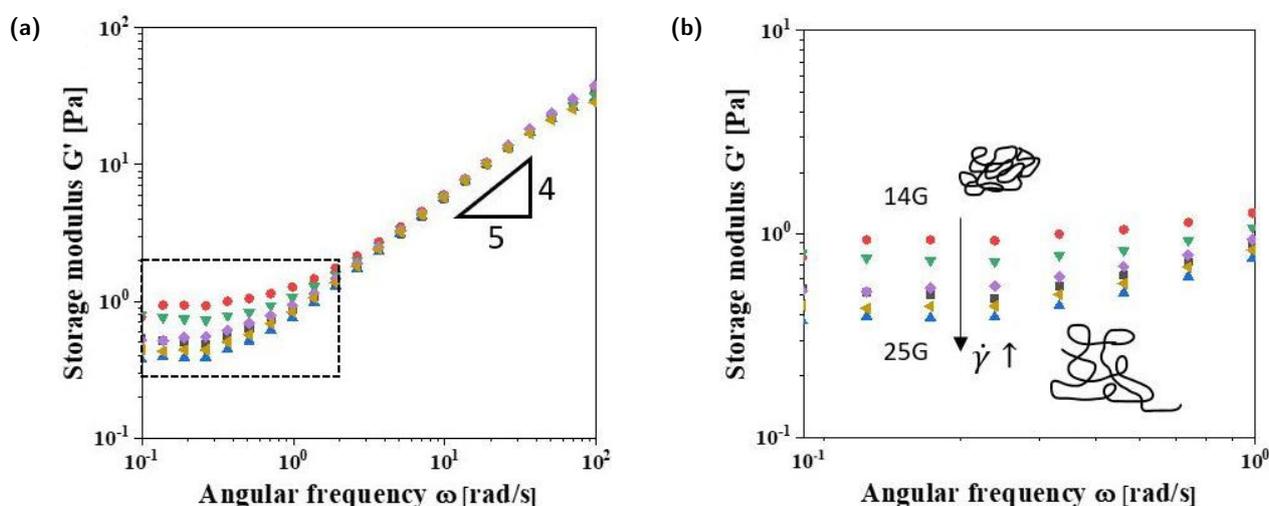
Figure 3. The dependence of the storage modulus on the angular frequency: full spectrum (a), narrowed range with observed changes (b).

proportional to the equilibrium modulus G_e:

$$M_C \approx G_e^{-1} \qquad (5)$$

Conversely, the crosslinking density is directly proportional to the equilibrium modulus:

$$n_e = \frac{G_e}{RT} \qquad (6)$$

Subsequently, the increase in the shear rate causes a gradual decrease in the value of the storage modulus responsible for the elastic properties and associated with polymer coils. As a consequence, this causes an increase in the average molecular weight of the polymer chain between neighboring crosslinks and a decrease in crosslinking density. Compared to chitosan sols, systems based on a cellulose derivative are characterized by about ten times lower values of the storage modulus G' determined both during the study of gelation kinetics and during three-interval thixotropic tests (Ryl and Owczarz, 2021a). The change in the elastic properties, quantitatively represented by the storage modulus G', demonstrated in the tests, clearly defines the state of the dispersed phase (polymer coils). The observed changes indicate a change in the conformation of the polymer coils, including their degree of entanglement and size. This has a significant impact both on the change of the initial state of the matter, which undergoes a further sol-gel phase transition, but also directly and indirectly affects its kinetics. The change of conformation consisting in the loosening of polymer coils facilitates access to the so-called junction zones, and consequently, it favors the creation of physical interactions responsible for the gelation of the tested systems. The shear-induced fragmentation, confirmed by rheo-optical tests, causes a reduction in the size of the aggregation nuclei, which, due to the reduction in the characteristic dimension, reduce the resistance to mutual diffusion. In the case of chitosan systems, despite the observed fragmentation of the coils (reduction of the gyration radius from 2.12 μm to 1.79 μm), their size exceeds the size of the critical aggregation nuclei, which enables further aggregation. For chitosan with a higher molecular weight, this phenomenon was even stronger (reduction of gyration radius from 2.28 μm to 1.52 μm) with simultaneous higher shear rates causing these changes. This is most likely due to the fact that polymer coils made of shrimp-derived chitosan were more strongly packed, and consequently much stronger cohesive interactions between the polymer chains were created inside the coils. According to the theory of Alexander and Netz (Alexander-Katz and Netz, 2008), it was only after instability and protrusion formation that chain fragmentation could occur. The daughter chain was characterized by a much smaller characteristic dimension due to the strong entanglement of the primary chain. Fig. 4 shows the schematic effect of the shear rate on changes in the conformation of polymer chains under the influence of the applied shear rate, depending on the molecular weight of the polymer, assuming constant biopolymer concentration (w/w).

Based on the results of rheo-optical measurements carried out for HPC sols (Ryl and Owczarz, 2021a), it was impossible to determine the change in gyration radius due to the obtained scattering patterns. However, their qualitative analysis suggests that fragmentation is also observed. Due to the lack of formation of a stable structure that does not show the phenomenon of flow, it can be assumed that the fragmented polymer coils do not reach the critical size (Fig. 5a) ensuring thermodynamic stability and enabling the formation of a spatial lattice (Fig. 5b).

Ultimately, both the initial state of the structure and the kinetics of aggregation will affect the final architecture of the formulated scaffold, which is crucial both in the design of smart drug carriers and scaffolds for cell cultures supporting the process of tissue regeneration. Considering the aggregation theory of colloidal systems, it should be expected that the lattices obtained from highly fragmented polymer coils will produce a much more compact structure defined by higher values of fractal dimensions. In the case of matrices intended to be used as smart drug carriers delivering medicinal substances with prolonged action, the reduction of the matrix size (mesh size/drug size ~ 1) will result in much slower diffusion than in the case of systems characterized by a larger mesh size (mesh size/drug size > 1) (Karvinen et al., 2019). In the case of the latter, the diffusion of the active substance will proceed much faster, narrowing the release profile, unfavorable in the case of designing systems with prolonged release. If the mesh of the polymer network
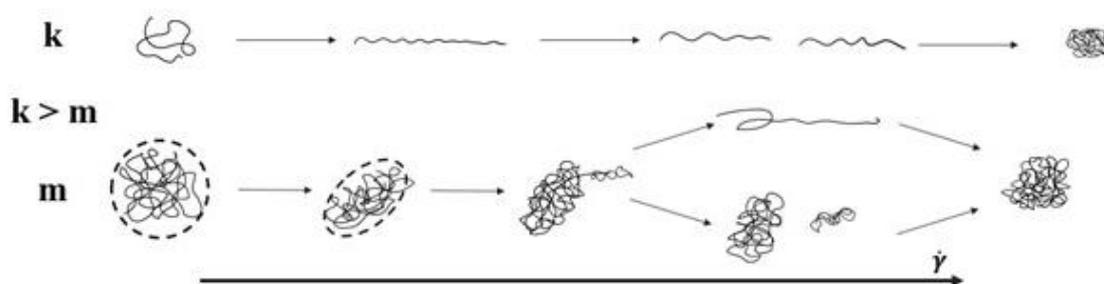
Figure 4. Influence of molecular weight on the change of the polymer coils state under the influence of shear rate increase, where k – the number of coils with a lower molecular weight, m – the number of coils with a higher molecular weight assuming constant polymer concentration.

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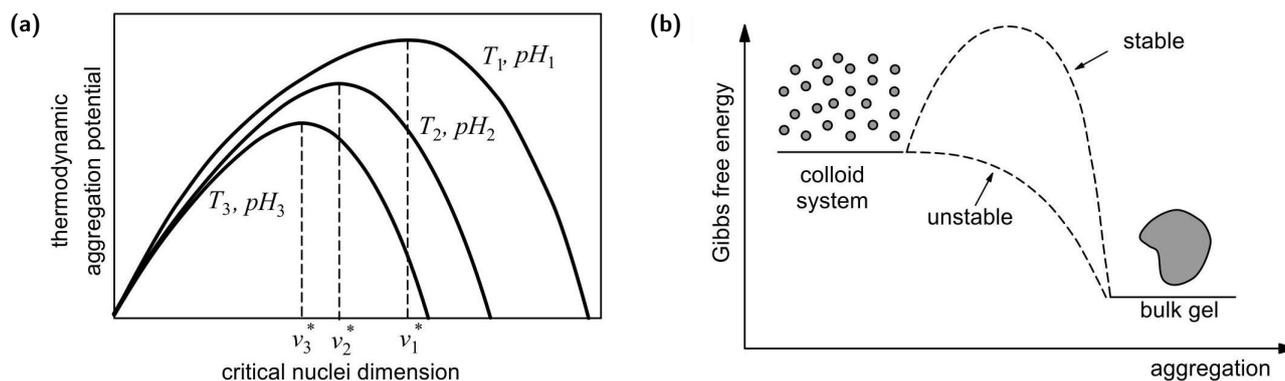
Figure 5. Dependence of the thermodynamic aggregation potential as a function of the embryo size (a), scheme of aggregation of colloidal systems (b) (Owczarz, 2019).

were significantly smaller than the characteristic dimension of the active substance (mesh size/drug size < 1), the only way to release it from the matrix would be to apply a trigger, for example, by swelling, mechanical deformation or degradation. The architecture of the polymer matrix produced is even more important when designing scaffolds supporting tissue regeneration. Although too compact a structure will ensure adequate mechanical strength, it may limit the ability to proliferate cells due to the lack of an appropriate environment and free space for the nutrients transport and the removal of matter products. In addition, under the above conditions, the diffusion of gases necessary for cell growth will be hindered. On the other hand, the open structure will not provide sufficient mechanical support for the proliferating cells.

Classically, the characterization of thermosensitive sols to be used in biomedical engineering from the rheological point of view focuses on three main stages according to Scheme 1. During it, the shear-thinning nature of the tested systems is determined, usually in the range of shear rates not exceeding 1000 1/s. Then, the phase transition temperature is determined based on the point where the equalization of the
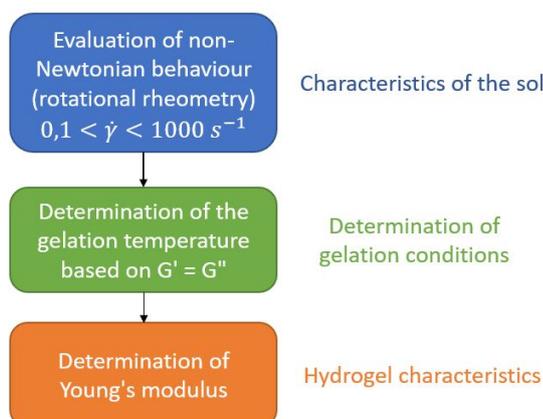
dynamic modulus values is observed. Finally, few papers describe the mechanical properties of the formulated hydrogels on the basis of the Young's modulus value.

However, this characteristic does not allow to determine the real application potential, considering the real ability to inject, conditions of gelation under the influence of an intense, short-term shear field and its impact on the final properties of the produced matrix. For this reason, the commonly used research methodology should be extended in accordance with Scheme 2. The key changes in the proposed methodology relate to the characteristics of media under high shear rate conditions, which will enable to determine the risk of reaching a plateau in the area of high shear rates occurring in small diameter injection needles. In addition, at the stage of research conducted for the polymer sol, it is recommended to perform instrumental injectability tests, which allow for a quantitative assessment of the possibility of application of the considered materials. In the stage of determining the conditions of the sol-gel phase transition, it is crucial to determine the gelation kinetics both in the orthokinetic and perikinetic regimes (Ryl and Owczarz, 2021b) under control conditions and after injection. Finally, due to the role of the designed matrices, advanced assessment of mechanical properties is recommended, using e.g. double compression tests (Owczarz et al., 2019b) or fatigue tests. The analysis of the results of the former will make it possible to determine parameters such as cohesiveness and adhesiveness, which will play an important role in the case of cell scaffolds and smart drug carriers.

On the basis of the conducted research, it can be concluded that the sols undergoing a thermoinduced sol-gel phase transition, which are to have the ability to form a spatial, unlimited polymer network after applying a short-term, intense shear field during injection, should be characterized by:

- strongly shear-thinning rheological behavior (as low as possible flow index value),
- the widest possible range of non-Newtonian behavior on the viscosity curve,

Scheme 1. Classical approach to characterization of injection scaffolds forming matrices as a result of thermoinduced sol-gel phase transition.

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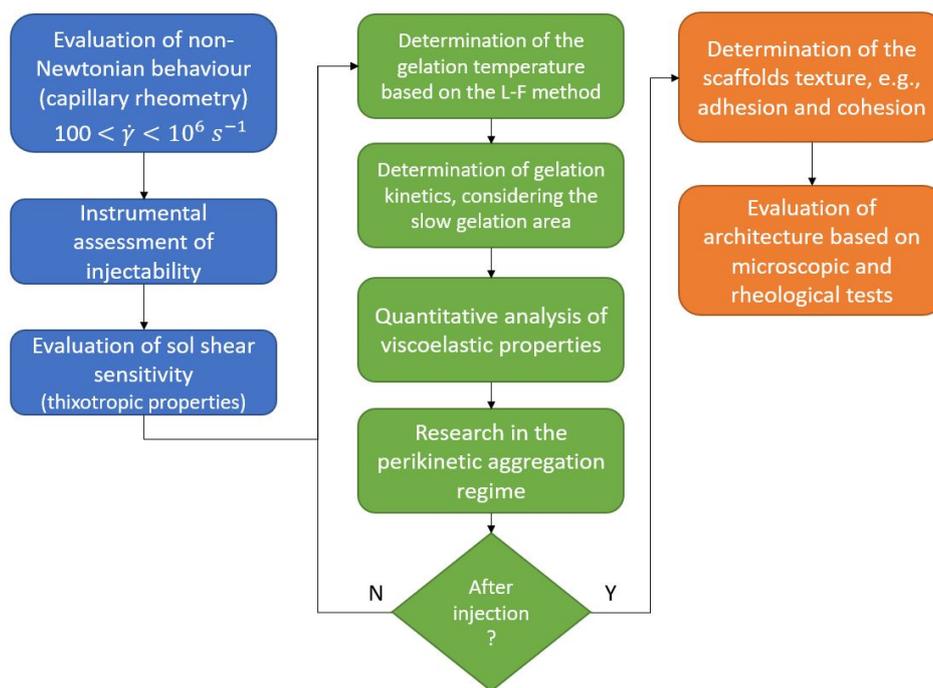
Scheme 2. Proposed research procedures for conscious design of injection scaffolds, taking into account the impact of injection on gelation conditions and the state of the created structure.

- values of the storage modulus G' at the level of several Pa (for $\omega = 5$ rad/s, $\gamma = 1\%$),
- the ability to structure recovery.

Maintaining the above parameters should enable injection application even with the use of needles with small diameters, while ensuring appropriate mechanical properties, making them sufficiently insensitive to mechanical stimuli.

4. CONCLUSIONS

Based on the analysis of rheological test results, it was shown that the injection application significantly affects the determined sol-gel phase transition times and cannot be omitted when designing scaffolds for biomedical applications. Particular attention should be paid to variations in control measurement test results that may lead to erroneous conclusions. As it was shown for the hydroxypropyl cellulose sol, which in the case of control measurements was characterized by the shortest gelation time, the use of injection needles with small diameters resulted in the extension of the phase transition time, and in the perikinetic regime, where only diffusive mass transport is observed, the spatial polymer matrix was not formed. In the case of using sols based on chitin derivatives, a favorable phenomenon of gelation acceleration and improvement of the final elastic properties was observed. Despite the shear-induced fragmentation of polymer chains observed in both cases, the size of chitosan coils exceeded the critical size of thermodynamically stable nuclei. The investigated sols, although obtained from polysaccharides with

a similar chemical structure, differed significantly in their initial viscoelastic properties (in particular, the storage modulus G' values). In addition, compared to the chitosan system, the sol obtained from the cellulose derivative, despite the 5-fold higher concentration, was characterized by a significantly higher flow index, which made its application impossible with the use of small-diameter injection needles. It should also be assumed that the initial viscoelastic properties will also significantly affect the architecture of the formed scaffolds, which in turn will affect the real potential of thermosensitive biopolymer sols. This aspect is undoubtedly a further direction of work devoted to this issue.

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SYMBOLS

N_A — the Avogadro constant, 1/mol
n_e — crosslinking density, mol/m^3
$\dot{\gamma}$ — shear rate, 1/s
c — polymer concentration, w/w
G' — storage modulus, Pa
G'' — loss modulus, Pa
G_e — equilibrium modulus defined as the plateau value of storage modulus measured by frequency sweep test, Pa
M_c — average molecular weight of the polymer chain between neighboring crosslinks, kg/mol

R	gas constant, J/(kg·K)
$t_{control}$	gelation time determined in control measurements in which the sample was not sheared before the actual gelation, s
t_{rel}	relative gelation time, –
T	temperature, K
ω	angular frequency, rad/s
ξ	mesh size, nm
ρ	density of water, kg/m³

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