# Assessment of rheological properties of selected viscosupplements used in knee osteoarthritis

## Ocena właściwości reologicznych wybranych wiskosuplementów stosowanych w chorobie zwyrodnieniowej stawów kolanowych

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**Abstrakt**

W pracy przedstawiono ocenę właściwości reologicznych wiskosuplementów opartych na bazie kwasu hialuronowego, a powszechnie stosowanych w chorobie zwyrodnieniowej stawów kolanowych. W oparciu o pomiary reometryczne wyznaczono moc i gęstość usieciowania oraz charakterystyczne wymiary liniowe sieci tych wiskosuplementów. Wykazano, że przebadane wiskosuplementy cechują się siecią luźno splątanych łańcuchów, które mogą tworzyć podobny, bądź zróżnicowany typ struktury.

**Abstract**

The paper presents an evaluation of the rheological properties of viscosupplements based on hyaluronic acid, commonly used in knee osteoarthritis. On the basis of rheometric measurements, cross-linking power and density of network as well as characteristic linear dimensions of the networks of these viscosupplements were determined. It has been shown that the tested viscosupplements are characterized by a network of loosely entanglement chains that can form a similar or different type of structure.

*Słowa kluczowe*: wiskosuplementy, kwas hialuronowy, właściwości reologiczne, moc usieciowania

*Keywords*: viscosupplements, hyaluronic acid, rheological properties, cross-linking power of network

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## 1. Introduction

Hyaluronic acid, also called hyaluronan, was discovered in 1934 by Meyer and Palmer in the vitreous fluid of the bovine eye [1,2]. Today it is known that it occurs in all vertebrates - it is part of the basic substance of connective tissues such as cartilage, dermis or umbilical cord, and is also a component of synovial fluid and the already mentioned vitreous fluid. On an industrial scale, hyaluronic acid is most often obtained by extraction from rooster combs, as well as biotechnologically, in a process consisting of bacterial fermentation, extraction and purification [3,4]. From a chemical point of view, hyaluronic acid is a glycosaminoglycan composed of repeating disaccharide subunits connected by a β1-4 glycosidic bond. Each of them consists of D-glucuronic acid and N-acetyl-D-glucosamine, between which there is a β1−3 bond [5-7] – fig.1.

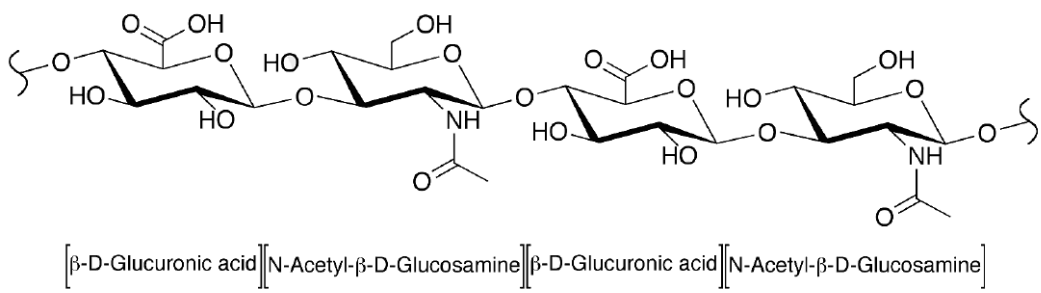


**Fig. 1.** Structure of hyaluronic acid [6].

Intensive research on hyaluronic acid, conducted from the moment of its discovery to the present, has shown that as a biocompatible, non-immunogenic and biodegradable compound, and at the same time having viscoelastic properties, it is perfectly suitable for use in medicine, cosmetics and pharmacy. Hyaluronic acid turned out to be particularly useful in the treatment of osteoarthritis of the synovial joints, and above all of the knee joint, improving its biomechanical conditions [2,6]. The concentration of hyaluronic acid in the synovial fluid decreases with age. This decrease is particularly pronounced when the degenerative process in the joint

progresses simultaneously with the aging process. Then, the ability of the synovial fluid to protect the cartilage and synovial membrane is reduced, which in turn leads to pain. Improvement of the patient's condition can be achieved by a series of several injections of preparations based on hyaluronic acid derivatives, i.e. viscosupplementation [8-10]. There are several preparations for viscosupplementation on the pharmaceutical market. However, they are not identical, which means that they differ in the form and origin of the hyaluronic acid contained in them and the molecular weight of the polysaccharide, but above all they differ in rheological properties.

Therefore, the aim of the presented study is to evaluate the rheological properties of selected hyaluronic acid preparations, commonly used in viscosupplementation during the treatment of knee osteoarthritis.

## 2. Materials and Methods

The research material consisted of three commercial preparations of hyaluronic acid - Ostenil, Hyalubrix and Orthovisc - see table 1.

**Tab. 1.** Properties of commercially viscosupplements

| **Commercially viscosupplements** | **Concentration [mg/ml]** | **Source** | **Molecular weight [kDa]** |
|---|---|---|---|
| OSTENIL (by Trb Chemedica) | 20mg/2ml | biofermentation | 1000-2000 (~1200) |
| HYALUBRIX (by Fidia Farmaceutici) | 30mg/2ml | biofermentation | >1500 |
| ORTHOVISC (by Anika Therapeutics) | 30mg/2ml | biofermentation | 1100-2900 |

Intra-articular injection solutions, directly from the syringe, were placed in the measurement system of the rotational rheometer and left at rest for 1 h at a constant temperature of $37^0$C to reach thermal and mechanical equilibrium. The rheological properties of the tested viscosupplements were determined using a Physica MCR 301 rotational rheometer by Anton Paar in a cone-plate measuring system with a

cone diameter of 50mm, an angle of inclination of $1^0$ and a distance between the measuring elements, i.e. a cone and a plate of 0,048mm. The basic tests were carried out in dynamic conditions in the controlled strain mode, determining the mechanical spectra by measuring the values of the storage modulus G' and the loss modulus G" and the complex viscosity η*. The tests were carried out in the range of changes in the oscillation frequency ω from 0,01rad/s to 300rad/s, taking 6 measurement points for each decade. For all oscillation frequencies, the same relative deformation value of 5% was assumed, determined in earlier studies on the range of linear viscoelasticity of the tested viscosupplements.

A comprehensive assessment of the rheological properties of the tested viscosupplements was based on determining the characteristic linear features of the network created by the viscosupplement. In order to know the linear dimensions of the network, it was additionally necessary to know such parameters as: the value of the plateau viscoelastic modulus $G_N^0$, i.e. the cross-linking power, the value of the cross-linking density $\omega_0$ and the value of the viscosity under steady-state flow conditions $\eta_0$. Taking into account the data presented in Table 1 regarding the molecular weight of the tested viscosupplements, they were treated as polydisperse materials, i.e. those in which the molecular weight distribution is statistically large. Therefore, the value of the viscoelastic modulus of the plateau $G_N^0$ [11-13] was determined from the following relationship:

$$G_N{}^0 = \frac{4}{\pi}\int_{-\infty}^{\omega_{max}} G"(\omega) d\, ln\, \omega \quad (1)$$

Obtaining the value of the viscoelastic modulus of the plateau $G_N^0$ made it possible to determine the characteristic linear dimensions of the network of the viscoelastic material tested (according to the markings presented in Fig. 2), which are:

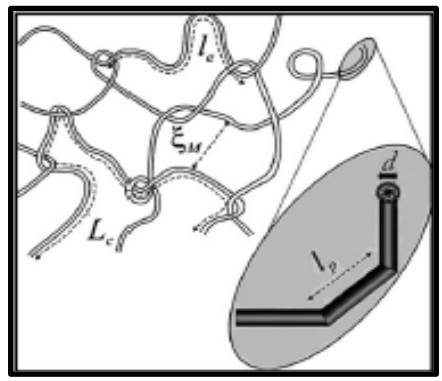


**Fig. 2.** Characteristic linear dimensions of the network of viscoelastic material [13].

- the mesh size of the network ξ:

$$\xi = \left(\frac{k_B \cdot T}{G_N{}^0}\right)^{\frac{1}{3}} \tag{2}$$

where: $k_B$ – Boltzmann constant ($1{,}38 \cdot 10^{-23}$J/K), T – temperature [K]

- the persistence length $l_p$:

$$l_p = \left(\frac{k_B \cdot T}{8 \cdot \omega_0 \cdot \eta_0}\right)^{\frac{1}{3}} \tag{3}$$

- the entanglement length $l_e$:

$$l_e = \frac{\xi^{\frac{5}{3}}}{l_p{}^{\frac{2}{3}}} \tag{4}$$

- the linear contour length $L_c$:

$$L_c = \frac{l_e}{\frac{G"min}{G_N{}^0}} \tag{5}$$

where: $G"_{min}$ – is the value of the local minimum of the loss modulus after the intersection of the curves of both modules, i.e. curve G' and G", impossible to obtain if such a point is missing,

- the network chain diameter $d_e$:

$$d_e = 19 \cdot l_p \tag{6}$$

The value of the viscosity at steady flow conditions $\eta_0$ was determined by extrapolating the complex viscosity to the value ω tending to 0. Thanks to the viscosity $\eta_0$ obtained in this way, the average relaxation time $\tau_0$ was numerically

determined from the relationship:

$$\tau_0 = \frac{\eta_0}{{G_N}^0} \tag{7}$$

The reciprocal of the relaxation time $\tau_0$ is also the cross-linking density of the structure $\omega_0$. In addition, the knowledge of the persistence length $l_p$ and the entanglement length $l_e$ made it possible to estimate the semi-elasticity of the network in the form of the parameter $\alpha_e$ [14] from the following relationship:

$$\alpha_e = \frac{l_e}{l_p} \tag{8}$$

Moreover, thanks to the knowledge of the viscoelastic modulus of the plateau ${G_N}^0$, the packing length p was also determined [15]:

$$p = \left(\frac{13{,}2}{{G_N}^0}\right)^{\frac{1}{3}} \tag{9}$$

and also, based on the relationship described by equation (10):

$${G_N}^0 = \frac{4}{5} \cdot \nu_s \cdot k_B \cdot T \tag{10}$$

the number of entanglements $\nu_s$ in ml of solution.

## 3. Results and Discussion

The graphs in Figure 3 show the oscillation curves obtained during rheometric measurements for all the tested viscosupplements, while Table 2 shows the values of the G' and G" modules at ω values corresponding to walking and running, and Table 3 shows the values of the parameters describing the network of these viscosupplements. The data presented in the graphs in Figure 3 show that the higher the molecular weight of the viscosupplement, the faster - with a lower value of the oscillation frequency ω - the transition from a viscous liquid to a solid occurs, i.e. the transition from viscous properties to elastic properties. So it should come as no surprise that it is for Orthoviscu that this oscillation frequency, denoted as $\omega_c$, has the lowest value of 1,42rad/s.

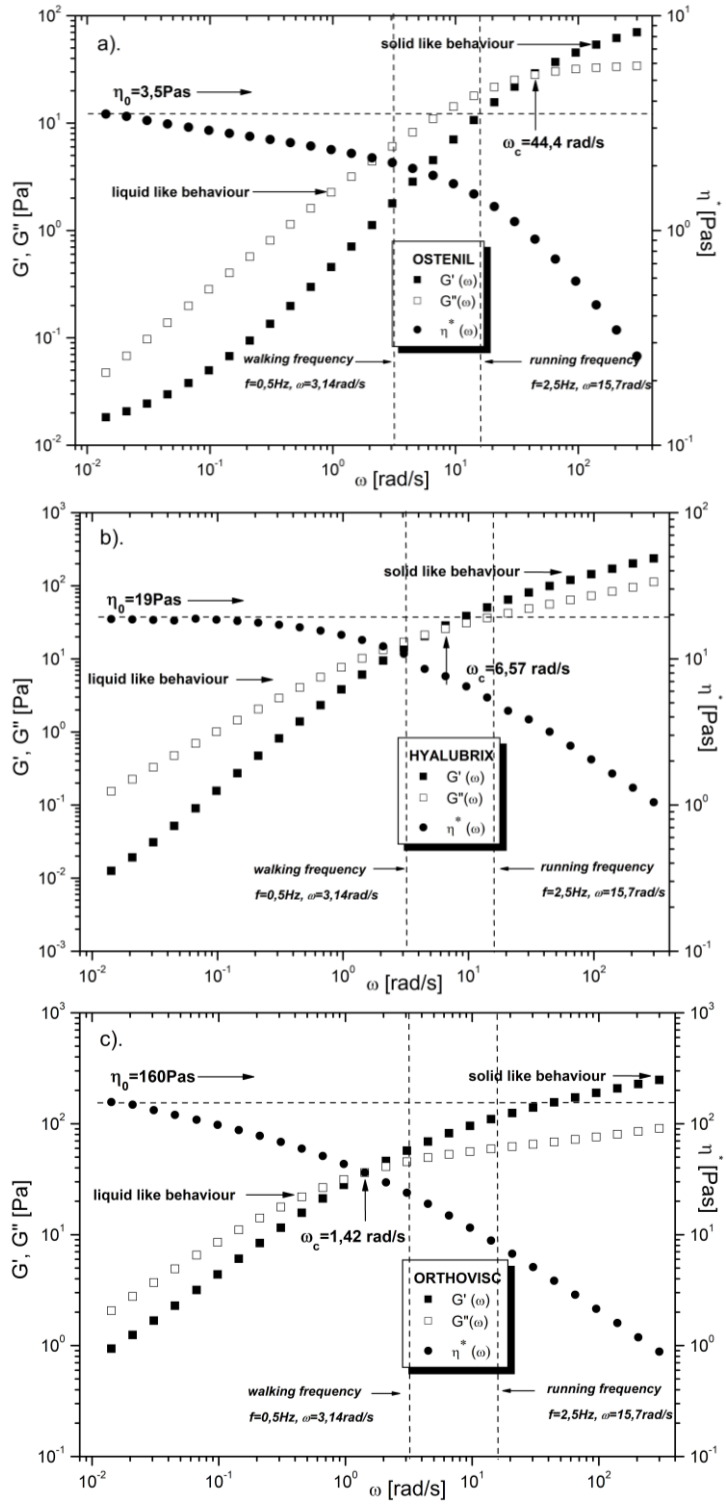


**Fig. 3.** Oscillation curves of tested viscosupplements at human body temperature $37^0$C.

Taking into account the work of the knee joint in real conditions, it is clearly visible on the graphs in Fig. 3 that Ostenil is characterized in a wide range of oscillation frequencies ω by the predominance of viscous over elastic features, this applies especially to this particular range ω which includes the work of the knee joint when walking (3,14 rad/s or 0,5 Hz) and running (15,7 rad/s or 2,5 Hz). In Hyalubrix, the transition from viscous to elastic characteristics takes place exactly in the range of work of the knee joint. This suggests that the viscosupplement behaves like a viscous liquid while walking - when the loads on the joint are lower - and the more the speed of movement increases, it starts to behave like an elastic solid. Orthovisc, meanwhile, is a viscosupplement which in this range of oscillation frequency is characterized by a definite predominance of elastic properties over viscous ones, i.e. high elasticity - see also table 2.

**Tab. 2.** Value of G' and G" while walking and running

| **Value of G' and G"** | **OSTENIL** | **HYALUBRIX** | **ORTHOVISC** |
|---|---|---|---|
| ω=3,14rad/s – f=0,5Hz | | | |
| G' [Pa] | 1.93 | 13.55 | 57.62 |
| G" [Pa] | 6.23 | 16.96 | 44.84 |
| ω=15.7rad/s – f=2.5Hz | | | |
| G' [Pa] | 12.22 | 54.56 | 113.43 |
| G" [Pa] | 19.07 | 38.27 | 58.82 |

The data presented in Table 3 shows that with the increase in the molecular weight of the viscosupplement, the cross-linking power of the structure in the form of the $G_N^0$ parameter increases, the viscosity in the conditions of steady flow $\eta_0$, and the relaxation time $\tau_0$. The reciprocal of the relaxation time $\tau_0$ is the cross-linking density of the viscosupplement structure $\omega_0$, which means that Orthovisc has the highest cross-linking density - at its lowest numerical value, it means that cross-linking occurs every $3{,}18s^{-1}$, so it is more frequent than in Ostenil, for which cross-linking occurs every $40{,}75s^{-1}$. Thus, as the molecular weight increases, the cross-linking density of the viscosupplement structure also increases.

**Tab. 3.** The rheological parameters of the tested viscosupplements

| **Rheological parameters** | **OSTENIL** | **HYALUBRIX** | **ORTHOVISC** |
|---|---|---|---|
| $G_N^0$ [Pa] | 142.64 | 340.52 | 509.27 |
| $\eta_0$ [Pas] | 3.5 | 19.0 | 160.0 |
| $\zeta$ [nm] | 31.07 | 23.25 | 20.33 |
| $l_p$ [nm] | 15.54 | 11.63 | 10.17 |
| $l_e$ [nm] | 49.33 | 36.91 | 32.27 |
| $d_e$ [nm] | 295.2 | 220.9 | 193.1 |
| $\tau_0$ [s] | 0.025 | 0.056 | 0.314 |
| $\omega_0$ [$s^{-1}$] | 40.75 | 17.92 | 3.18 |
| $\alpha_e$ [-] | 3.175 | 3.175 | 3.175 |
| p [Å] | 0.452 | 0.338 | 0.296 |
| $\nu_s$ [1/ml] | $4.17 \cdot 10^{28}$ | $9.94 \cdot 10^{28}$ | $1.48 \cdot 10^{29}$ |

Moreover, the more the cross-linking density $\omega_0$ is shifted to the left, i.e. towards smaller numerical values, the greater the mobility of the network structure elements. This property of the network translates into its ability to damp mechanical vibrations associated with movement, and thus the work of the knee joint. In practice, this means that all elements of the network structure are involved in the damping of mechanical vibrations, ensuring proper and painless functioning of the joint, if such a viscosupplement was administered by intra-articular injection. Of the three viscosupplements tested, Orthovisc is a viscosupplement that ensures such behavior of the network. At the same time, with the increase in the molecular weight of the viscosupplement, it was observed that the values of the characteristic linear dimensions of the network, such as: the mesh size $\xi$, the persistence length $l_p$ and the entanglement length $l_e$ decrease. This is additionally confirmed by the high cross-linking power of the structure, which is again in favor of Orthoviscu. In the data presented in Table 3, it is particularly significant that the semi-elasticity in the form of the $\alpha$e coefficient has the same value for all three viscosupplements tested, regardless of their $l_p$ and $l_e$ length values. Already Zou and Larson [14] showed in their research that if the coefficient $\alpha_e$>2, then we are dealing with a network of

loosely entanglement chains and such a network is characteristic of these three viscosupplements, which was guaranteed already at the stage of their production. However, the number of entanglements is the highest for the viscosupplement with the highest molecular weight, i.e. for Orthoviscu, for which it is $1{,}48 \cdot 10^{29}$ entanglements/ml with the lowest packing length p equal to 0,296Å.

Differences in the structure of the analyzed viscosupplements are additionally presented in the form of the so-called reduced curves in the relationship $G'/G_N^0=f(\omega/\omega_0)$ and $G''/G_N^0=f(\omega/\omega_0)$ - Fig. 4. This allowed to assess the superposition of the obtained experimental curves and to show that the share of shaping elements is very diverse viscous and elastic properties of the tested medium.

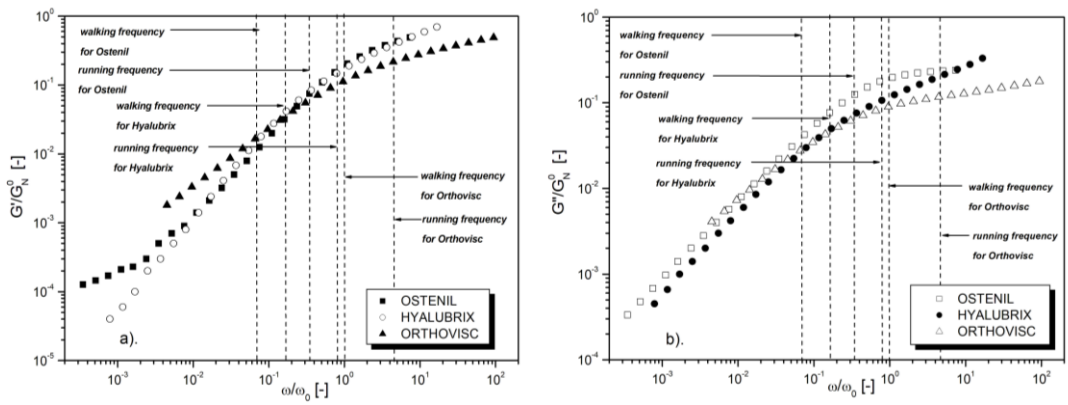


**Fig. 4.** Reduced curves of the tested viscosupplements.

The graph presented in Fig. 4a shows that both Ostenil and Hyalubrix form a similar type of structure in the scope of work of the knee joint, the elastic properties of these viscosupplements are not significantly differentiated - a full superposition of the reduced curves $G'/G_N^0=f(\omega/\omega_0)$ in this range. The lack of superposition of the reduced curves $G''/G_N^0=f(\omega/\omega_0)$ for these two viscosupplements can be seen in the graph in Fig. 4b, which proves the varied share of elements shaping the viscous properties. Orthovisc, in terms of the work of the knee joint, does not show any similarity in terms of the share of elements shaping the viscous and elastic

properties to Ostenil and Hyalubrix - because there is no superposition of the reduced curves $G'/G_N^0=f(\omega/\omega_0)$ and $G''/G_N^0=f(\omega/\omega_0)$ in this range. Thus, it creates a completely different type of structure than Ostenil and Hyalubrix.

## 4. Conclusions

The analysis of the rheological properties of three viscosupplements available on the pharmaceutical market - Ostenil, Hyalubrix and Orthoviscu - used in knee osteoarthritis showed that the molecular weight is responsible for these properties. The higher the molecular weight of the viscosupplement, the greater the power of the network, viscosity, crosslinking density of network and the number of entanglements of the viscosupplement. Although the tested viscosupplements had different characteristic linear dimensions of the network, all of them are characterized by a network of loosely entanglement chains that can form a similar or different type of structure. The obtained results may also indicate that in the case of significant advancement of knee osteoarthritis, Orthovisc turns out to be a more appropriate viscosupplement, due to the decisive advantage of elastic properties over viscous ones in the frequency range ω, covering the work of the knee joint. However, if the complaints related to the work of the knee joint are insignificant but noticeable and the patient wants to eliminate the discomfort, Hyalubrix and Ostenil seem to be good viscosupplements in the following order, which also ensure adequate lubrication of the knee joint.

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