Rheologic Properties of Some Current Hyaluronic Acid Products for Viscosupplimentation - New Trends for Amelioration

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Viscosupplementation (intra-articular supplementation of hyaluronic acid - HA) provides a useful alternative in treating osteo-arthritis patients and it is included in the professional guidelines for treatment of the disease in knee joint. Rheological investigations add an important element to viscosupplements characterization. In order to improve the hyaluronic acid concentration in currently commercial viscoelastic solution but also for targeted release on the clinical needs, we propose to use the magnetic nanoparticles functionalized with hyaluronic acid. In this paper, we analyze the rheological properties of some experimental viscoelastic solutions obtained after the modification of the commercial viscoelastic solution with a concentration of 40 mg/2mL HA (Arthrum) using different concentration of functionalized magnetic nanoparticles, using a Paar – Physica MC 301 Rheometer. The results indicate improved viscoelastic properties with increased HA concentration. Solutions with lower viscosity are much less effective in converting mechanical energy into elastic deformation and the effect being more visible with time.

Keywords: sodium hyaluronate, concentration, ferromagnetic nanoparticles, rheology

The treatment for osteoarthritis (OA) depends on the severity of the disease, the patient's age and the level of expected physical activity. A group that is difficult to treat consists in those patients which are suffering from moderate degenerative joint disease (as described on weight bearing radiographs with joint space narrowing F ≤ 50%) [1]; those patients are not yet candidates for surgery because they are often relatively young and wish to be active without problems such as chronic pain and drugs with associated side-effects. Viscosupplementation (intra-articular supplementation of hyaluronic acid - HA) provides a useful alternative in treating such patients and is included in the professional guidelines for treatment of the disease in several joints (knee, ankle, hip, shoulder). Hyaluronan (also referred to as hyaluronic acid or sodium hyaluronate) is a highly viscous polysaccharide found in the extracellular matrix, particularly in soft connective tissues and is a major component of the synovial fluid and of cartilage. HA in human synovial fluid has an average molecular weight of 3000 – 4000 kDa and a concentration of 2.5–4 mg/mL; its effects are closely linked to rheology. In addition to its elastic and viscous properties, the physical presence of HA supports its significant role in maintaining the rheological homeostasis of the synovial fluid in the joint, and also contributes to lubrication, shock absorption, hydration and nutrition for the articulation. HA in aqueous solutions is at low concentrations (< 1 mg/mL) as an extended random coil allowing free movement of the polymer chains. At high HA concentrations (> 1 mg/mL), a transient connecting network arises, where molecules interweave with each other and then separate after a period of time [2]. Hydrogen bonding and non-covalent intermolecular associations stabilize this network. The network properties of HA at high concentration affect the viscoelasticity of the solution, giving rise to enhanced viscosity and pronounced non-Newtonian behaviour, manifested by a higher extent of shear thinning. Also, similar effects have been noted as the molecular weight of HA in solution is increased for solutions of the same concentration [2].

A Cochrane meta-analysis of 76 trials (including 40 placebo-controlled trials of hylan) showed that viscosupplementation is effective in OA of the knee with beneficial effects on pain, function and patient global assessment, especially at the 5 to 13 week post-injection period when pain and function were improved from baseline by 28 - 54% and 9 - 32%, respectively [3]. Many current products are marketed with accent on the molecular weight class – as low and high. Short time data is available for high molecular weight compounds of hyaluronic acid – even there are some early clinical benefits, the molecules will eventually broke into low weight products with no late difference between the clinical effects. The viscous and elastic properties of HA solutions, in particular from synovial – articular fluid, have been studied since 1970's (and published by authors as E.A. Balazs since 1968) [4, 5]. Every decade brought new ways to modify hyaluronan so, current marketed products, came in a variety of derivatives, concentrations and degrees of cross-linking. As a result, the reported clinical results of intra-articular injections are quite difficult to be analyzed apart.

Experimental part

A series of experimental samples obtained after the modification of one current marketed products used in articular therapy, Arthrum® (40mg/ 2mL, molecular weight of more than 2400kDa), modified with functionalized magnetic nanoparticles, were analyzed from viscoelastic point of view using a Paar – Physica MC 301 Rheometer (cone - plate configuration, 40 mm diameter, at constant 20°C temperature). The technical approach involved also the investigation of the influence of ferromagnetic nanoparticles functionalized with HA. S-type magnetite nanoparticles were synthesized by co-precipitation process

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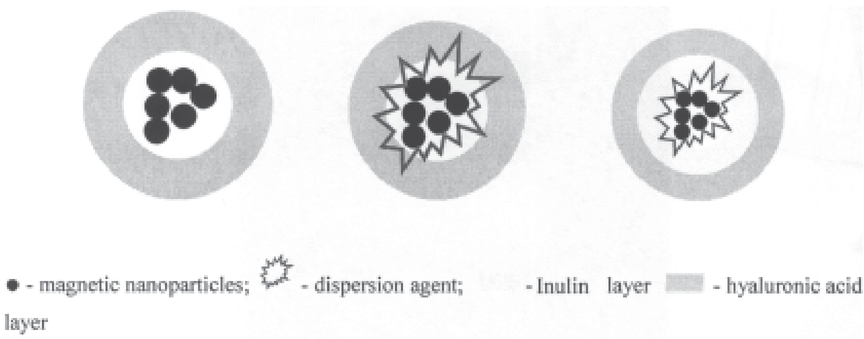

Fig. 1. Structure of the functionalized magnetic nanoparticles

Table 1
COMPOSITION AND CODIFICATION FOR THE EXPERIMENTAL FUNCTIONALIZED MAGNETIC NANOPARTICLES USED IN ORDER TO OBTAIN EXPERIMENTAL VISCOELASTIC SOLUTION FOR RHEOLOGICAL TESTS

Sample	Composition of the functionalized magnetic nanoparticles	Sample code - solutions prepared for rheological testing
1	NPM + HA	3N
2	(NPM + TMOH) + HA	3J
3	((NPM + TMOH) + INULIN) + HA	3A
4	NPM/10 + HA	4N
5	(NPM + TMOH)/10 + HA	4J
6	((NPM + TMOH)/10 + INULIN) + HA	4A
7	NPM/50 + HA	5N
8	(NPM + TMOH)/50 + HA	5J
9	((NPM + TMOH)/50 + INULIN) + HA	5A
10	NPM/100 + HA	6N
11	(NPM + TMOH)/100 + HA	6J
12	((NPM + TMOH)/100 + INULIN) + HA	6A

NPM – magnetic nanoparticles; TMOH – dispersion agent (tetramethylamonium hydroxide);

INULIN – coating agent used; HA – hyaluronic acid.

/10, /50, /100 – 10, 50, 100 times diluted regarding the synthesis concentration

in alkaline catalysis by Massart adaptation process and covered by a "layer by layer" technique [6]. The magnetic nanoparticles of iron oxide dispersible in solution have been obtained using ferric chloride (II), ferric chloride (III), 25% NH_4OH and 2M hydrochloric acid as starting materials. The dispersion of the magnetic nanoparticles of iron oxide was prepared by the classical method of co-precipitation, when adding a concentrated alkaline solution (NH_4OH 25%) into a mixture of iron salts in a molar ratio of $FeCl_2$: $FeCl_3$=1: 2. The magnetic nanoparticles of iron oxide were initially diluted to the desired concentrations and then were peptiser with tetramethylamonium hydroxide (TMOH) and peptiser with TMOH and covered with inulin, respectively. Finally, all samples were functionalized with hyaluronic acid in a volume ratio of 1:1. For a better evaluation, the solution of the magnetic nanoparticles was diluted in different concentrations.

The composition of the experimental functionalized magnetic nanoparticles is shown in table 1 and a schematic representation of these is shown in figure 1. The samples are gel-like and they were studied in oscillation mode by logging the answer to variable frequencies and strains. The strain-dependent response was followed as a sign of the viscoelastic response; this is why the cone/plate system was used and, also, some precautions of manipulation were taken in order to exclude the formation of a dry HA film on the analyzed surface.

Results and discussions

The viscoelastic response is presented in the figure 2, where the elastic and viscous modules are plotted against the function of frequency. Both modules vary in time and get stabilized around G'=150Pa and G"= 400Pa which denotes measurements close to physiologic values [4]. Balasz described an interesting aspect of the viscoelastic properties of the HA as proportion between elasticity and viscosity (Elasticity % =G'/ G'+ G" X100) [5].

Based on the diagram showed in the figure 2, the rheological testing of commercial viscoelastic solutions indicated a certain sensibility at air contact of these substances. Also, the modification of the rheological properties is in relation with the time (hysteresis on the returning curve). These are normal curves, comparable to current published data on the HA properties [4, 5].

In order to investigate the influence of functionalized ferromagnetic nanoparticles effect on the viscoelastic properties, we analyze following the same procedure the experimental solutions obtained after the loading the commercial HA based-solution with S-type magnetite nanoparticles at different concentrations. The results of rheological testing are shown in figures 3-5.

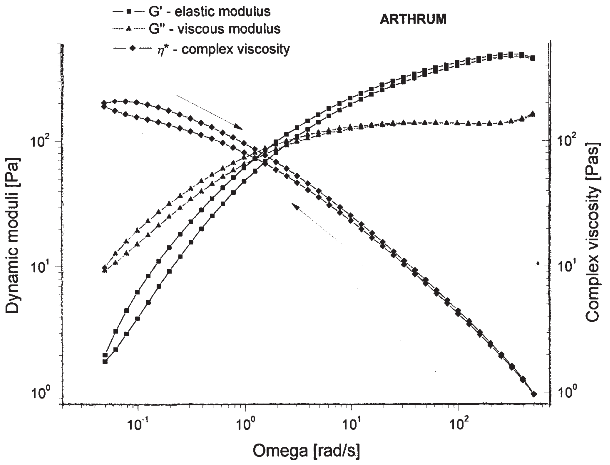

Fig. 2. Experimental diagram of rheological testing in the case of commercial HA based-solutions (ARTHRUM)

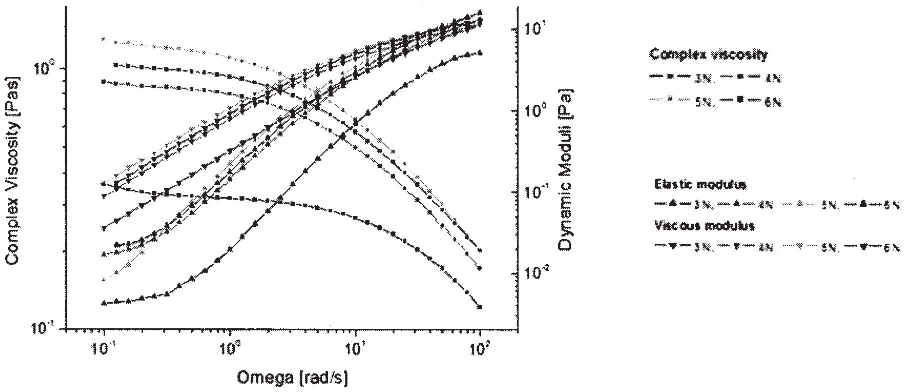

Fig. 3. Rheological test results for experimental solutions loaded with functionalized magnetic nanoparticles type (NPM+HA) in different concentration (3N- magnetic nanoparticles not diluted; 4N- magnetic nanoparticles 10 times diluted; 5N- magnetic nanoparticles 50 times diluted; 6N- magnetic nanoparticles 100 times diluted)

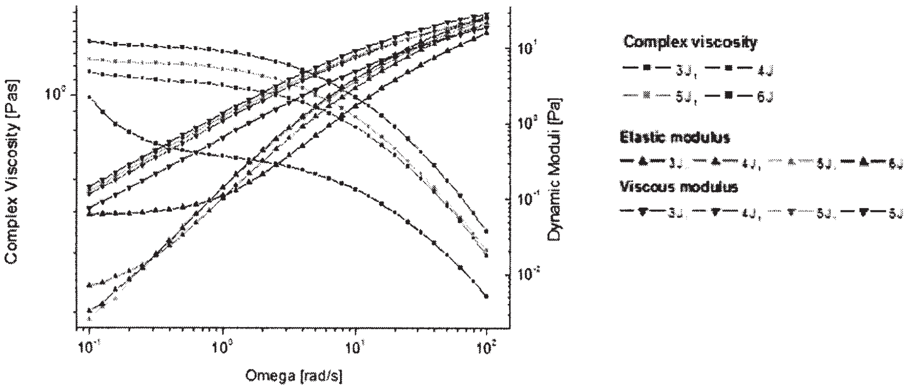

Fig. 4. Rheological test results for experimental solutions loaded with functionalized magnetic nanoparticles type (NPM + TMOH) + HA, in different concentration (3J-magnetic nanoparticles not diluted; 4J- magnetic nanoparticles 10 times diluted; 5J- magnetic nanoparticles 50 times diluted; 6J- magnetic nanoparticles 100 times diluted)

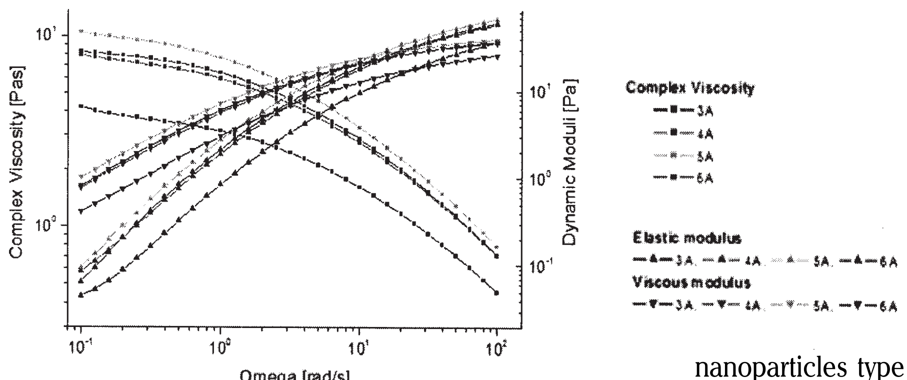

Fig. 5. Rheological test results for experimental solutions loaded with functionalized magnetic nanoparticles type ((NPM + TMOH) + INULIN) + HA, in different concentration (3A-magnetic nanoparticles not diluted; 4A- magnetic nanoparticles 10 times diluted; 5A- magnetic nanoparticles 50 times diluted; 6A- magnetic nanoparticles 100 times diluted)

The rheological results obtained for experimental solutions loaded with functionalized magnetic nanoparticles show that the use of functionalized magnetic nanoparticles induces a decrease of viscosity comparing with the original HA based viscoelastic solution. From the functionalized magnetic nanoparticles concentration point of view, solutions type 50 times and 100 times diluted show the better rheological properties. Because the experimental solutions not diluted have the lower properties in all cases, we consider that these solutions are not suitable for this kind of applications. Also, functionalized magnetic nanoparticles type (NPM + TMOH) + HA and ((NPM + TMOH) + INULIN) + HA appear to have optimal properties from rheological point of view.

These investigations add an important element to viscosupplements characterization, showing that the viscoelasticity could be improved by modulating the concentration and type of HA nanoparticle additive. Thus, the injectability of viscosupplements may be enhanced while maintaining high elasticity.

The experimental results show consistent lower (order 10 X) results for dynamic viscoelastic properties as well as the less important effect due to functionalized magnetic nanoparticles utilization (constant between the diagrams of the three products).

Solutions with lower viscosity are much less effective in converting mechanical energy into elastic deformation – the effect being more visible with time. It is the same effect observed in experimental conditions by Fam [7], where the higher molecular weight of HA lead to higher viscosities, closer to physiological conditions (described as shear thinning in steady-shear flow). It is a viscoelastic requested behaviour which makes the purpose of addition of viscosupplements to the synovial fluid.

The viscoelastic properties of synovial fluid, which are well suited to the joint, were first investigated by Ogston, according to Moreland [8]. The efficacy of intra-articular HA injections may be determined, in part, on the viscoelastic properties of the HA injected, this being a function of its molecular weight [8]. Some results from large animal models of OA indicate that HAs with molecular weight between 500-1000 KDa are more effective than high molecular weight HAs in reducing synovial inflammation and for restoration of the rheological properties of synovial fluid [9]. Other studies [10, 11] suggest that HA with high molecular weight may deliver better clinical benefits but the heterogeneity of these trials somehow dilute the results. We must be careful in assuming that the pain relief observed in human osteoarthritic knee joints after injection of HA preparations is directly comparable with viscoelastic properties. Recent studies confirm a local anti-inflammatory as well as a pain relieving effect, probably mediated through nociceptive receptors inhibition [12].

The viscoelasticity data obtained, demonstrate that viscoelastic solutions exhibit a HA gel–like behaviour, with constant response independent on frequency. Even after conditioning with ferromagnetic nanoparticles only slight variation in the response was observed, making this method a valuable improvement technique with potential applications in clinical practice. Experiments suggest that the commercially available products have a combination of viscoelastic properties comparable with literature results for synovial fluid [13].

Regarding to the HA molecular weight, Dahl et al. [14] asserted that normal synovial fluid HA molecular weight ($7x10^6$Da) is significantly higher than rheumatoid arthritis synovial fluid HA molecular weight ($4.8x\ 10^6$ Da). Another study made by Schurz and Ribitisch [15] reported that the molecular weight of HA in normal synovial fluid is 10^7 Da, while it is reduced to 10^6 Da in rheumatoid and osteoarthritic synovial fluid. The deterioration of rheological properties in diseased synovial fluids is likely related to the decrease in molecular weight of HA. The decrease in concentration and molecular weight of HA could be responsible for the altered in viscoelastic properties of pathological synovial fluid [16]. Mazzucco et al. [17] have investigated rheological properties of fluid from patients undergoing primary total knee arthroplasty and revision arthroplasty. The general behaviour of joint fluid samples was shear thinning. At low frequencies, loss modulus was found to dominate over shear modulus and both modules increased at higher frequencies. They also observed that crossover frequency during testing increased in arthroplasty fluids in comparison to normal synovial fluid.

In addition to these purely mechanical effects due to the viscosity of the products (restoration of viscoelastic properties of the synovial fluid: lubrication, elasticity), intra-articular HA viscosupplementation is thought to provide a range of biological actions including anti-inflammatory, analgesic and chondroprotective effects. The term "viscoinduction" has been created to describe the phenomenon of the clinical benefits of HA exceeding that of a physical lubricant/cushioning effect alone. Visco-induction ensures that clinical efficacy is maintained for several months despite the half-life of intra-articular HA being only a few days. It has been suggested that exogenous HA induces endogenous HA synthesis, possibly stimulating the regenerative process within the joint [18]. Indeed, *in vitro*, in studies of synoviocytes from joints of subjects with OA, exogenous HA was associated with new synthesis of HA so, potentially, the more HA concentration the better.

Conclusions

The present study confirmed the effect of the molecular weight of HA products on the viscoelastic properties. Comparative data suggest that increased concentrations and higher molecular weight of HA new viscoelastic solutions can lead to better viscoelastic properties and potentially better clinical results.

In addition, the study confirm that the functionalized magnetic nanoparticles can be added into HA polymer solutions in order to add more HA directly to the targeted specific articular areas without changing the viscoelastic properties. Future studies must be made in order to determine the influence of the functionalized magnetic nanoparticles concentration on the biocompatibility properties.

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