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Amphiphilic hyaluronic acid derivatives toward the design of micelles for the sustained delivery of hydrophobic drugs

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ABSTRACT

The idea of this study was to combine hyaluronic acid (HA) viscosupplementation and a local/controlled delivery of a hydrophobic anti-inflammatory drug. To this aim, we investigated the ability of an octenyl succinic anhydride (OSA) modified HA (OSA–HA), to act as a solubility enhancer and as a platform for slow release of hydrophobic drug(s). This novel HA derivative could act as a viscosupplementation agent and, for this reason, a rheological study was conducted along with calorimetric analysis. Differential scanning calorimetry (DSC) results revealed that the ability of HA to sequester water is enhanced by the introduction of lipophilic functions within HA molecules, resulting in a decrease of the fraction of free water able to freeze compared to the unmodified HA. Moreover, OSA–HA solutions appear to be an appropriate tool to be used in viscosupplentation therapy owing to their suitable viscoelastic features. Our results indicate that OSA–HA is able to self-assemble into micelles, load a hydrophobic drug and release the active molecule with controlled kinetics. In particular, the analysis of release profiles showed that, in all cases, drug diffusion into the gel is faster compared to gel/drug dissolution, being the dissolution contribution more relevant as the OSA–HA concentration increases.

1. Introduction

Amphiphilic copolymers, once in an aqueous solutions, can self-assemble into micelles owing to a high solubility difference between the hydrophilic and the hydrophobic segments (Cho et al., 2004; Pravata et al., 2008; van Hest, Delnoye, Baars, van Genderen, & Meijer, 1995). These materials can be used as drug delivery systems due to their potential high drug-loading capacity and possibility to load of a variety of drugs with diverse features into the hydrophobic core of the micelles to treat several diseases (Harada & Kataoka, 2006; Li et al., 2011).

Hyaluronic acid (HA) is a natural mucoadhesive polysaccharide, a main constituent of the extracellular matrix of connective tissues, which displays biodegradability and biocompatibility properties. It is composed of alternating D-glucuronic acid (GlcA) and *N*-acetyl-D-glucosamine (GlcNAc) repeating units linked together *via* β-(1,4) and β-(1,3) glycosidic junctions (Almond, 2007). HA is a major ligand of the adhesion receptor CD44, which is also expressed at the surface of chondrocytes (Savani et al., 2001). In solution, HA displays an extraordinary capability to retain water, and behaves as an expanded random coil occupying a large hydrodynamic volume. This causes neighboring molecules to overlap, thus forming the transient network structure with a marked viscoelasticity (Fusco et al., 2007). These compelling features make HA a ductile and versatile biopolymer used for several applications in the biomedical field. For instance, HA is commonly used in ophthalmology for the dry eye treatment and as a viscoelastic device in ophthalmologic surgery owing to the ability to form highly viscous solutions even at low concentrations (Borzacchiello et al., 2007; Maltese et al., 2006; Mayol, Quaglia, Borzacchiello, Ambrosio, & La Rotonda, 2008; Mayol et al., 2011). Moreover, intra-articular injection of HA (viscosupplementation) is one of the most used therapies for the treatment of knee osteoarthritis, its goal being to restore the elastic and viscous properties of the synovial fluid (SF) (Borzacchiello, Mayol, Schiavinato, & Ambrosio, 2010; Fakhari & Berkland, 2013). Pathologic alterations occurring in joint diseases, indeed, lead to a decrease of HA molecular weight and concentration in SF, and, consequently, a decline in SF viscoelastic properties (Gomez & Thurston, 1993). The beneficial improvements in SF viscoelastic properties and joint functions derive from both the intrinsic viscoelastic properties of HA and its potential stimulatory effect on the synthesis of high molecular weight HA by synoviocytes (Ghosh

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& Guidolin, 2002). Currently, several viscosupplemention products based on HA are commercially available and an important research effort has been devoted to chemically modify HA, through coupling or crosslinking reactions, preserving its biocompatibility. Indeed, native HA, once *in vivo*, undergoes a rapid degradation due to its sensitivity to hyaluronidase and high hydrophilicity. To avoid these drawbacks, among the possible chemical modification, various pendant chains have been grafted onto HA to increase its residence time and viscoelastic features (Fakhari & Berkland, 2013). In particular, long alkyl chain amine pendant chains have been used to obtain amphiphilic HA derivatives (Pelletier et al., 2001).

Moreover, to stimulate the production of healthy HA and facilitate the homeostasis in the joint region, oral administration of anti-inflammatory drugs is often necessary in combination, or as an alternative to HA viscosupplementation. However, the prolonged use of such drugs can cause important systemic adverse effects and, therefore, intra-articular injections of anti-inflammatory drug/s are often practiced (Gerwin, Hops, & Lucke, 2006). However, conventional dosage forms do not provide a prolonged release of the drug, thus leading to the necessity of frequent injections which can cause inflammations and lower patient compliance. To avoid these drawbacks, a local/sustained delivery of anti-inflammatory drug would be desirable.

In this frame, the idea of this study was to design a delivery system able to prolong the release of an anti-inflammatory drug into the joint cavity and, at the same time, able to restore the viscoelastic features of pathologic SF. To this aim, we designed an amphiphilic HA derivative able to self-assemble into micelles, load a hydrophobic drug and release the active molecule *in situ* with controlled kinetics, possessing suitable viscoelastic features to act as a viscosupplementation agent.

Recently, we have synthesized a new amphiphilic HA derivative and, in particular, an octenyl succinic anhydride (OSA) modified HA, through a simple reaction in an aqueous medium, which involves exclusively HA hydroxyl groups (Eenschooten, Guillaumie, Kontogeorgis, Stenby, & Schwach-Abdellaoui, 2010). The resulting derivatives present great potential since, first of all, no organic solvents are used in the reaction, thus avoiding environmental issues and allowing the upscalability of the preparation method; secondly, the reaction does not involve HA carboxyl groups which can neutralize negative charges along the polymer backbone. Actually, it is important to maintain the charge distribution that can confer an electrostatically-induced stability in the perspective of using the self-assembling properties of these derivatives toward the design of micelles for the drug delivery of poorly soluble drugs.

Here, the thermodynamic and the rheological properties of OSA–HA solutions at different concentrations were evaluated. Moreover, we investigated the ability of this novel derivative to self-assemble into micelles and to act as a solubility enhancer and as a modulator of release kinetics of triamcinolone acetonide (TA), a commonly used hydrophobic anti-inflammatory drug to treat joint pathologies.

2. Materials and methods

2.1. Materials

Hyaluronic acid (HA) with a weight-average molecular weight (MW_w) of 850,000 Da (HA850) was provided by Novozymes Biopharma (Denmark). Octenyl succinic anhydride (OSA; MW: 210.27 Da, purity P97%, mixture of *cis* and *trans* isomers) salts and HPLC-grade solvents were obtained from Sigma-Aldrich (USA). Distilled water from Milli-Q (Millipore, USA) was used, and triamcinolone acenotide (TA) was obtained from Farmalabor (Italy). OSA–HA derivatives, with a 6% degree of substitution, were provided by Novozymes. They were synthetized as previously reported (Eenschooten et al., 2010) and briefly summarized in Section 2.2.1.

2.2. Methods

2.2.1. Preparation of hyaluronic acid derivative

OSA-modified derivative at 6% degree of substitution was prepared by dissolving HA850 overnight at room temperature in de-ionized water. $NaHCO_3$ was added to the solution and mixed at room temperature (RT) for 1 h. Afterwards, the pH of the HA solution was adjusted to 8.5 with NaOH (0.5 M). OSA was added dropwise to the alkaline HA solution under vigorous stirring. The reaction medium was mixed at RT and the resulting crude product was dialysed against milli-Q water (7.5 L) using molecular porous membrane tubing (Spectra/Por®4, MWCO 12,000–14,000 Da; Spectrum Laboratories, Rancho Dominguez, California, United States) at 4 °C. The purified OSA–HA was finally freeze-dried. OSA–HA solutions were prepared by dissolving OSA–HA powder in bidistilled water or phosphate buffer saline (PBS; 120 mM NaCl, 2.7 mM KCl, 10 mM phosphate salts; pH 7.4) in the 0.1–5%, w/v concentration range.

2.2.2. Differential scanning calorimetry (DSC)

Thermoanalytical tests were carried out on solutions of HA and OSA–HA to study how the chemical modification could influence the interactions between polysaccharide chains and water. The heat involved in the solid-to-liquid phase transition of water within HA solutions was determined by a differential scanning calorimeter (DSC; DSC Q20, TA Instruments, U.S.A.), calibrated with a pure indium standard. The samples were prepared in flasks by simple stirring overnight at room temperature, until transparent and homogeneous solutions were obtained. The samples were placed in hermetically sealed aluminum pans, equilibrated at −30 °C and heated to 20 °C at 2 °C/min. Measurements were carried out under an inert nitrogen atmosphere, purged at a flow rate of 50 ml/min. The heat evolved by the fusion of water (W/g) within the solutions was calculated from the recorded DSC thermograms by integrating the endothermic melting peaks and normalized with respect to the actual content of water.

2.2.3. Morphology and size distribution of OSA–HA micelles

The morphology of OSA–HA micelles were investigated by transmission electron microscopy (TEM, FEI Tecnai G12 Spirit Twin) with emission source LaB6 (120 kV, spotsize 1) using 400 mesh carbon-coated copper grids at RT.

The OSA–HA solutions at 1 mg/ml, in water and PBS, were prepared. The carbon-coated copper grid was immersed in OSA–HA solution and, after the drying phase, the grid was placed on a rod holder for the TEM characterization. Three grids *per* OSA–HA solution were prepared and a minimum of four micrographs *per* grid were acquired.

Micelle mean size and size distribution were determined by laser light scattering (LS, ZetaSizer Nano ZS, Malvern Instruments, Malvern, UK) on a ultra-diluted suspensions in water (12 runs each sample).

2.2.4. Rheological properties

Small amplitude oscillatory shear tests were performed to evaluate the time-dependent response of OSA–HA and their linear viscoelastic properties. The frequency was in the range from 0.01 to 10 Hz. The measurements were carried out through a strain controlled rotational rheometer (Gemini Bohlin Instruments, UK), using a parallel plate geometry (PP30 cell). The tests were carried out at the controlled temperatures of 25 and 37 °C using a thermostatic bath.

In a dynamic test the material is subjected to a sinusoidal shear strain:

$$\gamma = \gamma_0 \sin(\omega t) \quad (1)$$

where γ_0 is the shear strain amplitude, ω is the oscillation frequency (which can be also expressed as $2\pi f$, where f is the frequency in Hz) and t the time. The mechanical response, expressed as shear stress τ of viscoelastic materials, is intermediate between an ideal pure elastic solid (obeying to the Hooke's law) and an ideal pure viscous fluid (obeying to the Newton's law) and, therefore, it is out of phase with respect to the imposed deformation as expressed by:

$$\tau = G'(\omega)\gamma_0 \sin(\omega t) + G''(\omega)\gamma_0 \cos(\omega t) \quad (2)$$

where $G'(\omega)$ is the storage or elastic modulus and $G''(\omega)$ is the loss or viscous modulus. G' gives information about the elasticity or the energy stored in the material during deformation, whereas G'' describes the viscous component or the energy dissipated as heat. Since viscoelastic properties strongly depend upon the time-scale of observation, the dependence of G' and G'' upon the frequency, the so called mechanical spectrum will be reported will be reported.

In order to identify the linear viscoelastic response range of the materials, preliminary strain sweep tests were performed on the samples, at the oscillation frequency of 1 Hz. The tests were repeated at least three times on each sample.

2.2.5. Release kinetics and dissolution tests

The solutions for the dissolution tests and drug release kinetics were prepared by adding OSA–HA, at 1, 2.5 and 5 mg/ml, to a suspension of TA in bidistilled water (100 μg/ml). A suspension of TA in bidistilled water at the same concentration (100 μg/ml) was used as control. In all the solutions the excess of TA was present and visible at the bottom of the vials.

Dissolution tests were performed by using the prepared solutions. At scheduled time intervals (2, 4, 6, 24, 48 h), the solutions were centrifuged (12000 rpm for 15 min) and supernatant analyzed by means of reversed-phase high-performance liquid chromatography (RP-HPLC).

As for the *in vitro* release kinetics of TA from OSA–HA-based gels, they were evaluated by immersing dialysis membranes (spectra/por biotech cellular ester, molecular cut off: 12 kDa) loaded with 5 ml of gels in 80 ml of PBS at 37 °C. At scheduled time intervals, the release medium was withdrawn, replaced with the same volume of fresh medium, and analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC). The chromatograph was equipped with a HPLC LC-10AD pump (Shimadzu, Milano, Italy), a 7725i injection valve (Rheodyne), a SPV-10A UV–vis detector (Shimadzu) set at the wavelength of 254 nm and a C-R6A integrator (Shimadzu). RP-HPLC experiments were carried out using a Luna C18 (150 × 4.6 mm) column (Phenomenex, Klwid, USA). The mobile phase was a mixture of water and acetonitrile (50:50 v/v). The flow rate was set at 1 ml/min, and the stop time was 10 min. Results were averaged on three independent batches and the experiments were run in triplicate.

Release data were fitted to the following semiempirical equations, adapted from methods reported in literature and describing drug release from HA-based systems as the result of a dissolutive and a Fickian diffusional mechanisms (Nastruzzi, Esposito, Cortesi, Gambari, & Menegatti, 1994; Peppas, 1985). The dissolutive contribution is expressed by:

$$F_{diss} = F_{diss,\infty} k_{diss} t^{0.5} \quad (3)$$

where F_{diss} and $F_{diss,\infty}$ are the drug fractions released by dissolution at time t and after an infinite time, respectively, k_{diss} is the kinetic dissolution parameter. The diffusional contribution to drug release is given by:

$$F_{diff} = (1 - F_{diss,\infty})(1 - \exp(-k_{diff} t)) \quad (4)$$

Here F_{diff} is the drug fraction released due to diffusion at time t and k_{diff} is the kinetic diffusionl parameter. The overall released fraction is given by the sum of diffusion and dissolution contributions, *i.e.*:

$$F = F_{diff} + F_{diff} = F_{diss,\infty} k_{diss} t^{0.5} + (1 - F_{diss,\infty})(1 - \exp(-k_{diff} t)) \quad (5)$$

3. Results and discussion

An amphiphilic HA derivative based on the reaction between HA and OSA in mildly alkaline aqueous media was previously developed through a novel and easily upscalable modification method (Eenschooten et al., 2010) (Fig. 1). The obtained polymer can be regarded as a fishbone-like macromolecule consisting of a linear backbone carrying randomly distributed hydrophobic octenyl succinate (OS) side groups with a 6% degree of substitution (DS).

3.1. Differential scanning calorimetry (DSC)

Fig. 2 shows DSC thermograms of water, HA and OSA–HA (DS = 6%) solutions. As shown in the figure, in all cases a single melting peak, caused by the fusion of free water was observed. Melting temperature (T_m) was here defined as the temperature at which the DSC curve shifts toward the endothermic direction. In particular, T_m obtained with HA and OSA–HA solutions were found to be lower than that of pure water. The heat of fusion was 335.9, 320.2 and 302.8 J/g for water, HA and OSA–HA, respectively, as reported in Table 1.

The decrease of T_m in the case of HA and OSA–HA solutions was associated to the occurrence of intermolecular interactions between HA and water. Likewise, the reduction of peak areas in the presence of HA, and hence of the melting enthalpies, could also be ascribed to the interactions of HA chains with water. It is well known that a certain number of water molecules are restrained in the junction zone where the polysaccharide chains interact among

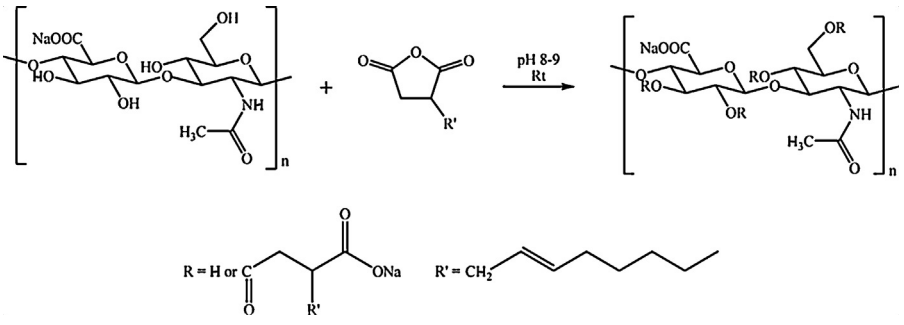

Fig. 1. Scheme of chemical modification of HA with OSA.

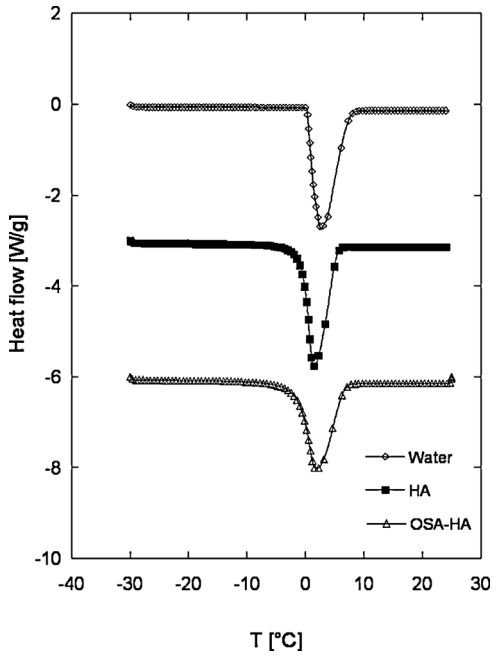

Fig. 2. Representative endotherms for pure water, unsubstituted HA, OSA–HA (DS = 4.6%). Exotherm is oriented upwards.

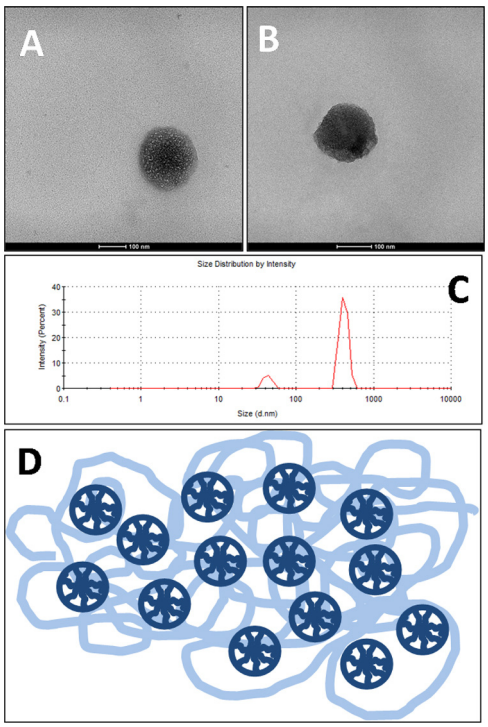

Fig. 3. Selected TEM images of OSA–HA micelles at 1 mg/ml in water (A) and PBS (B); LS results of OSA–HA micelles (C); graphical representation of the hypnotized structure of OSA–HA micelles (D).

each other to form a gel network structure, thus reducing the amount of free water available for freezing (Hatakeyama, Quinn, & Hatakeyama, 1996). In our case, both T_m and melting enthalpy of OSA–HA were found to be lower compared to unsubstituted HA. This result can indicate that the ability of HA to sequester water seems to be enhanced due the introduction of lipophilic functions within HA molecules, thus resulting in a further decrease of the fraction of free water able to freeze compared to the unmodified HA.

3.2. Morphology and size distribution of OSA–HA micelles

Fig. 3 summarizes the results of morphological and size distribution analysis of OSA–HA micelles. In particular, in Fig. 3A and B, selected TEM images of OSA–HA micelles at 1 mg/ml, in water and PBS respectively, are shown. As it can be seen, micelles are spherical objects with diameters around 100 nm in both water and PBS. Fig. 3C presents the size distribution of OSA–HA micelles obtained by LS measurements. This analysis evidenced the presence of a somewhat polydisperse population of objects with a mean diameter of about 400 nm. The discrepancy between the results of these techniques could be due to micelle aggregation. It is, indeed, likely to occur a dispersion of spherical hydrophobic domains, formed by the pendant OS groups, surrounded by a hydrophilic matrix of unmodified HA chains mutually interacting trough intra- and intermolecular hydrogen bonds. This hypothesis of micelle aggregation is also suggested by the difference in the molecular weight of HA chains and OS side groups. As a consequence, through TEM analysis it is possible to directly obtain a single micelle picture which appears spherical with a diameter in the order of 100 nm. In a LS experiments, instead, the Brownian motion of micelles in suspension causes the scattering of a laser light, at different intensities, which are correlated to particle size trough the Stokes–Einstein relationship. In this latter case, the laser light can be scattered by a single micelle or by a group of aggregated micelles, as previously described, thus giving higher apparent mean diameter values of the micelles. A schematic representation of the possible organization of the OSA–HA micellar system is depicted in Fig. 3D. Analogous results were obtained for the low molecular weight OSA–HA derivative, as previously reported (Eenschooten et al., 2012).

Table 1
Melting enthalpies and temperatures of water, HA and OSA–HA.

	ΔH_m (J/g)	T_m (°C)
Water	335.9 ± 2.7	0.21 ± 0.28
HA (DS = 0)	320.2 ± 5.9	−5.88 ± 1.07
OSA–HA (DS = 6%)	302.8 ± 9.2	−7.39 ± 1.05

3.3. Rheological properties

In the perspective of using the novel OSA derivatives as viscosupplementation products, the rheological features assume a crucial role since they must properly restore the biomechanical functions of the normal SF. To this aim, the rheological properties of unmodified and modified HA solutions were studied to evaluate the effect of the chemical modification, concentration and ionic strength of the dissolving medium.

In Fig. 4 the elastic and the viscous moduli curves as a function of the oscillation frequency (mechanical spectra) of HA and OSA–HA solutions at 50 mg/ml in PBS and distilled water are reported. Both HA and OSA–HA solutions behave as entangled solutions; indeed they are namely viscous at low frequency ($G'' > G'$) and prevalently elastic at high frequencies ($G' > G''$); the limit between the two regions is represented by the crossover frequency. These observations can be explained by consideration of the following. At low frequencies, the molecular chains can release stress by disentanglement and molecular rearrangement during the period of oscillation, and hence, the solution shows viscous behavior ($G'' > G'$). At high frequencies, however, molecular chains cannot disentangle during this short period of oscillation, and therefore, they behave as a temporarily cross-linked network, and the elastic behavior ($G' > G''$) is prevalent. Furthermore, from a quantitative point of view, both the viscoelastic parameters of OSA–HA solutions are lower than the

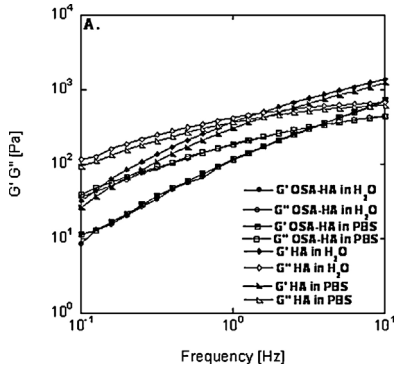

Fig. 4. Mechanical spectra of HA and OSA–HA solutions at 50 mg/ml in water and PBS.

corresponding values of the unmodified HA solutions. In particular, at 1 Hz, G' ad G'' values, at 50 mg/ml in water, decreases from 369 to 111 Pa and from 417 to 181 Pa respectively, and the cross over frequency increases from ~1 Hz to ~4 Hz. This result can be explained taking into account that the chemical modification leads to a shrinkage of the macromolecular coils which, in turn, reduce the probability of intermolecular interactions with a consequent

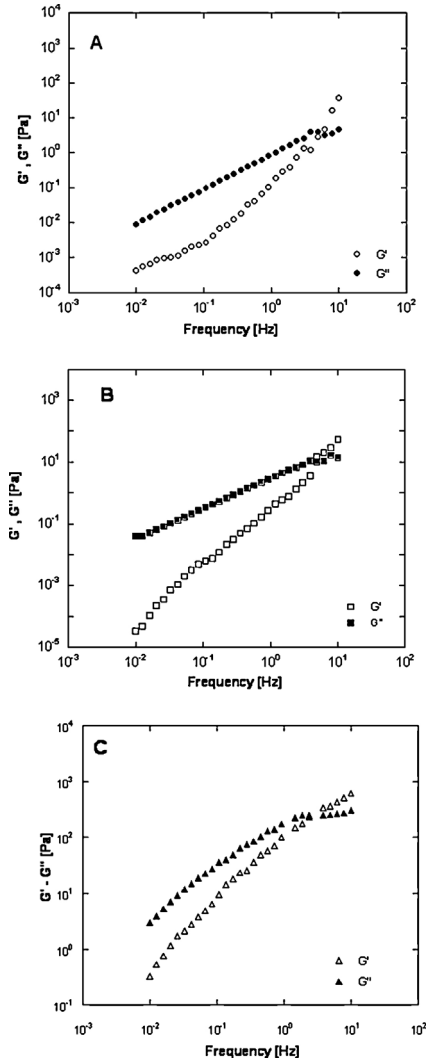

Fig. 5. Mechanical spectra of OSA–HA solutions at 10 mg/ml (A), 25 mg/ml (B) and 50 mg/ml (C).

Table 2
Viscoelastic parameters of OSA–HA solutions at $f = 2$ Hz.

HA_{osa}(mg/ml)	G′ (Pa)	G″ (Pa)
10	0.72	2.14
25	1.34	6.57
50	249	234

weakening of the polymeric network which become prevalently elastic at higher frequency.

Moreover, these results highlighted that ionic strength does not affect the rheological properties of HA and OSA/HA solutions. Indeed, the mechanical spectra in PBS and water were found to be identical.

Fig. 5A–C reports the mechanical spectra of OSA–HA solutions at 10 mg/ml, 25 mg/ml and 50 mg/ml and in Table 2 the viscoelastic parameters of OSA–HA solutions at $f = 2$ Hz are shown. It can be noticed that the increase of OSA–HA concentration leads to an increase of both the elastic and the viscous moduli. In particular, the values of both viscoelastic parameters increase of one order of magnitude by passing from 10 mg/ml to 25 mg/ml and of two orders of magnitude by passing from 25 to 50 mg/ml. This result can indicate that the increase of OSA–HA concentration leads to an enhanced organization of the polymer network.

The OSA–HA solutions exhibit a rheological behavior similar to the human synovial fluid, that is viscous at low frequencies and prevalently elastic at high frequencies and characterized by the presence of crossover frequency (Balazs, 2004).

Furthermore, the OSA–HA derivatives show higher values of both viscous and elastic moduli than those of healthy human synovial fluid; in particular in the case of the OSA–HA solutions at a concentration of 50 mg/ml, G' and G'' reach the values of 249 and 234 Pa, respectively.

The rheological features of OSA–HA solutions are particularly attractive for viscosupplementation applications aimed at restoration of the viscoelasticity of diseased SF. Several products are currently used in viscosupplementation which differ in HA source, molecular weight, concentration and chemical modification thus resulting in a wide array of rheological behaviors from both qualitative and quantitative standpoints. For example, at a frequency of 2 Hz, HYALGAN® is characterized by G' and G'' of respectively 0.1 and 0.8 Pa, HYADD4, a derivative of HA, presents an elastic modulus of 40 Pa and a viscous modulus of 10 Pa, and SYNVISC HYLAN G-F20®, made of cross-linked HA, shows the elastic and viscous moduli values of about 98 Pa and 20 Pa, respectively (Altman & Moskowitz, 1998; Borzacchiello et al., 2010; Petrella, DiSilvestro, & Hildebrand, 2002). It can be noticed that OSA–HA solutions, at a concentration of 50 mg/ml, show values of G' higher than those of viscosupplementation products currently used in clinical practice. The prevalent elastic character of an HA-based viscosupplementation products is not a problem being desirable in osteoarthritis applications. This is not only because of its capability to reduce the mechanical energy applied on the cartilage, but also because HA derivatives, with high elasticity, show an analgesic ability. It has been reported that substances with enhanced elastic characteristics reduce the effect of nociceptive stimulus on medial articular nerve activity and decrease the sensory response to passive movements of inflamed knee joints. Hypothetically, this positive effect is due to their capability to absorb a significant part of the mechanical energy of the stimulus, thus reducing the transmission to the mechano-transduction apparatus where pain signal originates.

3.4. Dissolution and release tests

In Fig. 6 the results of the dissolution tests are reported as the concentration of TA found into the supernatant of the solutions

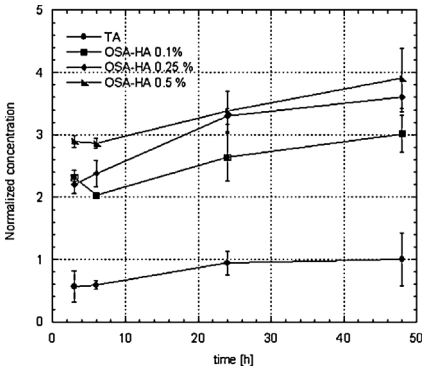

Fig. 6. Normalized solubilization profiles of TA.

prepared with different amount of OSA–HA (see Section 2.2) normalized to the water solubility of TA as a function of time. As it can be seen from the figure, after 48 h all the solutions reach an equilibrium solubility value. In particular, the value of TA solubility in water was found to be 18 μg/ml according to literature data (Miro et al., 2013). In the presence of OSA–HA in the solutions the TA concentration in the supernatant was found to increase as a function of OSA–HA concentration. In detail, the measured TA concentration was 4-fold higher compared to TA saturation concentration in water when OSA–HA concentration is 0.5%w/v. These results demonstrate the inclusion capability of OSA–HA toward TA.

Experimental and simulated *in vitro* fractional release profiles of TA from OSA–HA platforms in phosphate buffer are shown in Fig. 7. The release of the loaded TA could be sustained for at least 7 days. As shown in the graphs reported in Fig. 7, a 24 h-burst effect was noticed in all cases. In particular, the burst fraction was found to be decreasing with increasing OSA–HA concentration and the released drug fractions after 24 h were 0.835, 0.782, 0.695 and 0.571 for TA suspension, from 1, 2.5 and 5.0 mg/ml OSA–HA solutions, respectively.

Moreover, the applied model allowed to discriminate the diffusive and dissolution contributions to drug release. Experimental data were well fit by model equation. In the case of blank experiments (*i.e.* free TA released from solution loaded in dialysis membrane) the results of the model showed that TA is released from the membrane mainly by diffusion, as expected taking into account the poor solubility of the drug. It must also be underlined that an increase of the concentration of OSA–HA in the membrane led to increasing fractions of TA released by dissolution, as indicated by the increasing values of $F_{diss,\infty}$ (Table 3). These results confirm the solubility enhancement of the modified HA toward the hydrophobic TA. Furthermore, it should be noted that the kinetics of diffusion appears to be basically the same, irrespective of the OSA–HA concentration (Biondi, Fusco, Lewis, & Netti, 2013; Lustig & Peppas, 1988). Actually, k_{diff} values were variable between 0.230 h^{-1} in the case of free TA and 0.198 h^{-1} for the OSA–HA solution at 5.0 mg/ml, as expected in the case of the diffusion of low molecular weight molecules through the hydrated matrix of a polymeric solution.

Therefore, release data clearly show that a combined mechanism, based on parallel dissolution and diffusion, governs TA release from OSA–HA-based solution. The analysis of release profiles reported in Fig. 7 shows that, in all cases, diffusion is faster compared to dissolution, *i.e.* that at least initial release phases are governed by diffusion. The dissolution-controlled mechanism of TA released from the inner layer was regarded as a secondary, slower release.

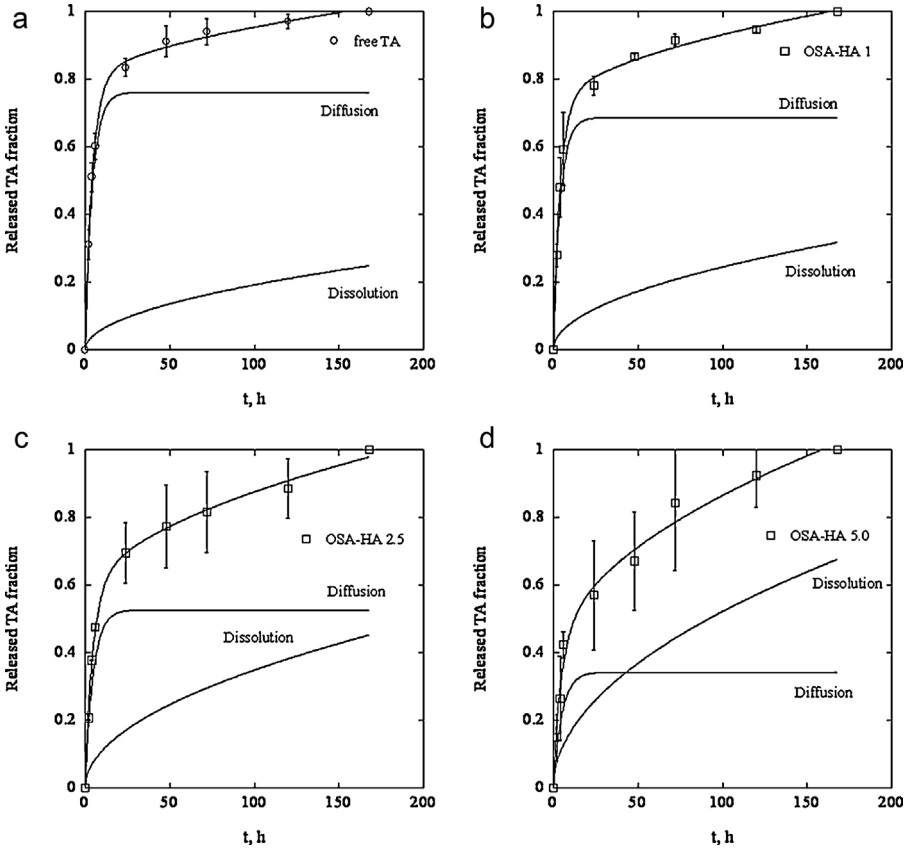

Fig. 7. TA release profiles from water suspension and OSA–HA solutions at different concentration: (A) TA suspension in water; (B) OSA–HA 0.1% w/v; (C) OSA–HA 0.25% w/v; and (D) OSA–HA 0.5% w/v. Solid lines represent model simulations. Fitting was performed by Eq. (5).

Table 3
Model parameters as calculated by fitting experimental release data to Eq. (5).

	Free TA	OSA–HA 1.0 mg/ml	OSA–HA 2.5 mg/ml	OSA–HA 5.0 mg/ml
$F_{diss,\infty}$	0.238 ± 0.078	0.313 ± 0.031	0.473 ± 0.182	0.657 ± 0.272
k_{diss}, $h^{-1/2}$	0.0808 ± 0.0030	0.0783 ± 0.0037	0.0738 ± 0.0055	0.0795 ± 0.0071
k_{diff}, h^{-1}	0.230 ± 0.018	0.241 ± 0.068	0.212 ± 0.100	0.198 ± 0.066

4. Conclusions

In this study we report the rheological, morphological and calorimetric study of a novel amphiphilic high molecular weight HA derivative and, in particular, an octenyl succinic anhydride (OSA) modified HA (OSA–HA). This novel derivative was found to be able to self-assemble into micelles, load a hydrophobic drug and release the active molecule with a controlled kinetic mechanism. Finally, owing to its suitable viscoelastic features, OSA–HA is potentially useful in restoring SF rheological properties during a viscosupplementation therapy.

References

Almond, A. (2007). Hyaluronan. *Cellular and Molecular Life Sciences*, *64*, 1591–1596.

Altman, R. D., & Moskowitz, R. (1998). Intraarticular sodium hyaluronate (Hyalgan) in the treatment of patients with osteoarthritis of the knee: A randomized clinical trial. Hyalgan Study Group. *Journal of Rheumatology*, *25*, 2203–2212.

Balazs, E. A. (2004). Viscosupplementation for treatment of osteoarthritis: From initial discovery to current status and results. *Surgical Technology International*, *12*, 278–289.

Biondi, M., Fusco, S., Lewis, A. L., & Netti, P. A. (2013). Investigation of the mechanisms governing doxorubicin and irinotecan release from drug-eluting beads: Mathematical modeling and experimental verification. *Journal of Materials Science: Materials in Medicine*, *24*, 2359–2370.

Borzacchiello, A., Mayol, L., Ramires, P. A., Pastorello, A., Di, B. C., Ambrosio, L., et al. (2007). Structural and rheological characterization of hyaluronic acid-based scaffolds for adipose tissue engineering. *Biomaterials*, *28*, 4399–4408.

Borzacchiello, A., Mayol, L., Schiavinato, A., & Ambrosio, L. (2010). Effect of hyaluronic acid amide derivative on equine synovial fluid viscoelasticity. *Journal of Biomedical Materials Research Part A*, *92*, 1162–1170.

Cho, B. K., Jain, A., Nieberle, J., Mahajan, S., Wiesner, U., Gruner, S. M., et al. (2004). Synthesis and self-assembly of amphiphilic dendrimers based on aliphatic polyether-type dendritic cores. *Macromolecules*, *37*, 4227–4234.

Eenschooten, C., Guillaumie, F., Kontogeorgis, G. M., Stenby, E. H., & Schwach-Abdellaoui, K. (2010). Preparation and structural characterisation of novel and versatile amphiphilic octenyl succinic anhydride-modified hyaluronic acid derivatives. *Carbohydrate Polymers*, *79*, 597–605.

Eenschooten, C., Vaccaro, A., Delie, F., Guillaumie, F., Tommeraas, K., Kontogeorgis, G. M., et al. (2012). Novel self-associative and multiphasic nanostructured soft carriers based on amphiphilic hyaluronic acid derivatives. *Carbohydrate Polymers*, *87*, 444–451.

Fakhari, A., & Berkland, C. (2013). Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. *Acta Biomaterialia*, *9*, 7081–7092.

Fusco, S., Borzacchiello, A., Miccio, L., Pesce, G., Rusciano, G., Sasso, A., et al. (2007). High frequency viscoelastic behaviour of low molecular weight hyaluronic acid water solutions. *Biorheology*, *44*, 403–418.

Gerwin, N., Hops, C., & Lucke, A. (2006). Intraarticular drug delivery in osteoarthritis. *Advanced Drug Delivery Reviews*, *58*, 226–242.

Ghosh, P., & Guidolin, D. (2002). Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: Are the effects molecular weight dependent? *Seminars in Arthritis and Rheumatism*, *32*, 10–37.

Gomez, J. E., & Thurston, G. B. (1993). Comparisons of the oscillatory shear viscoelasticity and composition of pathological synovial fluids. *Biorheology*, *30*, 409–427.

Harada, A., & Kataoka, K. (2006). Supramolecular assemblies of block copolymers in aqueous media as nanocontainers relevant to biological applications. *Progress in Polymer Science*, *31*, 949–982.

Hatakeyama, T., Quinn, F. X., & Hatakeyama, H. (1996). Changes in freezing bound water in water-gellan systems with structure formation. *Carbohydrate Polymers*, *30*, 155–160.

Li, G., Liu, J., Pang, Y., Wang, R., Mao, L., Yan, D., et al. (2011). Polymeric micelles with water-insoluble drug as hydrophobic moiety for drug delivery. *Biomacromolecules*, *12*, 2016–2026.

Lustig, S. R., & Peppas, N. A. (1988). Solute diffusion in swollen membranes.9. Scaling laws for solute diffusion in gels. *Journal of Applied Polymer Science*, *36*, 735–747.

Maltese, A., Borzacchiello, A., Mayol, L., Bucolo, C., Maugeri, F., Nicolais, L., et al. (2006). Novel polysaccharides-based viscoelastic formulations for ophthalmic surgery: Rheological characterization. *Biomaterials*, *27*, 5134–5142.

Mayol, L., Quaglia, F., Borzacchiello, A., Ambrosio, L., & La Rotonda, M. I. (2008). A novel poloxamers/hyaluronic acid in situ forming hydrogel for drug delivery: Rheological, mucoadhesive and in vitro release properties. *European Journal of Pharmaceutics and Biopharmaceutics*, *70*, 199–206.

Mayol, L., Biondi, M., Quaglia, F., Fusco, S., Borzacchiello, A., Ambrosio, L., et al. (2011). Injectable thermally responsive mucoadhesive gel for sustained protein delivery. *Biomacromolecules*, *12*, 28–33.

Miro, A., d'Angelo, I., Nappi, A., La, M. P., Biondi, M., Mayol, L., et al. (2013). Engineering poly(ethylene oxide) buccal films with cyclodextrin: A novel role for an old excipient? *International Journal of Pharmaceutics*, *452*, 283–291.

Nastruzzi, C., Esposito, E., Cortesi, R., Gambari, R., & Menegatti, E. (1994). Kinetics of bromocriptine release from microspheres-comparative-analysis between different in-vitro models. *Journal of Microencapsulation*, *11*, 565–574.

Pelletier, S., Hubert, P., Payan, E., Marchal, P., Choplin, L., & Dellacherie, E. (2001). Amphiphilic derivatives of sodium alginate and hyaluronate for cartilage repair: Rheological properties. *Journal of Biomedical Materials Research*, *54*, 102–108.

Peppas, N. A. (1985). Analysis of Fickian and non-Fickian drug release from polymers. *Pharmaceutica Acta Helvetiae*, *60*, 110–111.

Petrella, R. J., DiSilvestro, M. D., & Hildebrand, C. (2002). Effects of hyaluronate sodium on pain and physical functioning in osteoarthritis of the knee: A randomized, double-blind, placebo-controlled clinical trial. *Archives of Internal Medicine*, *162*, 292–298.

Pravata, L., Braud, C., Boustta, M., El, G. A., Tommeraas, K., Guillaumie, F., et al. (2008). New amphiphilic lactic acid oligomer-hyaluronan conjugates: Synthesis and physicochemical characterization. *Biomacromolecules*, *9*, 340–348.

Savani, R. C., Cao, G., Pooler, P. M., Zaman, A., Zhou, Z., & DeLisser, H. M. (2001). Differential involvement of the hyaluronan (HA) receptors CD44 and receptor for HA-mediated motility in endothelial cell function and angiogenesis. *Journal of Biology Chemistry*, *276*, 36770–36778.

van Hest, J. C., Delnoye, D. A., Baars, M. W., van Genderen, M. H., & Meijer, E. W. (1995). Polystyrene-dendrimer amphiphilic block copolymers with a generation-dependent aggregation. *Science*, *268*, 1592–1595.