

RELATION BETWEEN RHEOLOGICAL, STRUCTURAL AND DISSOLUTION PROPERTIES OF COVALENTLY AND IONICALLY MODIFIED HYALURONIC ACID-BASED DRUG CARRIERS

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Abstract

This paper presents some findings from the rheological and drug dissolution characterizations of hyaluronic acid (HyA)-based colloidal drug delivery systems. During this investigations the widely applied, non-steroidal, anti-inflammatory ketoprofen (KP) has been used as model drug. For the moderation of the hydrophilic character of HyA and encapsulation of the hydrophobic drug the cross-linked derivatives at different cross-linking ratio have been synthesized. To achieve the increasing encapsulation efficiency, the hydrophobized variants of HyA have also been prepared in aqueous medium by modifying the structure with positively charged cetyltrimethylammonium bromide (CTAB) at different polymer/surfactant mass ratios. It was established that the original coherent gel-like structure of the hydrophilic and well-solvated polymer chains change into an incoherent colloidal system due to modifications that were verified by rheological investigations. Taking into account the dissolution profiles of covalent and ionic systems it can be concluded that nearly 70 % of the encapsulated KP molecules dissolve from the totally cross-linked HyA systems after 8h, while the dissolved amount of KP is about 20 % from the surfactant modified colloidal system at near 1:1 molar ratio. It was also established that increasing the amount of surfactant the drug release turns to diffusion and erosion controlled way. It has been confirmed that the ionically modified HyA may be a potential candidate for controlled drug release of KP.

Keywords: Hyaluronic acid, ketoprofen, encapsulation, controlled drug release

1. INTRODUCTION

Novel colloidal drug delivery systems have become one of the most fascinating research areas in modern pharmaceutical developments. Numerous biocompatible macromolecules such as polyesters, proteins, polysaccharides, polyelectrolites, lipids *etc.* are used as drug carriers in order to achieve a controlled drug delivery and drug release process.[1] The hyaluronic acid is a well-known linear polysaccharide of alternating units of β -1,4-D-glucuronic acid and β -1,3-N-acetyl-D-glucosamine. [2] Thanks to the biocompatible, biodegradable, non-toxic, non-immunogenic and non-inflammatory features this polymer is a perfect candidate for a number of medical and pharmaceutical applications. At physiological conditions the mentioned monomer units have a negative charge (hyaluronate form) which results in a water soluble nature. Because of this hydrophilic properties of HyA it is present in several organic fluids, the highest mass being found in the extracellular matrix of the soft connective tissues. The central drawback of this hydrophilic character is that HyA molecules, without chemical modification, cannot be applied as colloidal drug delivery systems. Numerous techniques have been developed for the construction of cross-linked HyA, but one of the frequently used method is the carbodiimide technique.[2] An alternative technique is the neutralization of HyA by the creation of electrostatic interactions using cationic surfactants like cetyltrimethylammonium bromide (CTAB), which also results in associated colloid structure having hydrophobic nature.[3,4] In this work the hydrophobic KP,

as model drug molecule, has been used to synthesize and characterize diverse types of HyA-based colloids for controlled drug release. Depending on the drug dissolution results the possible application of a biopolymer founded and nano sized drug delivery systems for encapsulation of a NSAID drug have been reported.

2. MATERIALS AND METHODS

2.1. Materials

Hyaluronic acid sodium salt (HyA, 4000 kDa) was obtained from Gedeon Richter Plc. Ketoprofen (KP; C₁₆H₁₄O₃; ≥98 %) and hexadecyltrimethylammonium bromide, sodium phosphate dibasic dodecahydrate and sodium phosphate monobasic monohydrate were obtained from Sigma-Aldrich. Sodium chloride from Molar Chemicals was used to prepare isotonic (150 mM) NaCl solution. For the cross-linking reaction 2,2'(ethylenedioxy)bis(ethylamine) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimid methiodide (CDI) were purchased from Sigma-Aldrich. Highly purified water was obtained by deionization and filtration with a Millipore purification apparatus (18.2 MΩ·cm at 25 °C). All solvents and reagents used were of analytical grade and no further purification were made.

2.2. Experimental procedures of the covalent and ionic modifications

Cross-linked (cl) HyA derivatives were prepared according to the previously published procedure. [2] The synthesis was performed at room temperature. 200 mg HyA was dissolved in water to produce 1 mg/mL solution and then adjusted the pH to pH = 5.5. The stoichiometric ratio of cross-linking was 50 %, 75 % and 100 % resulting in cl-HyA/50 %, cl-HyA/75 % and cl-HyA/100 % samples. Accordingly, 1.88 mL, 2.82 mL and 3.76 mL 2,2'(ethylenedioxy)bis(ethylamine) solution (1 v/v%, pH = 5.5) was added to the HyA solution and mixed for 30 min. 80 mg, 120 mg and 160 mg CDI was dissolved in water and added to the mixture drop by drop, respectively. After an overnight stirring the product was purified by dialysis for 7 days against distilled water and the aqueous solution of the final product was freeze-dried. For CTAB modification different calculated amount of surfactant was added to the aqueous solution of HyA to change the phobicity. The mixture was stirred for 30 min before further use.

2.3. Preparation of drug containing systems

Because of the low solubility of KP in pure Milli-Q water ($C_{max} = 0.051$ mg/mL) all drug containing samples were prepared in phosphate buffer solution (PBS) at pH = 7.4 at 25 °C using constant ionic strength (0.9 % NaCl). In all cases constant KP ($C_{KP} = 20$ mg/mL) and constant HyA concentrations (100.0-100.0 mg lyophilized cl-HyA/ml) have been used. According to the preparation procedures the aqueous KP solutions were added to the different individual cross-linked HyA and HyA/CTAB samples which resulted in the formation of gel-like structure after 24 h.

2.4. Structural characterizations

The particle size and zeta potential of the prepared systems were determined by Dynamic Light Scattering (DLS) with a Zetasizer Nano ZS ZEN 4003 apparatus (Malvern Ins., UK) equipped with a He-Ne laser ($\lambda = 633$ nm). The measurements were performed at 25 ± 0.1 °C with an angle detection of 173° in clear disposable zeta cell. In order to determine the maximum amount of CTAB to be added prior to precipitation 0.02 M of CTAB was added stepwise (20-20 µl/ step) to 0.2 mg/mL concentration of HyA in PBS and the electrokinetic potential values were registered by DLS and parallel with this measurement the turbidity data were also registered. Turbidity measurements were performed by a Precision Bench Turbidity Meter LP2000 (Hanna Ins.), while the conductivity was measured by a Radelkis OK- 114 konduktometer equipped with an electrode with sheet plates.

2.5. Rheological studies

All rheological measurements were performed using an Anton Paar Physica MCR 301 Rheometer (Anton Paar, GmbH, Germany) at 25 °C. The measuring system equipped with a 25 mm diameter parallel cone-plate geometry (CP25-1-SN12204), a double-gap- (DG26.7-SN12740) and a concentric cylinder geometry (CC27-SN12793). The rheometer utilized a temperature controlled water bath in combination with a Peltier heating system for accurate control.

2.6. In vitro drug release experiments

The spectrophotometric properties of KP was studied by using an UV-1800 (Shimadzu) double beam spectrophotometer, using 1 cm quartz cuvette in the range of 200-500 nm. The concentration of KP was determined at 260 nm. The *in vitro* drug release experiments were carried out in phosphate buffer (PBS, pH = 7.4) at 25 °C. A cellulose membrane (Sigma-Aldrich) was used as a dialysis membrane. The release process was followed for 480 minutes (8 hours). Samples were taken every 10 minutes in the first hour and then once per hour.

3. RESULTS AND DISCUSSION

Both the cross-linked (cross-linking ratio of 50, 75 and 100 %) and hydrophobized derivatives (chemical modification of the HyA polymer chains and neutralization with positively-charged amines) have been successfully prepared by different methods.

3.1. Structural characterization of the different HyA-based carriers

We established that increasing cross-linking ratio in the formation of nanosized particles with increasing average particle diameter (ca. 45 nm (50 %), ca. 100 nm (110 %)). However the particle size increases with the cross-linking ratio, the presence of more polydispersed system can be confirmed by DLS. Besides the syntheses of cross-linked variants of HyA the hydrophobization of the polymer has been performed by using positively-charged CTAB surfactant and aminodecane primer amine as well. Firstly, conductometric studies have been carried out in aqueous solution at 25 °C in order to determine the critical micelle concentration (cmc) of CTAB. According to the parallel measurements 0.94 ± 0.01 mM of cmc value is obtained, while in presence of 0.1 mg/ml of HyA the cmc of CTAB shifts to 1.16 ± 0.02 mM. Besides, the zeta potential of the negative charged polymer reaches the zero value at 0.5 mM surfactant concentration which corresponds to 1:1 surfactant/monomer molar ratio.[5] Both the turbidity measurements and the change of the average particle diameter of this system confirm the aggregation of the polymer chains at and above that surfactant concentration (0.5 mM in the case of 0.2 mg/ml HyA in PBS) which necessary for the charge compensation of the carboxylate groups of HyA monomers.

3.2. Rheological characterization of aquatic HyA solutions and hydrogels

Based on the rheological characterization of aquatic hyaluronic acid solution we found that, the increase in concentration of the polymer solution resulted in dramatically change of the rheological behavior. Namely, the HyA solutions shows Newtonian, shear-thinning and viscoelastic behavior as the polymer concentration in aquatic solutions and hydrogels rises up from 0.05 to 100 mg mL⁻¹. In the case of very dilute solutions the linear flow curves prove the validity of the Newton law, in this way these solutions were characterized by constant (shear rate-independent or Newtonian) viscosity. Viscosities of moderately concentrated solutions of high-molecular weight polymers are known to be variable quantities which decreases with increasing shear rate. This shear-thinning effect has been known for a long time and same behavior was observed above 1.00 mg mL⁻¹ HyA concentration (**Figure 1A**).

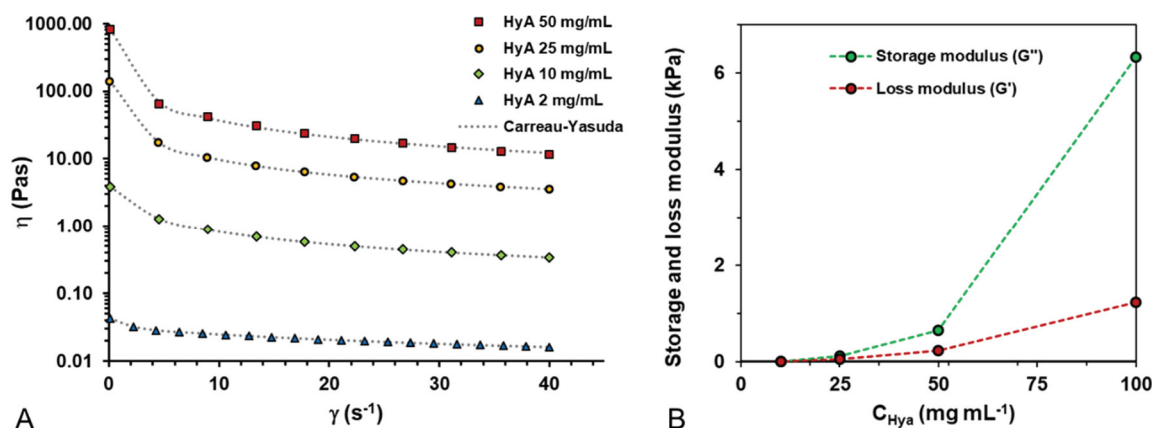


Figure 1 A: Measured and Carreau-Yasuda model fitted viscosity curves of HyA solutions ($C_{\text{HyA}} = 2, 10, 25$ and 50 mg mL^{-1}), B: concentration dependence of the viscoelastic of HyA hydrogels

Instead of the viscosity/shear rate profile (such as that shown in **Figure 1A**) is more suitable the determination of both loss and storage modulus (G' and G'') as a function of amplitude, under 10 s^{-1} oscillation frequency via amplitude sweep measurements. Amplitude scans of the linear HyA gels shown that the G''/G' ratio does not change as strain increases from 0.1 to 10.0 %, it means that the internal friction is independent of strain in this region in this way the viscoelastic behavior can be described by constant G' and G'' values as presented in **Figure 1B** against the polymer concentration.

3.3. Rheological characterization of ionically- and covalently modified HyA solutions and hydrogels

To characterize the ionically modified form of polymer, a constant (0.1 mg/mL) initial HyA concentration was chosen for steady shear rate measurements of CTAB solution diluted HyA samples and a larger concentration (50 mg/mL) was applied in the case of amplitude sweep measurements. As it can be seen in **Figure 2A**, the steady shear rate tests determined apparent viscosity of polymer solution continuously decreased due to the added surfactant and a visible breakpoint can be observed. Above the 1:1 surfactant/monomer molar ratio the viscosity of the neutralized and thus hydrophobized polymer containing solution shows a rising trend. After this observation it can be stated that in addition to conductivity and ITC measurement a modified rheological investigation also suitable for detection the structural change of the polymer - surfactant colloid system.

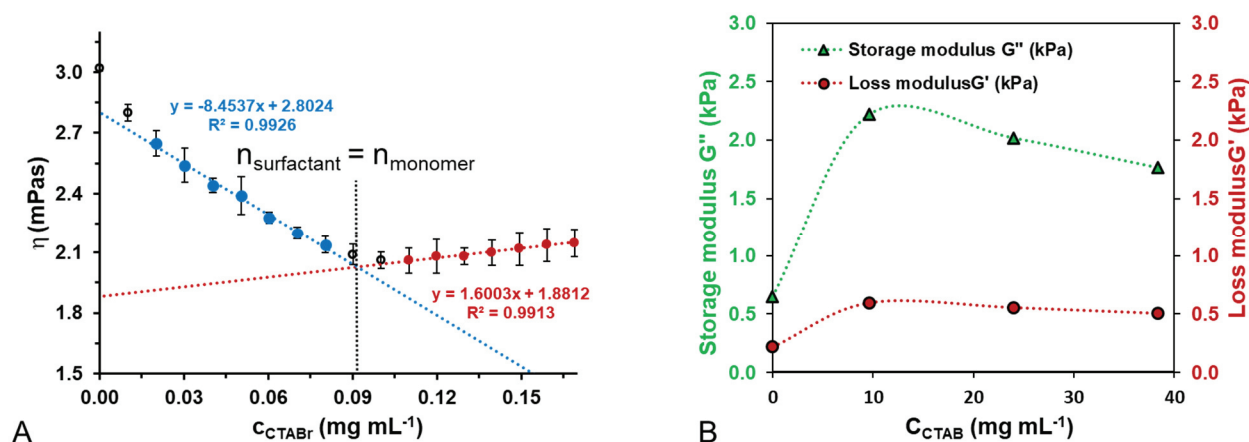


Figure 2 A: Steady shear rate determined apparent viscosity of linear HyA solution (0.1 mg mL^{-1}) titrated by CTAB ($c = 25.0 \text{ mM}$) solution, B: Amplitude sweep determined rheological parameters of linear HyA hydrogels ($C_{\text{HyA}} = 50 \text{ mg mL}^{-1}$) as a function of CTAB concentration

As illustrated in the **Figure 2B** the varying degrees hydrophobized polymer based hydrogels are show elastic character at low strain, while at high strain range the loss modulus dominates ($G'' > G'$) as the above reported linear hyaluronic acid hydrogels. Same trend was observed in the case of the varying degrees cross-linked polymer hydrogels (**Figure 3A**) where the storage modulus almost reaches zero value due to the structure modification of polymer chains. The latter outlined two observations confirm the assumption that both the added CTAB and cross-linking agent break the coherent structure which spontaneous formed from the solvated biopolymer.

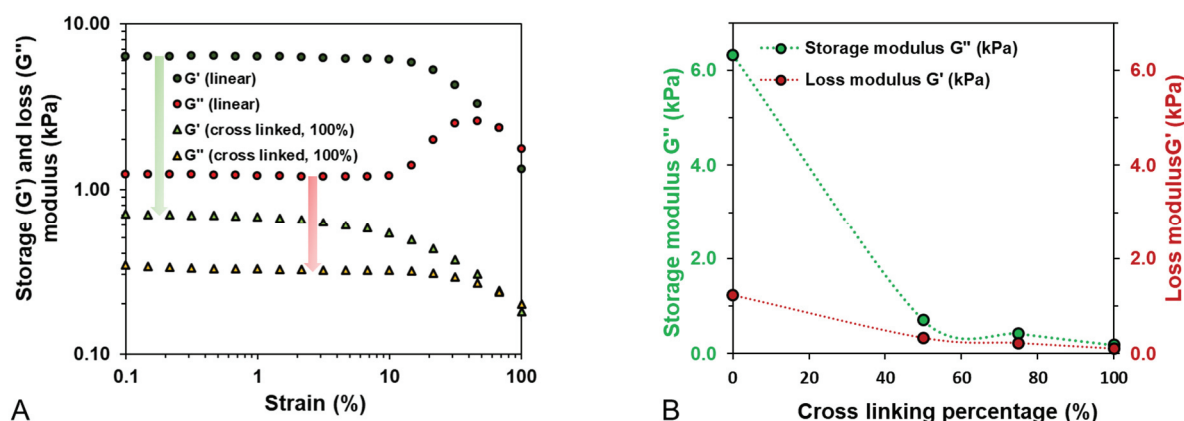


Figure 3 Changing of the amplitude sweep determined rheological parameters (storage and loss modulus) of linear and cross-linked hyaluronic acid hydrogels ($C_{HyA} = 50 \text{ mg mL}^{-1}$) as a function of applied strain (A) and the degree of cross-linking (B)

3.4. Evaluation of in vitro drug release experiments

The evolution of KP dissolution from the HyA based carriers was investigated using first-order kinetic model.[6] Drug release curves of KP from varying degrees hydrophobized or crosslinked polymer based carriers are depicted in **Figure 3**. As it can be seen, all the formulations are able to sustain KP release for more than 6 h, but the release of the drug from the cross-linked polymer is almost complete within 7 h. By adding the linker from 50 % to 75 and 100 % into the polymer it was possible to achieve a more accurate control over drug release. However, it should be noted that nearly 80 % is released from the total drug content in the case of completely cross-linked polymer at the end of the seventh hour (**Figure 4B**). On the other hand, using the least amount of surfactant (**Figure 8A**) is causing that only 60 % of the active ingredient dissolved during the experiment.

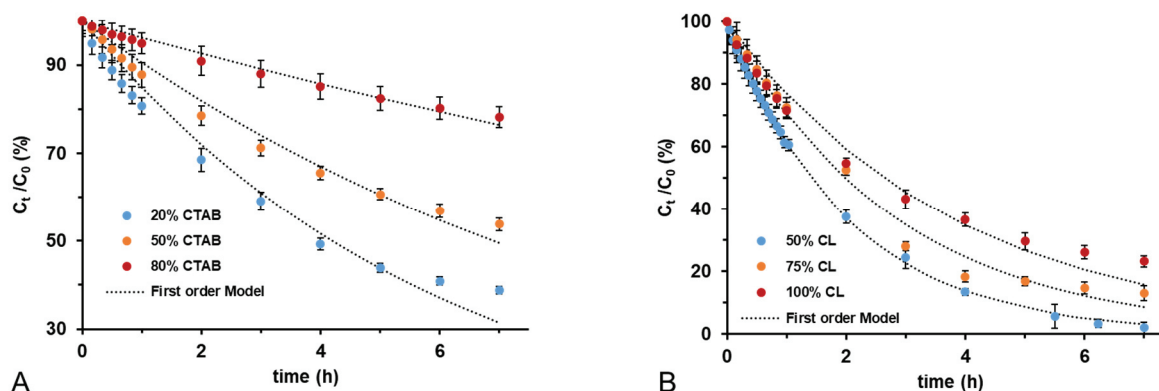


Figure 4 Photometric measured release profiles and first-order kinetic model predicted release curves of KP from varying degrees neutralized (A) or cross-linked (B) HyA-based drug carriers

Based on the observation of previous parts (change of the zeta potential, turbidity and particle size values as a function CTAB amount), it can also be established that the release of KP from the carrier faster and rather diffusion controlled when relatively small amount electrostatic adsorbed surfactant molecules are present in the system. As the polymer-ionic surfactant interactions lead to changes in polymer structure the dissolution of drug becomes slower and the release turns to diffusion and erosion controlled way.

4. CONCLUSION

During this study we have demonstrated the preparation and characterization of a biopolymer based colloidal drug delivery systems. Because of the hydrophilic character of the polymer, cross-linked derivatives have been synthesized to moderate the water soluble nature and produce polymer nanoparticles. The neutralized variants of polymer have also been produced by modifying with ionic surfactant to reach an incoherent „particle like” colloid system. Due to modifications the coherent structure of the polymer changes into an incoherent colloidal system that were verified by dynamic light scattering and rheological investigations. In the case of the cross-linked carrier, we could not significantly reduce the rate of drug dissolution but in the surfactant modified system the release rate has increased near four times larger in proportion to the amount of surfactant. It has been verified that the modified hyaluronic acid may be a potential candidate for controlled drug release of hydrophobic molecules.

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