

"CHEMICAL MODIFICATION OF HYALURONIC ACID: ALKYLATION"

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Introduction

Hydrophobically modified (HM) water-soluble polymers, e.g. cellulose, starch or guar exhibit enhanced solution viscosity and unique rheological behaviour. These properties are explained in terms of intermolecular hydrophobic associations [1]. The general solution viscosity behavior of HM polymers of different hydrophobic chain length (most frequently in the range of C_8 to C_{24}) is characterized by the peak viscosity region observed for a certain alkyl group content (between 1 to 5 wt. %). After reaching the maximum increase in viscosity (peak region) at a certain hydrophobic level, the viscosity dramatically decreases with increasing number of the hydrophobic moieties on the polymer chain.

The monomer unit of HA consists of N-acetyl-D-glucosamine and D-glucuronic acid bonded via $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ interglycosidic bonds [2]. In general, alkylation of cellulosic derivatives proceeds via corresponding alcoholates. Dialkyl sulfates are not suitable due to their toxicity and carcinogenicity. Preparation of alkyl-modified HA chains therefore was achieved upon the alkylation with alkyl halides. In both procedures the selectivity in the attachment of the alkyl chain is limited.

The aim of this study was to prepare and characterize physico-chemical properties of aqueous solutions of hydrophobically modified hyaluronic acid derivatives (FM HA).

Experimental

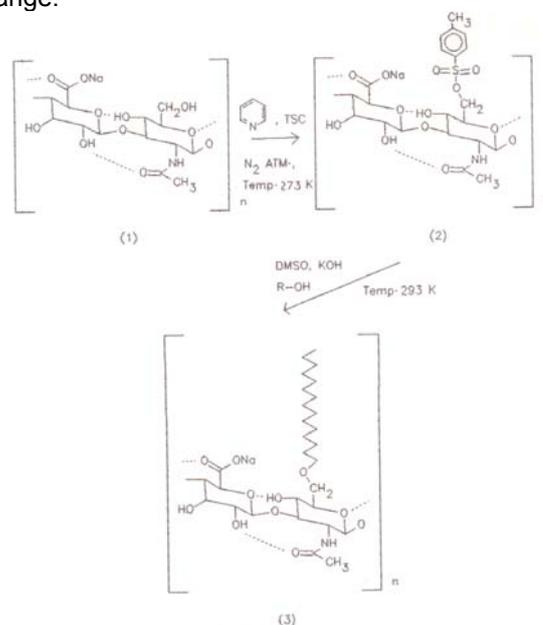
Materials and methods:

Sodium hyaluronate (M_w 1.15 MDa) extract from rooster combs was obtained from Diosynth, Netherlands. All reagents used were of ACS purity grade purchased from Janssen Chimica, Sigma-Aldrich and UCB.

Synthesis of HM HA. We were primarily interested in preserving the polymerization degree of the polymer, while maintaining the biologically and physiologically important side chain groups, i.e. the negatively charged carboxyl group and the acetyl-amide. For these reasons, a more selective method for

modifying the primary alcohol group was preferred (Scheme 1). The secondary hydroxyl groups are less acidic than the primary alcohols.

In the first step, alkyl sulfate and the polymer alkoxide form and alkoxy-sulfonyloxy-complex. Under strict temperature control this reaction is highly selective for primary alcohols [3]. In the second step, the complex and the alcohol are added to powdered KOH and stirred in DMSO at room temperature for several hours [4,5]. To ensure that no degradation of the main HA polymer chain occurs during the synthesis, a blank experiment was performed. By the latter blank experiments was confirmed that the M_w of the original sample did not change.



Scheme 1. (1) – Sodium Hyaluronate (HA), (2) – HA-Tosyl, (3) Pentadecyl-oxy-HA [5].

IR Spectroscopy. All measurements were performed on samples prepared in the form of KBr discs or the solid films and measured against air as reference.

DSC Measurements. Standard aluminum sample pans (Perkin Elmer) for volatile liquids were used.

Thermograms of the samples were recorded on Du Pont Instruments 9900 Computer/Thermal Analyzer 910 Differential Scanning Calorimeter in the temperature range of -25°C to $+50^{\circ}\text{C}$ at a scanning rate of $3^{\circ}\text{C}/\text{min}$. The samples were prepared by cooling from $+23^{\circ}\text{C}$ to -30°C at $5^{\circ}\text{C}/\text{min}$ cooling rate.

Size Exclusion Chromatography. All experiments were carried out on coupled Ultrahydrogel 1000 and Ultrahydrogel 2000 columns (Millipore) connected with Waters 510 Pump. For detection the Waters 410 Differential Refractometer was used. The SEC column was calibrated by the standard calibration method using the pullulan standards (Sigma).

NMR Spectroscopy. ^1H NMR experiments were performed in D_2O (Aldrich) under nitrogen atmosphere by dissolving 12-16 mg/mL of the polymer. The spectra were recorded on 360 MHz and 500 MHz Bruker spectrometers at room temperature.

Rheological Measurements. For measuring the shear rate dependence of dynamic viscosity and for oscillatory measurements, a Thermo-HAAKE Rotovisco rotational viscometer was used. All measurements were carried out at 25°C . For each measurement, the solutions were prepared by dissolving freeze-dried HA two days prior to the measurements.

Results and discussion

The main characteristic of HA hydrogels is the ability to bind extremely high amounts of water in the swollen state due to complex inter-molecular hydrogen bonding system [2, 6, 7].

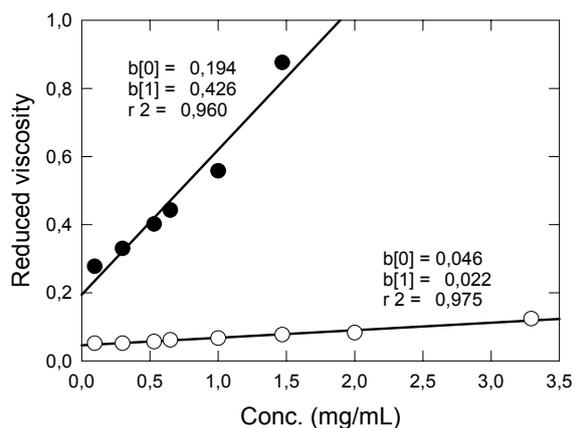


Fig. 1. Huggin's plot of the $\eta_{sp}/c = f(c)$ dependencies of the 100 mol.% modified hexyl-oxy-HA as measured by the Ubbelohde capillary viscosimeter at 25°C .

Introduction of the alkyl pendant groups on main HA macromolecular chain may trigger preferential intra-molecular association, rather than inter-molecular,

what was reflected both in rheological as well as turbidity measurements. The presence of the hydrophobic microdomains on the polymer chain was indicated by the decrease of the slope of the reduced viscosity dependencies, i.e. the decrease of the corresponding Huggin's constants.

Previous studies have shown that the transport properties of tightly bonded water differ from those of free water. The nature of the water present in the native and modified polymer influences drug release from such matrices. The DSC pattern for hexyl-oxy-HA was characteristic with endothermic peak at $+0.96^{\circ}\text{C}$ (free water) with broad exothermic peak at -3.96°C (water hexameric structure).

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