

## Nanoscaled Polysaccharides in Solution: Scaling Laws of Hyaluronan

Elashhab Fathi, Sheha Lobna, Alfazani Tahani\*, Youssef Abdelsallam EA and Elsupikhe Randa Fawzi

Chemistry Department, Faculty of Science, University of Benghazi, Benghazi, Libya.

**\*Correspondence:**

Alfazani Tahani, Chemistry Department, Faculty of Science, University of Benghazi, Benghazi, Libya, E-mail: tahananialfazani19@gmail.com.

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**ABSTRACT**

Ionic polysaccharides (e.g. hyaluronan (HA)) are currently used in a range of sizes, or molar masses, in an increasing number of nanomaterials for biocompatible applications. The aim of this work is to predict the scaling law of HA, in aqueous salt solution, by applying simple and low-cost methods of green degradation and molar mass determination. In this respect, moderately concentrated solutions of native and different ultraviolet (UV)-degraded HA in NaCl aqueous solution were prepared. The corresponding molar masses were obtained via one-point method of viscosity measurements. It has been observed that the progress of molar mass production, via HA degradation, was UV-dose dependent. The graphical interpretation method of kinetic analysis confirmed the first order degradation rate, which is an indication of a random session on the glycosidic bonds. The viscosity data were treated via Flory-Fox's theory to get molecular parameters such as: the hydrodynamic radius ( $R_{H,n}$ ), radius of gyration ( $R_{G,n}$ ), coil density ( $\rho_{c,n}$ ), critical concentration ( $C_n^*$ ) and second virial coefficient ( $A_{2,n}$ ). It has been found that:  $R_{H,n}$  and  $R_{G,n}$  were UV-dose decreases; while  $\rho_{c,n}$ ,  $C_n^*$  and  $A_{2,n}$  were UV-dose increases. The results were explained according to the influence of size and excluded-volume.

**Keywords**

Viscosimetry, Coil properties, Scaling laws, Nanoscaled hyaluronan, UV- degradation rate.

**Introduction**

Polysaccharides are an important class of polymeric biomaterial, especially in the domain of biocompatible applications [1,2]. Polysaccharides consist of carbohydrate monomers chained by O-glycosidic bonds in a linear or branched configuration; they can be divided into two classes: noncharged and charged chains [3]; in solutions, the noncharged chains are gyrate in a randomly coiled conformation, while the charged chains are in an extended form [3,4]. Not surprising, then, if the extended conformation is converted to randomly coil one by adding ionic salt to the solution.

The relative viscosity molar mass  $M_{\eta,r}$  of polysaccharides in solution is directly related to their coil properties CPs, such as those of coil dimensions (hydrodynamic radius  $R_{H,n}$ , and radius of gyration  $R_{G,n}$ ), coil density  $\rho_{c,n}$ , critical concentration  $C_n^*$ , and second virial coefficient  $A_{2,n}$ , via the following relationship [5-13]:

$$CP = K_{cp} M_{\eta,r}^{a_{cp}} \quad (1)$$

where  $K_{cp}$  is the proportionality constant and  $a_{cp}$  is the scaling coefficient. A practical application of these laws is that, once we know the law and the value of the property, we can design the molar mass.

Hyaluronan (HA) is a class of negatively charged polysaccharides composed of glucuronic acid and N-acetylglucosamine repeating monomers [14]. HA in solution, is used in a range of coil dimensions, from  $R_{G,n} \sim 28$  nm to 0.3  $\mu$ m, in a considerable number of biocompatible applications [15]. However, nowadays the nanoscaled HA ( $R_{G,n} < 100$  nm) has been recognized to have novel features, such as: free radical scavenging activities, antioxidant activities and dietary supplements [15,16].

Tailoring the microscaled HA to nanoscaled fragments can be performed by several methods; among them is the green-degradation by irradiation technology, which is the simplest one to truncate the HA backbone into nanoscaled fragments; the method is environmentally friendly, and is capable to tailor the HA to the applicants in one-step truncation [16-24].

Almost no data on the CPs of micro and nanoscaled hyaluronan

are available. In this work, we will deduced their scaling laws by applying the photo-degradation method backed with the viscosity measurement.

## Experimental

### Material

The HA-powder and sodium chloride solution (0.5M NaCl) were supplied by Sigma–Aldrich, and used without further purification.

### Sample preparation and irradiation

A mother solution of aqueous HA with concentration ( $C_p$ ) equal to  $0.7 \text{ mg cm}^{-3}$  was prepared, by adding a known weight of the HA powder to the affixed 0.5M NaCl solution, and gently agitated with magnetic stirrer to complete the polymer solubility. The mother solution was divided into two parts and labelled Micro-HA and Nano-HA. The Micro-HA was used natively, while the Nano-HA has been exposed to UV-radiation in air at room temperature, for different period of time, by a low pressure mercury lamp ( $\lambda=185 \text{ nm}$  and  $P = 6\text{W}$ ), made by spectral lamp company. Further experimental details are given in [25].

### Density and viscosity methods

All measurements were made into thermo stated water bath at  $24.99 \pm 0.01^\circ\text{C}$ . The solvent and solution densities ( $\rho_0$  and  $\rho$ , respectively) were measured by 25 ml- calibrated glass pycnometer (a specific gravity bottle, accuracy:  $\pm 0.02 \text{ g cm}^{-3}$ ). The solvent and solution flow times  $t_{f,0}$  and  $t_f$ , respectively) were measured by pipetting 10 ml of each liquid in a glass viscometer of U-pipette type, suspended in a thermostatically controlled water bath with accuracy equal to  $\pm 0.02^\circ\text{C}$ . Running the equilibrated flow out of the bulb was measured in triplicate, with accuracy equal to  $\pm 0.015$ . More details are given in [5,26].

### Data treatment

The measured data were treated via Eqs. 2-17 in Table 1.

**Table 1:** Equations for the data treatment.

Eq	No	Ref	Eq	No	Ref
$\rho = \rho_0 m/m_0$	(2)	[5,26]	$R_{G,\eta} = K_{FF} M_\eta^{1/3}$	(11)	[5-7]
$\eta_r = \eta/\eta_0$	(3)	[5,26]	$K_{FF} = ([\eta]/\Phi_{RG})^{1/3}$	(12)	[5-7]
$\eta = t_f x \rho$	(4)	[5,26]	$\Phi_{RG} = 4.17 \times 10^{24} \text{ mol}^{-1}$	(13)	[5-7]
$\eta_0 = t_{f,0} \times \rho_0$	(5)	[5,26]	$\rho_{c,\eta} = 2.5/[\eta]$	(14)	[5-7]
$\ln \eta_r = ([\eta]C)^{2/3}$	(6)	[27]	$C_\eta^* = 0.77/[\eta]$	(15)	[5]
$[\eta] = K_{[\eta]} M_\eta^a$ $K_{[\eta]} = 1.81 \times 10^{-3} \text{ g}^{-1} \text{ cm}^3$ $a = 0.93$	(7)	[28-30]	$A_{2,\eta} = \frac{[\eta] - [\eta]_\theta}{5 \times 10^{-5} M_\eta}$	(16)	[8,9]
$\frac{1}{M_\eta^*} = \frac{1}{M_\eta^0} + \frac{kt}{M_\eta}$	(8)	[31]	$[\eta]_\theta = \frac{[\eta][1 - \exp(-\frac{C_p}{C_\eta^*})]}{0.773 \rho_{c,\eta} - \exp(-\frac{C_p}{C_\eta^*})}$	(17)	[10-12]
$\tau = 1/k$	(9)				
$R_{H,\eta} = (\frac{3[\eta]M_\eta}{10\pi N_A})^{1/3}$	(10)	[5-7]			

## Results and Discussion

Figures 1 and 2 represent the degradation progress and first order rate of HA respectively. This is an indication on the random chain

scission of HA glycosidic bonds, via the mechanism of free radical oxidation [16-24]. The rate  $k$  and lifetime  $\tau$  of the scission were  $\sim 12.96 \text{ min}^{-1}$  and  $0.077 \text{ min}$ , respectively.

As indicated in Figure 3, the increases in the irradiation dose of solid HA brought about decreases in  $R_{G,\eta}$ ,  $R_{H,\eta}$  and increases in  $\rho_{c,\eta}$ ,  $C_\eta^*$  and  $A_{2,\eta}$ . The linear plots in Fig.4 represent the respective logarithmic scaling laws of those parameters. This was expected sense the molecular size should be reduced by the degradation, while the solute solvent interaction must increase [32]. Consequently, one could deduce that,

$$R_{H,\eta} = 0.019 M_r^{0.58} \quad (18)$$

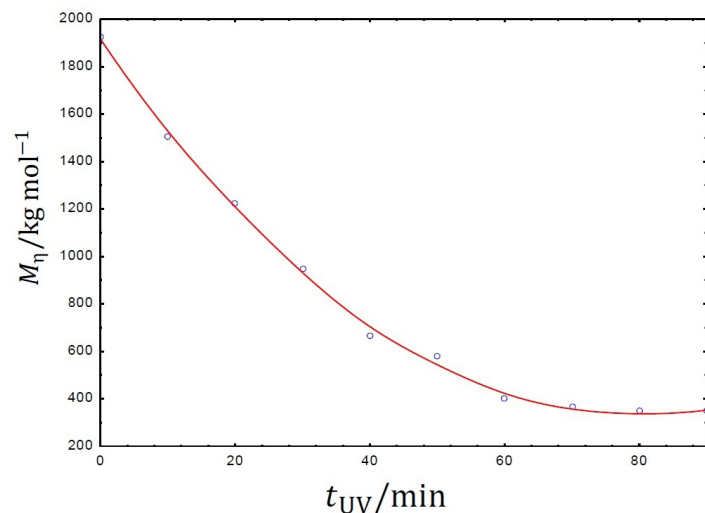
$$R_{G,\eta} = 0.020 M_r^{0.59} \quad (19)$$

$$\rho_{c,\eta} = 87.553 \times 10^3 M_r^{-0.78} \quad (20)$$

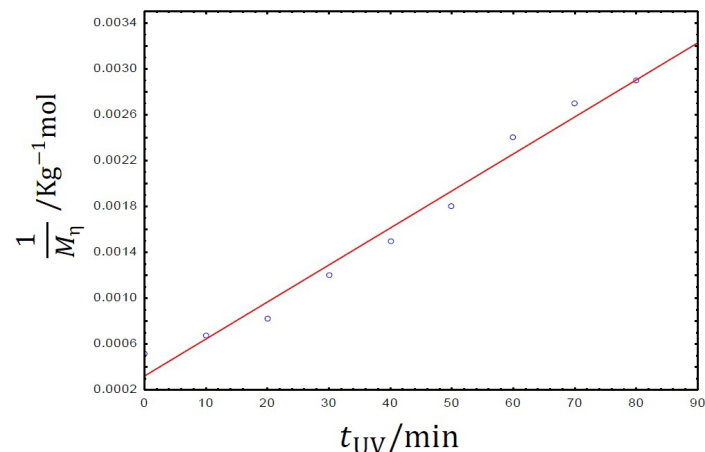
$$C_\eta^* = 25.848 \times 10^3 M_r^{-0.78} \quad (21)$$

$$A_{2,\eta} = 2246.2 M_r^{-0.38} \quad (22)$$

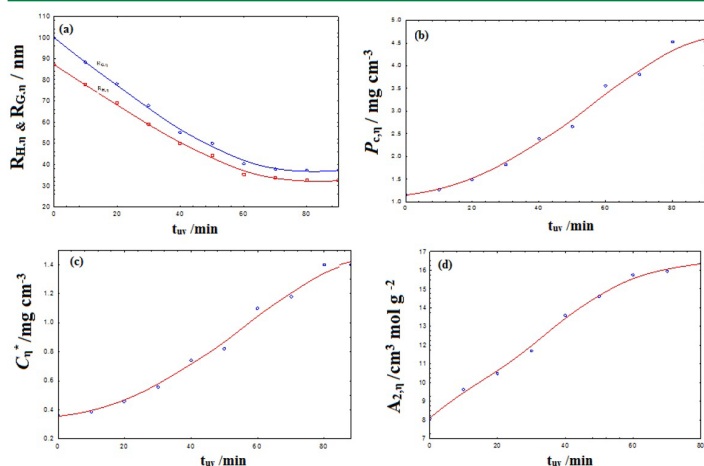
Moreover, it's worth mentioning that, the  $R_{G,\eta}$  scaling law, in Eq. 19, is in a good agreement with that one, which was obtained by light scattering method in the literature [15].



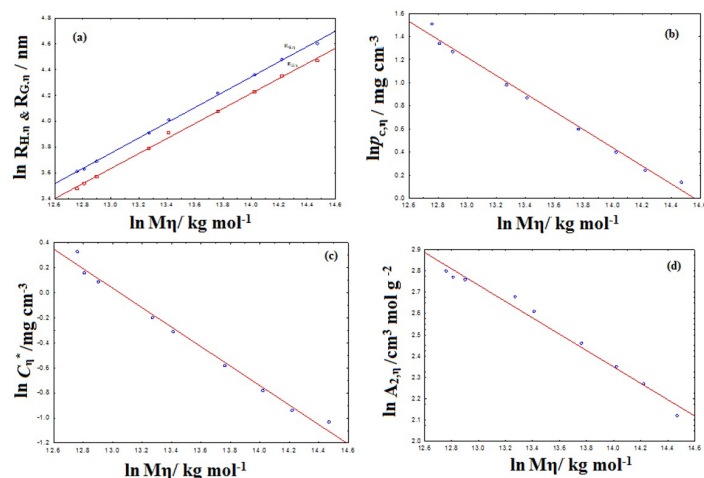
**Figure 1:** Exposure time with  $M_\eta$  of HA in 0.5M NaCl solution at  $25^\circ\text{C}$ .



**Figure 2:** First order degradation of HA in 0.5 M NaCl solution at  $25^\circ\text{C}$ .



**Figure 3:** The time of HA by UV- irradiation dependences of HA molecular parameters in NaCl solution at 25°C.



**Figure 4:** Logarithmic  $M\eta$  dependences of HA molecular parameters in NaCl solution at 25°C.

## Conclusion

- The HA-scaling laws are of practical use for designing nanoscaled HA to the applicants.
- The green degradation by UV technology is an efficient method for the production of nanoscaled HA.
- One point method of viscosity measurement can be employed, as an efficient method, to determine the molar mass of the micro and nanoscaled HA.
- The native molecular HA has a larger size than the nanoscaled one.
- The quality of nanoscaled HA solution is better than that of microscaled one.

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