ADVANCES IN LIGHT SCATTERING DETECTION FOR THE CHARACTERIZATION OF DRUG DELIVERY SYSTEMS

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INTRODUCTION

Multi-Angle Light Scattering (MALS) coupled with separation techniques has proven indispensable for characterizing critical quality attributes in drug delivery systems. Among these attributes, molecular weight (MW) determination, size distribution (radius of gyration – Rg), aggregation profiling, and structural conformation are necessary for ensuring safety, efficacy, and reproducibility (S.Đorđević et al, Drug Deliv Transl Res. 2022;12(3):500-525)

Current methodologies of MALS detectors face limitations in detecting particles smaller than 12 nm due to their weak scattering signals and minimal angular dependence. Therefore, highlighting the need for more sensitive detectors to capture critical size differences that can impact overall behavior and efficacy.

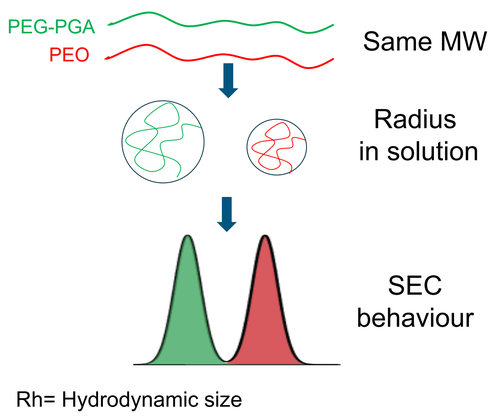
RESULTS

ABSOLUTE Molar Mass Determination by MALS

Table 1. Molecular weight of poly-ethylene-glycol – poly(glutamic) acid copolymer (PEG-PGA) determined by light scattering (LS) and conventional column calibration method (CC)

	Time (min)	Mn (g/mol)		Mw (g/mol)		Mz (g/mol)		PDI	
		LS	CC	LS	CC	LS	CC	LS	CC
	27.05	7,167	8,036	7,215	9,241	7,493	10,511	1.01	1.15
CV (%)*	0.02	0.57	0.34	0.38	0.77	4.1	1.5	0.17	0.41

*coefficient of variation calculated from three replicates



Rh= Hydrodynamic size

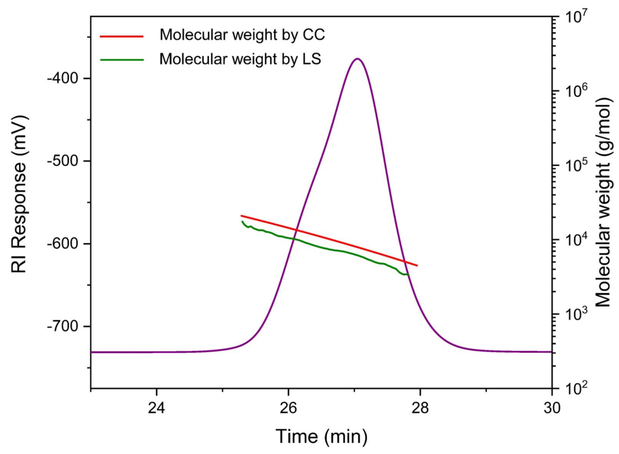


Figure 1. MW distribution of PEG-PGA determined by LS and CC

Conventional column calibration for MW determination leads to overestimated Mw and PDI

Light scattering MW determination does not depend on the elution profile but on the amount of light scattered.

Method: EcoSEC Elite (HLC-8420) GPC, 2x TSKgel GMPWxl columns, 0.01M NaNO3 and 0.02% NaN3 as mobile phase, 0.7 ml/min flow rate, refractive index (RI) and LenS3 MALS detector.

ACCURATE Low Rg Determination by MALS

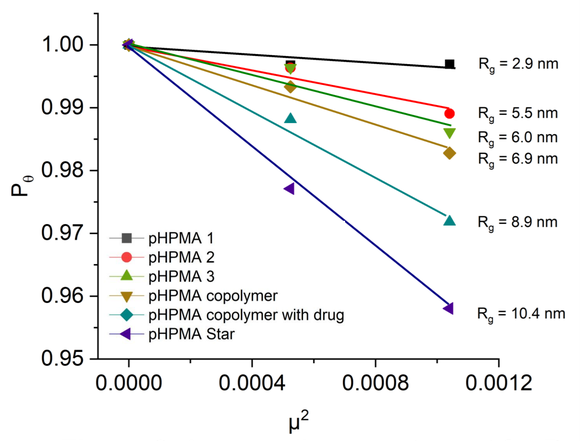


Figure 2. Angular dissymmetry plot for Rg determination of pHPMA* polymers

Table 2. Mw and size of pHPMA* polymers

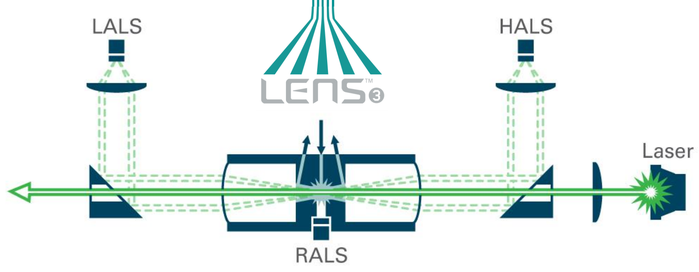
	M_w (kDa)	R_g (nm)	R_{h**} (nm)	R_g/R_h
pHPMA 1	9,8	2.9	2.33	1.247
pHPMA 2	52,1	5.5	5.25	1.048
pHPMA 3	109,8	8.9	8.17	1.089
pHPMA cop	51,4	6.0	5.03	1.194
pHPMA-drug conjugate	61,4	6.9	5.15	1.340
Star pHPMA copolymer	361,9	10.4	11.93	0.872

*pHPMA - poly(N-(2-hydroxypropyl)methacrylamide)
**radius of hydration

The LenS3 MALS detector detects a 0.3% difference in particle scattering function (P_θ) between the 10° and 170° detectors, determining an Rg below 10 nm.

Higher MW of pHPMA shifts solution conformation from random coil to compact structures

Drug conjugation to a pHPMA increases the Rg/Rh from 1.194 to 1.34, which is indicative of a random coil structure.



Method: EcoSEC Elite (HLC-8420) GPC, TSKgel Alpha-4000, 10 µm, + TSKgel Alpha-3000, 7 µm, (7.8 mm x 30 cm), MeOH/0.3 M CH3COONa (80/20), pH 6.5 as mobile phase, 0.7 ml/min flow rate, RI and LenS3 MALS detector.

CONFORMATIONAL Changes in Solution Characterized by MALS-Viscometer

Conventional column calibration for MW determination of star shaped PGA leads to overestimated Mw and PDI due the lack of suitable calibration standards.

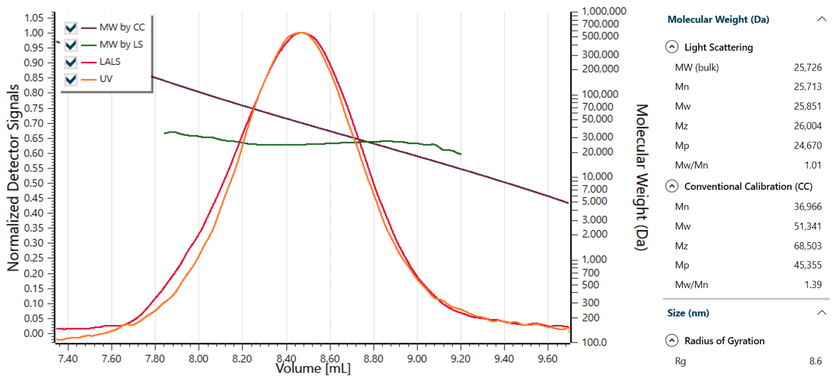
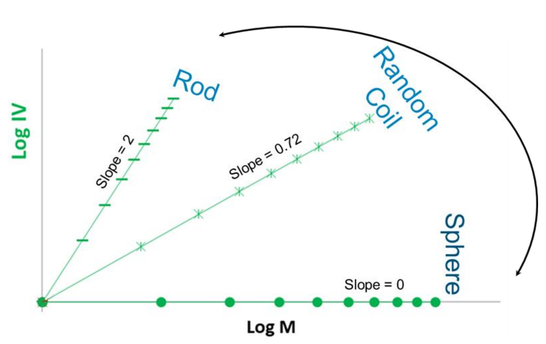
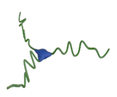


Figure 3. MW distribution of StPGA determined by **LS** and **CC**

Mark Houwink (MH) plot



Rg/Rh ~0.77
Sphere



Rg/Rh >1
Random coil

Drug conjugation to StPGA leads to Rg/Rh > 1 suggesting random coil conformation, while the MW increase leads to higher viscosity and more expanded structure in the solution (hence $Rg_{StPGA-D1} > Rg_{StPGA}$)

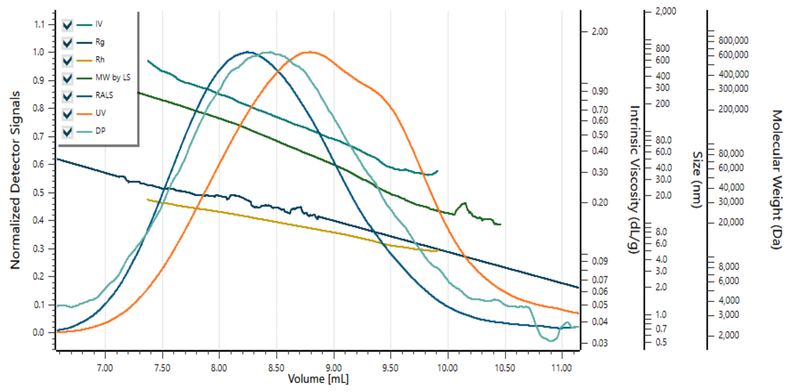


Figure 4. **MW**, **Rg**, **Rh**, and **IV** distribution of StPGA-drug conjugate determined by LS and viscometer.

Table 3. Rg, Rh, Mark Houwink (MH) slope, and intrinsic viscosity (IV) of StPGA and its conjugates

	R_g (nm)	R_h (nm)	R_g/R_h	MH Slope	IV
1. StPGA	8.6	4.8	1.8	0.84	0.29
2. StPGA-D1	15.9	9.3	1.71	0.65	0.58
3. StPGA-D2	20.7	7.0	2.95	0.57	0.22
4. CSS StPGA-D1-D2	16.8	32.0	0.53	0.04	1.67

Method: EcoSEC Elite (HLC-8420) GPC, TSKgel G5000PWxl column 10 µM, 7.8 x 300 mm, phosphate buffer pH 7.4 and 0.02% NaN3 as mobile phase, 0.8 ml/min flow rate, ultra-violet (UV) and LenS3-Viscometer MALS detector.

Drug conjugation and crosslinking results in increased Rg, and Rh in solution. Simultaneously, **the Rg/Rh ratio** and **MH slope decrease** – indicating a transition toward a more **spherical morphology** in crosslinked StPGA-drug conjugates, compared to the random coil observed in non-crosslinked counterparts.

The observed **increase in IV** suggest the crosslinked StPGA-D1-D2 structures are less compact than the unimeric StPGA.

Combined, IV, MH slope and Rg/Rh ratio suggest that the crosslinked particles adopt **spherical architecture.**

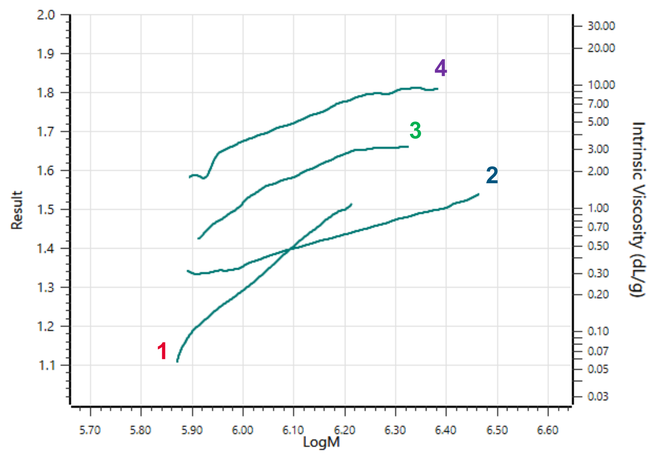


Figure 5. **IV** distribution of **StPGA (1)**, **StPGA-D1 (2)**, **StPGA-D2 (3)**, and **CSS StPGA-D1-D2 (4)** determined by viscometer

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS. TOUCHING LIVES.

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