The value of size exclusion chromatography in hydrodynamic characterization of molecules

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Abstract

Statement of the Problem: In size exlusion chromatography (SEC), macromolecules are sorted according to their size distribution. Therefore, SEC is also a measure for the hydrodynamic volume and yields valuable analytical information. Column calibration with well-defined polymer standards allows for estimation of apparent molecular weights. However, SEC analysis is usually considered an isolated methodology, overlooking interconnections with related methods. The present study aimed in the detailed hydrodynamic characterization of a novel recombinant biopolymer in comparison with poly(ethylene glycol) by elaborating the correlation between various techniques, including SEC, in order to obtain an integrated overall picture of hydrodynamic molecular properties. Methodology & Theoretical Orientation: In polymer sciences, a universal SEC calibration procedure is used, keeping in mind the dependence of the hydrodynamic volume on both the molecular weight and the intrinsic viscosity. Though essentially defining the solution viscosity of a macromolecule, the latter parameter is often neglected in biochemical sciences. It can be obtained from viscosity data and yields an expectation value for the hydrodynamic radius r_h, assuming an ideal spherical molecule shape. Dynamic light scattering (DLS), as a shape-sensitive technique, leads to diverging r_h values for non-ideal, elongated molecular shapes. Findings: The discrepancy between the r_h data obtained by DLS and viscometric methods was evaluated for molecular shape estimation. SEC for itself is less sensitive to molecular shape and conformation than DLS, but provided useful indications for non-ideal molecule shapes in combination with DLS. In addition, SEC data confirmed independently obtained results from microviscometry. Conclusion & Significance: In measuring the hydrodynamic volume, SEC data holds relevant conformational information exceeding the mere molecular weight information. To access shape information, it is advisable to use complementary hydrodynamic techniques. Especially the biosciences can only benefit from changing their view on SEC from an isolated standard technique to an integrated hydrodynamic characterization tool.

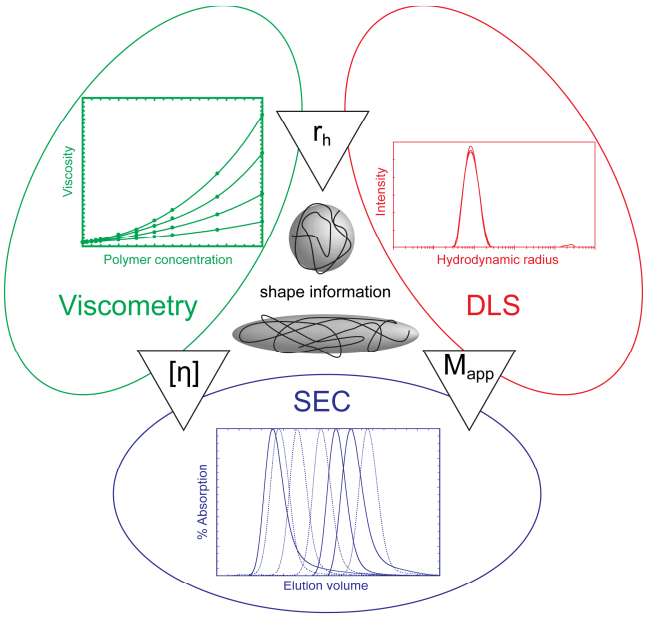


Figure 1: Comprehensive approach for shape estimation of macromolecules. DLS: dynamic light scattering; SEC: size exclusion chromatography; r_h: hydrodynamic radius; [η]: intrinsic viscosity; M_{app}: apparent molecular weight

Recent Publications

1. Breibeck, J., Serafin, A., Reichert, A., Maier, S., Küster, B. & Skerra, A. (2014) PAS-cal: a Generic Recombinant Peptide Calibration Standard for Mass Spectrometry. *J. Am. Soc. Mass Spectrom.*, 25:1489-1497.
2. Breibeck, J. (2016) Studies on the biophysical behaviour of recombinant amino acid polymers with defined composition (PhD thesis).
3. Breibeck, J. & Skerra, A. Insights into the polypeptide biophysics of proline/alanine-rich sequences (PAS): a recombinant biopolymer with PEG-like properties. (manuscript in preparation)
4. Breibeck, J., Stellner, N., Friedrich, L. & Skerra, A. Chemical PASylation – a biomimetic PEGylation approach based on recombinant proline/alanine-rich polypeptides. (manuscript in preparation)



Biography

With a strong interest in the interdisciplinary contact zone linking chemistry and biosciences, Joscha Breibeck has complemented his chemistry studies at Technical University of Munich (TUM, Germany) with a PhD in biotechnology and protein biochemistry (Institute for Biological Chemistry, TUM). His projects were centred around the detailed biochemical and biophysical characterization of a novel class of recombinant polypeptides. During those studies and especially in viewing the polypeptides as (bio)polymers rather than proteins, Breibeck gained in-depth experience with various preparative and analytical techniques, with a strong focus on chromatographic applications. Further investigations of the novel biopolymers involved their use for the hydrodynamic volume enlargement of (bio)pharmaceuticals by either genetic fusion or chemical conjugation and even as a calibration standard for mass spectrometry. Breibeck currently studies the bioscientific applicability of inorganic polyoxometalate clusters, in particular for improved protein crystallization, at the Institute for Biophysical Chemistry at University of Vienna (Austria).

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