Profile

Dendritic-polymer drug delivery structures characterized by a novel triple detection method

A novel multi-detector analysis method known as size-exclusion chromatography cubed (SEC^3), which accurately characterizes the molecular weight, size and architecture of biopolymers, is playing a major role in the development of novel polymer structures for drug delivery. Large macromolecular assemblies with linear segments in the periphery and perfectly branched blocks called micelles in the core have a tremendous potential for use as extended drug delivery systems. Many of these molecules, however, are 500–1000 Da and conventional methods are unable to accurately measure this molecular weight and size. Researchers at the Polymer Chemistry Department at the State University of New York (SUNY, Syracuse, NY, USA), report that they have overcome this problem by combining SEC, a laser light-scattering detector, differential viscometer detector and a differential refractive-index detector to accurately characterize macromolecular properties.

Tree-like dendritic polymers, whose structure can be controlled at a nanoscopic level, have the potential to revolutionize the field of drug delivery. These new materials represent the first examples of structure-controlled polymer architectures that rival the control seen in natural biopolymers such as protein, DNA and RNA. Dendritic polymers have three major architectural components:

(1) a core,
(2) an interior, and
(3) a surface.

Their architecture mimics the dendritic branching of trees. Dendrimer polymers are produced by a unimolecular branching process, in which commercial feedstock monomers are introduced into ordinary laboratory glassware to produce covalently bonded monomer arrays. Since 1990, research in dendritic polymers has rapidly increased; in the past five years, there have been at least 600 publications and patents in this field.

New type of copolymer

Starting initially in close cooperation with Jean M-J. Fréchet (University of California, Berkeley, CA, USA), the SUNY group has developed convenient methods for the synthesis of a new type of hybrid block copolymer containing both flexible linear (A) and rigid dendritic (B) blocks. The flexible, hydrophobic and amorphous A blocks consist of linear aliphatic polyesters or polystyrene, and the semi-rigid hydrophilic crystalline B blocks consist of dendritic aliphatic polyethers. These copolymers (Fig. 1) are able to form macromolecular assemblies, or micelles, that have chemical compositions influenced only by the type of solvent, and not by its concentration or temperature[1]. By changing the size of the dendrimers and the length of the linear block, as well as its chemical nature, products with a wide range of useful properties might be developed[2]. Dendritic polymers of this type can host several times their weight of pharmaceuticals, and the interior space has unique shape-specific recognition properties for guest molecules; these features offer exciting new options for smart drug delivery.

An important issue, and up until now a major obstacle, in dendritic polymer research is the need to accurately characterize molecular properties. When producing these micelles, it is important to control their molecular weight, size and architecture because this determines their strength and how much material they can hold.

Molecular characterization is particularly crucial when dealing with hybrid structures whose behavior is intermediate between flexible linear polymers and rigid dendritic polymers. These macromolecules can form a wide range of different architectures while maintaining a constant chemical composition. An instrument that is sensitive enough to trace the smallest change in molecular properties is required to assign such properties to these architectures and to determine which are the most appropriate for specific applications.

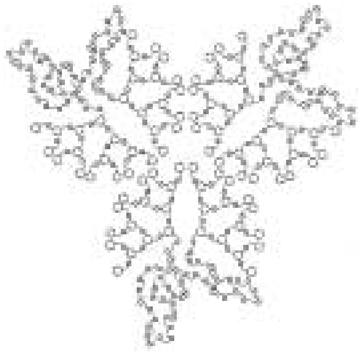

Figure 1. The scheme of the supramolecule constructed out of linear-dendritic block copolymers.

Twilight zone

Many of the macromolecules produced by these methods are 500–1000 Da and conventional methods cannot provide an accurate characterization of their molecular size and weight. The light-scattering method, which involves passing monochromatic light, typically of a wavelength of either 488 or 633 nm, through a polymer solution, is inaccurate for characterizing macromolecules of less than 1000 Da. However, classical methods based on either the boiling-point elevation, freezing point depression or osmotic pressure of a solution are typically not effective with macromolecules larger than 500 Da. Furthermore, even when molecules are produced of a size that can be measured by one of these conventional methods, the inability of these methods to characterize either the architecture or level of branching is unsatisfactory.

For the past several years, Gitsov has coupled differential viscometry to increasingly sophisticated supplementary

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detectors to address this problem. A Model 110 differential viscometer from Viscotek (Houston, TX, USA) was first used in his Cornell laboratory in 1990. The differential viscometer is used to measure the inherent viscosity of the polymer. The polymer or macromolecule of interest is dissolved in the same solvent that flows through the bridge, which consists of four stainless steel capillaries. The polymer solution is injected into one side of the bridge while pure solvent flows through the other side. Pressure transducers measure the pressure of the solvent at the inlet of the bridge and the pressure differential between the solvent side of the bridge and the polymer solution side of the bridge. The differential pressure, inlet pressure and sample concentration are then used to calculate the inherent viscosity of the polymer solution.

Adding special detectors for SEC

The viscometer used alone is capable of generating relatively accurate molecular weight measurements, even in the crucial 500–1000 Da range. However, because Gitsov required a more complete molecular comparison, he coupled a Model 250 viscometer to an existing differential refractometer. Measuring a solution with both of these instruments makes it possible to use the Flory-Fox and Ptytsin-Eizner equations to determine the hydrodynamic radius of the polymer molecules, providing another crucial parameter. However, a method of obtaining an accurate characterization of the architecture of the molecule still remained to be found.

This was accomplished by using a four-channel data manager and TriSEC software (Viscek), and integrating the viscometer and refractometer with existing SEC and multi-angle laser light-scattering detector to achieve a three-dimensional approach that completely and accurately characterizes molecules of any size. The first dimension comes from the chromatographic process that separates polymer molecules according to molecular size. The second dimension is obtained by the light-scattering detector, which determines the molecular weight, and the third dimension comes from the viscometer detector, which gives a parameter that is inversely proportional to molecular density. Taken together, these variables provide a detailed picture of the molecular structure.

Universal calibration approach

Generally, the light-scattering detector measures molecular weight directly, without the need to run a calibration curve. Instead, an injection of a standard of known molecular weight is required to calibrate the response of the light-scattering detector. Sometimes, however, the light-scattering detector is inappropriate for the sample of interest.

In such situations, the on-line differential viscometer offers an alternative approach to determining molecular weight. The viscometer measures the intrinsic viscosity (IV) of the sample and a polymer standard of known molecular weight. The IV is measured as volume per unit mass and represents the specific volume of the molecules in solution, which is inversely proportional to the molecular density. As the molecular weight of the standard is known, it is possible to calculate the molecular weight of the sample using the more reliable universal calibration approach. Unlike the conventional column calibration approach of SEC, this provides accurate molecular weight data while eliminating the requirement that the sample and standard have the same molecular composition and structure.

Molecular-structure determinations

Quantitative calculations using the information from all three detectors yield both the molecular-weight distribution and the intrinsic viscosity distribution. The relationship between these variables is shown most usefully in a Mark-Houwink plot, which is a log-log plot of IV against molecular weight for a polymer sample. This plot demonstrates how the intrinsic viscosity, that is, the molecular density, changes as the molecular weight increases. It clearly indicates differences in branching even for samples with the same molecular weight. Being more compact in structure, branched molecules appear to have much lower IV values than linear molecules of the same molecular weight. Using a linear standard as a reference, it is possible to calculate the number of branches and branching frequency.

Conclusions

This triple detection method has enabled SUNY researchers to accurately and quickly characterize dendritic polymers of various sizes, including those within the crucial 500–1000 Da range. The accuracy of these measurements has been verified using reliable NMR instruments (I. Gitsov, unpublished). The ability to accurately characterize polymer weight, size and architecture has assisted SUNY researchers in several significant developments, including the formation of rarely reported micelles containing dendritic blocks in their cores.

References

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