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Analysis of aromatic polyether dendrimers and dendrimer-linear block copolymers by matrix-assisted laser desorption ionization mass spectrometry

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Summary.

Polyether dendrimers with differing chain end functionalites and molecular topologies were analyzed by MALDI-TOF mass spectrometry. The success of this analytical method is highly dependent on the type of matrix and sample preparation used. Ester and perdeuterophenyl terminated dendrimers with molecular weights up to 14,000 amu were successfully analyzed using an indoleacrylic acid matrix in tetrahydrofuran. Hybrid block copolymers consisting of linear polystyrene or poly (ethylene glycol) chains attached to one or two dendrimers gave spectra with oligomeric resolution in matrices of *t*-retinoic acid and 2-nitrophenyl octyl ether respectively. The MALDI-TOF results provide access to molecular weight distribution data confirming the results of size exclusion chromatography (SEC) analysis.

Introduction.

In addition to finding wide use in the analysis of biological macromolecules, the technique of matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry has been used increasingly in the characterization of synthetic polymers. A significant amount of work has been done with water soluble polymers[1-4] and the discovery of organic solvent based matrix systems has allowed the application of this method to low and medium molecular weight organic-soluble polymers[3,5] and dendrimers based on phenylacetylene[6] or phenyl ester[7] moieties. We now report the application of MALDI-TOF mass spectrometry to the analysis of a variety of polyether dendrimers. These dendrimers, based on a repeat unit (Figure 1a) derived from 3,5-dihydroxybenzyl alcohol, are prepared by the convergent growth approach.[8] In these studies, success is highly dependent on the choice of matrix and on sample preparation. The MALDI technique is particularly attractive for dendrimers that are prepared in stepwise processes involving intermediate purifications because the precise analysis of these high molecular weight (MW) materials by classical techniques is difficult. For example NMR or elemental analyses fail to detect minute amounts of structurally related impurities, while SEC is of limited value since the dendrimers themselves are much less polydisperse than the polymer standards used to calibrate the columns.

Results and Discussion.

The matrix system of indoleacrylic acid (IAA) in THF solvent was found to be the most universally applicable for a variety of polyether dendrimers with varying chain-

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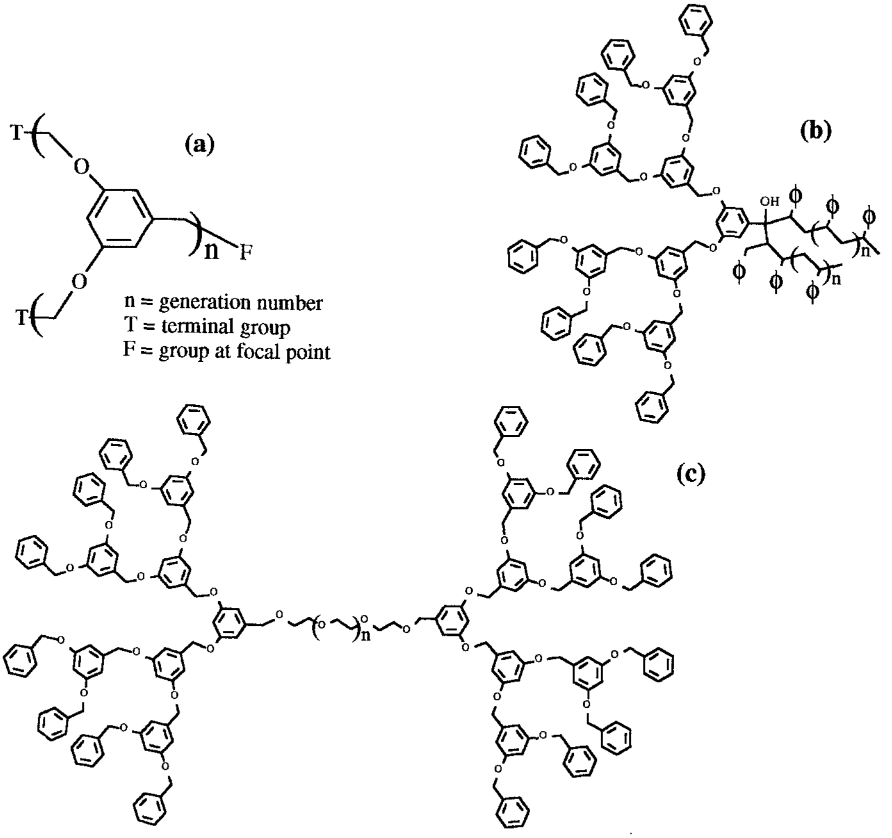


Figure 1. Structures of the various dendrimers analyzed by MALDI.: (a) generic dendrimer structure; (b) [G-3]-$(PSTY)_2$; (c) [G-3]-PEG-[G-3].

end functionalities, molecular topologies, and MW of up to 14,000 amu. Mass accuracies were typically 0.01 - 0.05 %. The THF solutions of sinapinic acid, 3,5-dihydroxybenzoic acid, and α-cyano-4-hydroxycinnamic acid that are commonly used as matrices for water-soluble biopolymers, gave no measurable signal with the majority of the polyether dendrimers we tested. The use of the IAA matrix rarely afforded the $(M+H)^+$ mass ion, instead giving the $(M+Na)^+$ or $(M+K)^+$ ion, or quite often both. Others have successfully dealt with this problem by doping the samples with sodium[3,7] or lithium[1,4] salts so as to simplify the spectrum by suppressing all other residual cations. Most of these studies, however, used aqueous (or partially aqueous) based matrix solutions in which the salts were soluble. The addition of small amounts LiCl and sodium dodecyl sulfate, which are at least partially soluble in THF, to the IAA matrix solution appeared to interfere with the crystallization process and often resulted in a drastically reduced signal. In the latter case, even when signals could be obtained, peaks corresponding to the potassium adduct (M+39), could sometimes still be observed, particularly for dendrimers or molecular weight greater than 3000. Storage of the matrix solution over a small amount of insoluble NaCl gave the same results.

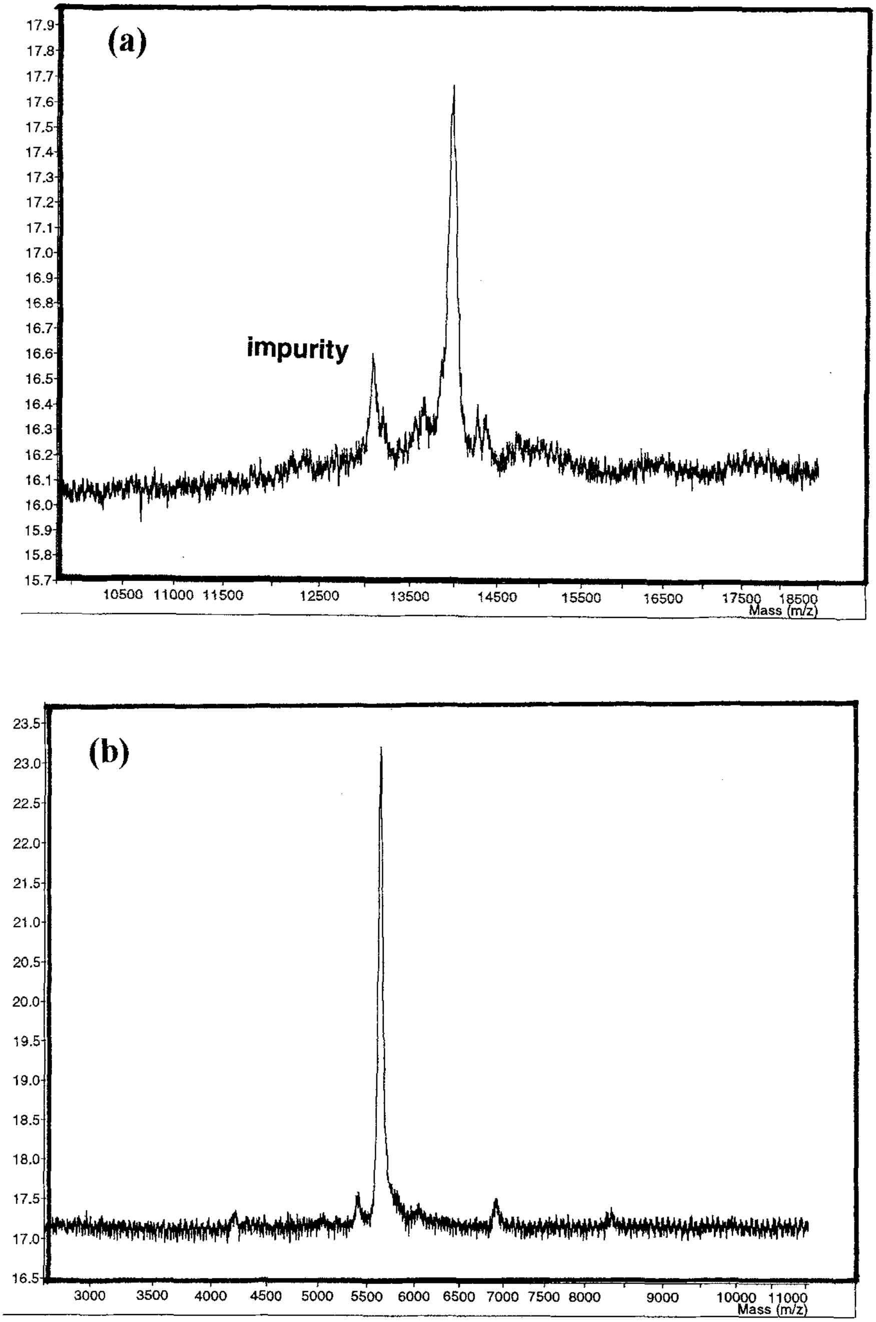


Figure 2. Maldi-TOF mass spectra of selected dendrimers: (a) D_{448}-[G-6]-OH with lower molecular weight impurity, (b) $(EtO_2C)_{32}$[G-4]-OH

Table 1 provides MALDI-TOF molecular weight data for four representative polyether dendrimers prepared by the convergent growth approach using 3,5-diester or perdeuterobenzyl chain ends. For all of these compounds, the molecular weights fall within 2.5 amu of the calculated values. The nomenclature $(EtOCO)_{32}$-[G-4]-OH describes the dendrimer chain end functionality (32 ethyl ester groups attached to the terminal aromatic rings) followed by the generation number (fourth), followed by the focal point group (hydroxyl).

A typical sample preparation is as follows. 5 μl of a solution of the analyte in THF (1-10 mg/ml) was combined with 20 μl of IAA matrix solution (0.2-M/THF) in a microcentrifuge tube and agitated on a touch mixer. The optimum concentration of the analyte solution varied slightly among different compounds. Higher concentrations (10 mg/ml) typically gave the best results for dendrimers of low molecular weight (<3000). The matrix solution was freshly prepared before each use as it yellows upon standing over 48 hours. Spectra taken using aged matrix solution were usually of drastically reduced quality. A 1 μl aliquot of this matrix/sample solution was transferred to a stainless steel sample plate via automatic pipette. All analyses were performed on a Finnigan MAT LASERMAT instrument equipped with a 337 nm nitrogen laser. Laser power settings of 15-25% were found to be optimal. It was observed that higher laser power settings gave greater signal to noise ratios at the expense of peak resolution, while very low settings gave the opposite result. Spectra were averaged over 30 - 60 laser pulses. The instrument was calibrated using bovine pancreatic trypsin inhibitor (BPTI) in an α-cyano-4-hydroxycinnamic acid matrix, and was generally recalibrated after every ten spectra.

Table 1. MALDI-TOF Molecular Weight Data of Selected Dendrimers

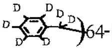
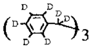
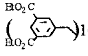
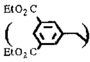
Dendrimer	Calculated Molecular Weight	Experimental Molecular Weight
 $_{64}$-[G-6]-OH	13,966.8 $(M+K)^+$	13965.0
 $_{32}$[G-5]-OH	6934.8 $(M+Na)^+$	6933.9
 $_{16}$-[G-4]-OH	5621.0 $(M+Na)^+$	5623.4
 $_{8}$-[G-3]-OH	2769.8 $(M+Na)^+$	2769.6

Figure 2 shows the spectra obtained for two different dendrimers using the conditions outlined above. The spectrum shown in Figure 2a was obtained using a sixth generation dendrimer sample that was prepared several years ago.. This spectrum, which reveals the presence of a lower molecular weight impurity, confirms the usefulness of the method to ascertain the purity of a specific dendrimer sample. Despite the presence of this impurity, the dendrimer is much "purer" than any polymer obtained by a conventional polymerization technique. While the exact origin of this impurity is not known, it may well have arisen by cleavage of one of the many acid-labile benzyl ether groups of the original dendrimer. Figure 2B shows a reactive dendron used in the preparation of water-soluble dendrimers. At present, MALDI-TOF mass spectrometry, when applicable, appears to be the best way to test a dendrimer's true purity.

We have also used MALDI-TOF mass spectrometry to characterize a series of hybrid linear-dendritic block copolymers such as those shown in Figure 1. For copolymers consisting of hydrophilic poly (ethylene glycol) (PEG) blocked at both extremities with hydrophobic dendrimer end groups,[9] neither the IAA/THF matrix, used successfully for the analysis of dendrimers, nor 3,5-dihydroxybenzoic acid/5% EtOH:H_2O, used successfully for PEG, [3] gave detectable signals. However, a retinoic acid matrix with CH_2Cl_2 solvent has been used by Moore and Wilkins[6] for both phenylacetylene hydrocarbon dendrimers and PEG. This matrix was used successfully with the dendrimer-PEG-dendrimer triblock copolymers, affording spectra that show signals for each individual oligomer (Figure 3). This spectrum is also in good agreement with data obtained by SEC using universal calibration (Table 2).

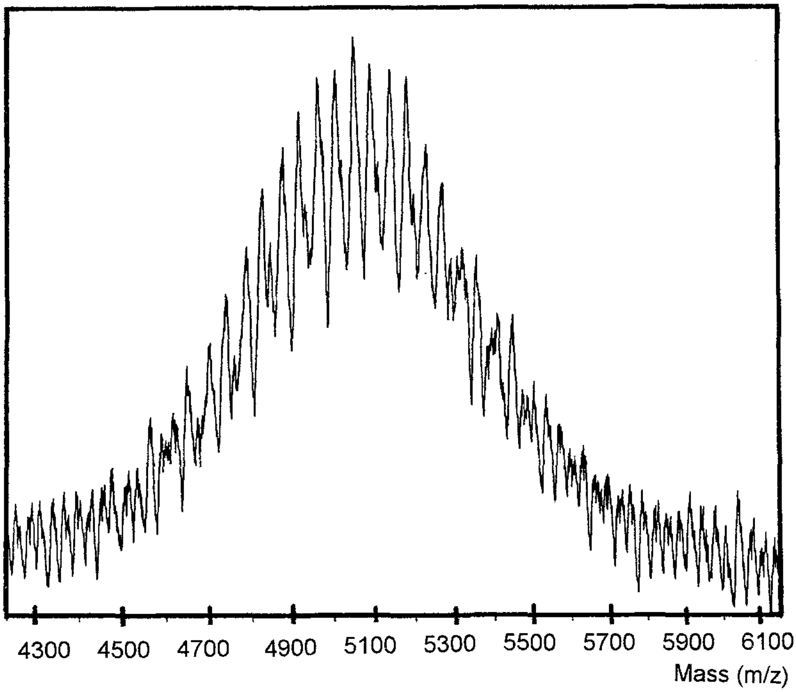


Figure 3. MALDI-TOF mass spectrum of [G-3]-PEG-[G-3]

A liquid matrix system, 2-nitrophenyl octyl ether with added silver trifluoroacetate, has been reported for the analysis of polystyrene.[3] This matrix was also found to be useful for a polystyrene-dendrimer block copolymer prepared by the reaction of living polystyrene with a [G-3]-COOMe dendrimer having a methyl ester functionality at its focal point.[10] Using higher power fluences (~40%) a broad peak showing resolution of individual oligomers was obtained after the averaging of 200 laser pulses. The MALDI spectrum also shows good agreement with SEC data (Table 2). A small peak corresponding to the $[2M+Ag]^+$ cluster is evident, this is not unusual in view of the higher power used to obtain this spectrum.

In conclusion, we have demonstrated that MALDI-TOF MS is an extremely useful technique for the analysis of a wide variety of dendritic architectures. The degree of accuracy is high and sample preparation is quick and simple. The success of this method for different classes of compounds is highly specific to the type of matrix used.

The widespread use of the technique will require further developments in both instrumentation, software, and new matrix systems in order to increase the range of molecular weight that is accessible with a broad spectrum of synthetic polymers.

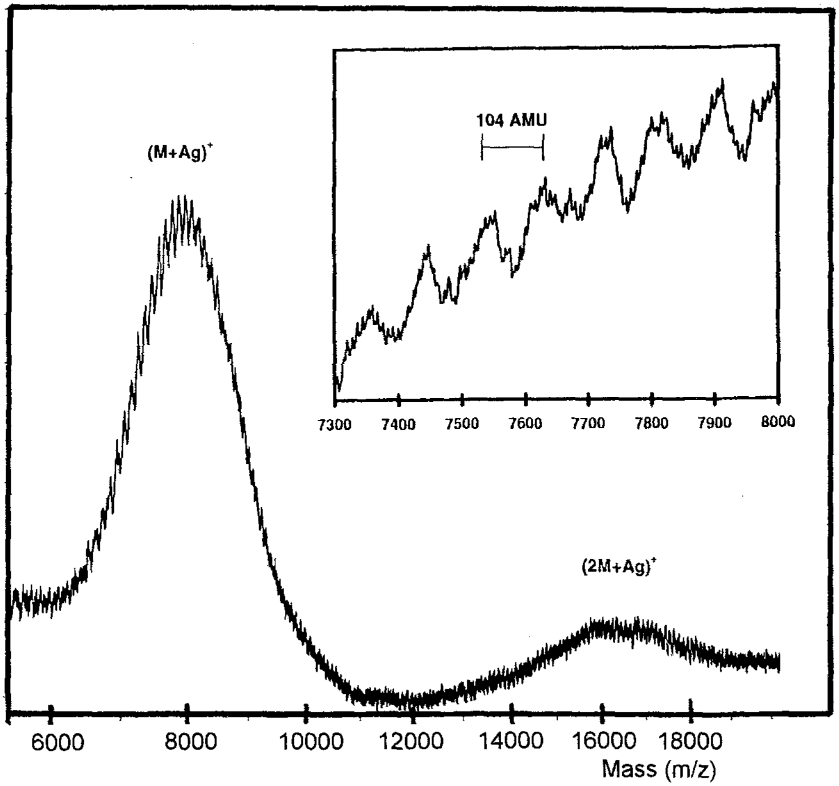


Figure 4. MALDI-TOF mass spectrum of [G-3]-(PSTY)$_2$

Table 2. Comparison of Molecular Weight Data Derived from MALDI and GPC Analysis of Dendrimer-Linear Copolymers.

	MALDI Data			GPC Data		
Polymer	M_n	M_w	M_p	M_n	M_w	M_p
[G-3]-PEG-[G-3]	5038	5095	5033	5499	5554	5000
[G-3](PSTY)$_2$	8341	8496	8073	8023	8215	8010

Acknowledgements.

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