

Matrix Assisted Laser Desorption (MALDI), a versatile tool for the characterization of Dendrimers

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I. ABSTRACT

Matrix Assisted Laser Desorption (MALDI) is a soft ionization technique used in modern day chemistry to analyze polymers with high precision and speed. This technique does not require any polymer standard for mass calibration (as in the case of gel – permeation chromatography GPC). Protocols require only minimum amounts of solvent and consumables. Measurements of the masses of individual oligomers facilitate the determination of masses of monomers and end groups. This overview discusses the application and use of MALDI for the determination of molecular mass distribution of oligomers and end groups. However, biomolecules and other polymers with a polydispersity index (PDI) greater than 1.2 are not suitable candidates for this analysis owing to signal intensity discrimination against higher mass oligomers.

Keywords: Dendrimers, MALDI, TOF, MS, DHB matrix

The scientific innovations led by the advent of nanotechnology involved the construction nano – sized materials that include biomacromolecules and other polymeric architectures. The synthesis of dendrimers was one such unique discovery brought about by Tomalia in 1983 and Dewald by U.S. Patent 4507466 (Tomalia et al. 1985). Dendrimers are nano – sized, hyperbranched macromolecules that are monodisperse in nature and globular in shape with iterating successive generational levels that emerge or grow outwards from a central core. The origin of the term ‘dendrimer’ can be attributed to the Greek word ‘dendron’ which means a tree and ‘meros’ meaning ‘a part of’. They are also termed as ‘arborols’ from a Latin word ‘arbor’ which also connotes ‘a tree’ (Barbara & Maria, 2001).

The synthesis of dendrimers can be carried out using either the divergent or the convergent method. The divergent method involves starting synthesis with a reaction sequence between a central core/focal point and a branch cell monomer unit to form



a first generation level. Iterative synthesis using controlled reactions results in further growth in an outward expansion of polymeric design. The anchoring focal point (dendrimer nucleosome) provides information related to size, shape and core multiplicity. It also gives us an idea about the number of dendrons that may grow from the core. The surface of the dendrimer contains terminal functional groups that are characteristic to it. The functional groups can be either hydrophobic or hydrophilic or may even exhibit electrical conductivity. Moreover, dendrimers developed by divergent synthesis mimics biological proteins and hence they are nicknamed 'artificial proteins' (Tomalia et al. 1997). Convergent synthesis involves the formation of a construct by attaching all individual monomer units or groups present at the periphery to form branch assemblies (polymeric arms) and finally getting them attached to the central core of the dendrimer. Preparations of lesser quantities of sample or lower generation dendrimers are carried out by convergent synthesis as it generates defect free, high purity samples with reduced steric effects (Hawker & Fréchet, 1990).

The peculiarity in the molecular architecture of dendrimers makes it a perfect candidate for diverse biological and industrial applications. Structural elucidation using various analytical techniques is required to explain the critical nanoscale design parameters that include size, shape, surface chemistry and elemental composition (Morawetz, 1985). These techniques include separation techniques (Chang, 2003), spectroscopic methods (Heigl et al. 2007), X – ray (Medhioub et al. 2007) and mass spectrometry (Montaudo et al. 2006). Among these, mass spectrometry along with MALDI has become indispensable tool for the analysis of dendritic polymers, their composition, molecular mass distribution, presence of impurities, surface functional groups and how they undergo fragmentation depending on the variety of matrices and laser pulse energy used for such fragmentation (Juhan et al. 2005)

MALDI is a soft ionization technique used in modern day chemistry to analyze polymers with high precision and speed. This technique does not require any polymer standard for mass calibration (as in the case of gel – permeation chromatography GPC). Protocols require only minimum amounts of solvent and consumables. Measurements of the masses of individual oligomers facilitate the determination of masses of monomers and end groups. This review discusses the application and use of MALDI for the determination of molecular mass distribution of oligomers and end groups (Rémi et al. 2007) However, biomolecules and other polymers with a



polydispersity index (PDI) greater than 1.2 are not suitable candidates for this analysis owing to signal intensity discrimination against higher mass oligomers. A very desirable mass analyzer for characterization of polymers is the Time of Flight (TOF) device. It offers unparalleled resolving power; ion detection and mass range and the same may be coupled with the MALDI instrumentation to provide excellent results (Liang Li et al. 2010).

II. FRAGMENTATION OF POLY (AMIDOAMINE) (PAMAM) DENDRIMERS

Different generations of PAMAM dendrimer fragmentation by MALDI were studied by Juhan et al (2005) using 2, 5 – Dihydroxybenzoic acid (DHB), 4 – hydroxy – 3 – methoxycinnamic acid (ferulic acid) (FER), α – cyano – 4 – hydroxycinnamic acid (ACH), 2, 4, 6 – trihydroxyacetophenone (THAP) and 3 – hydroxypicolinic acid (HPA) as matrices. The study concluded that DHB was the softest of the lot (Peterson et al. 2003). Multiple fragmentation channels with relative intensities depended on the choice of matrices. However, no correlation was observed in the case of pulse energy used for such fragmentation. It was concluded that for a given quanta of pulse energy fragmentation could occur along different protonation pathways and further that cationized fragments produced were more stable than the protonated fragments.

Later in 2007, Rémi et al studied structural deviation of PAMAM fan – shaped dendrimers from generations 0 – 3 using MALDI – TOF with N – laser at 337 nm (10 Hz) and electrospray mass spectra (ESI). Formation of new impurities either from perfect or defective synthesis of upper generations were detected apart from the commonly reported ‘missing arm’ and ‘molecular loop’ and some signal peaks remained unknown and required further investigation using mass spectrometry (Rémi et al. 2007).

The molecular weight M_n of a dendrimer of generation n is calculated as

$$M_n = \text{mass of dendrimer precursor} + \text{mass of desired dendrimer} \left[\sum_{i=0}^n 2^{i+1} \right]$$

Mallory et al (2013) analysed generation and branch defects in G5 PAMAM dendrimer using MALDI – TOF. Molecular weights measured for G5 PAMAM including fractional sets such as trailing generations, dimers, trimers and tetramers that were eluted via Reverse Phase High Performance Liquid Chromatography (RP – HPLC) and using Gel Permeation Chromatography (GPC) were found to be in agreement to that



calculated by MALDI measurements. Branch defects usually cause a decrease in molecular weight and total number of peripheral primary amine groups (from the theoretical standard of 128) in the case of G5 PAMAM. But, the inference from the available RP – HPLC data shows that early elution of the chromatogram contains defective dendrimer components and G5 PAMAM with 128 primary amine groups would elute later.

Holl et al (2013) used MALDI to identify the molecular weights of different components eluted by Reverse Phase Ultra High Performance Liquid Chromatography (RP – UPLC) and samples were further characterized by GPC to corroborate the molecular weights calculated. It was observed that MALDI – TOF – MS was consistent with the RP – UPLC structure assignments.

Hans et al (2015) characterized homologous series of polyphenylene (PP) dendrimers up to generation 9 using MALDI. Structural integrity was studied with theoretical molecular weights against measured molecular weights. A narrow polydispersity index (PI) less than 1.005 was obtained which confirmed high structural integrity of the megadalton range of the PP dendrimer under study.

Fragmentation using MALDI by Xiawen Lou et al (2017) analysing a series of synthetic organic ions bearing fixed multiple charges resulted in the conclusion that only ions with single charges were produced. They deduced that this was because such organic ions could undergo rearrangement/fragmentation during the process of reduction in charges. This observed phenomenon lends credence to the fact that misinterpretation of the MALDI – MS spectra could be a likely result with these generated ions being mistaken for impurities. To sum up their conclusion, a thorough foresight of the MALDI induced fragmentation pathways is highly essential for proper interpretation of the MALDI – MS spectra.

Naganath et al (2019) attempted the design of stimuli – responsive heterofunctional degradable G2 polyethylene glycol dendrimer exploiting Passerini multicomponent reaction (P – MCR) and click chemistry. MALDI was used to analyze structural integrity and purity of the dendrimer. Fragmentation at the phenylboronic ester – amide linkage and breaks at the benzylic carbon and oxygen (PhCH – O) bond and benzylic bond (PhCH – O) from the ONB group were observed. Fragmentation peaks were observed yet again in linear mode MALDI – TOF – MS analysis due the cleavage of the



dendrimer with a difference between cleavages around 1700 g/mol and for inefficient clicks with a difference of 625 g/mol indicating dendrimer disintegration.

III. CONCLUSION

The numerous scientific works produced from the fourth architectural class of polymers (dendrimers) is due to their unique characteristic features. This overview illustrates the potential of MALDI along with TOF and MS spectrometry techniques in the characterization of dendritic polymers. However, biomolecules and other polymers with a polydispersity index (PDI) greater than 1.2 are not suitable candidates for this analysis owing to signal intensity discrimination against higher mass oligomers.

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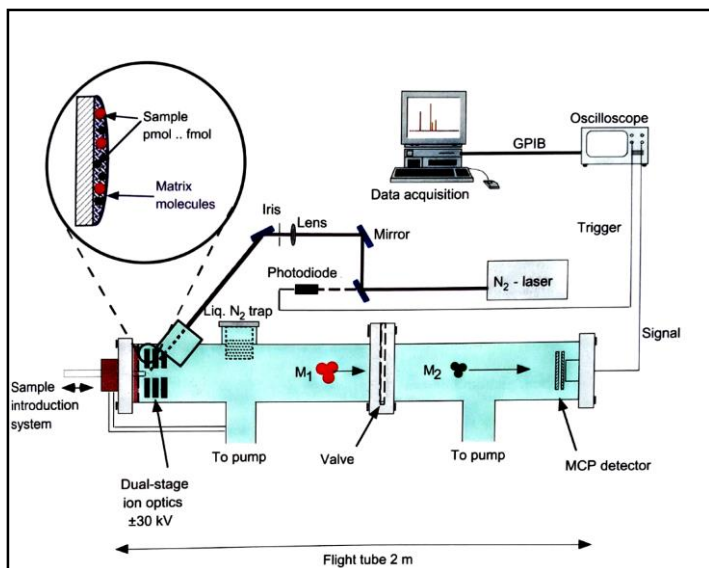


Figure 1: Schematic diagram of a MALDI – TOF mass spectromete

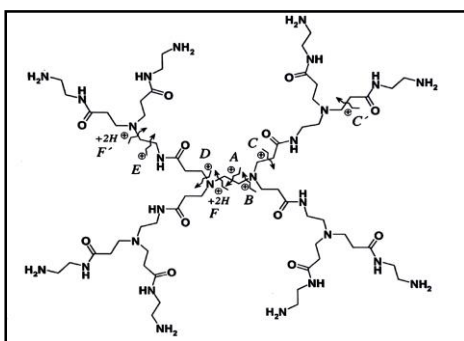


Figure 2:: G1 PAMAM dendrimer with fragmentation sites

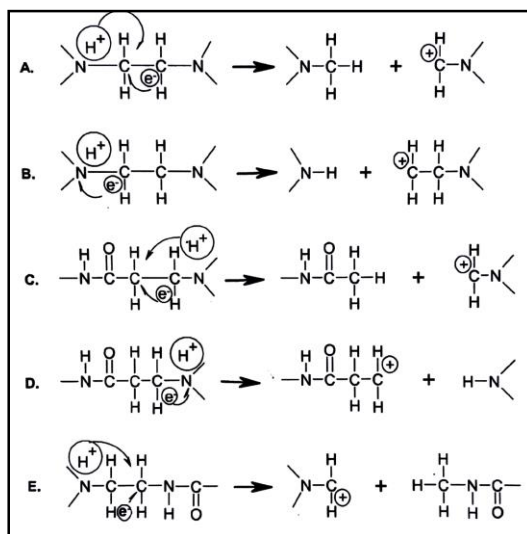


Figure 3:Mechanisms for observed fragmentation channels

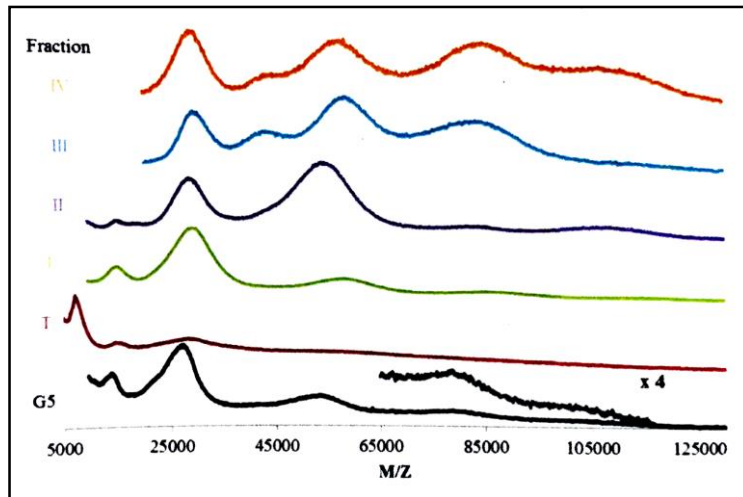


Figure 4: MALDI – TOF MS Spectra of G5 PAMAM showing trailing generations

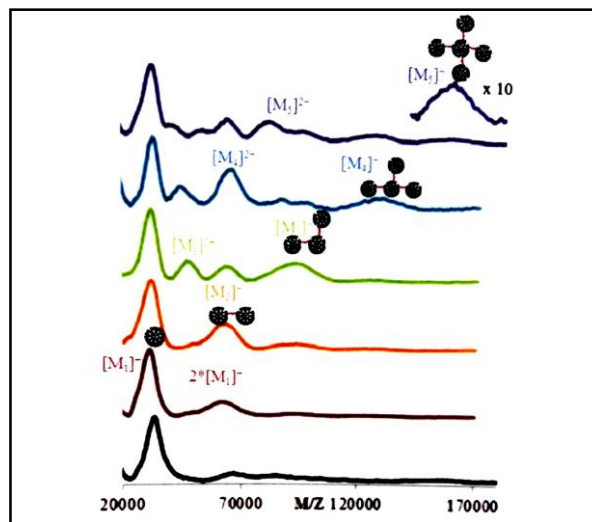


Figure 5: Normalized MALDI – TOF – MS spectra of commercial G5 PAMAM, G5 monomer (M_1^+), dimer (M_2^+), trimer (M_3^+), tetramer (M_4^+) and pentamer (M_5^+) peaks