Characterization of Linear and Branched Polyethylenimine (PEI) by ESI-MS and MALDI-TOF-MS

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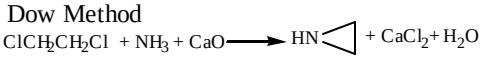
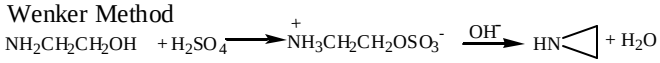
Polyethylenimine (PEI) is being used as a DNA transfection agent[1-5]. Complexes of DNA and PEI are known to pass through cell walls, and studies have reported that PEI's efficiency as a gene delivery vehicle depends on its molecular mass[1]. These assays were performed with commercial PEI that was poorly characterized. This study attempted to characterize commercial PEI, both linear and branched, by the use of mass spectrometry. Branched PEI can be synthesized from an aziridine, which undergoes homopolymerization by ring opening (Sn2-type cleavage). The aziridines are synthesized by one of the two methods below.

Dow Method

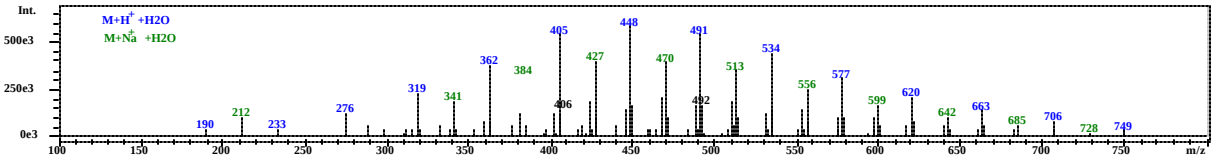


Wenker Method

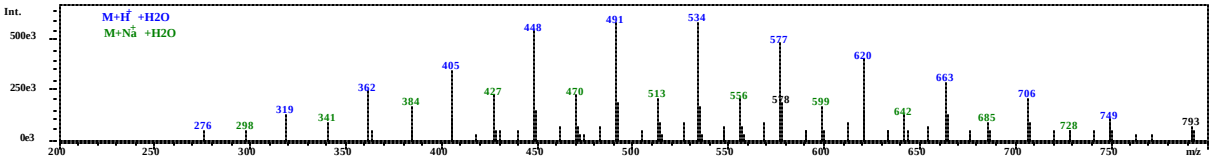


Three commercial branched PEI samples with average molecular masses of 600 u, 1200 u and 1800 u were analyzed by electrospray ionization mass spectrometry (ESI-MS). The molecular mass distribution (MMD) of each sample, shown below, was found to be very similar, with an average MMD of approximately 450 u. The averages of the MMD obtained by ESI-MS and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) have an estimated standard uncertainty of 50 u.

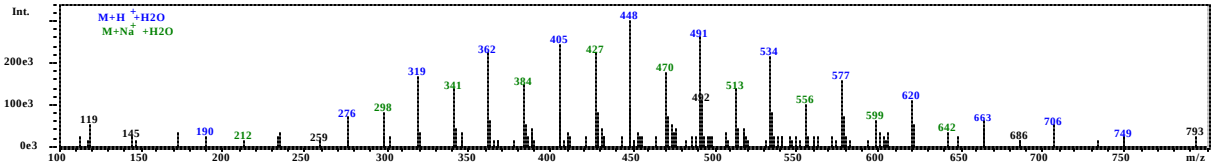
PEI-600



PEI-1200



PEI-1800

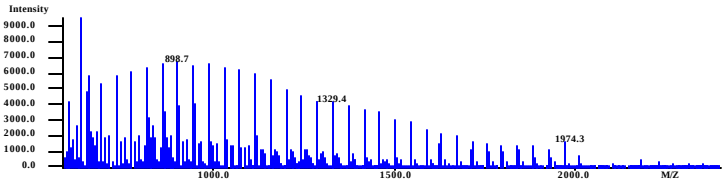


Branched PEI analyzed by ESI shows ion attachment to most molecules. The results also indicate that the branched PEI is predominately a cyclic material. A study with six cations of varying radii showed that there was preferential ion attachment that was not correlated with ionic radius of the cation, but rather the Lewis-acid strength of the cation.

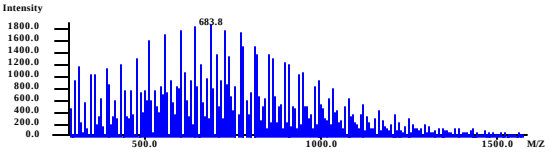
The effect of matrix and laser energy in MALDI on the MMD of four PEI samples of low polydispersity and varying degrees of polymer branching were studied. Two common matrices were considered: sinapinic acid, and 2,5-dihydroxybenzoic acid (DHB). Sinapinic acid and DHB formed solid complexes with PEI. The complexation was carried out in methanol and both the complex and the uncomplexed filtrate were analyzed. Analysis of the complex formed with DHB and branched PEI, with average molecular mass of

1200 u, and the uncomplexed filtrate can be seen below. The DHB complexes with PEI and removes the polymer from the solution leaving behind some polymer and most of the impurities in the sample.

PEI-1200 (DHB-COMPLEX)

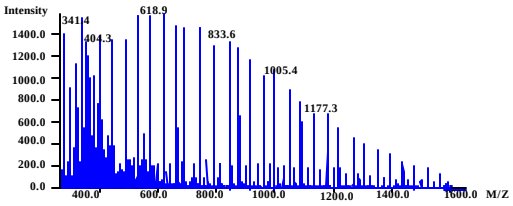


PEI-1200 (DHB-FILTRATE)



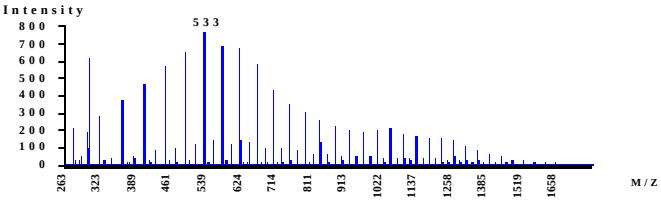
Previous work in our laboratory showed that retinoic acid was a matrix that required much less laser energy than sinapinic acid or DHB. Retinoic acid was not a matrix that was compatible with PEI because its solublity in solvents that would dissolve PEI was too low. Caffeic acid, on the other hand was found to be an ideal matrix for the analysis of PEI. With caffeic acid, the laser energy could be lowered, and the amount of laser damage reduced.

PEI-1200 (CAFFEIC ACID MATRIX)



PEI-600 (CAFFEIC ACID MATRIX)

Analyses of the molecular mass distributions, obtained by varying laser energy and matrices, reveal trends



in the data. These trends can be used to describe the influences of matrix and laser energy on MALDI-TOF-MS data measurement of polyethylenimine polymers. A statistical analysis revealed that matrix has a greater effect on the polymer MMD than was expected.

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