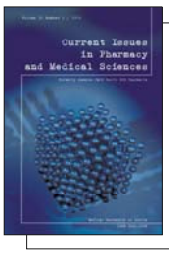

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Identification of polyamidoamine dendrimers (PAMAM-NH_2) by ESI-Q-TOF method

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ABSTRACT

The method of identification of poly(amidoamine) dendrimers (PAMAM-NH_2) G1.0, G2.0 and G3.0 generation with the use of high resolution mass spectrometry and hybrid quadrupole-time-of-flight analyzer with electrospray ionization (ESI-Q-TOF) was developed. The analysis was performed in positive ionization mode in the range m/z 100-3200 where MS spectra (TOF) as well as fragmentation spectra (MS/MS) were recorded simultaneously. The investigated substances were injected to the MS analyzer by the use of UHPLC system in a direct way (with no chromatographic separation). After deconvolution MS spectra were used for accuracy estimation of the mass of the analyzed dendrimers (PAMAM G1.0 - 1430.0153 Da, PAMAM G2.0 - 3256.2696 Da, PAMAM G3.0 - 6908.6850 Da). Registered fragmentation spectra were used for the confirmation of the structure of the analyzed three generation PAMAM-NH_2 dendrimers.

Keywords: PAMAM, dendrimers, ESI, TOF, mass spectrometry

INTRODUCTION

Dendrimers, also called "cascade molecules" or "arborols", are a relatively new class of compounds which are characterized by unique molecular architecture and dimensions in comparison to traditional linear polymers [1]. These hyperbranched molecules were first discovered by Donald Tomalia and co-workers in the early 1980s and, at the same time but independently, by George R. Newkome [7, 11, 12]. Dendrimers are large, complex molecules with well-defined chemical structures. They are characterized by a multibranched, three-dimensional architecture, low polydispersity and high functionality [6]. A typical dendrimer consists of three components: an initiator core (focal core), interior generations composed of repeating units, radially attached to the initiator core and multiple peripheral functional groups which are attached to the outermost interior generation. The type of initiator core determines the dendrimer's shape, e.g., ammonia core dendrimers are spheroid, whereas alkyleneamine dendrimers are ellipsoid-shaped molecules. Furthermore, this part of dendrimer's structure has the ability, related to the special nanoenvironment surrounded by extensive dendritic branching, to encapsulate different chemical species [5, 6]. The next component of dendrimers — an interior layer, due to a flexible space that is created within the voids of dendritic building blocks, gives an opportunity to attach various small guest molecules. Dendrimer's macroscopic properties are defined also by peripheral functional groups, which may react with the surrounding of the molecule [6].

Polyamidoamine (PAMAM) dendrimers, the first synthesized type of dendrimers, receive widespread attention and are under the most active investigation [3, 7, 10]. The core molecule in PAMAM dendrimers is ammonia or ethylenediamine (EDA) particle. Physical properties of PAMAM dendrimers change according to the generation number. Dendritic molecules' diameter tends to increase linearly with increasing dendrimers generation [5]. Generation influences also the morphology of dendrimers [9]. Dendrimers generation from zero to two present highly asymmetric shape and have more open structure than dendrimers of higher generation. The longer and more branched is the dendrimer, the more its shape resembles globular structure. When the generation number increases, the branches of dendrimer become densely packed and form tight membrane-liked structure [4].

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Qualitative analysis of PAMAM dendrimers due to their complex structure and high molecular weight is usually performed by means of mass spectrometry techniques (MS), combined with soft ionization method (MALDI - matrix assisted laser desorption ionization, FAB – fast atom bombardment). More popular techniques such as electrospray ionization (ESI) is used less frequently due to the larger fragment in the source of these compounds, resulting in the recording of spectra much more difficult to interpret [2, 8, 13].

The aim of this study was to develop a method for the identification of poly(amidoamino) dendrimers (PAMAM-NH_2) generation G1.0, G2.0 and G3.0 using high resolution mass spectrometry with electrospray ionization source (ESI-Q-TOF).

MATERIALS AND METHODS

The methanolic standard solutions of PAMAM-NH_2 G1.0, G2.0 and G3.0 dendrimers (20% concentration) were obtained from Sigma-Aldrich (St. Louis, USA). Methanol hypergrade for LC-MS was purchased from Merck (Darmstadt, Germany) and 98% formic acid for mass spectroscopy was obtained from Fluka (Taufkirchen, Germany). Water for GC and LC was purchased from Honeywell Burdick & Jackson (Muskegon, USA).

ESI-Q-TOF analysis was carried out with the use of Agilent Accurate-Mass Q-TOF LC/MS G6520B system with dual electrospray source and Infinity 1290 ultra-high-pressure liquid chromatography system consisting of: binary pump G4220A, FC/ALS thermostat G1330B, autosampler G4226A, DAD detector G4212A and TCC G1316C module (Agilent Technologies, Santa Clara, USA). A mixture (50:50, v/v) of methanol (A) and water (B) with addition of 0.1% solution of formic acid in both media was used as a mobile medium (constant flow 0.01 ml/min) and direct analysis (without column) was performed. The working solutions of dendrimers (0.4 mg/ml) were injected in to the ESI-Q-TOF system in volume 0.5 µl.

Q-TOF detector was tuned in a positive mode with the use of Agilent ESI-L tuning mix in high resolution mode (4 GHz) and no mass correction was performed. The main parameters were optimized and the following settings were applied: gas temp.: 150°C, drying gas: 10 l/min, nebulizer pressure: 20 psig, capillary voltage: 4500 V, fragmentor voltage: 300 V, skimmer voltage: 20 V, octopole 1 RF voltage: 250 V. Data acquisition was performed in auto MS/MS mode with spectral parameters: mass range: 100-3200 m/z and acquisition rate: 1 spectra/s (for MS and MS/MS data). Collision energy was in the range 35.5-46V.

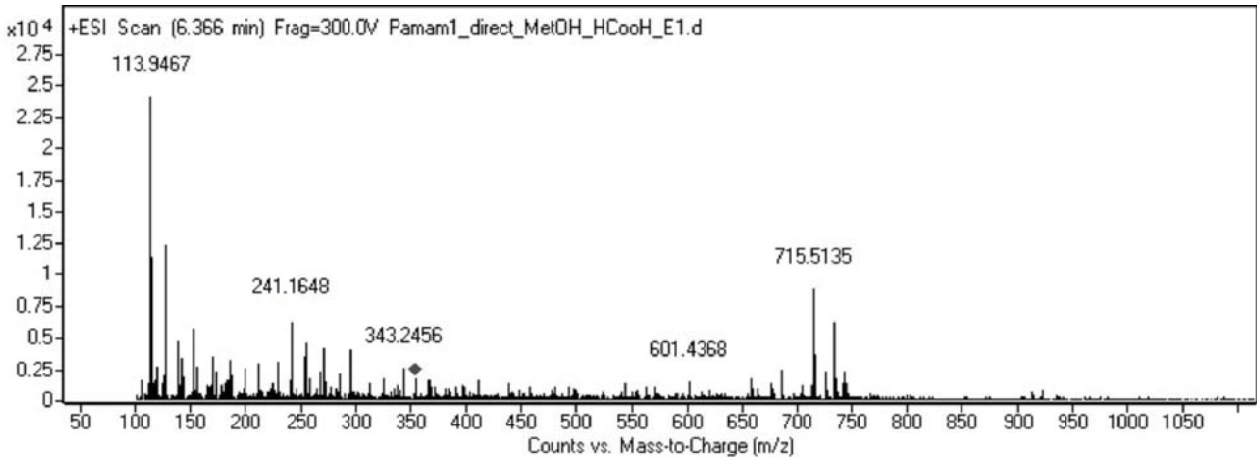

Fig. 1. ESI-MS TOF spectrum of PAMAM-NH_2 G1.0 dendrimer

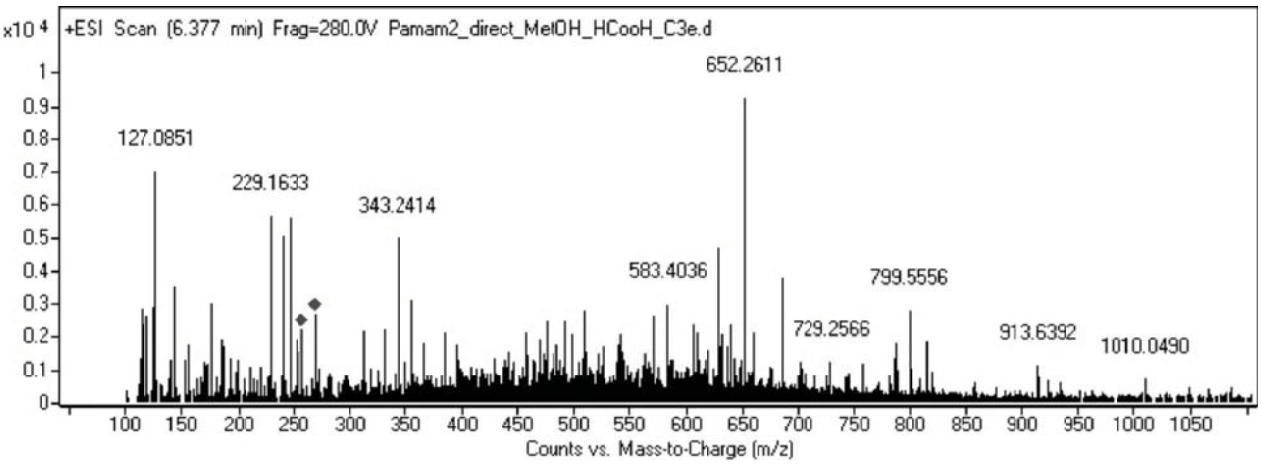

Fig. 2. ESI-MS TOF spectrum of PAMAM-NH_2 G2.0 dendrimer

RESULTS AND DISCUSSION

The ESI mass spectra of dendrimers is characterized by the presence of multi-charged ions and molecular single-charged ions are usually not observed. In this case the interpretation of these spectra is more difficult and additional software calculations (e.g. deconvolution) are required. In this study high resolution MS TOF spectra were registered for all G1.0-G3.0 dendrimers (Figures 1-3) and their deconvolution was performed by the use of Mass Hunter software (Figures 4-6). This procedure allowed the determination of the mass of the analyzed compounds directly from the recorded ESI-MS TOF spectrum. As presented in Table 1 accurate masses of PAMAM-NH_2 G1.0 (1430.0153 Da), G2.0 (3256.2696 Da) and G3.0 (6908.6850 Da) were obtained.

The main ions registered in MS TOF spectra of the analyzed dendrimers were next selected for MS/MS fragmentation. For the first generation of PAMAM-NH_2 the m/z 715.5136 ion with charge state z = 2 was chosen for the collision inducted fragmentation. As shown in Figure 7 the most characteristic fragments for this dendrimer are ions: m/z 1087.7980 (z = 1), 704.4554 (z = 2), 613.9465 (z = 2) and 343.2483 (z = 1). The proposed fragmentation pathway of PAMAM G1.0 is presented in Figure 8. In the case of the second generation of PAMAM- NH_2 the m/z 652.0632 ion with charge state z = 5 was selected for MS/MS fragmentation and the characteristic fragmentation ions were also observed: m/z 752.5116 (z = 3), 583.3936 (z = 5) and 498.3503 (z = 1) (Figure 9). The proposed fragmentation pathway of this dendrimer is shown in Figure 10. For the third generation of PAMAM-NH_2 the m/z 864.3508 (z = 8) ion was selected for the collision inducted fragmentation. The most characteristic fragmentation ions for this dendrimer are: m/z 1176.3181 (z = 3), 1039.3253 (z = 5) and 852.3032 (z = 2). MS/MS spectrum and the proposed fragmentation pathway for G3.0 generation of PAMAM are presented in Figure 11 and 12. The obtained fragmentation ions, their charge state and the loss fragments for all the analyzed dendrimers are presented in Table 2.

It should be noted that some of the fragmentation ions are common to the course of fragmentation of the analyzed PAMAMs and are also helpful in determining the structure of these substances. To these fragmentation ions belong ions with relatively large abundance and charge state z = 1: m/z 325.2307 (e fragment) and 241.1639 (f fragment).

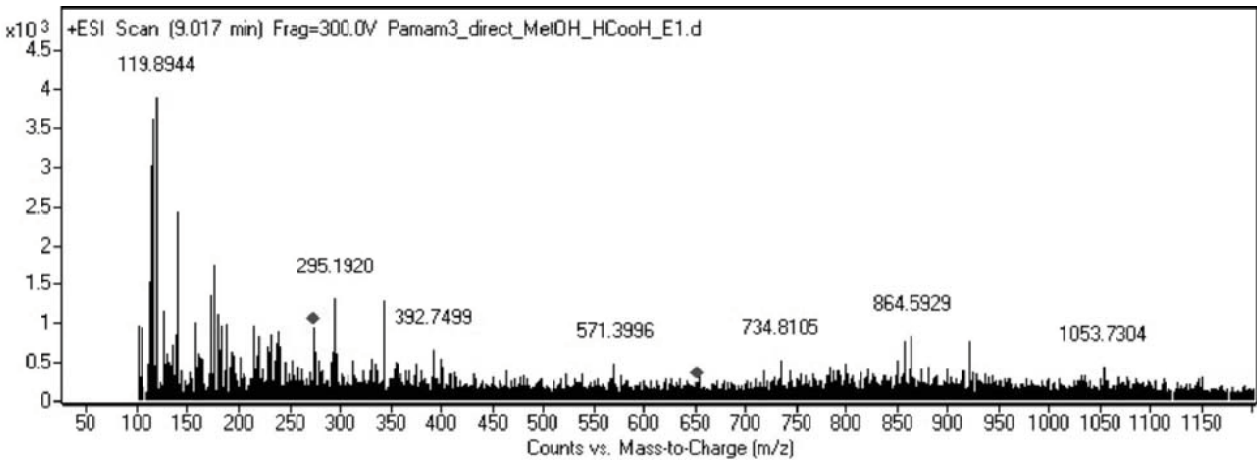

Fig. 3. ESI-MS TOF spectrum of PAMAM-NH_2 G3.0 dendrimer

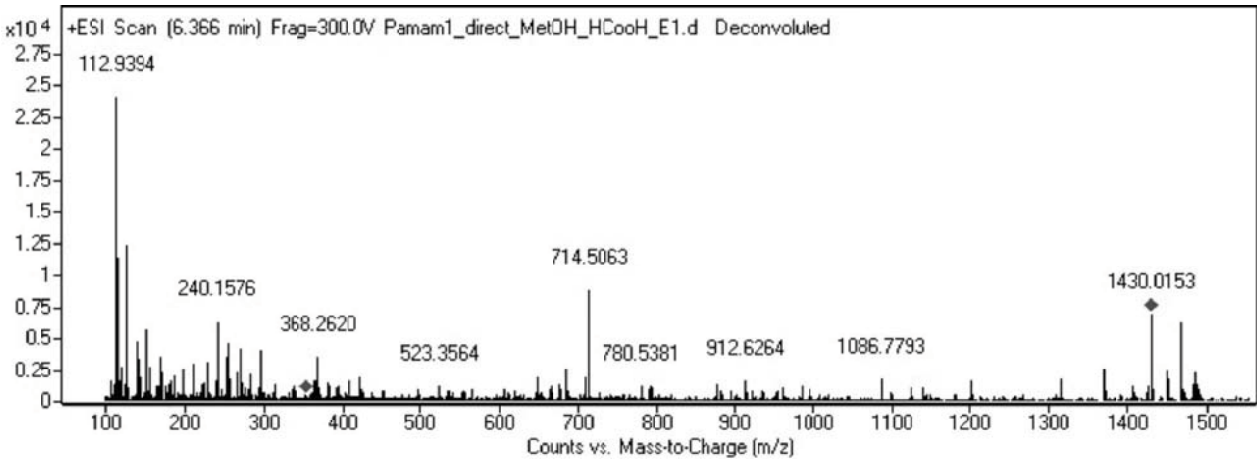

Fig. 4. Deconvoluted ESI-MS TOF spectrum of PAMAM-NH_2 G1.0 dendrimer

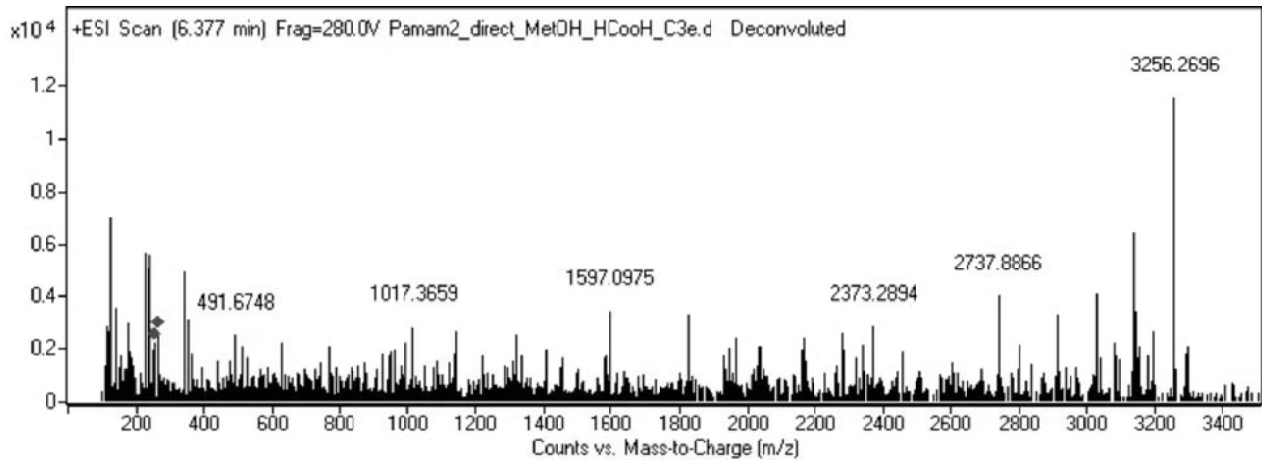

Fig. 5. Deconvoluted ESI-MS TOF spectrum of PAMAM-NH_2 G2.0 dendrimer

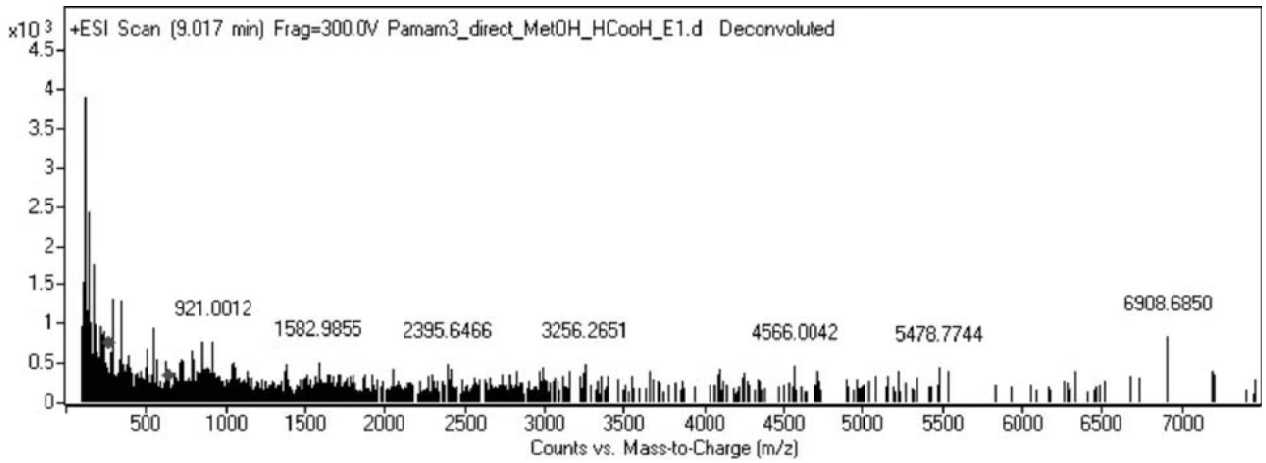

Fig. 6. Deconvoluted ESI-MS TOF spectrum of PAMAM-NH_2 G3.0 dendrimer

Table 1. Accurate mass of the analyzed PAMAM-NH_2 dendrimers

No.	Name	Molecular formula	Theoretical mass[a]	Mass defect[b]	Measured mass[c]
1	PAMAM G1.0	$C_{62}H_{128}N_{26}O_{12}$	1429.0205	1.0205	1430.0153
2	PAMAM G2.0	$C_{142}H_{288}N_{58}O_{28}$	3254.2895	2.2895	3256.2696
3	PAMAM G3.0	$C_{302}H_{608}N_{122}O_{60}$	6904.8275	4.8275	6908.6850

[a] monoisotopic mass (Da)
[b] theoretical defect (Da)
[c] average mass from deconvoluted MS-TOF spectra (Da)

CONCLUSIONS

Electrospray ionization (ESI) combined with high resolution mass spectrometry was found to be a powerful analytical tool for the accurate analysis of dendrimers.

The developed ESI-Q-TOF method can be used for fast identification of PAMAM-NH_2 dendrimers generation G1.0 to G3.0 as well as to qualitative analysis of new dendrimers obtained by its modification.

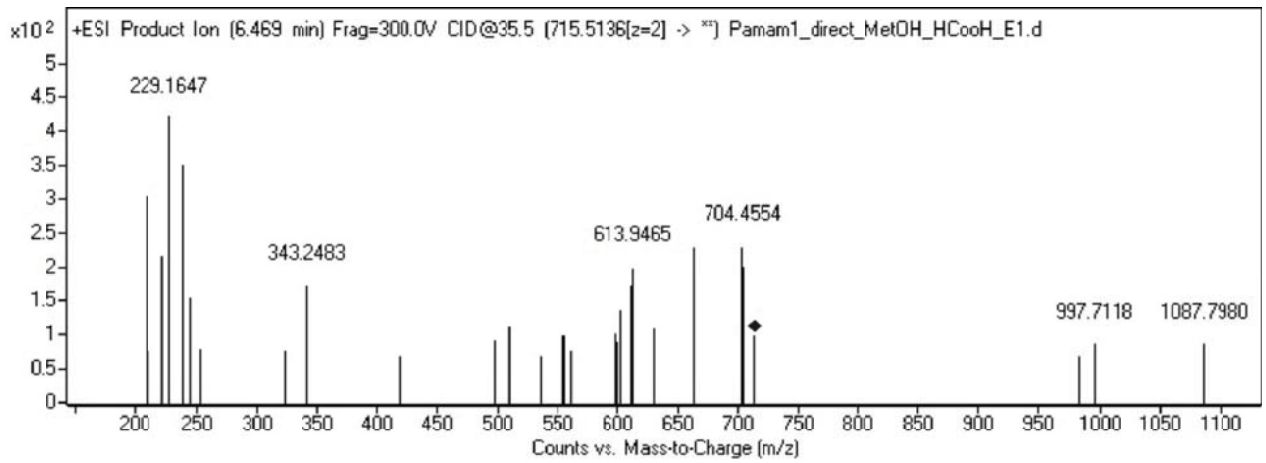

Fig. 7. ESI-MS/MS spectrum of PAMAM-NH2 G1.0 dendrimer

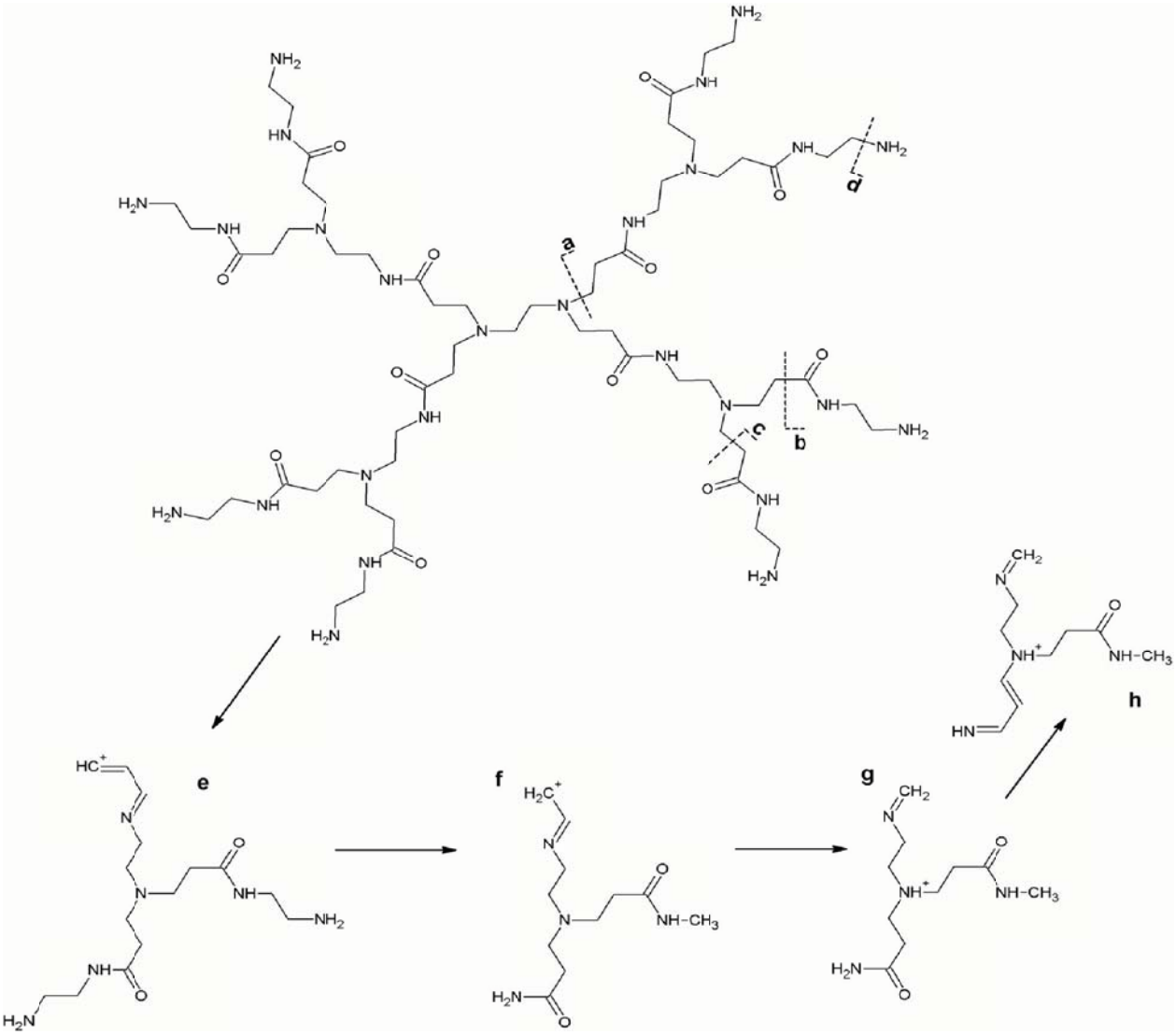

Fig. 8. Structure and proposed fragmentation pathway of PAMAM-NH_2 G1.0

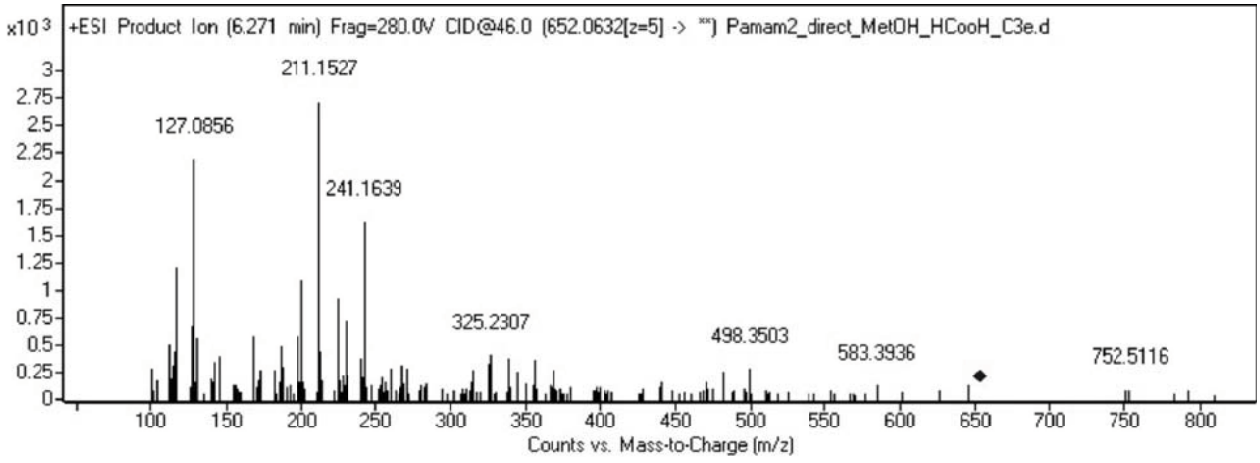

Fig. 9. ESI-MS/MS spectrum of PAMAM-NH_2 G2.0 dendrimer

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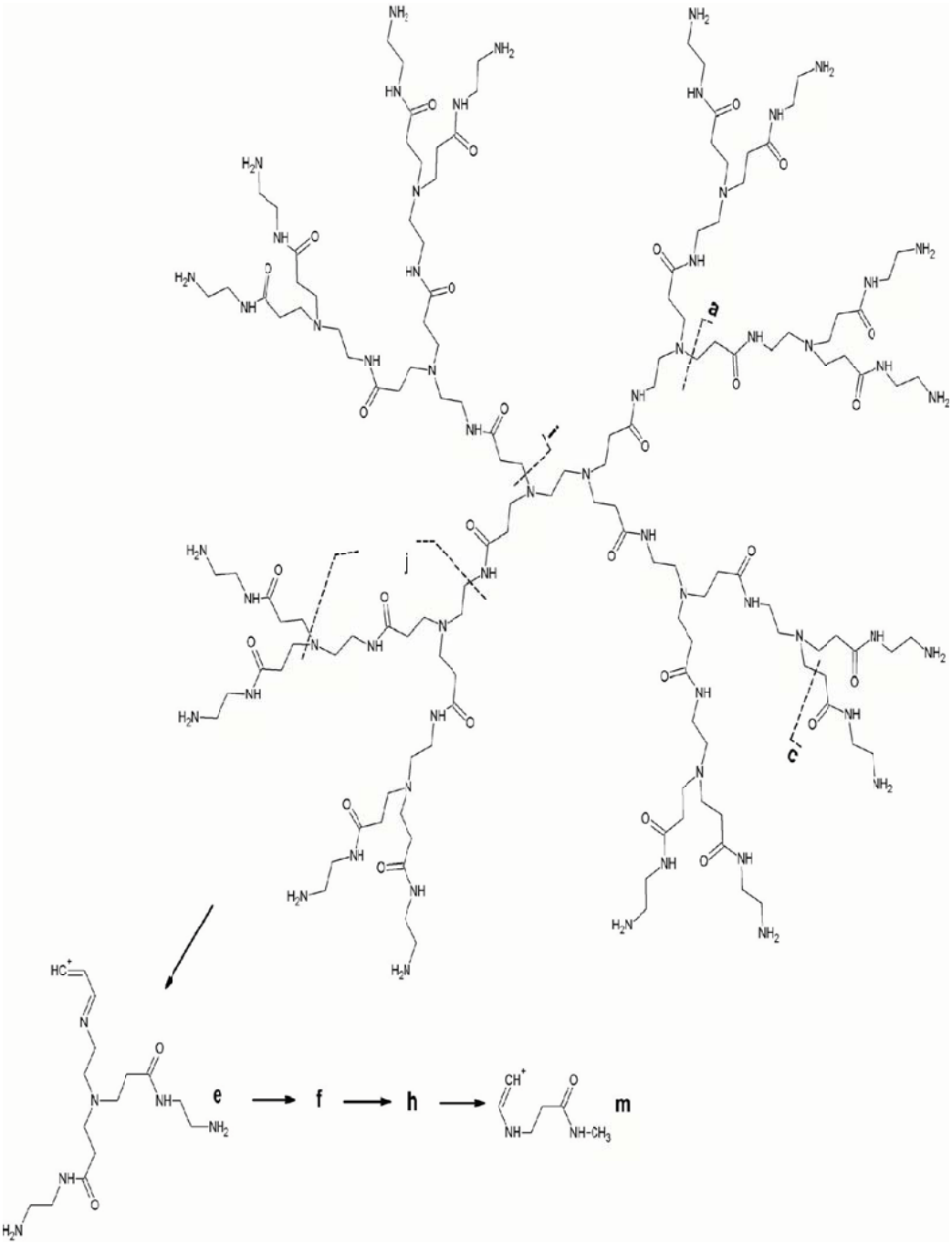

Fig. 10. Structure and proposed fragmentation pathway of PAMAM-NH_2 G2.0

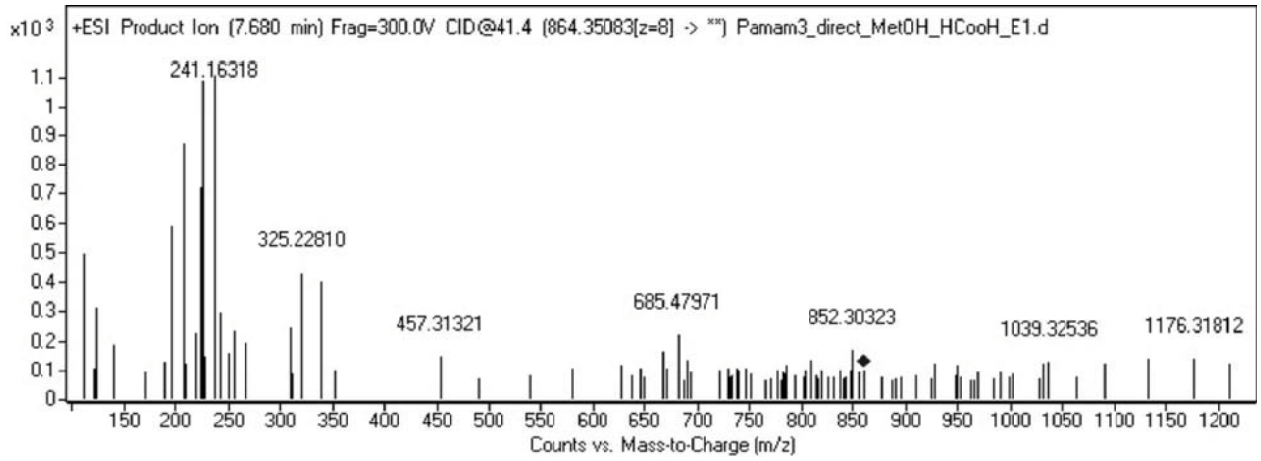

Fig. 11. ESI-MS/MS spectrum of PAMAM-NH_2 G3.0 dendrimer

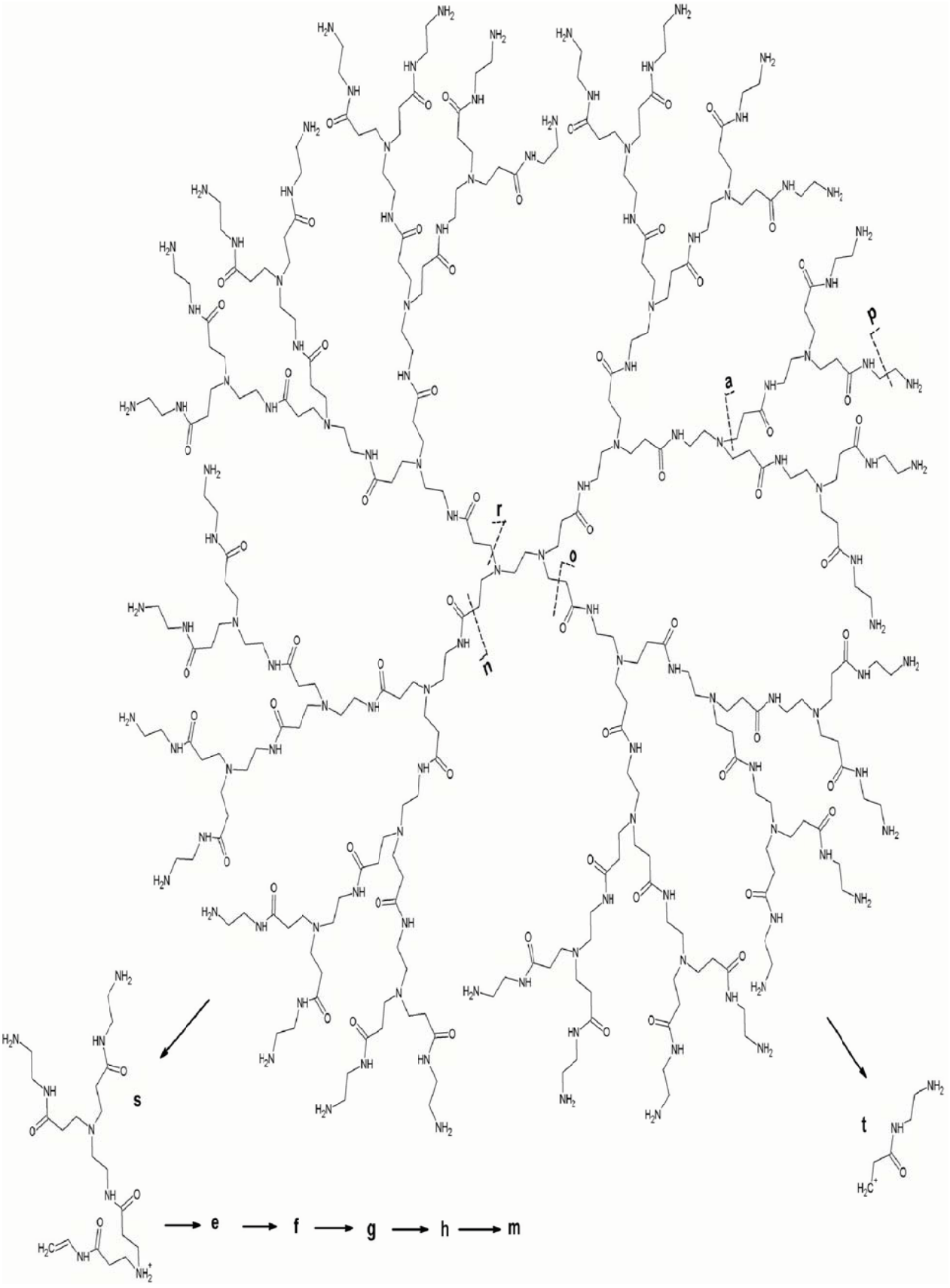

Fig. 12. Structure and proposed fragmentation pathway of PAMAM-NH_2 G3.0

Table 2. Observed main fragmentation ions in MS/MS spectra of analyzed PAMAM-NH_2 dendrimers

No.	Name	Precursor ion (*m/z*)	Fragmentation ions (*m/z*)	Proposed structure[a] (loss fragments)
1	PAMAM G1.0	715.5136 (*z* = 2)	1087.7980 (*z* = 1)	G1-a
			997.7118 (*z* = 1)	G1-a-b
			704.4554 (*z* = 2)	G1-d
			664.4794 (*z* = 2)	G1-c
			613.9465 (*z* = 2)	G1-b-c-d
			343.2483 (*z* = 1)	a
			325.2307 (*z* = 1)	e
			241.1647 (*z* = 1)	f
			229.1647 (*z* = 1)	g
			211.1555 (*z* = 1)	h
2	PAMAM G2.0	652.0632 (*z* = 5)	752.5116 (*z* = 3)	G2-i-2c
			583.3936 (*z* = 5)	G2-a
			498.3503 (*z* = 1)	j
			325.2307 (*z* = 1)	e
			241.1639 (*z* = 1)	f
			211.1527 (*z* = 1)	h
			127.0856 (*z* = 1)	m
3	PAMAM G3.0	864.3508 (*z* = 8)	1176.3181 (*z* = 3)	G3-n-o
			1039.3253 (*z* = 5)	G3-n-p
			852.3032 (*z* = 2)	o
			685.4797 (*z* = 2)	r-a
			457.3132 (*z* = 1)	s
			325.2281 (*z* = 1)	e
			241.1631 (*z* = 1)	f
			229.1642 (*z* = 1)	g
			211.1523 (*z* = 1)	h
			127.0876 (*z* = 1)	m
			115.0859 (*z* = 1)	t

[a] according to pathways and marks showed in Fig. 8, 10 and 12.

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