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CHARACTERIZATION OF FULLERENE DERIVATIVES BY MALDI LIFT-TOF/TOF MASS SPECTROMETRY

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MALDI LIFT-TOF/TOF mass spectra of fullerene C_{60} and six of its derivatives, methano[60]fullerene carboxylic acid, its ethyl ester, diethyl methano[60]fullerene dicarboxylate, and three isomeric tetraethyl bis-methano[60]fullerene tetracarboxylates (compounds **I–VII**, respectively) as model analytes were recorded and discussed. This technique used in mass spectrometry for the first time is proposed for the characterization, structure elucidation, and non-target screening of fullerenes.

Keywords: Mass spectrometry, MALDI, LIFT-TOF/TOF mass spectra, fullerene derivative, reference mass spectra.

1. Introduction

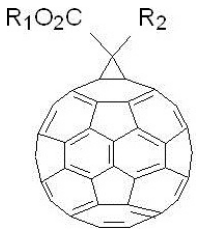
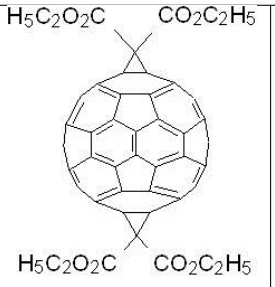
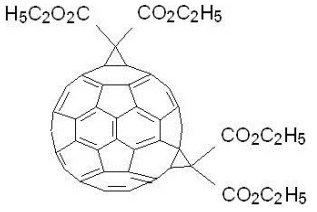
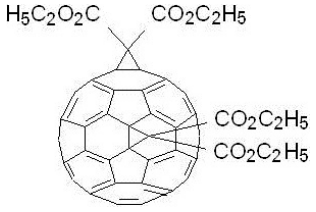
Fullerene derivatives have clear theoretical and applied relevance [1] and, correspondingly, call for new developments in their analysis. These compounds have been routinely characterized by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) [2–6]. However, the corresponding mass spectra may be rather complicated and may contain a number of analyte- and matrix-associated ion peaks, as well as ionized adducts and products of their decomposition. So, the identification of molecular ions or protonated analyte molecules may be hindered. There may also be cases of simple MALDI spectra, where only intense peaks of molecular ions present. Those are certainly insufficient for both substructure elucidation and identification of complex fullerene compounds. For this reason, characteristic fragment ions are also needed.

There have been two generation techniques of characteristic MALDI mass spectra of fullerenes. First, the instruments combining MALDI ion sources, CID cells, and different mass analyzers were used [3, 5, 7]. Second, the technique of post-source decay (PSD) was introduced having such disadvantages as long acquisition times for spectra and rather low fragmentation efficiency [8]. The PSD spectra were recorded for some fullerene compounds [6].

In this research, we propose to use the commercial technique of MALDI LIFT-TOF/TOF MS [8] as one choice to obtain clean product-ion spectra for a reliable characterization/identification of fullerene derivatives. The LIFT process (a) selects a ‘family’ of precursor and its product ions generated from laser-induced ionization and succeeding fragmentation and (b) additionally accelerates these ions to detect them. To the best of our knowledge, this is the first time that this mass spectrometry technique has been used for fullerenes.

In the report, MALDI LIFT-TOF/TOF mass spectra acquired for six fullerene derivatives: methano[60]fullerene carboxylic acid **II**, its ethyl ester **III**, diethyl methano[60]fullerene dicarboxylate (**IV**), three isomeric tetraethyl bis-methano[60]fullerene etracarboxylates (**V–VII**), and also fullerene itself **I** as model analytes (see Table 1), will be briefly described and discussed.

TABLE 1. Fullere derivatives

				
I	**II:** $R_1=R_2=H$ **III**: $R_1=C_2H_5$, $R_2=H$ **IV**: $R_1=C_2H_5$, $R_2=CO_2C_2H_5$	**V**	**VI**	**VII**

2. Experimental

The fullerene **I**, purity of 99.7–99.8%, was obtained from NeoTechProduct (Saint-Petersburg, Russia). Compounds **II–VII** were prepared and purified by known methods described in the literature.

A 10 μl aliquot of the 0.02% and 0.002% w/v solution of every fullerene compound in toluene was mixed with a 10 μl 0.1 % w/v solution of 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB, Sigma-Aldrich) in acetone. A 0.5 μl volume of the mixture was deposited onto an AnchorChip MALDI plate (Bruker Daltonics) for analysis.

MALDI MS and LIFT MS/MS spectra were obtained by means of UltrafleXtreme MALDI TOF/TOF mass spectrometer (Bruker Daltonics). MALDI-ToF spectra were acquired by 300–4000 shots in the positive and negative ion reflector mode at 1000 Hz UV laser frequency and 10–40 % its power in a mass range from 400 to 1200–5000 Da. MALDI TOF/TOF spectra were composed from precursor ion peaks recorded at the above conditions to which were added product ion spectra acquired by 500–2500 shots in the corresponding ion mode at the same laser frequency and 14–35 % power in a mass range from 40 Da to a parent ion mass.

This matrix [2] (common ones such as α-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid were also tested) and this MALDI plate, with special patches in the centers of spots, were of critical value to obtain ToF mass spectra with abundant molecular ion peaks.

3. Results and discussion

Mass spectra of compounds under consideration are given in Fig. 1 and in Table 2.

The ToF spectra were of a varied complexity. In most spectra, molecular ion peaks were the principal ones, with the rest of a few other peaks (Fig. 1c). The exclusion were mass spectra of the acid **II**, especially in positive mode, which consisted of a lot of peaks comparable in intensity to each other (Fig. 1a).

All our LIFT ToF/ToF spectra were of the same type, with molecular ions and series of product ions terminating mainly in C_{60} itself (Fig. 1b and d, Table 2). Fragmentation of the fullerene core was only observed in the case of **I**. Far lower mass fragments were very rare excluding the isomeric molecules **V–VII** (Table 2).

The LIFT spectra (Table 2) characterize all structural details of compounds **II–VII**, i.e. structure of addends bonded to the C_{60} core. Ester groups are eliminated as neutral species of $CO_2C_2H_5$ and $HCO_2C_2H_5$. There is no strong evidence to suggest that these are one-step

TABLE 2. MALDI LIFT-TOF/TOF spectra. Principal ions

Compound	m/z (composition), %	
	Positive mode	Negative mode
I	720 ($M^{+\cdot}$) 696 ([M–C_2]$^{+\cdot}$), 100% 672 ([M–C_4]$^{+\cdot}$), 6%	720 ($M^{-\cdot}$)[1] 696 ([M–C_2]$^{-\cdot}$), low peak
II	778 ($M^{+\cdot}$) 733 ([M–CO_2H]$^{+}$)[2], 100% 720 ([M–$CHCO_2H$]$^{+\cdot}$)[3], 6%	778 ($M^{-\cdot}$) 733 ([M–CO_2H]$^{-}$)[2], 100%
III	806 ($M^{+\cdot}$) 778 ([M–C_2H_4]$^{+\cdot}$), 100% 734 ([M–C_2H_4–CO_2]$^{+\cdot}$)[4], 30% 733 ([M–$CO_2C_2H_5$]$^{+}$)[2], 30% 720 ([M–$CHCO_2C_2H_5$]$^{+\cdot}$)[3], 5%	806 ($M^{-\cdot}$) 762 ([M–CO_2]$^{+\cdot}$), 9% 733 ([M–$CO_2C_2H_5$]$^{-}$)[2], 100% 720 ([M–$CHCO_2C_2H_5$]$^{-\cdot}$)[3], 5%
IV	878 ($M^{+\cdot}$) 804 ([M–$HCO_2C_2H_5$]$^{+\cdot}$), 70% 778 ([M–$2C_2H_4$–CO_2]$^{+\cdot}$), 5% 764 (?), 17% 760 ([M–$HCO_2C_2H_5$–CO_2]$^{+\cdot}$), 42% 734 ([M–$2C_2H_4$–$2CO_2$]$^{+\cdot}$)[4], 8% 733 ([M–C_2H_4–CO_2–$CO_2C_2H_5$]$^{+}$)[2,4], 9% 732 ([M–$2CO_2C_2H_5$]$^{+\cdot}$), 12% 720 ([M–$C(CO_2C_2H_5)_2$]$^{+\cdot}$)[3], 100%	878 ($M^{-\cdot}$) 834 ([M–CO_2]$^{+\cdot}$), 2% 805 ([M–$CO_2C_2H_5$]$^{-}$), 30% 765 (?), 9% 761 ([M–$CO_2C_2H_5$–CO_2]$^{-}$)[4], 22% 760 ([M–$HCO_2C_2H_5$–CO_2]$^{-\cdot}$), 17% 733 ([M–C_2H_4–CO_2–$CO_2C_2H_5$]$^{-}$)[2], 13% 720 ([M–$C(CO_2C_2H_5)2$]$^{-\cdot}$)[3], 100%
V–VII	1036 ($M^{+\cdot}$) 991 ([M–CO_2H]$^{+}$), 10–21% 962 ([M–$HCO_2C_2H_5$]$^{+\cdot}$), 70–72% 935 ([M–C_2H_4–$CO_2C_2H_5$]$^{+}$), 4–6% 918 ([M–CO_2–$HCO_2C_2H_5$]$^{+\cdot}$), 5% 891 ([M–C_2H_4–CO_2–$CO_2C_2H_5$]$^{+}$), 7–9% 888 ([M–$2HCO_2C_2H_5$]$^{+\cdot}$), 5% 878 ([M–$C(CO_2C_2H_5)_2$] $^{+\cdot}$), 100% 804 ([M–$C(CO_2C_2H_5)_2$–$HCO_2C_2H_5$] $^{+\cdot}$), 7–8% 748 ([M–$4C_2H_4$–$4CO_2$] $^{+\cdot}$), 8–10% 733 ([M– $C(CO_2C_2H_5)_2$–C_2H_4–CO_2–$CO_2C_2H_5$]$^{+}$)[2], 4% 720 ([M–$2C(CO_2C_2H_5)_2$] $^{+\cdot}$)[3], 77–80%	1036 ($M^{-\cdot}$) 963 ([M–$CO_2C_2H_5$]$^{-}$), 5–9% 919 ([M–CO_2–$CO_2C_2H_5$]$^{-}$), 2–3% 878 ([M–$C(CO_2C_2H_5)_2$]$^{-\cdot}$), 23–38% 758 (?), 10–16% 720 ([M–$2C(CO_2C_2H_5)_2$]$^{-\cdot}$)[3], 100% 112 ([$C_6H_8O_2$]$^{-\cdot}$), 5–6%

[1]Insignificant fragmentation at the set experimental conditions. [2]$C_{60}CH^{\pm}$. [3]$C_{60}^{\pm}$. [4]Intensity of the isotope peak of the ion with one less mass unit is subtracted.

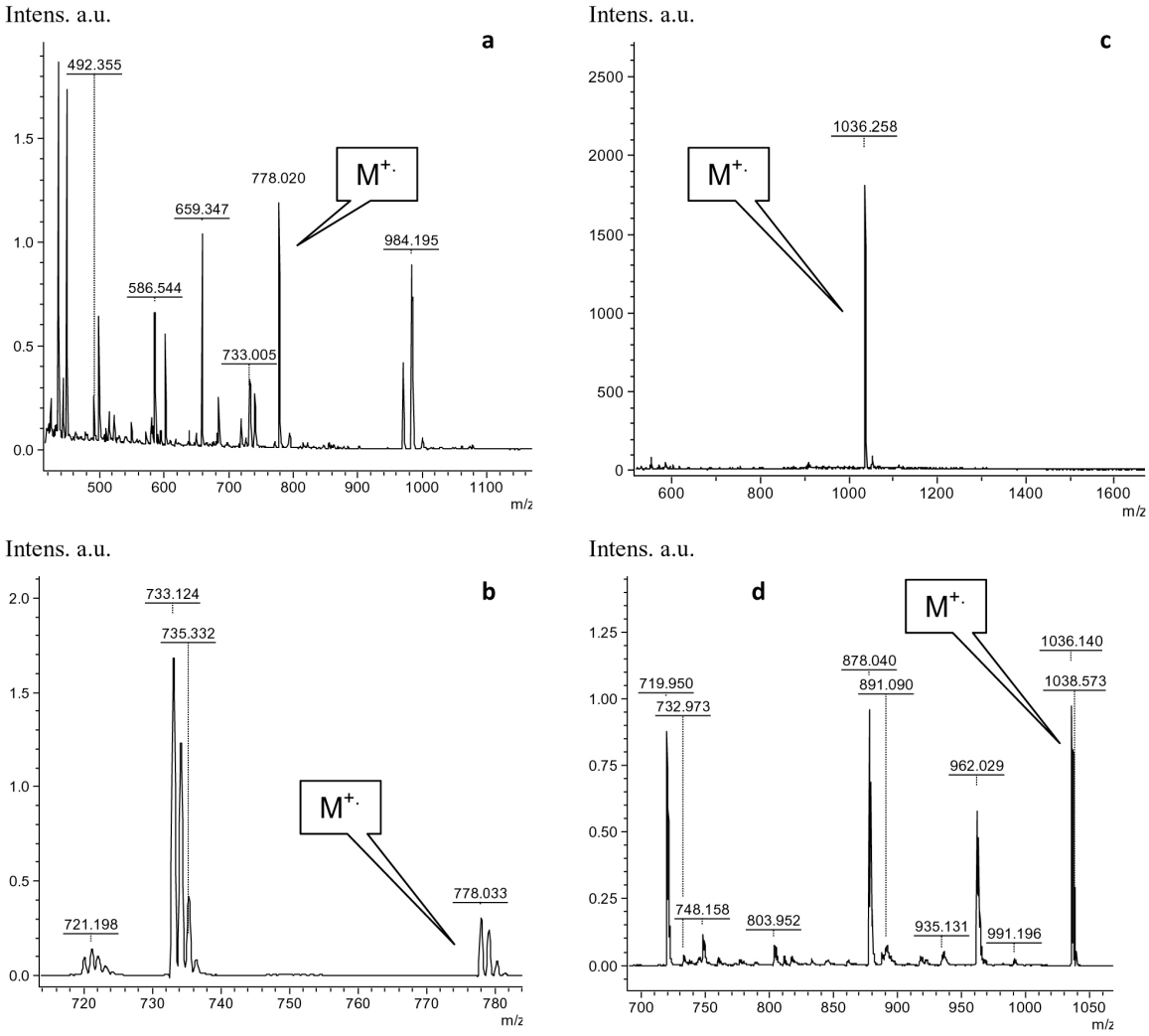


FIG. 1. ToF spectra of the compounds II (a) and V (c) in the positive mode. ToF/ToF spectra of the compounds II (b) and V (d) in the positive mode

processes. In concurrent reactions, carboxylate and ethyl groups were removed individually in the form of CO_2 and C_2H_4, respectively. The presence of geminal carboxy ethyl groups in compounds **IV–VII** was correlated with the formation of $[M\text{-}C(CO_2C_2H_5)_2]^{\pm}$ fragments. The fullerene core of the molecules was observed as the ion with m/z 720 ($C_{60}^{\pm}$).

We did not find any significant difference between the spectra of regioisomers **V–VII**. Probably, a common intermediate is formed in the course of fragmentation of these compounds, which leads to the same product ions. Mass spectral discrimination between these fullerene derivatives calls for a special search for appropriate technique/method.

There are the distinct differences between the spectra of positive and negative ions (see Table 2). First, positive spectra contain more abundant peaks of different ion compositions. Second, some negative ion types are not available in positive spectra and *vice versa*. For example, peaks of fragments formed by means of elimination of ethylene molecules are more intensive in positive than negative spectra (see Table 2). Then, even- and odd-electron ions are present in spectra. The odd-electron ions dominate, especially in the case of positive ions: they account for an average of 3/4 of the peak intensity sum.

4. Conclusion

The MALDI LIFT-TOF/TOF technique known for its efficiency in proteomics, has been shown to provide single-type product-ion spectra of fullerene derivatives, enabling their characterization, structure elucidation, and non-target screening. This mass spectrometry technique used for fullerenes for the first time is suitable for building reference libraries of product-ion mass spectra [9] in the cases of these and other fullerene compounds.

Acknowledgments

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