

Laser Desorption/Ionization Time-of-flight Mass Spectrometric Analysis of Some Synthetic Flavonoids and Their Complexes with Zn and Fe

Tetiana FESENKO, Iryna LAGUTA, Pavlo KUZEMA *, Oksana STAVINSKAYA

Chuiiko Institute of Surface Chemistry of NAS of Ukraine, 17 General Naumov Str., Kyiv 03164, Ukraine

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Three antioxidants: flavonol (3-hydroxyflavone) (L^1), 4'-(N,N-dimethylamino)flavonol (L^2), 4'-[N,N-di(2-hydroxyethylamino)]flavonol (L^3) and their complexes with Zn and Fe prepared in aqueous-alcoholic solutions were examined by means of UV-Vis spectroscopy and laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF MS) using the standard stainless steel targets. It was possible to obtain the protonated molecules in the mass spectra of all flavonoids under study, practically no fragmentation being achieved in the case of unsubstituted flavonol. The identified positively charged ions in the mass spectra of L^1 -metal complexes correspond to the ligand:metal ratio 2:1, 3:2 for L^1 -Zn and 2:1, 3:2, 4:2 for L^1 -Fe. The presence of the electron donating substituent in 4'-position of flavonol molecule enhanced fragmentation and affected the contribution and ligand:metal stoichiometry of the minor ions in the LDI mass spectra. Namely, the compounds with ligand:metal ratio 2:1, 3:2 for L^2 -Zn, 2:1, 3:2, 4:2 for L^2 -Fe, 1:1, 2:1, 3:2 for L^3 -Zn, 2:1, 3:1, 4:2 for L^3 -Fe, respectively, were identified. The discrepancy in the composition of the complexes derived from the spectroscopic data (ligand:metal ratio 1:1) and MS analysis may be attributed to differences in the environment for the complexes in solution and in solid state, as well as to peculiarities of laser desorption/ionization from the solid sample condensed on steel target.

Keywords: antioxidant, flavonoids, flavonol, metal complex, laser desorption ionization (LDI), mass spectrometry.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds of plant origin possessing antioxidant activity [1–5] and chelating properties [6, 7]. In order to improve the antioxidative efficiency of flavonoids synthetic analogues are obtained [8]. Besides, enhancement in antioxidant and anti-inflammatory activity was shown to occur when flavonoids are coordinated with transition metal ions [9, 10]. It is suggested [10] that such enhancement is related to the acquiring of additional superoxide-dismuting metal center. However, depending on biological environment, transition metal complexes with flavonoids might exhibit both anti- and pro-oxidant effects [10]. Moreover, in biological systems flavonoids undergo extensive chemical transformations generating new compounds of as yet unknown properties [11]. Therefore, in order to elucidate structure-properties relationship it is important to be able to perform rapid and reliable examination of the flavonoids, their metal complexes and transformation products.

Mass spectrometry (MS) with different ionization techniques, as well as in combination with various chromatographic methods has proved to be highly successful in the analysis of flavonoids [11–16]. For instance, electrospray ionization (ESI) MS in couple with liquid chromatography has been successfully utilized for identification of flavonoids and tannins in bioactive extracts [12, 17–19] and for the analysis of the stable free radical scavenging capability of artificial polyphenol mixtures and plant extracts [20]; matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS

was used for structural determination of isolated anthocyanin and flavonol components [21].

Relatively little information on MS analysis of flavonoid-metal complexes is found in literature. For identification and structural characterization of metal complexes mainly ESI MS is used [9, 22, 23]. Although MALDI MS is a high-throughput method and has proved to be a powerful tool for analysis of a wide class of biomolecules, there is no wide application of this method for the analysis of small molecules, in particular, flavonoids and their complexes. The main reason for that is the complications related to low resolution and high matrix background signals [11]. To avoid the latter, matrix-free laser desorption/ionization (LDI) techniques have been developed including desorption/ionization on porous silicon (DIOS) [24], silicon dioxide (DIOSD) [25], mesoporous silicate (DIOM) [26], surface assisted LDI from porous films (SALDI) [27] and nanoparticles (SPALDI) [28]. Such techniques have proved to be successful for the analysis of small molecules (aminoacids, oligopeptides, benzylpyridinium salt derivatives). No reports, however, were found regarding the analysis of flavonoid-metal complexes neither by MALDI nor by LDI MS.

The aim of our work is to estimate the performance of LDI-TOF MS in the analysis of flavonoids and their metal complexes. Analysis of Zn and Fe complexes is of special interest because of their biological importance [29]. Flavonol (3-hydroxyflavone) possesses the appropriate proton donating ability and UV absorbability to be a convenient analyte for matrix-free LDI MS analysis even using the standard stainless steel targets. Evaluation of the efficiency of this approach for the analysis of flavonol and some of its derivatives, as well as their Zn and Fe complexes, is the scope of this paper.

*Corresponding author. Tel.: +380-44-4248232; fax: +380-44-4243567.
E-mail address: sci-worker@yandex.ru (P. Kuzema)

EXPERIMENTAL

Investigated flavonoids (structures are given in Fig. 2–4, a) were synthesized according to [30, 31]. Their purity of not less than 99% was confirmed by chromatographic and quantitative elemental analysis, fluorescence and $^1\text{H-NMR}$ spectroscopy, EI mass spectrometry [30, 31]. Zinc acetate and ferric chloride were obtained from Sigma-Aldrich Corp. Ethanol and distilled water were used as solvents.

To determine the complexes stoichiometry the method of isomolar series [32] was used. For this method, a solution of zinc acetate (0.5 mM) or ferric chloride (0.5 mM) in 70% ethanol was mixed in the different volume ratios (1:9–9:1) with the solution of flavonoid (0.5 mM) in the same solvent. Immediately after mixing during the stirring at room temperature the color changes in the solutions were observed indicating that complexation reactions occurred.

UV-Vis absorption spectra (Fig. 1) were recorded with a Lambda 35 Perkin-Elmer double-beam spectrophotometer (scanning speed – 480 nm/min; slit – 2); interfaced to a PC for data processing (software: UV-Win Lab, from Perkin-Elmer). Quartz cells (Perkin-Elmer) with 10 mm pathlength were employed.

Positive ion LDI mass spectra were acquired on an Autoflex II (Bruker Daltonics Inc., Germany) mass spectrometer in the linear or reflectron mode. The instrument was equipped with a pulsed nitrogen laser emitting at 337 nm. The laser energy was varied within the limits of 20 μJ –30 μJ . Ionization was produced by 3 ns impulses with frequency 20 Hz. The accelerating voltage was held at 20 kV and the delay time was 10 ns. Each mass spectrum was the smoothed average of 100–150 laser pulses. For m/z calibration fullerene C_{60} was used.

The flavonoids and the metal salts were dissolved in 70% ethanol at a concentration of 0.5 mM. After the salt and flavonoid were mixed in solution in 1:1 molar ratio in an eppendorf tube, the dried-droplet standard preparation was used by depositing 1 μL of resulting mixture onto a standard stainless steel target plate.

RESULTS AND DISCUSSION

UV-absorption spectrum of flavonol (see the structure in Fig. 2, a) has broad bands situated between 220 nm–270 nm and 270 nm–370 nm (Fig. 1, a). The peak with a maximum around 340 nm (“Band I”) is attributed to the cinnamoyl portion of the molecule (B-ring); the one with a maximum around 240 nm (“Band II”) is related to the benzenic moiety (A-ring); the absorption band with a maximum around 305 nm corresponds to the pyronic ring (C-ring) [33]. The presence of the electron donor substituents ($-\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$ and $-\text{N}(\text{CH}_3)_2$) in para-position of the B-ring (structures in Fig. 3–4, a) induces the shift of a maximum at 340 nm to the long-wave region (410 nm) and appearance of a new peak with a maximum around 280 nm (Fig. 1, b, c). The complexation in all cases causes a bathochromic shift of absorption band I (Fig. 1). This shift can be explained by the extension of the conjugated system with the complexation [34]. The method of isomolar series gives the 1:1 stoichiometry for all the complexes under study.

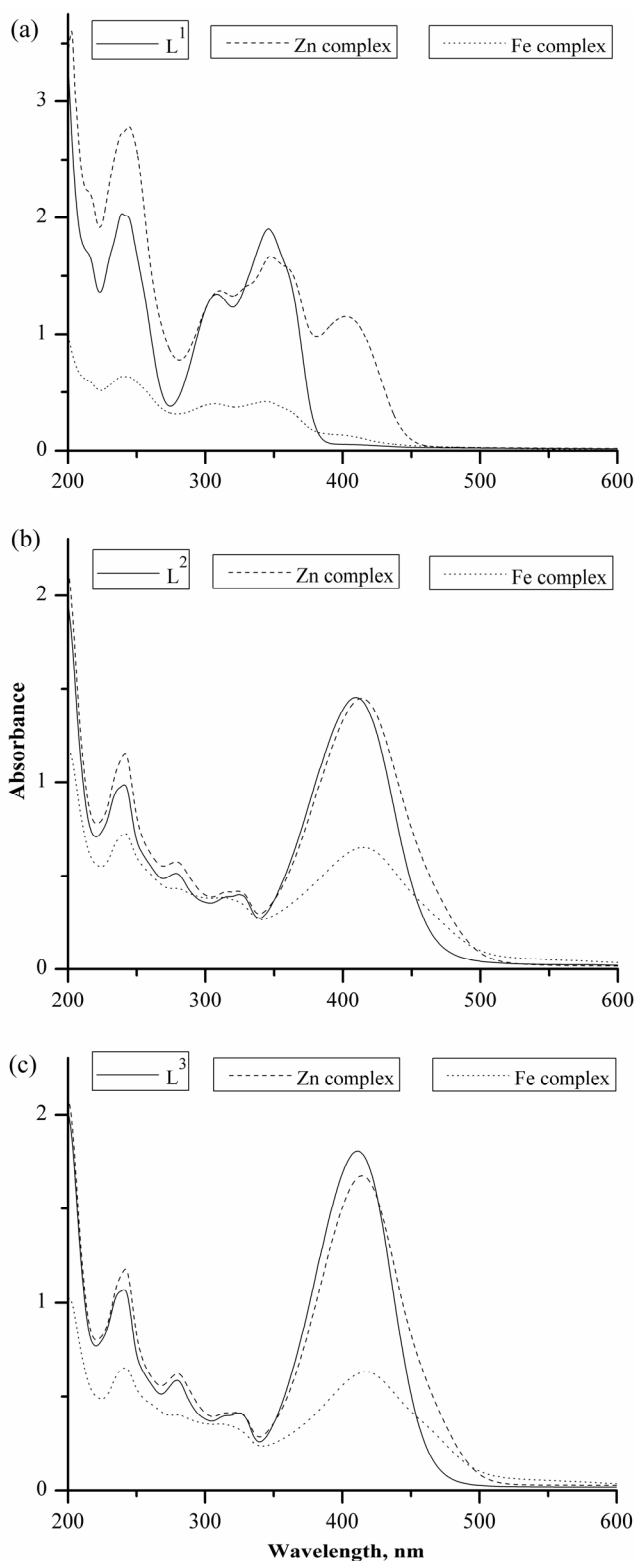


Fig. 1. UV-Vis spectra of flavonol (L^1) (a), 4'-(N,N-dimethylamino)flavonol (L^2) (b), 4'-[N,N-di(2-hydroxyethylamino)]flavonol (L^3) (c) in aqueous-alcoholic solution and their corresponding Zn and Fe complexes (metal-ligand molar ratio 1:1)

This is in agreement with the results obtained by other researchers for flavonol-Zn(II) [35] and flavonoid-Fe(III) [10] complexes.

Laser desorption/ionization of flavonol (L^1) condensed on a steel target results in the observation of ion with m/z 239 in mass spectrum (Fig. 2, a) corresponding to the

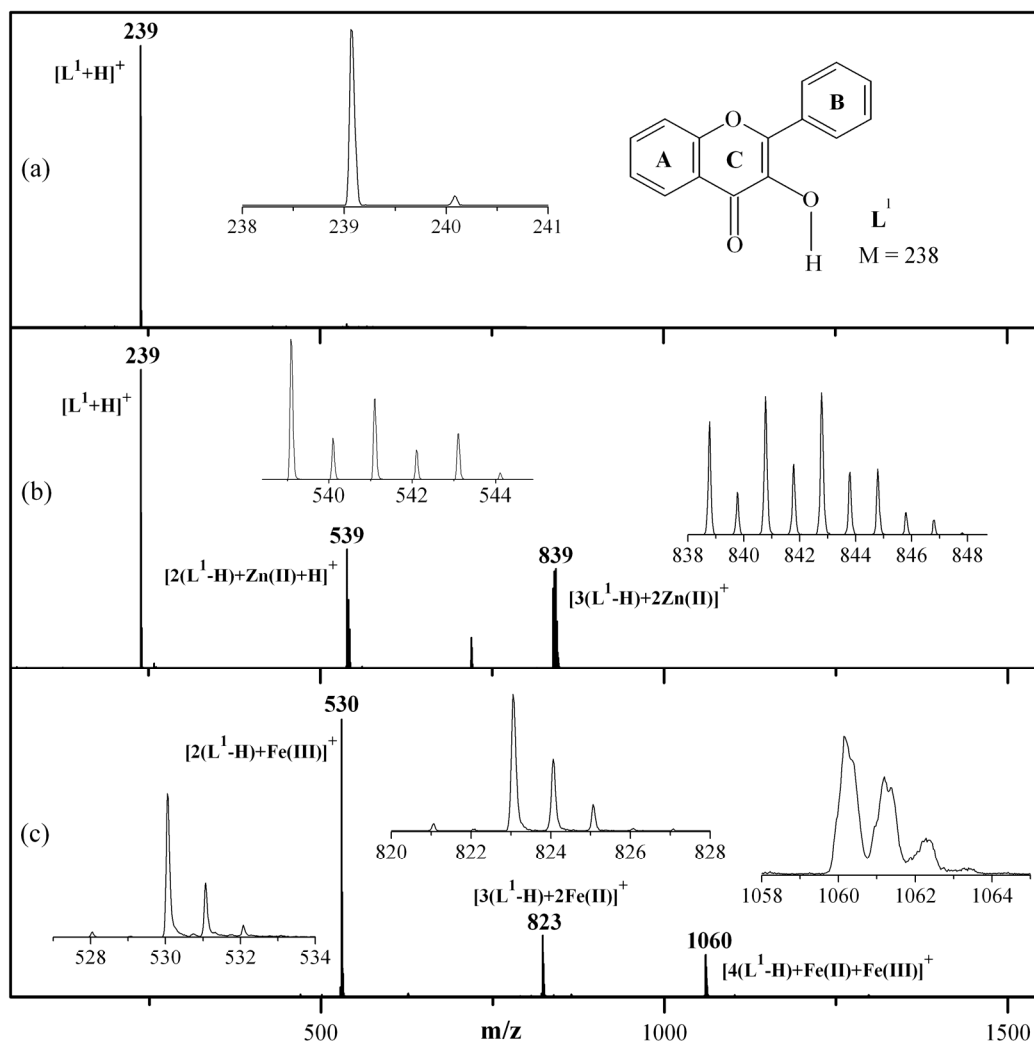


Fig. 2. LDI mass spectra of flavonol (a) and its complexes with Zn (b) and Fe (c)

protonated form $[L^1+H]^+$, practically no fragmentation being achieved. In LDI mass spectrum of flavonol-Zn(II) complex (Fig. 2, b), besides the $[L^1+H]^+$ ions induced by the flavonol, a set of ions in the range of m/z 539–544 and 839–848 was clearly observed indicating the formation of L^1 -Zn species. Analysis of the mass/charge ratio and isotopic distribution gives the following composition of the ions observed: $[(L^1-H)_2Zn+H]^+$ and $[(L^1-H)_3Zn_2]^+$.

The ions corresponding to the ligand:metal stoichiometry 2:1 and 3:2 ($[2(L^1-H)+Fe(III)]^+$ and $[3(L^1-H)+2Fe(II)]^+$, respectively, with a set of ions in the m/z range around 530 and 823) were also observed in LDI mass spectrum of flavonol-Fe complex (Fig. 2, c). Additionally, L^1 -Fe species with a ligand:metal ratio 4:2 were found as $[4(L^1-H)+Fe(III)+Fe(II)]^+$ ions (m/z around 1060). The presence of the Fe(II) species indicates that Fe(III) may be reduced by flavonol in a solution or, as it was stated in [36], Fe(III) in chelate complexes may be converted to Fe(II) under LDI conditions.

It should be noted that, bearing in mind the 1:1 stoichiometry of the flavonol-metal complexes, determined by UV-Vis spectroscopy, we expected to detect the signals of ions with the same ligand:metal ratio in positive ion LDI mass spectra. This, however, is not the case neither for L^1 -Zn nor for L^1 -Fe complexes. It could be explained by both the state of the sample subjected to laser exposure and

peculiarities of ions formation under these conditions. Condensation and crystallization of the analyte on the surface of steel target plate may lead to the formation of the flavonoid-metal complexes with structure and stoichiometry different from those in solution. The evidence of a ligand:metal ratio different from 1:1 in solid state is, for instance, the crystal flavonol-Fe(III) complex with 2:1 stoichiometry synthesized and examined by El Amrani et al. [37]. Besides, every laser exposure of a solid sample leads to the removal of many monolayers (ablation process) – under these conditions the formation of aggregates as neutral compounds and mainly singly charged ions is likely [38, 39]. As it can be seen from the mass spectra in Fig. 2, b, c, the metal complexes ions do not contain neither counter ions nor solvent adducts. The complexes with such structure in the case of ligand:metal ratio 1:1 appear to be highly unstable. If these ions are formed upon sample laser exposure, they may participate in aggregation/dissociation processes. The overall result is the observation of relatively stable singly charged positive ions in LDI mass spectra of flavonol-metal complexes with ligand:metal stoichiometry 2:1, 3:2 or more.

LDI mass spectrum of 4'-(N,N-dimethylamino)flavonol (L^2) (Fig. 3, a) contains the protonated molecule $[L^2+H]^+$ with m/z 282. Ionization of this compound was always accompanied by the formation of some

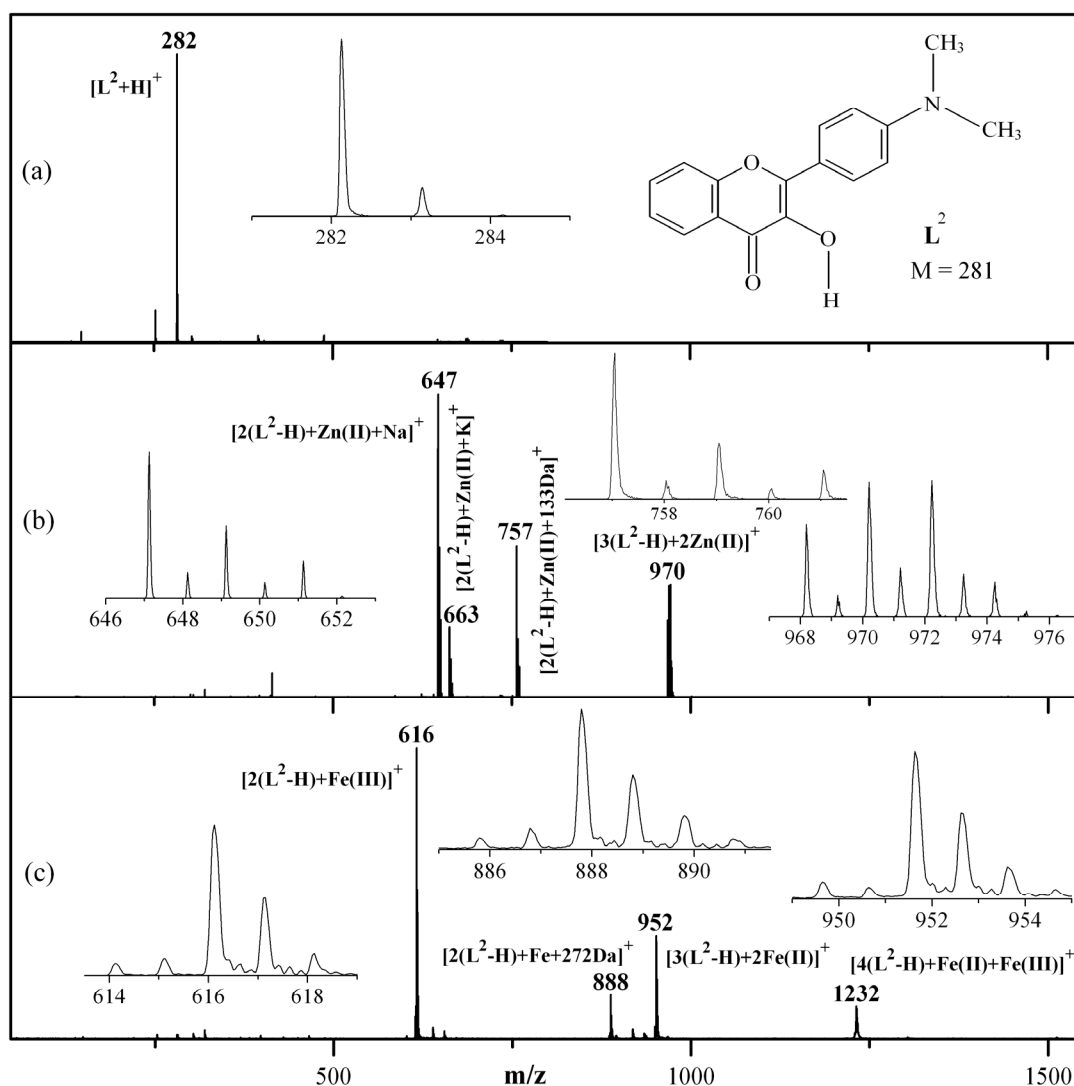


Fig. 3. LDI mass spectra of 4'-(N,N-dimethylamino)flavonol (a) and its complexes with Zn (b) and Fe (c)

fragment ions. As in the case of unsubstituted flavonol, L^2 complex with Zn also gives in mass spectrum (Fig. 3, b) the ions with ligand:metal stoichiometry 2:1 (cationized ions containing Na and K, m/z around 647 and 663, respectively) and 3:2 (a set of ions in m/z range 968–976). The aggregate ion 2:1 containing also flavonoid fragment (m/z 757–761) was observed, as well. In the case of L^2 -Fe complex (Fig. 3, c), aside from the ions with the same stoichiometry as for the L^1 -Fe complex (2:1, 3:2 and 4:2), the aggregate ion 2:1 containing additional flavonoid fragment (m/z 888) was registered.

Fragmentation of the substituted flavonoid could be due to a several reasons. Firstly, UV absorbability at 337 nm for the solution of dimethylaminated flavonol diminishes approximately sixfold comparing to the unsubstituted one (Fig. 1, a, b). This affects the efficiency of the sample molecules excitation and makes it necessary to increase the instrumental laser power for the analyte to be desorbed and ionized. Therefore, the probability of fragmentation is increased, as well. Secondly, the presence of the electron donating substituent may induce the formation of relatively stable fragment ions under the conditions of laser desorption/ionization experiment.

LDI mass spectra of 4'-[N,N-di(2-hydroxyethylamino)]flavonol (L^3) and its Zn and Fe complexes (Fig. 4) are

characterized by the presence of both protonated (m/z 342) and cationized ($[L^3+Na]^+$, m/z 364; $[L^3+K]^+$, m/z 380) forms of the flavonoid, as well as a number of fragment ions, both of flavonoid (Fig. 4, a) and flavonoid–metal complex origin (Fig. 4, b, c). As in the case of L^2 -Zn complex, the ions with ligand:metal stoichiometry 2:1 (m/z 745–750) and 3:2 (m/z around 1151) were also identified in mass spectrum of L^3 -Zn complex (Fig. 4, b). The main difference is the registration of a set of ions (m/z 464–469) identified by isotopic distribution as $[L^3-3H+Zn(II)+Na+K]^+$ structure and related to complex with ratio $L^3:Zn = 1:1$. This is the only case when stoichiometry determined from UV spectroscopic and MS data coincides. It appears that the registration of the 1:1 complex is only possible when L^3 , coordinated with Zn atom *via* the deprotonated hydroxyl of the α -hydroxy-carbonyl group, is cationized *via* substitution of two hydrogens of hydroxyethylaminogroups by the alkali metal ions.

The difference in the structure of the substituent in B-ring of flavonol molecule affects also the stoichiometry of flavonoid-Fe complexes. Instead of 3:2 ligand:metal ratio (determined for L^1 and L^2) a set of the ions (around m/z 1115) related to the structure having the ratio 3:1 was detected for the L^3 -Fe complex (Fig. 4, c).

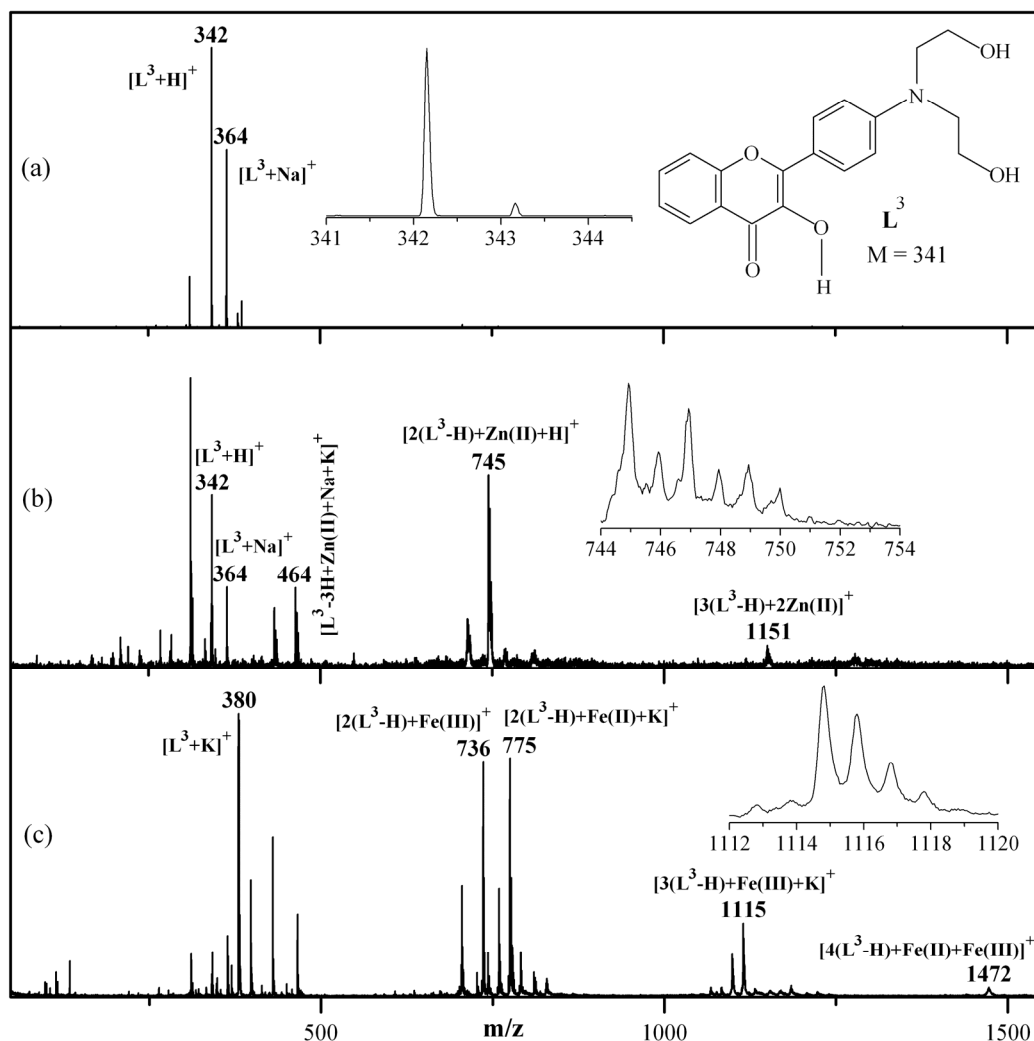


Fig. 4. LDI mass spectra of 4'-[N,N-di(2-hydroxyethylamino)]flavonol (a) and its complexes with Zn (b) and Fe (c)

CONCLUSIONS

Matrix-free laser desorption/ionization mass spectrometry on steel target has been used for the analysis of some synthetic flavonoids and their complexes with Zn and Fe. We have succeeded to obtain LDI mass spectrum of flavonol containing mainly the signals related to the intact protonated molecules. The presence of electron donating substituents ($-\text{N}(\text{CH}_3)_2$, $-\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$) in the para-position of side ring (B-ring) of the flavonol molecule renders the compound less stable under the conditions of laser desorption/ionization experiment and makes it hard to avoid fragmentation using the standard steel targets.

Zn and Fe complexes of the flavonoids under examination were observed in LDI TOF mass spectra as positive ions mainly having the ligand:metal ratio 2:1. The nature of the B-ring substituent affected the contribution and stoichiometry of the minor ions in mass spectra. The discrepancy in the composition of the complexes derived from the spectroscopic data (ligand:metal ratio 1:1) and MS analysis may be attributed to differences in the environment for the complexes in solution and in solid state as well as to peculiarities of laser desorption/ionization from the sample condensed on steel target.

In order to decrease or avoid fragmentation and/or aggregation under the conditions of laser desorption/

ionization of flavonoid–metal complexes it is necessary to elaborate special targets and special techniques of sample preparation. The promising could be the use of the appropriate porous material providing “wet” environment [40] for the analyte molecules. Improvement of LDI approach to analyze the complexes of flavonoids with metals is the subject of further research.

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