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Flavonoids as matrices for MALDI-TOF mass spectrometric analysis of transition metal complexes

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ABSTRACT

Matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a suitable method for the analysis of inorganic and organic compounds and biomolecules. This makes MALDI-TOF MS convenient for monitoring the interaction of metallo-drugs with biomolecules. Results presented in this manuscript demonstrate that flavonoids such as apigenin, kaempferol and luteolin are suitable for MALDI-TOF MS analysis of Pt(II), Pd(II), Pt(IV) and Ru(III) complexes, giving different signal-to-noise ratios of the analyte peak. The MALDI-TOF mass spectra of inorganic complexes acquired with these flavonoid matrices are easy to interpret and have some advantages over the application of other commonly used matrices: a low number of matrix peaks are detectable and the coordinative metal–ligand bond is, in most cases, preserved. On the other hand, flavonoids do not act as typical matrices, as their excess is not required for the acquisition of MALDI-TOF mass spectra of inorganic complexes.

1. Introduction

Transition metal complexes have attracted much attention as therapy for various types of tumors [1,2]. The challenge for scientists is to develop new generations of these anti-tumor agents in order to overcome the resistance developed against some metallo-drugs [3] and to develop new therapeutic approaches with less serious side-effects [1,2,4]. From this point of view, various analytical approaches that can be used to control the synthesis and monitor the interaction of metallo-drugs with biomolecules are of great importance.

Mass spectrometric methods have previously been applied for the analysis of transition metal complexes. For instance, fast atom bombardement (FAB) has been used for the analysis of Pt(II)/Pt(I) dinuclear hybrids [5], whereas electron ionization (EI) has been used for the assesment of the mass spectrometric behavior of Pt-complexes [6]. The soft ionization mass spectrometric approach, electrospray ionization mass spectrometry (ESI MS), has also been applied for the analysis of Ru [7,8] and Pt(II) complexes [9]. Another soft ionization technique, matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used to monitor the interaction of inorganic complexes with various molecules, including nucleotides [10], proteins [11] and certain synthetic polymers [12,13]. From the outcome of these studies, it appears that Pt-complexes are detectable mostly as protonated adducts in MALDI-TOF mass spectra.

However, there are few applications of MALDI-TOF MS for the analysis of low-molecular mass transition metal complexes. 2,5-Dihydroxybenzoic acid (DHB) and sinapinic acids have generally been applied as matrices [12,14]. On the other hand, certain problems arise with the α-cyano-hydroxy-cinnamic acid (CHCA) matrix that are related to unreliable peak identification arising from the transition metal complexes [15]. These problems are probably related to the formation of clusters with the matrix molecules.

We have recently shown that quercetin and rutin are suitable matrices for the MALDI-TOF mass spectrometric analysis of Pt(II) and Pd(II) complexes [15]. It has been stated that these flavonoids can be applied for MALDI-TOF MS of transition metal complexes. The spectra acquired with these molecules are easy to interpret because they show only a small number of peaks, mostly no fragmentation occurs and these matrices are highly tolerant of inorganic salts [15]. These results have implicated the potency of

Abbreviations: CHCA, α-cyano-hydroxy-cinnamic acid; DHB, 2,5-dihydroxybenzoic acid; EI, electron ionization; FAB, fast atom bombardement; LDI, laser desorption and ionization; MALDI-TOF MS, matrix-assisted laser desorption and ionization time-of-flight mass spectrometry; UV, ultraviolet.

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this group of natural products in assisting analytical (instrumental) methods.

The aim of this study was to test the suitability of other flavonoids as matrices for the MALDI-TOF mass spectrometric analysis of Pt(II), Pt(IV), Pd(II) and Ru(III) complexes. We have demonstrated that apigenin, luteolin and kaempferol can also be applied as matrices for MALDI-TOF mass spectrometric analysis of other transition metal complexes, together with Pt(II) and Pd(II), although they provide different quality spectra.

2. Experimental

2.1. Materials

The following flavonoids were used in this study without further purification: apigenin (powder, purity ≥95%), luteolin (powder, ≥98%), kaempferol (powder, ≥ 90%) and quercetin dihydrate (≥98%). They were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany). Transition metal complexes were synthesized according to the procedures described in the literature [16–18].

The following complexes were used: dichlorido (ethylenediamine)platinum(II) ([Pt(*en*)Cl_2] (*Mr* = 324.1); dichlorido(diaminocyclohexane)platinum(II), [Pt(*dach*)Cl_2] (*Mr* = 380.2); chlorido(diethylenetriamine)palladium(II) chloride, [Pd(*dien*)Cl]Cl (*Mr* = 280.5); tritetrachlorido (ethylenediamine)platinum(IV), [Pt(*en*)Cl_4]; and dichlorido (ethylenediemine)ruthenium(III) chloride, [Ru$(en)_2Cl_2$]Cl. They were analyzed with UV/vis, NMR and HPLC. Methanol, HPLC grade, was purchased from Sigma–Aldrich GmbH (Munich, Germany).

2.2. Methods

2.2.1. Preparation of the samples for MALDI-TOF MS

Metal complexes were dissolved in a combination of methanol/physiological salt solution (0.9% NaCl) at the following concentrations: [Pt(*en*)Cl_2] 6.79×10^{-3} M (in 75% methanol/25% physiological salt solution); [Pt(*dach*)Cl_2] 5.29×10^{-3} M (75% methanol/25% physiological salt solution); [Pt(*en*)Cl_4] 6.045×10^{-3} M (75% methanol/25% physiological salt solution); [Pd(*dien*)Cl]Cl 8.62×10^{-3} M (50% methanol/50% physiological salt solution); [Ru$(en)_2Cl_2$]Cl 6.1×10^{-3} M (50% methanol/50% physiological salt solution).

Apigenin (2.48×10^{-3} M), luteolin (6.92×10^{-3} M), kaempferol (1.75×10^{-3} M) and quercetin (1.08×10^{-3} M) were prepared in methanol. For all experiments, freshly prepared solutions of the flavonoids were made.

A small volume (0.5 μL) of the solution of metal complex was applied onto the MALDI target, which was followed by immediate addition of the same volume of a particular flavonoid. The mixture was then left at room temperature to co-crystallize. This approach was shown to result in the best quality of MALDI-TOF mass spectra (data not shown).

2.2.2. MALDI-TOF MS

MALDI-TOF mass spectra were acquired on a Voyager Biospectrometry DE Pro Workstation (Perseptive Biosystems, Framingham, MA, USA). The system utilizes a 20 Hz pulsed nitrogen laser emitting at 337 nm. The spectra were acquired without a low mass gate and under delayed extraction conditions in the reflector mode. All spectra represent the average of 400 single laser shots. The laser intensity was kept sufficiently low to prevent the degradation of the flavonoids and to obtain a good signal-to-noise ratio of the analyte.

2.2.3. Theoretical presentation of the mass spectra

Theoretical presentation of the spectra was performed with assistance of the Selket program, version 1.4, available online [19].

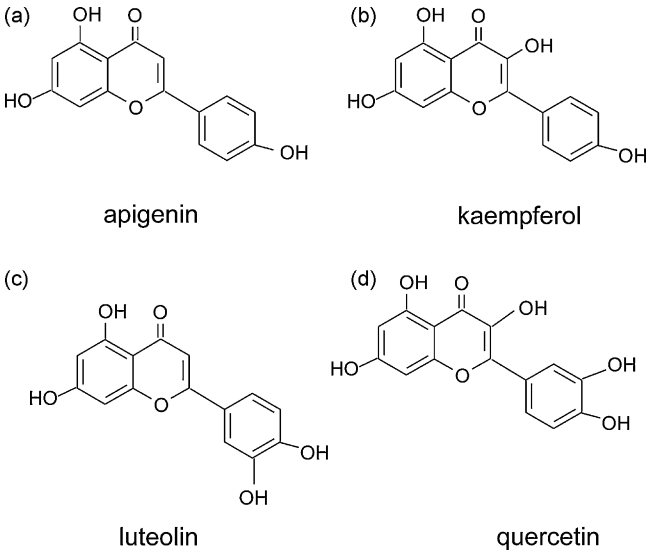

Fig. 1. Structural formulae of flavonoids-matrices: (a) apigenin; (b) kaempferol; (c) luteolin and (d) quercetin.

3. Results and discussion

The flavonoids studied for their applicability as matrices for the MALDI-TOF MS of transition metal complexes differ either in the number or position of OH groups (Fig. 1), which is slightly reflected in the pattern of their UV/vis spectra (data not shown). The molar absorption coefficients of the flavonoids at 337 nm (the emission wavelength of the nitrogen MALDI laser) are listed in Table 1. The tested flavonoids have absorption coefficients in the range from 13,169.9 (quercetin) to 20,829 (apigenin). Therefore, it appeared that the position of the OH groups slightly affected the absorbance at 337 nm (*cf.* Table 1), and it was reasonable to assume that the LDI-TOF mass spectra of these flavonoids would differ.

3.1. MALDI-TOF mass spectrometry of flavonoids

The positive and negative ion laser desorption and ionization time-of-flight (LDI-TOF) mass spectra of the flavonoids are given in Fig. 2. All tested flavonoids gave signals as positive and negative ions without matrix assistance. Apigenin, with a molecular mass of 270.4 g/mol, gives a signal arising from the proton adduct at $m/z = 271.2$ (Fig. 2a), whereas in the negative ion mode, it generates a peak at $m/z = 269.2$ (Fig. 2e). There were also some unidentified low-intensity peaks detectable in both the positive and negative ion mode LDI-TOF mass spectra of apigenin. They probably arose from the photodegradation of apigenin (at lower m/z ratios) or polymerization of the flavonoid. The intensity of those peaks decreased with decreasing laser intensity to a greater extent than those of the peaks arising from apigenin (data not shown). Since luteolin and kaempferol have the same molecular mass and differ only in the position of the OH group (*cf.* Fig. 1), it was interesting to examine whether the position of OH group would affect the spectral pattern and products detectable in their LDI-TOF mass spectra.

The positive ion mode LDI-TOF mass spectrum of kaempferol is given in Fig. 2b, whereas the negative ion mode spectrum is pre-

Table 1
Molar absorption coefficients ($m^2\ mol^{-1}$) of methanol solutions of flavonoids (3×10^{-5} M) at 337 nm.

Flavonoid	a_{337} ($m^2\ mol^{-1}$)	Flavonoid	a_{337} ($m^2\ mol^{-1}$)
Apigenin	2082.9	Kaempferol	1926.2
Luteolin	2246.0	Quercetin	1316.9

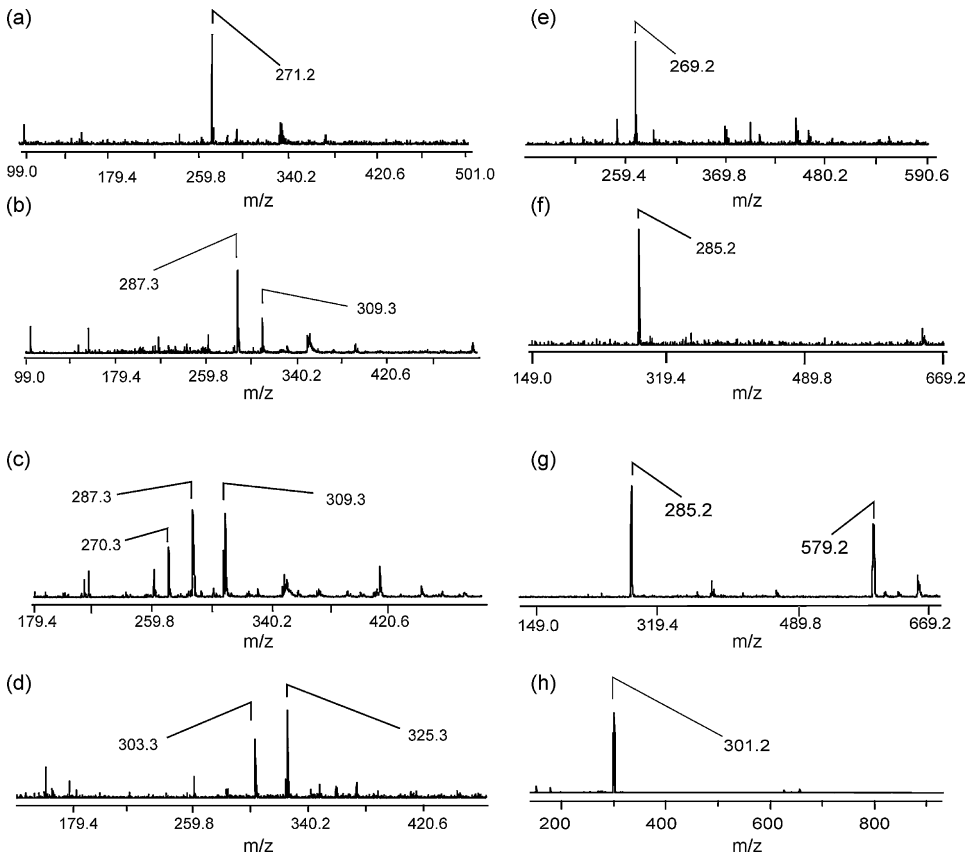

Fig. 2. LDI-TOF mass spectra of flavonoids: (a) and (e) apigenin, (b) and (f) kaempferol, (c) and (g) luteolin and (d) and (h) quercetin. Spectra (a, b, c, and d) are recorded in the positive ion mode and those given in (e, f, g and h) represent the negative ion LDI-TOF mass spectra of flavonoids. All spectra are acquired in the reflector mode and under delayed extraction conditions and represent the summary of 400 single laser shots.

sented in Fig. 2f. The positive ion mass spectrum of kaempferol is characterized by two peaks at $m/z = 287.3$ and $m/z = 309.3$, corresponding to the proton adduct and the sodium adduct of the analyte, respectively. It should be pointed out that the flavonoids were investigated as methanol solutions, which might be the source of sodium, since traces of this metal cation are always present in the solvents.

The same molecular mass flavonoid, luteolin, gives three peaks in the positive ion mode at $m/z = 270.3$, 287.3 and 309.3 (spectrum is presented in Fig. 2c). The peak at $m/z = 270.3$ is generated by loss of the OH group followed by charge compensation during proton incorporation. The second peak at $m/z = 287.3$ is generated by the incorporation of one proton into the complex, whereas the peak at $m/z = 309.3$ is generated by the addition of one sodium cation to the kaempferol molecule. The negative ion mode LDI-TOF mass spectra of kaempferol are simple, since they contain only one peak generated by the abstraction of one proton (at $m/z = 285.2$). On the other hand, the negative ion mode mass spectra of luteolin contains only one peak arising from the negatively charged monoanion (at $m/z = 285.2$). There was, however, an additional signal arising from a thus far unknown product of luteolin (at $m/z = 579.2$). However, only this flavonoid yielded such a peak in the negative ion mode LDI-TOF mass spectra, the intensity of which depended on the laser intensity (data not shown). Fortunately, the metal complexes tested in this study yielded spectra only as positive ions (see below), and this unidentified peak did not further complicate the spectra.

The LDI-TOF mass spectra of quercetin are shown in Fig. 2d (positive ion mode) and 2 h (negative ion mode). The properties of the quercetin spectra have been discussed in our previous report [15] and, therefore, will not be discussed in detail here. It is only worth mentioning that the peak arising from the quercetin sodium adduct ($m/z = 325.3$) is of somewhat higher intensity in comparison to the proton adduct ($m/z = 303.3$), which is in contrast to our previous report [15]. The reason might be the higher content of sodium in methanol (used as the solvent in the present study) compared to distilled water (used in our previous report). Finally, being a negative ion, quercetin gives only one signal at $m/z = 301.2$ (Fig. 2h) in the negative ion mode LDI-TOF mass spectra.

3.2. MALDI-TOF MS of Pt(II) and Pd(II) complexes

Three neutral ([Pt(*en*)Cl_2], [Pt(*dach*)Cl_2] and [Pt(*en*)Cl_4]) and two cationic complexes ([Pd(*dien*)Cl]Cl and [Ru(*en*)$_2Cl_2$]Cl) were tested. The spectra of each tested complex with each of the four flavonoids were recorded.

Before passing to a detailed analysis of the mass spectra of the transition metal complexes, it should be indicated that each peak arising from the complex consisted of a group of isotopically resolved peaks, the identity of which can be explained as a combination of different isotopes in the complexes, *i.e.*, isotopes of Pt (or Pd or Ru), C, N, O, H and Cl. Since transition metals have a large number of naturally abundant isotopes, which are listed in Table 2, the peaks arising from Pt, Pd or Ru complexes (tested in this work) can be recognized by the characteristic spectral pattern. On the one hand, this fact further complicates the spectra, but on the other hand, it makes the identification of peaks in the MALDI-TOF mass spectra of transition metal complexes much easier. The most intense peak in each group arising from the transition metal complex is indicated and considered for analysis.

The positive ion MALDI-TOF mass spectra of one of the Pt(II) complexes analyzed — [Pt(*en*)Cl_2] — are presented in Fig. 3a–d for

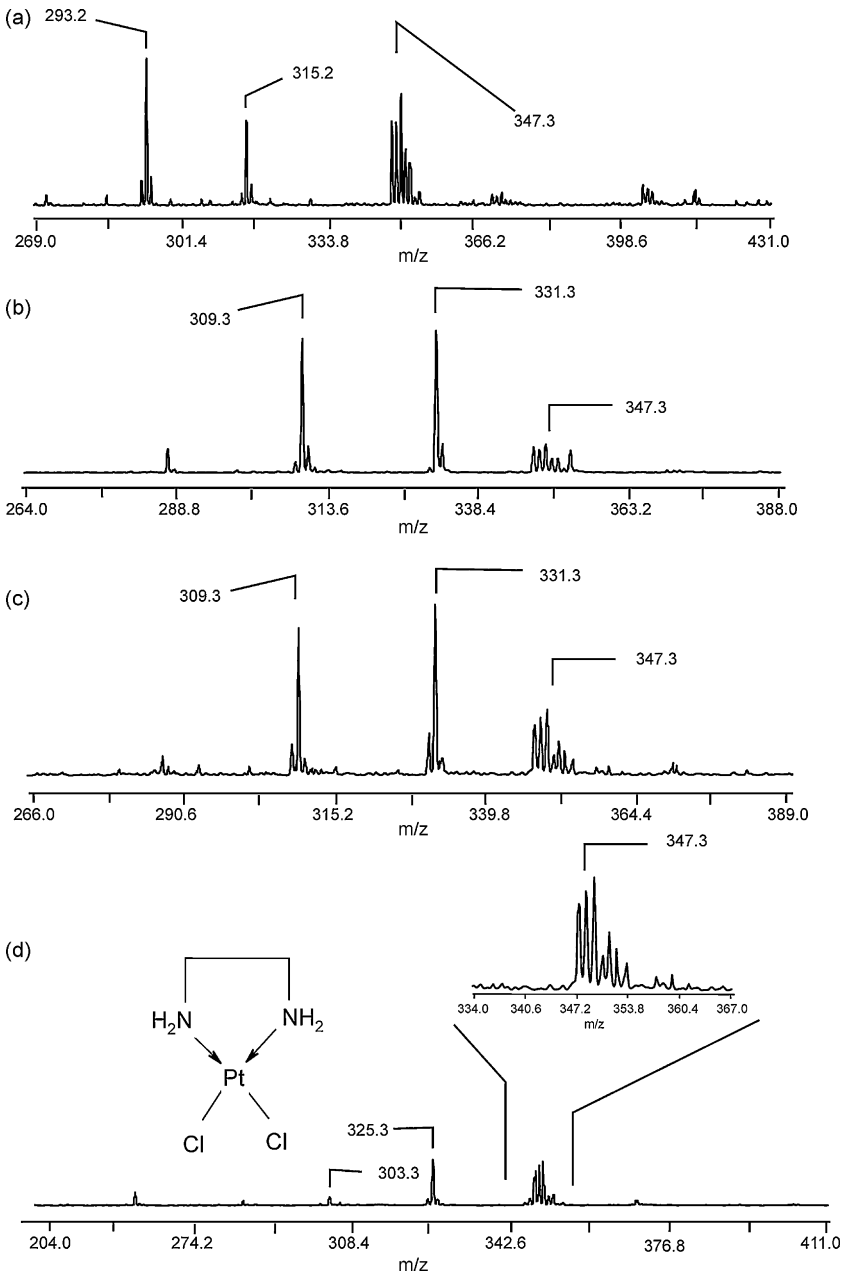

Fig. 3. Positive ion MALDI-TOF mass spectra of [Pt(*en*)Cl_2] recorded with apigenin (a), kaempferol (b), luteolin (c) and quercetin (d) as matrices. Inset in spectrum trace (d) represents emphasized peak arising from the analyte. The structural formula of the analyzed complex is also given in (d). The spectra are acquired in the reflector mode and under delayed extraction conditions; the peaks arising from the flavonoid-matrices are indicated with an asterisk.

the apigenin, kaempferol, luteolin and quercetin matrixes, respectively. The structural formula of the complex analyzed is given in trace (d). Peaks arising from the matrices are also indicated in the spectra, but they are not discussed in more detail here.

The peak detected at $m/z = 347.3$ in each of the spectra corresponds to the sodium adduct of the complex. In our previous report [15], two peaks of this complex were detectable: one arising from the sodium and one from the potassium ($m/z = 363.2$) adduct of the complex. The reason we failed to detect the potassium adduct of the complex in this work was probably because the solution used for the complexes (20% physiological salt solution) contained a large amount of NaCl. Under these conditions, the peaks that would be generated by cationization with K^+ are suppressed.

The mass region of the detection of the peak is emphasized in trace (d) to demonstrate the complexity of the peaks arising from the transition metals, *i.e.*, Pt-complexes.

The spectra of [Pt(*en*)Cl_2] recorded with flavonoid matrices presented in Fig. 3 are simple, since they contain only a small number of peaks: two of them arise from flavonoids and one from the Pt(II) complex. Other matrices commonly applied for MALDI-TOF MS give a greater number of peaks in this mass region, making the application of flavonoids, with two well-defined peaks that do not overlap with the peaks of interest, advantageous.

Differences in the peak pattern of the flavonoids presented in Fig. 2 arise from the higher sodium content; the transition metal complexes were dissolved in a solution that contained NaCl. Therefore, apigenin (Fig. 3a) gave two peaks under these conditions: one at $m/z = 293.2$ and one at $m/z = 315.2$, corresponding to the ion generated by ionization with Na^+ and replacement of one proton with sodium and further ionization with Na^+, respectively. In similar manner, kaempferol (Fig. 3b) and luteolin (Fig. 3c) also yielded two peaks at $m/z = 309.3$ and $m/z = 331.3$. The former ion corresponds

to $M+Na^+$, whereas the latter one corresponds to $M-H^+ + 2Na^+$, where M indicates molecule, *i.e.*, bears no charge. Quercetin also yielded two peaks, which are indicated in Fig. 3d and which were discussed above. It is noteworthy, however, that the sodium adduct (at $m/z = 325.3$) of quercetin is much more intense compared to the proton adduct of the same molecule (at $m/z = 303.3$) under these conditions.

All of the investigated flavonoids appeared to be suitable matrices for MALDI-TOF mass spectrometric analysis of yet another neutral complex of Pt(II)—[Pt(*dach*)Cl_2]. To illustrate this, the positive ion mode MALDI-TOF mass spectra of [Pt(*dach*)Cl_2], recorded with apigenin and luteolin, are given in Fig. 4a and b, respectively. The structural formula of this complex is given in trace (b). There were two peaks detectable in both spectra of [Pt(*dach*)Cl_2] at $m/z = 345.2$ and at $m/z = 309.2$. These peaks are generated upon the loss of one Cl^- ion and subsequent elimination of neutral HCl. This is somewhat surprising since one would expect the addition of sodium to the neutral complex, as previously observed [15]. The presence of physiological salt solution (*i.e.*, high content of Cl^- ions) clearly prevents hydrolysis and Cl^-/OH^- replacement within the complex [20]. The elimination of Cl^- from the complex, instead of the addition of Na^+ to the complex, occurs upon laser irradiation, leading to generation of the detectable ion in the mass spectra. The peak of considerably lower intensity at $m/z = 309.2$ is probably generated from the elimination of Cl^- and HCl. Although neutral, this Pt(II) complex showed characteristic spectral behavior similar to the cationic complexes used in this study.

In contrast to this finding, when the [Pt(*dach*)Cl_2] complex and the matrix quercetin were applied onto the MALDI target as water suspensions, only peaks corresponding to the sodium and potassium adducts of the complex were detectable [15], with no elimination of the Cl^- observed. Why the MALDI-TOF spectral pattern of this complex differs from the one previously described is not known, and it should be better investigated. The behavior was not different, even with lower laser intensities (data not shown); therefore, it cannot be attributed to increased fragmentation.

In the spectra recorded with luteolin (Fig. 4b), there was also a very low-intensity peak at $m/z = 456.2$. Judging from the characteristic 6-peak group, this peak could be assigned to the Pt-complex.

In Fig. 4c and d, the spectra of the [Pd(*dien*)Cl]Cl complex with kaempferol and quercetin as matrices are presented, respectively.

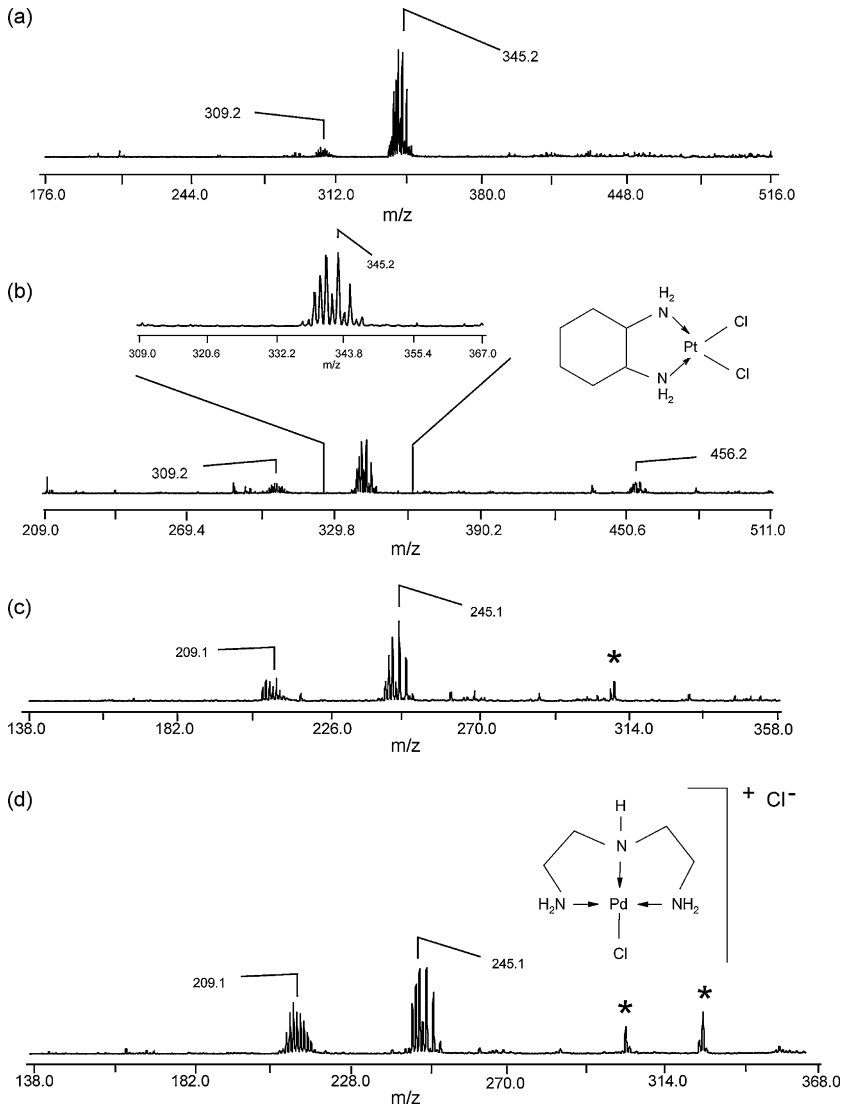

Fig. 4. Positive ion MALDI-TOF mass spectra of [Pt(*dach*)Cl_2] complex recorded with apigenin (a) and luteolin (b) as matrices. The positive ion mode MALDI-TOF mass spectra of [Pd(*dien*)Cl]Cl complex recorded with kaempferol (c) and quercetin (d) as matrices. The structural formulae of [Pt(*dach*)Cl_2] and [Pd(*dien*)Cl]Cl are given in traces (b) and (d), respectively. All spectra are acquired in the reflector mode and with delayed extraction conditions.

The structural formula of the analyzed complex is depicted in the latter trace and the matrix peaks are indicated by asterisks. Similar to Pt, Pd also has 6 natural isotopes, with ^{106}Pd being the most abundant. Therefore, a group of isotopically resolved peaks was detectable in the MALDI-TOF mass spectra.

There were two peaks detectable in both spectra of the [Pd(*dien*)Cl]Cl complex, one at $m/z = 245.1$ and one at $m/z = 209.1$ (Fig. 4c and d) generated by the loss of Cl^- and subsequent elimination of HCl, respectively. These two peaks were detectable in the MALDI-TOF mass spectra of the complex recorded using all of the investigated flavonoids as matrices. This spectral pattern of the same complex was also observed in our previous report [15], in which quercetin and rutin were used as matrices. The signal intensity of both peaks arising from the complex was good with all of the other flavonoids used in this study (data not shown). It is possible that the mass of the Pd(II) complex, which is lower than the mass of flavonoids, also contributes to the good detectability of this complex in the MALDI-TOF mass spectra.

3.3. MALDI-TOF MS of Pt(IV) and Ru(III) complexes

Another complex of Pt(IV) — ([Pt(*en*)Cl_4]) — was studied with MALDI-TOF MS using the same four flavonoids as matrices. The positive ion MALDI-TOF mass spectra of this complex recorded with apigenin and luteolin as matrices are given in Fig. 5a and b, respectively. Obviously, a somewhat worse quality of the spectra was obtained when the Pt(IV) complex was recorded with apigenin as a matrix (Fig. 5a). Peaks arising from the complex were rather low in intensity and a number of additional peaks were observed. A similar pattern was obtained with other flavonoids (data not shown), with the exception of luteolin (Fig. 5b). The low detectability compared to the MALDI-TOF mass spectra of the Pt(II) complexes acquired with flavonoids and the greater number of peaks can be explained by the somewhat different chemical properties of Pt(IV), *i.e.*, different interaction of this complex with the flavonoids. Specifically, it is possible that partial reduction of Pt(IV) to Pt(II) occurs upon its interaction with the flavonoids, par-

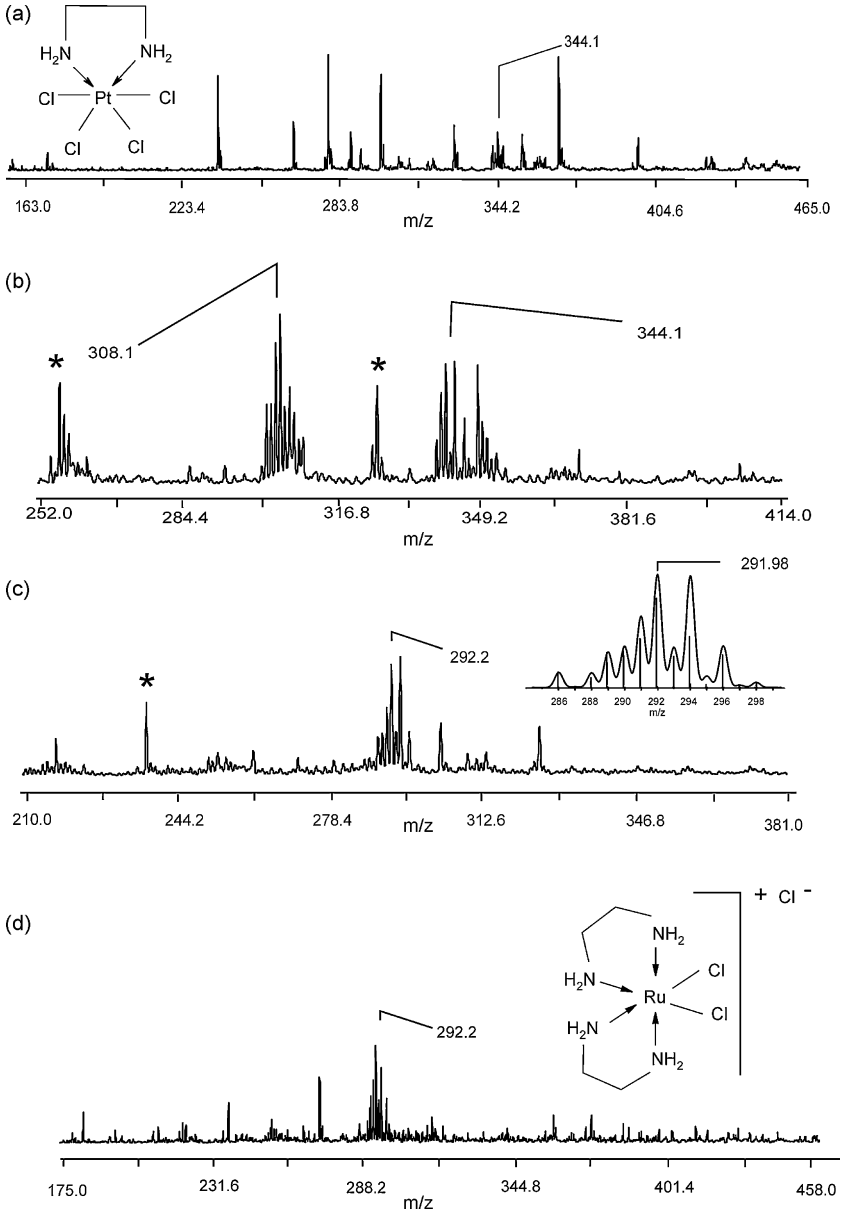

Fig. 5. Positive ion MALDI-TOF mass spectra of [Pt(*en*)Cl_4] complex with apigenin (a) and luteolin (b) as matrices and the spectra of [Ru(*en*)$_2$ Cl_2]Cl complex recorded with quercetin (c) and kaempferol (d) matrices. Inset in trace (b) represents theoretical representation of the mass spectrum of [Ru(*en*)$_2$ Cl_2]Cl complex. The structural formulae of the complexes investigated are also given in the figure. Spectra are acquired in the reflector mode and under delayed extraction conditions.

Table 2
List of isotopes of transition metals and Cl with their abundance in the nature.

Metal	Relative molar masses	Probability (abundance)
Pt	189.96	0.00029585798816568
	191.96	0.0233727810650888
	193.96	0.973372781065089
	194.96	1
	195.96	0.748520710059172
	197.97	0.21301775147929
Pd	101.91	0.0373216245883644
	103.90	0.407610684229784
	104.91	0.817050859860959
	105.90	1
	107.90	0.968166849615807
	109.91	0.428832784485913
Ru	95.91	0.175
	97.91	0.0591772151898734
	98.91	0.401898734177215
	99.90	0.39873417721519
	100.91	0.541139240506329
	101.90	
	103.91	0.588607594936709
Cl	34.97	1
	37.00	0.319783555496898

The data have been taken from the Selket programme.

ticularly with apigenin, which has been observed in other reports [21].

In the spectra of the Pt(IV) complex recorded with luteolin as the matrix (Fig. 5b), two peaks were present at m/z = 344.1 and m/z = 308.1. The former ion was generated by the loss of Cl^- ion and elimination of ammonium from the ligand. This behavior has been demonstrated for some other ligands coordinatively bound to Pt [15]. Another elimination of HCl from the complex resulted in a fragment at a lower m/z ratio. Analogous ions were detectable in the MALDI-TOF mass spectra of [Pd(*dien*)Cl]Cl recorded with flavonoids (cf. Fig. 4).

Finally, the MALDI-TOF mass spectra of a Ru(III) complex — ([Ru(*en*)$_2$Cl$_2$]Cl) — were also acquired with the investigated flavonoids. In Fig. 5c and d, quercetin and kaempferol were applied for MALDI-TOF MS analysis of [Ru(*en*)$_2$Cl$_2$]Cl, respectively. There was only one peak at m/z = 292.2, generated from the loss of Cl^- ion, leaving a singly charged positive ion. The same result was obtained with all of the other flavonoids.

The group of peaks that arose from the Ru(III) complex seemed to be even more complex, since this metal has seven isotopes. This is clearly indicated in the inset in trace 5c, which represents the theoretical presentation of the mass spectrum of the positive ion of the Ru(III) complex generated from the loss of Cl^-. The mass of the combination of the most abundant isotopes is indicated in the figure and corresponded to the mass detected in the MALDI-TOF mass spectra of the same complex.

3.4. Relationship of the signal-to-noise ratio to the matrix/analyte ratio

In the last part of this work, the suitability of individual flavonoids as matrices for different inorganic complexes for MALDI-TOF MS was compared in terms of different matrix/analyte ratios. The relationship of the S/N ratio of the peak at m/z = 347.3 arising from the [Pt(*en*)Cl$_2$] and of the peak at m/z = 292.2 from the peaks detectable in the spectra of [Ru(*en*)$_2$Cl$_2$]Cl are shown in Fig. 6a and b, respectively. These two complexes were selected because they posses the same ligand bound to a different metal.

Quercetin seemed to be better matrix for the detection of [Pt(*en*)Cl$_2$], since the S/N ratio for this complex was comparably higher (Fig. 6a). Judging from this, apigenin appeared to be the worst matrix for this compound. As for the Ru(III) complex, luteolin gave better results, whereas kaempferol seemed to yield a considerably lower S/N ratio of the signal arising from the complex (Fig. 6b).

Although no clear "flavonoid structure–analyte signal intensity" relationship could be established at this stage, it is advisable to individually test all of the combinations of transition metal complexes/flavonoids in order to find the combination resulting in the best complex detectability. It seems that not only the ligand bound to the metal causes the differences in detectability, but also the metal itself.

Surprisingly, the best detectability of the complexes was achieved with lower matrix/analyte ratios, which was also noticed in our previous report [15]. On the other hand, a very good detectability of all flavonoids, as well as the peaks that arise from them, would probably lead to the suppression of further analyte peaks. Since this is contrary to the behavior of the "traditional" matrices for MALDI-TOF MS, which have to be applied in 500–1000 molar excess, it was assumed that the temporary clusters of metal complexes and flavonoids that are generated are sufficiently stable until the ion from the complex reaches the mass analyzer. This assumption is based on the well-known fact that flavonoids are

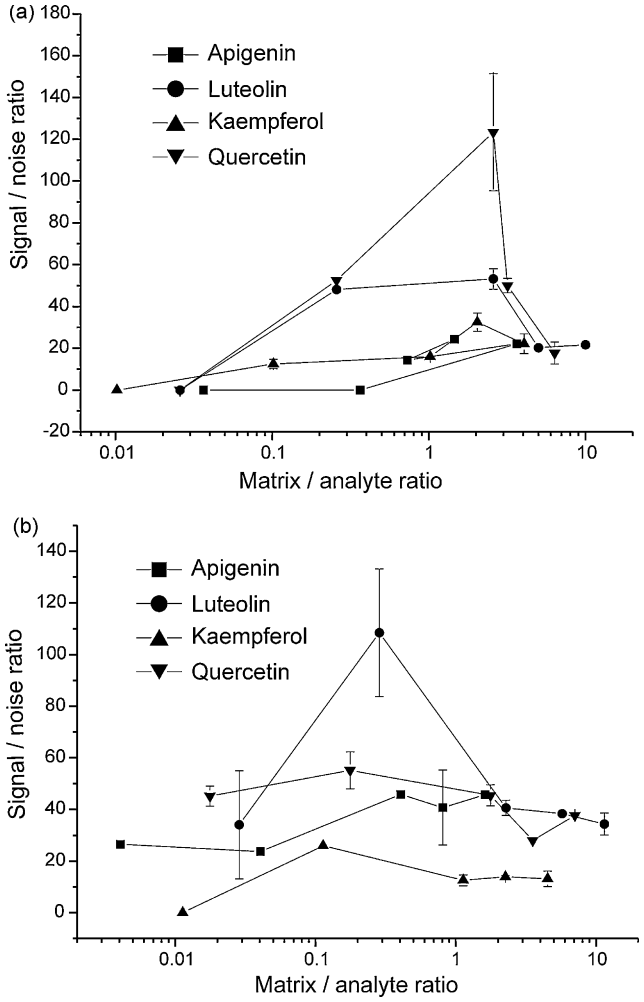

Fig. 6. Relationship between the signal-to-noise ratio and the matrix-to-analyte ratio of the peak at m/z = 347.3 arising from the [Pt(*en*)Cl$_2$] complex (a) and peak at m/z = 292.2 arising from [Ru(*en*)$_2$Cl$_2$]Cl complex.

able to interact with transition metals [21]. Since these temporary metal complex/flavonoid clusters are not detectable in the MALDI-TOF mass spectra, we tried to document new complexes by recording the UV/vis spectra of the mixture of flavonoids and transition metal complexes. Unfortunately, it was not possible to confirm the formation of any new complex between the flavonoid matrices and the analytes (data not shown). We assume that further elucidation of the mechanism of the flavonoid-transition metal complex interaction may result in their more efficient exploitation as matrices.

4. Conclusions

Naturally occurring flavonoid molecules are suitable matrices for MALDI-TOF MS analysis of transition metal complexes, which are used as metallo-drug anti-tumor agents. In this work, it has been demonstrated that the described approach has broad applicability for the analysis of Pt(IV) and Ru(III) complexes. This approach also has great potential for the reliable analysis of new metallo-drugs.

The application of flavonoids as matrices is also recommended for monitoring of synthesis processes because the acquired spectra are simple, and they have a low background signal; furthermore, the metal–ligand bond is, in most cases, preserved. In addition, the flavonoid matrices appeared to have a higher tolerance for MALDI-TOF mass spectrometric analysis of samples with higher amounts of inorganic salts, as demonstrated in our previous work [15]. However, it is necessary to examine individual transition metal complex with selected flavonoids in order to obtain the best analytical system.

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