

Electrospray Ionization Mass Spectrometric Study on Complexation of Flavone With Divalent Metal Ions

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Abstract: The analytical potential of the complexation of flavone with Zn(II), Cd(II), and Co(II) was investigated by positive-ion electrospray ionization Fourier transform ion cyclotron resonance (ESI-FTICR) mass spectrometry. The binding selectivity of the flavone with three different divalent metal ions Cd(II), Co(II), and Zn(II), reveals that the flavone favors binding to Zn(II) over Co(II) and Cd(II) and that the order of relative binding affinity is Zn(II) > Co(II) > Cd(II). This can be attributed to the polarizability, or relative hardness of the metal ions. The counter-ion binding selectivities measured by FTICR mass spectrometry of flavone with ZnCl₂, Zn(NO₃)₂, and Zn(ClO₄)₂ show that the order of selectivity for the complexation of flavone metal complex with the counter-ions is ClO₄⁻ > Cl⁻ > NO₃⁻. The exact reason for such selectivity order is not known, so that further experiments and calculation are required to understand these effects of counter-ion selectivities.

Keywords: flavonoids, surfactants, heavy metals, mass spectrometry.

Introduction

Flavonoids hold promise for preventing age-related diseases including heart disease and cancer.¹ Beside being antioxidants, flavonoids present other biological activity, including antibacterial, antifungal, anti-inflammatory and antimicrobial activity.²⁻⁵ Mass spectrometry has become an important analytical technique for flavones, particularly when small quantities are involved. The reactivity between flavone and metal ions has been widely studied in solution because of the involvement of metal ion-flavonoids interactions in key biological processes. Several groups have also reported the gas phase mass spectrometric study of the complexation of flavone with transition metal ions.⁶⁻⁷ All these studies showed that gas-phase metal ion chemistry is a powerful tool to determine the binding strengths of metal ion-flavonoids. With the development of soft ionization methods, mass spectrometry has been playing a more and more important role in flavonoid studies, including structural characterization and enhanced detection. The first metal complexation strategy involved the use of

ESI mass spectrometry to study transition metal and an auxiliary ligand complexes of the type [M(II)(flavonoid-H) auxiliary ligand]⁺.⁸ These transition metal complexes of flavonoids exhibit altered fragmentation pathways of the isomeric flavonoids which allow more effective differentiation of them.

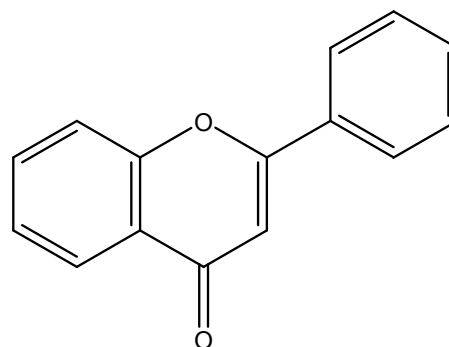


Figure 1 Chemical structure of flavone

ESI-FTICR mass spectrometry has been used to investigate the reaction between flavone (Figure 1) and the divalent metal ions Co(II), Zn(II) and Cd(II), and how divalent metal ions chelate to the oxygen. Since the attachment of flavone to divalent metal ions is associated with a counter-ion, a series of experiments using different zinc salts was carried out to examine whether counter-ions have any effect on the complexation reactions. The selectivity of flavone binding with Zn(II), Cd(II), and Co(II) and the effects of experimental parameters such as capillary-skimmer potential difference (CSPD) on the signal intensities of the divalent metal ion/flavone complexes are also studied.

Experimental

Raw Material

The flavone was purchased from Aldrich Chemical Co. Cadmium nitrate, cobalt nitrate, and zinc nitrate were obtained from Sigma-Aldrich and were used without any further purification.

Sample Preparation

The metal complexes were produced by mixing solutions of the appropriate metal salt $[MX_2]$ where $M = \text{Co(II), Zn(II), Cd(II)}$, and $X = (\text{NO}_3)_2$ and $(\text{ClO}_4)_2$ and the flavone in a methanol (HPLC grade). Solutions were then injected to FTICR mass spectrometry such that the flavone : metal concentration ratio was 1:5 and only the positive ion mass spectra were recorded. Data acquisition and processing together with the comparison of the experimental isotope patterns and mass values observed and the theoretical patterns for all major product ions are performed using Xmass version 6.2 Bruker software. A Bruker BioApex-II 7T FTICR mass spectrometer with an on- and off-axis Analytica ESI spray source was used in this study. Stronger signals were observed using the off-axis configuration.

Results and Discussions

Positive-ion FTICR mass spectrometry of the attachment of divalent metal ions to flavone.

The interaction of flavone with metal ions, studied by FTICR mass spectrometry, indicates that complexes were formed between flavone and each metal Co(II), Zn(II) and Cd(II) and all formed flavone complexes having the flavone : metal ratios 1:1, 2:1, and 3:1. Figure 2 show positive-ion FTICR mass spectrum of the reaction products of flavone with $\text{Zn(NO}_3)_2$ at CSPD 30 V.

Figure 2 shows that the dominant base peak is assigned to the complex ion $[(\text{flavone})_2 + \text{ZnNO}_3]^+$. In this case of flavone, where no mobile proton is available, it is mainly ionized by forming the ion pairs involving flavone-Zn(II) often in combination with the counter-ion, NO_3^- . This ion pair formation was also observed in

a previous quadrupole ion trap study by Vachet and Callahan in their study on transition metal–bipyridine ligand systems.⁹⁻¹⁰ The inset expansion in Figure 2 shows small peaks assigned to singly and doubly charged clusters formed between the flavone and Zn(II) ions. For example, $[\text{Flavone} + \text{Zn} + \text{H}_2\text{O} - \text{H}]^+$, $[\text{flavone} + \text{ZnNO}_3]^+$, $[(\text{flavone})_3 + \text{Zn}]^{2+}$, $[(\text{flavone})_4 + \text{Zn}]^{2+}$ and $[(\text{Flavone})_2 + \text{Zn} - \text{H}]^+$ are observed as well. Each spectrum obtained within a particular set of experiments was based on an average of 120 scans under a selected constant CSPD value (30 V). All FTICR mass spectrometry experiments were repeated at least seven times to ensure the results were reproducible.

The effect of experimental parameters on complexation of flavone with M(II)

The reaction of flavone and $\text{Zn(NO}_3)_2$ where the CSPD is varied from 30 to 300 V is studied to determine the effect of this parameter on the ions produced. Whilst the CSPD was varied all other parameters were held constant through the experiments. Figure 3 shows the positive-ion FTICR mass spectra of the products of the reaction of flavone with $\text{Zn(NO}_3)_2$ at CSPD setting of (a) 30 V, (b) 120 V and (c) 300 V. Figure 3a at CSPD of 30 V shows that singly and doubly charged ions are produced for various monomer, dimer, trimer, and tetramer flavone/metal complexes. These complexes were detected through peaks at m/z 302.998748, 347.984433, 365.066424, 507.056928, and 570.052540 corresponding to the ions $[\text{Flavone} + \text{Zn} + \text{H}_2\text{O} - \text{H}]^+$, $[\text{Flavone} + \text{ZnNO}_3]^+$, $[(\text{Flavone})_3 + \text{Zn}]^{2+}$, $[(\text{Flavone})_4 + \text{Zn}]^{2+}$, $[(\text{Flavone})_2 + \text{Zn} - \text{H}]^+$ and $[(\text{Flavone})_2 + \text{ZnNO}_3]^+$ respectively. Table 1 show a comparison of the experimental and calculated isotopes distributions for the complex ions $[(\text{Flavone})_2 + \text{Zn} - \text{H}]^+$, $[\text{Flavone} + \text{Zn} + \text{H}_2\text{O} - \text{H}]^+$ and $[(\text{Flavone})_3 + \text{Zn}]^{2+}$. Also, the relative mass deviations (ppm) for all assigned ion products are listed in Table 1. The presence of H_2O in the $[\text{Flavone} + \text{Zn} + \text{H}_2\text{O} - \text{H}]^+$ complex may be due to the reaction of the flavone with one of the H_2O molecules present in the hydrated nitrate salt.

As shown in Figure 3a, $[(\text{Flavone})_2 + \text{ZnNO}_3]^+$ is the base, most intense peak observed at CSPD 30 V. Very small peak intensities for the rest of the ions are observed. It can be seen that the intensity of the complex ion $[\text{Flavone} + \text{ZnNO}_3]^+$ is very small at 30 V. This peak is believed to be due to the results of the fragmentation of the base peak $[(\text{Flavone})_2 + \text{ZnNO}_3]^+$. This is confirmed after increasing the CSPD to 300 V. By increasing the capillary-skimmer potential difference to 120 V (Figure 3b), the complex ion $[(\text{Flavone})_2 + \text{ZnNO}_3]^+$ remain as the base peak while the intensities of the complex ions $[\text{Flavone} + \text{ZnNO}_3]^+$, $[(\text{Flavone})_3 + \text{Zn}]^{2+}$ and $[(\text{Flavone})_2 + \text{Zn} - \text{H}]^+$ increase.

At high CSPD 300 V, (Figure 3c), the complex ion $[(\text{Flavone})_2+\text{ZnNO}_3]^+$ is still the base peak and the intensities of peaks assigned to $[\text{Flavone}+\text{ZnNO}_3]^+$ and $[\text{Flavone}+\text{Zn-H}]^+$ increased to ~25% and 7.5% respectively with respect to the base peak. The loss of neutral flavone molecule from $[(\text{Flavone})_2+\text{ZnNO}_3]^+$ via CSPD processes to produce $[\text{Flavone}+\text{ZnNO}_3]^+$ is expected because the electrostatic interactions between divalent metal ions and the nitrate anion makes this interaction stronger in the gas phase than the interaction between divalent metal ions and flavone and so flavone is lost instead of NO_3^- . The absence of solvent very likely forces the NO_3^- to position itself more closely to the divalent metal ion than it might in solution. In general, the increase in the intensity of $[\text{Flavone}+\text{ZnNO}_3]^+$ and $[\text{Flavone}+\text{Zn-H}]^+$ is attributed to the increased kinetic energy of the ions in the capillary-skimmer region of the ESI source so that the collisions result in the fragmentation of the dimer and trimer ions $[(\text{Flavone})_2+\text{ZnNO}_3]^+$, $[(\text{Flavone})_3+\text{Zn}]^{2+}$ to the monomer ion $[\text{Flavone}+\text{ZnNO}_3]^+$ which is further fragmented to the complex ion $[\text{Flavone}+\text{Zn-H}]^+$.

It has been noted that at higher CSPD, the complex ion $[(\text{Flavone})_2+\text{ZnNO}_3]^+$ undergoes dissociation to produce $[\text{Flavone}+\text{ZnNO}_3]^+$ and $[\text{Flavone}+\text{Zn-H}]^+$. The loss of HNO_3 from the complex ion $[(\text{Flavone})_2+\text{ZnNO}_3]^+$ can be attributed to the fact the flavone does not contain a labile proton. Flavone is normally ionized in ESI by forming ion pairs involving the flavone-M(II) complex in combination with the counter-ion, in this case NO_3^- . Further, Vachat and Callahan indicate that the Coulombic interactions that exist between the counter-ion NO_3^- and divalent metal ions M(II) where M(II) = Co(II), Zn(II) and Cd(II) in the gas phase are stronger than the electron-sharing interactions between the neutral glucose and divalent metal ions.¹¹ Desolvation of the dimer in the ESI process may be easier than for a 1:1 complex, meaning that the ionic charge in the dimer complexes is better distributed by two flavone molecules rather than by one. Overall, the dimer complexes are expected to have higher surface activities and lower solvation energies which lead to larger ESI signals.^{12,13} In summary, at low CSPD a variety of singly and doubly charged cluster ions are produced with the major ion being $[(\text{Flavone})_2+\text{ZnNO}_3]^+$. However, as might be expected, as the translational kinetic energy of the ions in the capillary skimmer region is increased the multiply charged and cluster ions are removed and while $[(\text{Flavone})_2+\text{ZnNO}_3]^+$ remains the major ion produced in the experiment, fragment ions such as $[\text{Flavone}+\text{ZnNO}_3]^+$ and $[\text{Flavone}+\text{Zn-H}]^+$ are seen to increase in concentration.

Binding selectivity of flavone with M(II) ions

Two experiments for binding selectivity of flavone with divalent metal ions were carried out. The first experiment aimed to evaluate the binding selectivity of the flavone with three different divalent metal ions namely Cd(II), Co(II) and Zn(II) whereas the second experiment was designed to evaluate the binding selectivity of flavone with Zn(II) in the presence of the different counter-ions NO_3^- , Cl^- , and ClO_4^- . The two selectivity experiments were carried out at CSPD at 300 V and with the hexapole trapping time at 1 s.

For the purpose of selectivity experiments to select one mode of ion attachment (singly charged ion), harsh in-source collision induced dissociation has been carried out to completely eliminate any cluster complexes. The ratio of the products obtained in the ESI spectra of the multi-component mixture was then corrected based on the relative intensities (spray efficiencies) measured from the single component solutions.

Flavone competing for three divalent metal ions

For the quantitative studies, the ESI intensity coefficients for flavone with an excess of each of the three divalent metal ions, Cd(II), Co(II) and Zn(II) ions are evaluated first. Table 2 lists the assigned ions, intensities, spray efficiencies and relative binding affinities of flavone with $\text{Co}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$. From the Table, the relative peak intensities for $[(\text{Flavone})_2+\text{CoNO}_3]^+$: $[(\text{Flavone})_2+\text{ZnNO}_3]^+$: $[(\text{Flavone})_2+\text{CdNO}_3]^+$ are measured to be 10×10^8 : 8×10^8 : 3.7×10^8 and therefore the "ESI Intensity Coefficients" for $[(\text{Flavone})_2+\text{CoNO}_3]^+$: $[(\text{Flavone})_2+\text{ZnNO}_3]^+$: $[(\text{Flavone})_2+\text{CdNO}_3]^+$ are 2.7: 2.2: 1.0. The corrected relative binding affinity of flavone for Co(II): Zn(II): Cd(II) in multi-component solution is then evaluated as 1.9: 3.6: 1.0. The results in Table 2 reveal that, relative to binding with Cd(II), flavone shows at least a 3.5-times preference for Zn(II) and 2-fold preference for Co(II). Therefore the metal ion selectivity of the relative binding affinity for the divalent metal ions with flavone is Zn(II) > Co(II) > Cd(II). The selectivity might be correlated more strongly to polarizability. The flavone, as an oxygen donor ligand is considered 'hard'. The metal ions are classified as Zn(II) (hard), Co(II) (intermediate) and Cd(II) (soft). The binding selectivity of flavone is therefore consistent with this polarizability classification, although cation size must also play a role with the ionic radius of Zn(II) (0.074 nm) much smaller than that of Cd(II) (0.097 nm). Alternatively the binding of the more strongly-basic anions could lead to dissociation of ZnL_2^{2+} , by their acting as competing ligands. It may be feasible to describe the

relative stabilities in terms of the ability of the various counter-ions to stabilize Zn(II) in the form ZnL_2^{2+} . Then the weaker the anion is as a Lewis Base (e.g. ClO_4^- weakest), the more likely the Zn(II) is to exist in the most highly ligated form. This is the logic that is used for the observation of unusual coordination numbers and/or unusual binding of weak ligands with metal ions when they exist as strong-acid salts in poorly-coordinating solvents.

The effect of counter-ions on the attachment of flavone with divalent metal ions

In this experiment, we examine the effect of the counter-ion on the binding affinity of Zn(II) to flavone. To do this we examine three counter-ions namely ClO_4^- , Cl^- and NO_3^- . First the relative spray efficiencies for each of the different salts dissolved in methanol with flavone were measured individually to determine the spray correction factors. That is, in the single metal-ion complexation experiments, a comparison of the absolute peak intensities for $[(\text{Flavone})_2+\text{ZnNO}_3]^+$: $[(\text{Flavone})_2+\text{ZnCl}]^+$: $[(\text{Flavone})_2+\text{ZnClO}_4]^+$ gives values of 4.6×10^8 : 4.0×10^8 : 2.10×10^8 and therefore the “ESI intensity coefficients” for $[(\text{Flavone})_2+\text{ZnNO}_3]^+$: $[(\text{Flavone})_2+\text{ZnCl}]^+$: $[(\text{Flavone})_2+\text{ZnClO}_4]^+$ are 1: 0.9 : 0.5. Next, the multicomponent mixture was examined. Figure 4 shows the FTICR mass spectrum of this mixture which contains all three counter-ions (CSPD 120 V). The relative binding affinities corrected by the “ESI intensity coefficients” of flavone for $[(\text{Flavone})_2+\text{ZnNO}_3]^+$: $[(\text{Flavone})_2+\text{ZnCl}]^+$: $[(\text{Flavone})_2+\text{ZnClO}_4]^+$ in multi-component solution are then evaluated as 1.0 : 1.3 : 7.4. The calculations reveal that, relative to binding with ZnNO_3^+ , flavone shows at least a 7-times preference for ZnClO_4^+ and 1.3-times preference for ZnCl^+ . Therefore, the selectivity data shows that the order of relative binding affinity for counter-ions with flavone bound Zn(II) is $\text{ClO}_4^- > \text{Cl}^- > \text{NO}_3^-$.

Table 3 lists the assigned ions, intensities, spray efficiencies and relative binding affinities of flavone with ZnCl_2 , $\text{Zn}(\text{NO}_3)_2$ and $\text{Zn}(\text{ClO}_4)_2$. Previous studies reported that counter-ions do have an effect on the conformations of host-guest complexes due to the degree of ion pairing between the counter-ion and the metal, even when the metal is complexed to the host.^{14,15} The differences in the type of complexes formed when using various salts can be related to the strength of the metal-anion bonds. Therefore it seems that the major increase in the relative metal binding can occur if a suitable anion is chosen. This of course has application to metal remediation from soils where various anions might co-exist with the metals. This result is not surprising as increased binding by the counter-ion ligand through atom such as oxygen no doubt stabilize the cation complexes.

The larger, symmetrical anions, such as the perchlorates and nitrates, allow for negative charge to be more delocalized, thus creating a weaker ion pair between the counter-ion and metal.^{13,14} This weaker bond found in the perchlorate or nitrate ion pairs enhances the displacement of the counter-ion by the host because the host is able to more effectively compete with the anion for complexation with the cation. Based on the stability constants compiled by Smith and Martell, nitrates and perchlorates are known to be weaker coordinating anions as compared to chloride.¹⁶

From their study we should predict the order of selectivity as follows $\text{Cl}^- > \text{NO}_3^- > \text{ClO}_4^-$. However the order of selectivity observed in this study for the complexation of flavone with the counter-ions is $\text{ClO}_4^- > \text{Cl}^- > \text{NO}_3^-$. The exact reason for such a difference is not known, however the binding selectivity does not always follow the expected order of selectivity. For example, in the presence of nitrate or perchlorate, diaza-15-crown-ether prefers to bind mercury over the slightly smaller metals, copper and cadmium. The ion pairs for copper or cadmium with the perchlorate and nitrate ions are tighter than those for the slightly larger mercury cation. This difference makes binding of the crown ether with mercury more favorable than binding of the crown ether with copper or cadmium because the bulky perchlorate or nitrate counter-ion is able to locate farther away from the binding cavity, causing reduced steric hindrance, while the metal is still small enough to be bound by the polyether cavity.¹⁷

The interpretation of the selectivity of the flavone-Zn(II) complex with counter-ions in this study does not correlate to the results observed in the Smith and Martell study and may simply be related to the difference in the structure of the flavone which is quite different to the crown ether as a ligand. In a fast bombardment mass spectrometry study of the complexation of glycerin with nickel salts [NiCl_2 , $\text{Ni}(\text{ClO}_4)_2$, and $\text{Ni}(\text{NO}_3)_2$] the order of selectivity for forming NiCl_2 , $\text{Ni}(\text{ClO}_4)_2$, and $\text{Ni}(\text{NO}_3)_2$ obtained by Victoria et al.¹⁸ is $\text{NO}_3^- > \text{Cl}^- > \text{ClO}_4^-$. The order of selectivity obtained by Victoria et al. is again not in accordance with Smith and Martell as well. In fact further experiments are required to investigate counter-ion binding in the flavone system. For example, the complex ion $[(\text{Flavone})_2+\text{ZnNO}_3]^+$ can be isolated and reacted with various reagent gases to examine the counter-ion binding strength relative to the flavone. It would also be worth investigating solvents other than methanol, since solvation energy must also play a role.

Conclusion

Complexation of flavone with metal ions

Ion pairs of the form $[(\text{Flavone})_2+\text{MX}]^+$ are the base species observed in the FTICR mass spectra of the

divalent metal ions complexed to the simplest flavonoid, flavone. High CSPD is used to reveal the coordination chemistry of flavone in the ion pair complexes.

Binding selectivity of flavone with M(II)

The counter-ion binding selectivities measured by FTICR mass spectrometry of flavone with ZnCl_2 , $\text{Zn}(\text{NO}_3)_2$, and $\text{Zn}(\text{ClO}_4)_2$ show that the order of selectivity for the complexation of flavone metal complex with the counter-ions is $\text{ClO}_4^- > \text{Cl}^- > \text{NO}_3^-$. The exact reason for such selectivity order is not known, so that further experiments and calculation are required to understand these effects of counter-ion selectivities.

The binding selectivity of the flavone with three different divalent metal ions Cd(II), Co(II), and Zn(II), reveals that the flavone favors binding to Zn(II) over Co(II) and Cd(II) and that the order of relative binding

affinity is $\text{Zn(II)} > \text{Co(II)} > \text{Cd(II)}$. This can be attributed to the polarizability, or relative hardness of the metal ions.

Effect of CSPD on the flavone/M(II) system

At high CSPD voltage (300 V), $[(\text{Flavone})_2 + \text{ZnNO}_3]^+$ is forced to undergo dissociation to produce $[\text{Flavone} + \text{ZnNO}_3]^+$ and $[\text{Flavone} + \text{Zn-H}]^+$. However the intensities of these ions remain low (about 5% of the base peak). Flavone does not lose HNO_3 easily because it does not have labile protons and thus there is no means of readily losing a proton with the counter-ion NO_3^- . The loss of a proton from flavone increases at high CSPD. At high CSPD when one molecule of flavone is lost, Zn(II) becomes coordinatively unsaturated so that there is the possibility of loss of a proton from the benzene ring. However due to the very low concentration of the fragment ion $[\text{Flavone} + \text{Zn-H}]^+$, it is clear that the loss of a proton here is not a favourable process.

Table 1 Comparison of observed and calculated masses for the most abundant isotopes of assigned ions in Figures 2 and 3.

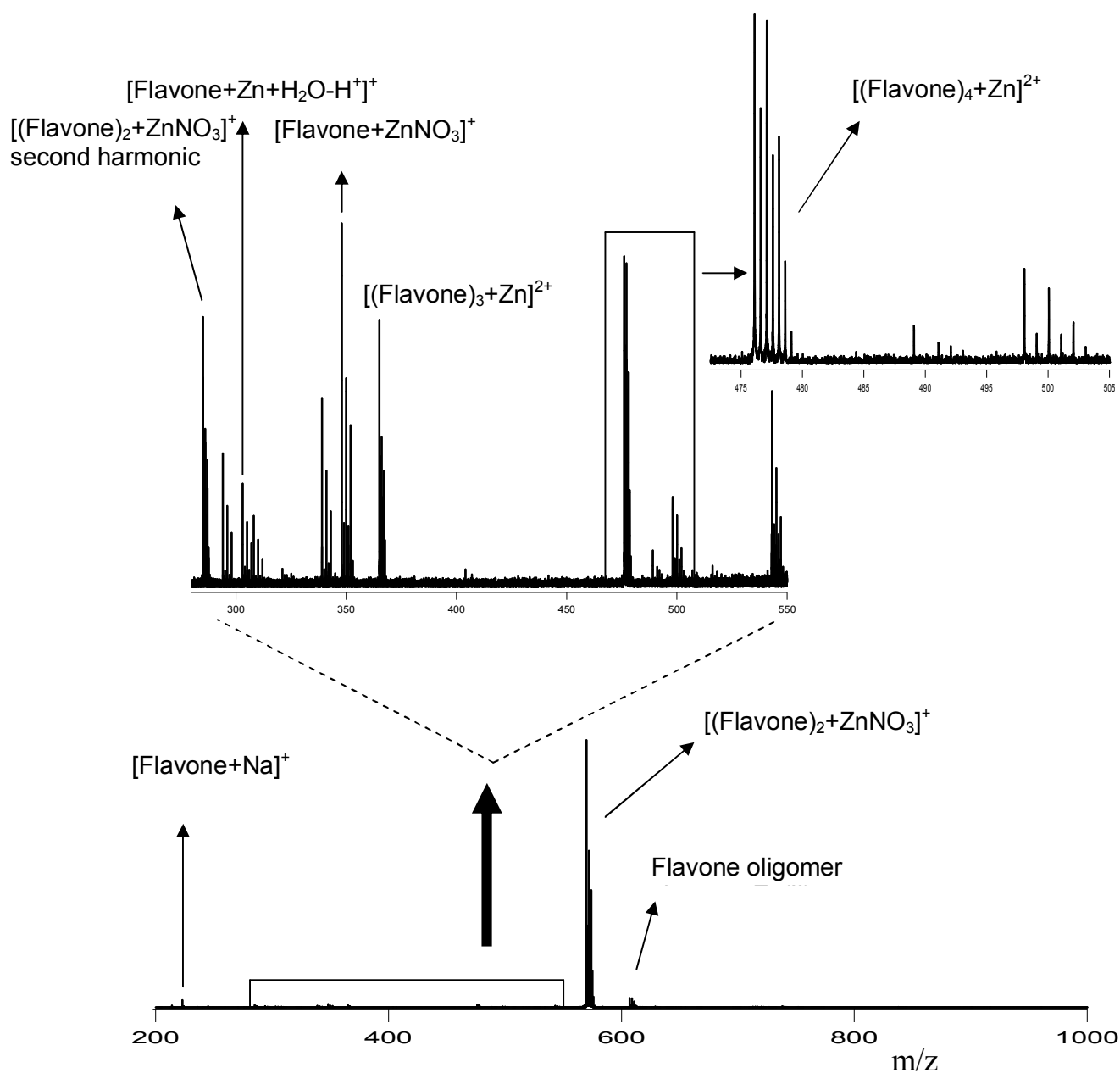
Assigned ion product	Observed mass (m/z)	Calculated mass (m/z)	Mass deviation (ppm)
$[\text{Flavone} + \text{Zn} + \text{H}_2\text{O} - \text{H}]^+$	302.998748	302.999482	2.4
$[\text{Flavone} + \text{ZnNO}_3]^+$	347.983288	347.984433	3.2
$[(\text{Flavone})_3 + \text{Zn}]^{2+}$	365.066658	365.066424	1.4
$[(\text{Flavone})_4 + \text{Zn}]^{2+}$	476.100190	476.100098	0.2
$[(\text{Flavone})_2 + \text{Zn-H}]^+$	507.057149	507.056928	0.4
$[(\text{Flavone})_2 + \text{ZnNO}_3]^+$	570.052894	570.052540	0.6

Table 2 Assigned ions, intensities, spray efficiencies and relative binding affinities of flavone with $\text{Co}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$.

Assigned ion product	Absolute intensity ($\times 10^8$) (Fig. 6-9)	Relative intensity (Uncorrected) (Fig. 6-9)	Absolute intensity ($\times 10^8$)	ESI intensity coefficient	Relative binding affinity (Corrected) $\pm$ Standard deviation
$[(\text{Flavone})_2 + \text{CdNO}_3]^+$	0.35	1.0	3.7	1.0	1.00 (± 0.12)
$[(\text{Flavone})_2 + \text{ZnNO}_3]^+$	2.8	8.0	8.0	2.2	3.60 (± 0.11)
$[(\text{Flavone})_2 + \text{CoNO}_3]^+$	1.8	5.1	10	2.7	1.90 (± 0.10)

Table 3 Assigned ions, intensities, spray efficiencies and relative binding affinities of flavone with ZnCl_2 : $\text{Zn}(\text{NO}_3)_2$: $\text{Zn}(\text{ClO}_4)_2$ from data in Figure 4.

Assigned ion product	Integrated intensity ($\times 10^8$) (Fig. 6-10)	Relative intensity (Uncorrected) (Fig. 6-10)	Integrated intensity ($\times 10^8$) (Fig. 6-5)	ESI intensity coefficient (Fig. 6-5)	Relative binding affinity (Corrected) $\pm$ Standard deviation
$[(\text{Flavone})_2+\text{ZnNO}_3]^+$	0.44	1.0	4.6	1.0	1.0 (± 0.14)
$[(\text{Flavone})_2+\text{ZnCl}]^+$	0.46	1.10	4.0	0.87	1.3 (± 0.12)
$[(\text{Flavone})_2+\text{ZnClO}_4]^+$	1.5	3.41	2.1	0.46	7.4 (± 0.11)

**Figure 2 Positive-ion FTICR mass spectrum of the reaction products of flavone with $\text{Zn}(\text{NO}_3)_2$ showing assignment of singly and doubly charged ions. (CSPD = 30 V).**

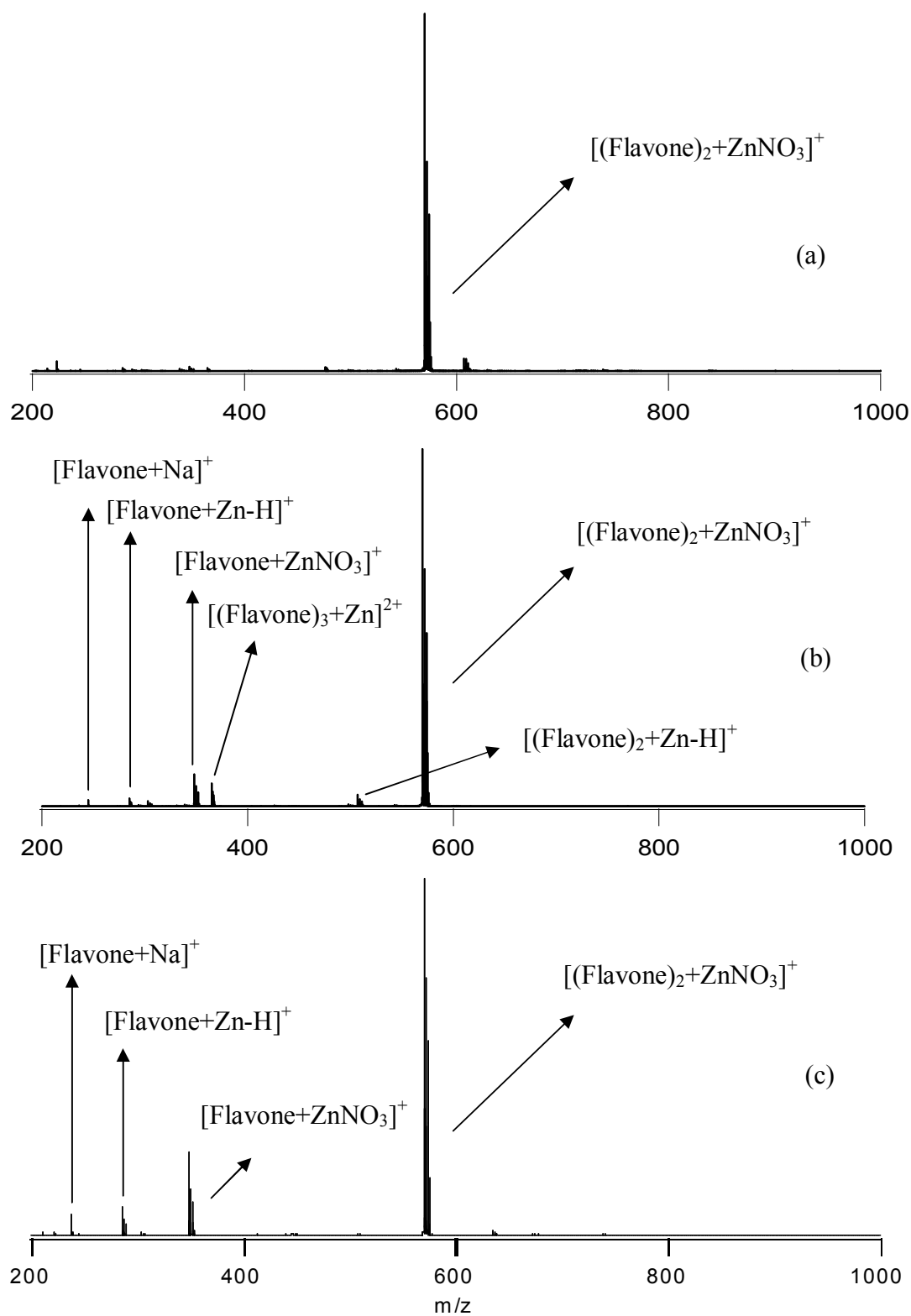


Figure 3 Positive-ion FTICR mass spectra of the products of the reaction of $\text{Zn}(\text{NO}_3)_2$ and flavone in methanol. (CSPD (a) 30 V, (b) 120 V, (c) 300 V). (ion residence-time in the hexapole 1 s).

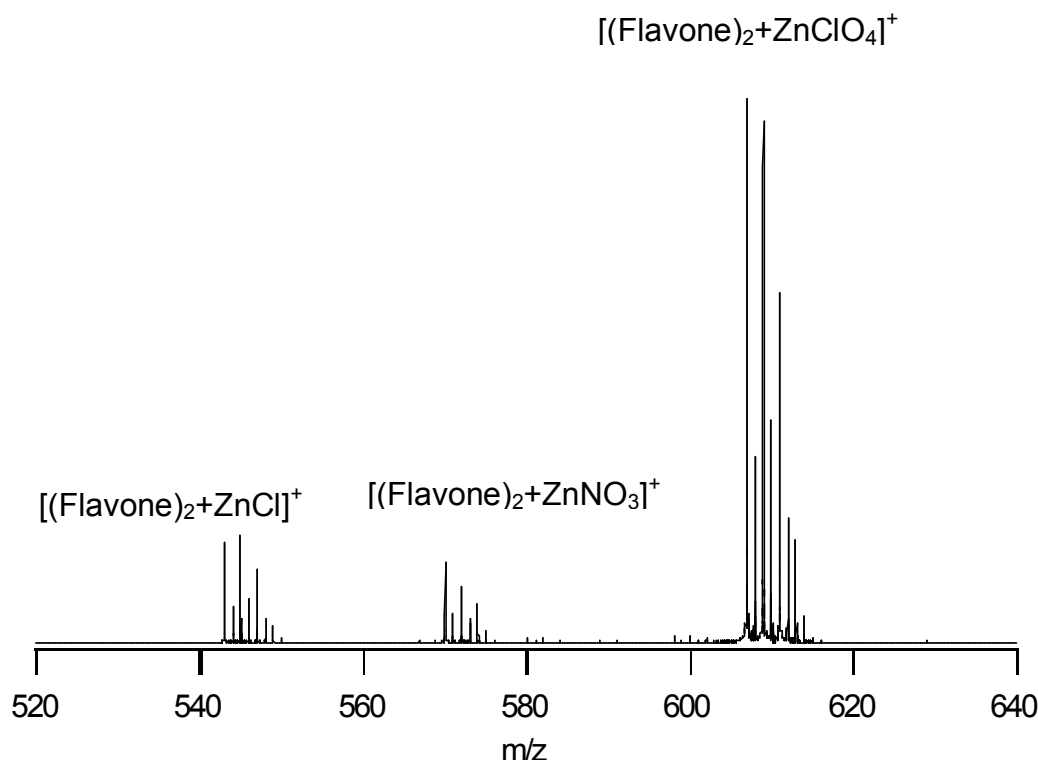


Figure 4 Positive-ion FTICR mass spectra of the reaction products of flavone with divalent zinc salts at a 1:5:5:5 mole rat

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