ORIGINAL ARTICLE

Characterization of second coordination shell ionophore–siderophore host–guest assemblies and binding selectivities in binary and complex mixtures by electrospray ionization mass spectrometry

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Abstract An ESI-MS method for the determination of host–guest association constants and binding selectivities for systems of binary and complex mixtures, respectively, is presented in an effort to quantitatively study the strength of host–guest interactions, while accounting for the different ESI-MS spray efficiencies of each component. Association constants were obtained for host–guest complexes formed between a series of crown ether and linear ionophore hosts and various guests, including protonated amines, and an apo- and ferri-siderophore, ferrioxamine B.

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Introduction

Molecular recognition plays an important role in biological and chemical processes, in many cases in the form of host–guest complex assemblies. As such, there has been a growing interest in analytical techniques that can aid in the characterization of host–guest complexes, and the determination of association constants and binding selectivities. Association constants have been traditionally measured by NMR titrimetry, potentiometry, extraction, and UV–Visible spectrophotometry [1–11]. These techniques suffer from large sample consumption, lack of applicability in certain solvents, and relatively long analysis time. Recently, electrospray ionization mass spectrometry (ESI-MS) has been successfully used to measure the association constants of various complexes formed in solution upon their transfer to the gas phase [12–18]. This technique requires little sample, is applicable in a wide range of solvents, provides rapid data analysis, and as such has become a promising technique for the analysis of association constants and selectivities for intramolecular interactions. Although a wide range of host–guest complexes have been examined by ESI-MS, including small organic host–guest complexes and large biological assemblies [19–21], the systems most studied by ESI-MS center around the determination of binding selectivities of simple and modified crown ethers with alkali metal ions [22–25]. To our knowledge, the use of ESI-MS for the determination of association constants and selectivities involving small metal complexes has not been pursued. This is an objective of our study.
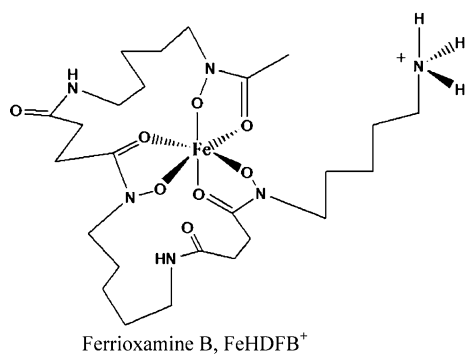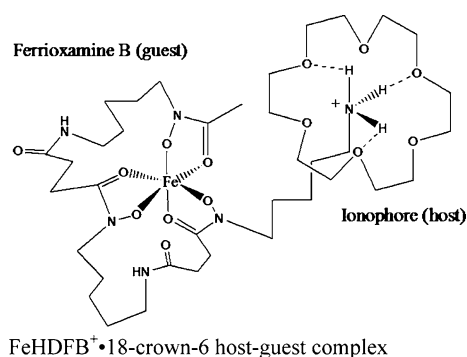
We have previously extensively studied the second-sphere host–guest complexation of the siderophore ferrioxamine B, $FeHDFB^+$, with a series of crown ethers as a model relevant to molecular recognition associated with microbial iron transport [26–32].

Ferrioxamine B, FeHDFB$^+$

From crystallographic studies, we have determined that FeHDFB$^+$ has the ability to form a series of host–guest complexes with macrocycles through hydrogen bonding of its pendant amine tail to ether oxygen atoms present in the macrocycle [33].

Ferrioxamine B (guest)

Ionophore (host)

FeHDFB$^+$·18-crown-6 host-guest complex

We have obtained association constants for these complexes through liquid–liquid extraction and microcalorimetry studies [26–30, 32, 34, 35]. However, as previously discussed, the extraction technique is both time-consuming and requires a large amount of sample to be utilized.

Herein we present a method by which ESI-MS has been used to determine association constants and binding selectivities for host–guest systems of binary and complex mixtures, including a metal complex guest. Our study involves the complexation of ammonium, methylammonium, pentylammonium, and desferriferrioxammonium B with 18C6, B18C6, DC18C6, and DB18C6 macrocycles, and the linear ionophore lasalocid. The complexation of a metal complex guest, i.e. ferrioxamine B, with these macrocycles and the linear ionophore host was also studied.

Experimental

Materials

All referenced crown ethers, 18-crown-6 (18C6), benzo-18-crown-6 (B18C6), dibenzo-18-crown-6 (DB18C6), dicyclohexano-18-crown-6 (DC18C6), methylamine, and pentylamine were purchased from Acros Organics. Anhydrous MeOH and tetra-n-butyl ammonium acetate were purchased from Alfa Aesar. Potassium chloride and ammonium chloride were purchased from Fisher Scientific. Ferrioxamine E, FeDFE, was purchased from Fluka, and desferriferrioxamine B, H$_4$DFB$^+$, was obtained from Sigma Aldrich. Ferrioxamine B, FeHDFB$^+$, was prepared as previously described [30], using MeOH as the solvent. Lasalocid A was purchased from Sigma Aldrich. Stock solutions for all other reagents were prepared in MeOH, with the exception of that of KCl, which was prepared in 0.1 M aqueous HCl.

Sample preparation

For systems of binary mixtures, a representative preparation is as follows: a series of samples was prepared containing an excess of guest (1 mM), and increasing concentrations of host (as low as 6 μM, and as high as 400 μM) in MeOH. For systems of complex mixtures, a similar series of samples was prepared, in addition to a competition sample, containing an excess of guest (1 mM), and two hosts (each at 50 μM) in MeOH. All samples contained tetra-n-butyl ammonium acetate (242 m/z) or FeDFE (658 m/z) as an internal calibration standard (13 μM, 0.1 or 1 mM). Each individual sample was filtered before analysis using PTFE membrane syringe filters with a 0.45 μm pore size, and 13 mm diameter.

ESI-MS

All mass spectrometry experiments were performed using an Agilent 1100 SL LC/MSD-Trap instrument in positive ion mode at a flow rate of 60 μL/min, a nebulizer pressure of 15 or 20 psi, a dry gas flow rate of 6 or 7 L/min, and an ionization chamber temperature of 50 °C, 125 °C, or 300 °C, depending on the host–guest system of interest. In addition to the conditions mentioned above, several different nebulizer pressure, dry gas flow rate, and ionization chamber temperature conditions were investigated in order to maximize the output intensity of the host–guest complex. A series of combinations of the nebulization pressure and dry gas flow rate were tested, ranging from a nebulizer pressure of 15–22 psi, and a dry gas flow rate of 5–10 L/min. Two settings were found to work best; i.e. 20 psi, 7 L/min for host–guest systems of lower molecular weight cations, and 15 psi, 6 L/min for systems involving apo- or ferri-siderophores. Lower ionization chamber temperatures were found to favor the formation of host–guest complexes with a higher mass to charge ratio, whereas higher temperatures worked well for systems involving smaller cations as guests. All system lines were purged with MeOH before injection to eliminate any residual aqueous solvent present

in the system. Samples were either directly injected, or injected through the LC using an auto-sampler. In the case of the latter, a restrictor was used to provide sufficient back-pressure to the instrument, since no column was in place. Relative intensities of the host–guest complexes were obtained by accounting for the intensity of the standard in each mixture. Tetra-n-butyl ammonium acetate (242 m/z) was used as the standard for the system of binary mixtures involving crown ether hosts and potassium, ammonium, alkyl ammonium, and desferriferrioxamine B, H_4DFB^+, guests. Ferrioxamine E, FeDFE (658 m/z), was used as the standard for binary mixtures involving crown ethers or lasalocid with H_4DFB^+ and $FeHDFB^+$, as well as complex mixtures involving crown ethers and $FeHDFB^+$. FeDFE is a highly stable tris-hydroxamate siderophore complex similar to $FeHDFB^+$, but with a cyclic structure shown below and no opportunity for host–guest complex formation.
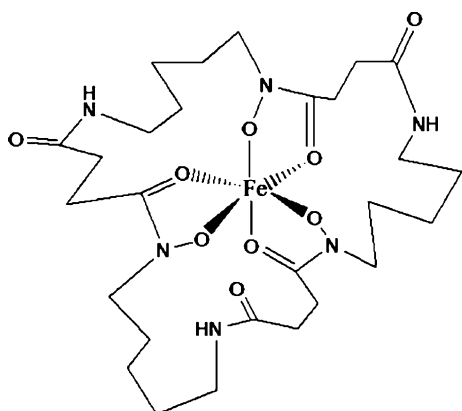
Ferrioxamine E, FeDFE

Tetra-n-butyl ammonium acetate and FeDFE were chosen as standards as they had no interaction with the complexes being studied, had high spray efficiency, and a constant ESI response throughout the experiments.

All samples were prepared and run in 100% MeOH. The host–guest base peak was identified by the expected m/z and the expected isotope distribution. For samples containing $FeHDFB^+$ as the guest, the isotopic distribution of the host–guest complex peak, arising from Fe and C isotopes, was used as further evidence of the presence of Fe in the complex. A spectrum simulation program was used to verify the isotopic distribution obtained experimentally.

Method validation

In order to determine the accuracy and precision of our experimental method, the association constants of several cation–crown ether host–guest complexes, whose association constants were previously reported in the literature using different experimental methods, were compared with

association constants derived using our method. The equilibrium reaction under investigation is shown below,

$$H + G \underset{}{\overset{k_a}{\rightleftharpoons}} H \cdot G \tag{1}$$

where H refers to the host component, i.e. the crown ether, G refers to the guest component, in this case the cation, $H \cdot G$ refers to the host–guest assembly, and K_a, the equilibrium constant for the formation of the host–guest assembly. A series of samples was analyzed at a constant K^+ concentration, and varying 18C6, B18C6, and DC18C6 concentrations, respectively, in MeOH. A representative ESI-MS spectrum for the $18C6 \cdot K^+$ host–guest complex is shown in the Supplementary materials, Fig. S1. Each of these samples contained tetra-n-butyl ammonium acetate or FeDFE as a relative intensity reference. These relative host–guest complex intensities were then plotted as a function of host concentration. The data were fit to a nonlinear regression for single-site saturation ligand binding (Eq. 2), using SigmaPlot 9.0 software,

$$I = \frac{I_{max}[\text{host}]}{1/K_a + [\text{host}]} \tag{2}$$

where I is the intensity of the host–guest complex relative to the standard, I_{max} is its maximum intensity, [host] is the molar free concentration of the crown ether host, and $1/K_a$ is the reciprocal of the equilibrium constant for the association of the host–guest complex, as shown in Eq. 1 (Supplementary materials, Fig. S2). This was the best model for the data, as no other model with different stoichiometries fit the data as well. Association constants (K_a) were obtained for systems involving K^+ with 18C6, B18C6, and DC18C6. These are generally in agreement with the values available in the literature in MeOH (Table 1). The only literature value available for comparison obtained by ESI-MS is that for K^+ and 18C6, but it is in MeCN. Our data yield a lower K_a value in MeOH as expected for a hydrogen-bonding solvent, which would compete with the guest for the ether oxygen atoms of the host.

All of the results presented in Table 1 were analyzed in this way using Eqs. 1 and 2. Other stoichiometries do not fit the data obtained.

Results and discussion

Determination of host–guest complex association constants in systems of binary mixtures

Protonated amine guests

As a prelude to our study of the second coordination shell complexation of ferrioxamine B, $FeHDFB^+$, we

Table 1 Formation constants (K_a) for binary host–guest mixtures in methanol solvent

Guest[a]	Host[b]	Log K_a[c]	Lit. values
K^+	18C6	4.29 ± 0.06	4.34^d, 4.8^e
K^+	B18C6	4.51 ± 0.04	5.05^f
K^+	DC18C6	4.26 ± 0.06	5.63^g
NH_4^+	18C6	3.70 ± 0.06	4.10^h
NH_4^+	B18C6	4.00 ± 0.06	
$CH_3NH_3^+$	18C6	4.49 ± 0.03	4.25^i
$CH_3NH_3^+$	B18C6	4.50 ± 0.03	
$CH_3(CH_2)_4NH_3^+$	18C6	5.21 ± 0.03	
$CH_3(CH_2)_4NH_3^+$	B18C6	4.00 ± 0.04	
$CH_3(CH_2)_4NH_3^+$	DC18C6	4.40 ± 0.05	
H_4DFB^+	18C6	4.07 ± 0.05	
H_4DFB^+	B18C6	3.69 ± 0.06	
H_4DFB^+	DB18C6	4.03 ± 0.13	
H_4DFB^+	DC18C6	4.27 ± 0.05	
H_4DFB^+	Lasalocid	4.13 ± 0.09	
$FeHDFB^+$	18C6	3.70 ± 0.07	
$FeHDFB^+$	B18C6	3.40 ± 0.24	
$FeHDFB^+$	DB18C6	4.00 ± 0.13	
$FeHDFB^+$	DC18C6	4.03 ± 0.07	
$FeHDFB^+$	Lasalocid	4.53 ± 0.07	

[a] H_4DFB^+ refers to desferriferrioxamine B; $FeHDFB^+$ refers to ferrioxamine B

[b] 18C6, B18C6, DC18C6, and DB18C6 are 18-crown-6, benzo-18-crown-6, dicyclohexano-18-crown-6, and dibenzo-18-crown-6, respectively

[c] Host–guest association constants obtained in MeOH in this work. Error values were obtained from the non-linear least squares fit of Eq. 2 to the data using SigmaPlot software

[d] Obtained by conductivity in MeOH [10]

[e] Obtained by ESI-MS in MeCN [23]

[f] Obtained by ion-selective electrode measurements in MeOH [10, 11]

[g] Obtained by calorimetry in MeOH [10]

[h] Obtained by conductivity in MeOH [10]

[i] Obtained by calorimetry in MeOH [11]

investigated protonated amine guest/crown ether host assemblies involving NH_4^+, $CH_3NH_3^+$, and $CH_3(CH_2)_4$ NH_3^+. A representative ESI-MS spectrum for the 18C6 · NH_4^+ system is shown in Fig. 1A, where the intense peak at 282 m/z corresponds to the host–guest complex. Our K_a value for 18C6 · NH_4^+ (Fig. 2) is in reasonable agreement with the reported value obtained by conductivity measurements in MeOH (Table 1). Data are reported for this system in other non hydrogen-bonding solvents where the K_a is higher than in MeOH [9–11]. This reflects the hydrogen-bonding competition between the guest and solvent for the ether oxygen atoms.

Alkyl ammonium guests were investigated in MeOH with 18C6, B18C6, and DC18C6 hosts. Representative

spectra for methylammonium and pentylammonium guests are shown in Fig. 1 (B and C) with intense signals at 296 and 352 m/z corresponding to the 18C6 · $CH_3NH_3^+$, and 18C6 · $CH_3(CH_2)_4NH_3^+$ host–guest complexes, respectively. Fits of Eq. 2 to the data are shown in Fig. 2 and the derived K_a values are listed in Table 1. We find reasonable agreement between K_a values for 18C6 · $CH_3NH_3^+$ in MeOH obtained here by ESI-MS and elsewhere [11] by calorimetry (Table 1).

Siderophore guests

The host–guest complexes formed between 18C6, B18C6, DB18C6, and DC18C6 and H_4DFB^+ were studied. A representative ESI-MS spectrum of the 18C6 · H_4DFB^+ system is shown in Fig. 3. The 18C6 · H_4DFB^+ complex gives an ESI-MS base peak centered at 825 m/z, as expected. The relative host–guest complex intensities for all apo-siderophore–crown ether systems were plotted as a function of host concentration from which association constants were obtained (Fig. 4, Table 1). No values exist in the literature for the host–guest complexes formed between 18C6, B18C6, and DB18C6 and H_4DFB^+ in any solvent. Our obtained K_a for the host–guest complex formed between DC18C6 and H_4DFB^+ in MeOH is lower than the literature value in $CHCl_3$, as expected due to solvent–crown ether interactions [9–11].

Host–guest complexes between a series of crown ethers and the iron-loaded siderophore $FeHDFB^+$ were studied. A representative ESI-MS spectrum of the 18C6 · $FeHDFB^+$ system is shown in Fig. 5. This complex gives an ESI-MS base peak centered at 878 m/z, as expected. The isotopic distribution centered on this peak is consistent with the natural isotope distribution of Fe ($^{56}Fe(91.8\%)$, $^{54}Fe(5.8\%)$, $^{57}Fe(2.1\%)$) which provides further evidence of the presence of Fe in a host–guest complex with $z = 1$ [36]. A spectrum simulation based on the singly-charged 18C6 · $FeHDFB^+$ host–guest complex produced an isotopic distribution consistent with that observed experimentally. Following the procedure mentioned previously, host–guest association constants were obtained (Fig. 6, Table 1). As with host–guest complex systems of crown ethers and H_4DFB^+, no literature values exist for host–guest complexes of crown ethers and $FeHDFB^+$ in MeOH. However, our obtained association constants again fall within the range that would be expected for MeOH based on literature association constants in other solvents [9–11].

Open chain ionophore hosts

The host–guest complexes formed between lasalocid A, a polyether carboxylic acid antibiotic, and H_4DFB^+ and $FeHDFB^+$ were studied. Unlike cyclic crown ethers,

Fig. 1 ESI mass spectrum of crown ether · ammonium host–guest systems. **A**. 18C6 · NH$_4^+$ host–guest system: (1) [Tetra-*n*-butyl ammonium acetate + H]$^+$, (2) [18C6 + H]$^+$, (3) [18C6 + NH$_4$]$^+$.
B 18C6 · CH$_3$NH$_3^+$ host–guest system: (1) [Tetra-*n*-butyl ammonium acetate + H]$^+$, (2) [18C6 + CH$_3$NH$_3$]$^+$.
C 18C6 · CH$_3$(CH$_2$)$_4$NH$_3^+$ host–guest system: (1) [Tetra-*n*-methyl ammonium acetate + H]$^+$, (2) [18C6 + Na]$^+$, (3) [18C6 + K]$^+$, (4) [18C6 + CH$_3$(CH$_2$)$_4$NH$_3$]$^+$.
Conditions: [Crown Ether] = 0.15 mM, [amine] = 10 mM, [tetra-*n*-butyl ammonium acetate] = 1 mM (for **C**) or 13 μM (for **A** and **B**), in MeOH, T = 200 °C, Nebulizer = 20 psi, Dry Gas = 7 L/min
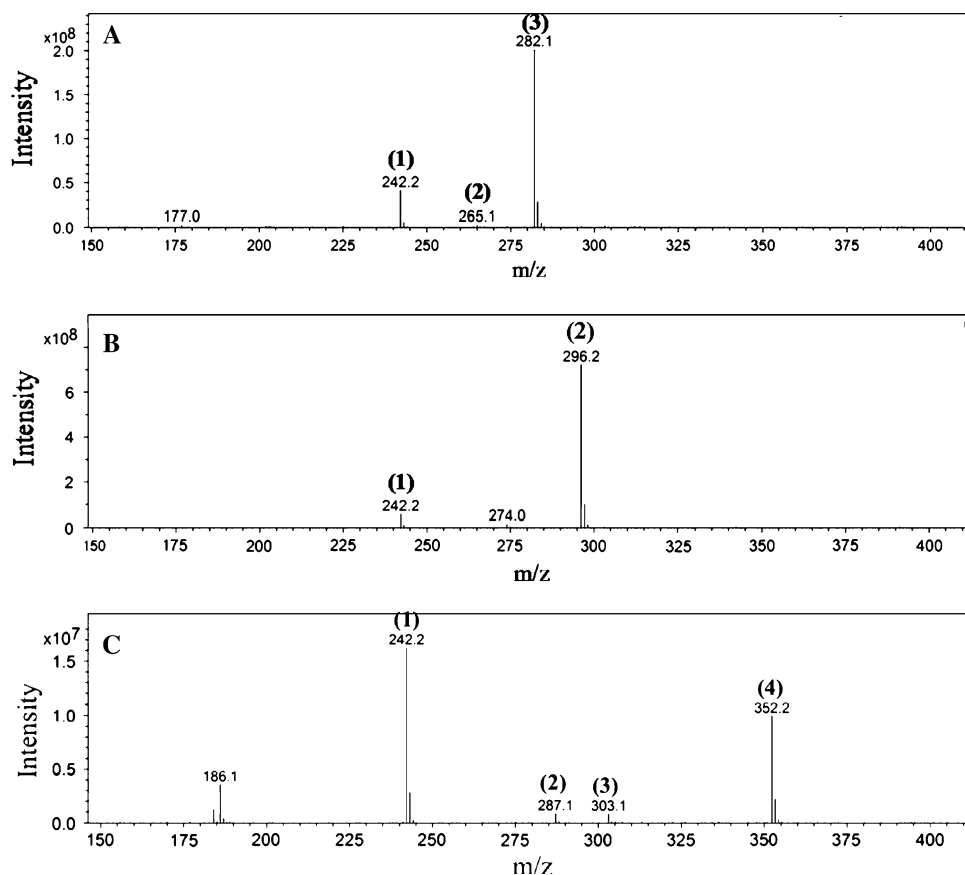
lasalocid A has an open chain structure and acidic hydrogen, which can lead to vastly different host–guest complexation behavior [37].

Lasalocid A

Lasalocid•FeHDFB$^+$ host-guest complex
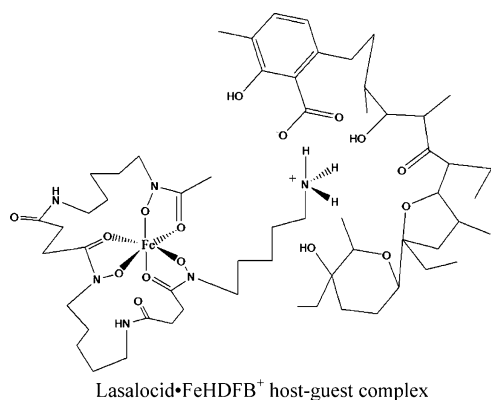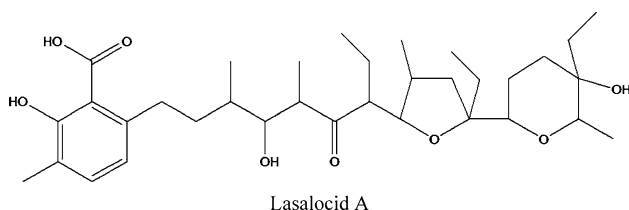
Lasalocid alone was observed as a Na$^+$ adduct in the ESI-MS spectrum. The lasalocid · H$_4$DFB$^+$ host–guest complex was observed at 1,151 *m/z* and the lasalocid · FeHDFB$^+$ host–guest complex was observed as a Na$^+$ adduct at 1,226 *m/z* (Supplementary materials, Fig. S3). Following the procedure previously described, the host–guest complex intensities were monitored and association constants were obtained (Fig. 7, Table 1). Although we do not have direct proof, we propose the structure shown above for the lasalocid · FeHDFB$^+$ host–guest assembly, based on the similarity of the K_a value with those observed for the macrocyle hosts, and our previous studies [37].

Determination of binding selectivities in systems of complex mixtures

The applicability of the ESI-MS method for systems of complex mixtures, i.e. mixtures containing two hosts competing for one guest, was explored. This method is illustrated in Eq. 3 and described below using 18C6, B18C6, and DC18C6 as the competing hosts, and FeHDFB$^+$ as the guest.

$$H_1 + G \stackrel{K_{H_1}^{\mathrm{mix}}}{\rightleftharpoons} H_1 \cdot G$$
$$H_2 + G \stackrel{K_{H_2}^{\mathrm{mix}}}{\rightleftharpoons} H_2 \cdot G$$

(3)

A sample containing 18C6, DC18C6, and FeHDFB$^+$ in a 1:1:20 ratio was analyzed. The spectrum is shown in
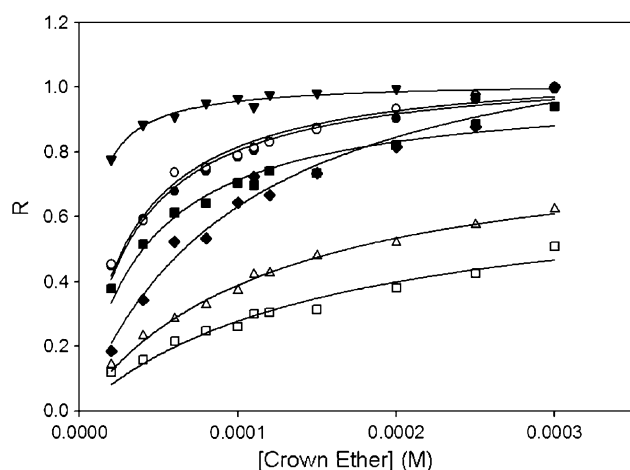
Fig. 2 Plot of the ESI mass spectrum peak intensity ratio (R) between host–ammonium complex and standard peaks as a function of crown ether concentration. The *solid line* represents a non-linear least squares fit of Eq. 2 to the data points. Each data point represents one experimental trial. (▼) 18C6 · $CH_3(CH_2)_4NH_3^+$ complex (monitored at 352 *m/z*) (◯) B18C6 · $CH_3NH_3^+$ complex (monitored at 344 *m/z*), (●) 18C6 · $CH_3NH_3^+$ complex (monitored at 296 *m/z*), (◆) DC18C6 · $CH_3(CH_2)_4NH_3^+$ complex (monitored at 460 *m/z*), (■) B18C6 · NH_4^+ complex (monitored at 330 *m/z*), (△) B18C6 · $CH_3(CH_2)_4NH_3^+$ complex (monitored at 400 *m/z*), (□) 18C6 · NH_4^+ complex (monitored at 282 *m/z*). Conditions: [Crown Ether] = 20–300 μM, [NH_4Cl] = [CH_3NH_2] = [$CH_3(CH_2)_4NH_2$] = 10 mM, [tetra-*n*-butyl ammonium acetate] = 13 μM, T = 200 °C, Nebulizer = 20 psi, Dry Gas = 7 L/min
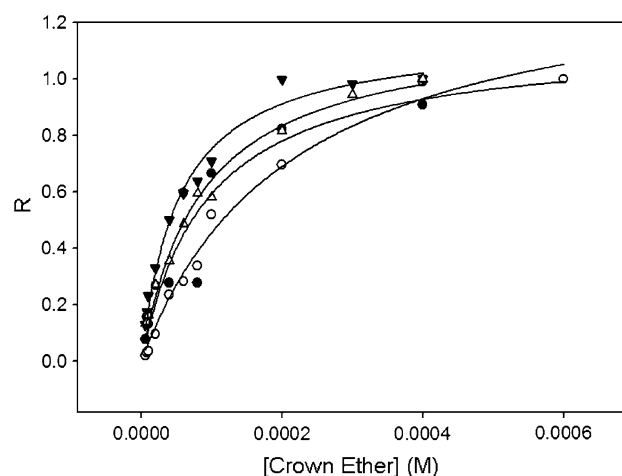
**Fig. 4** Plot of the ESI mass spectrum peak intensity ratio (R) between host–H_4DFB^+ complex and standard peaks as a function of crown ether concentration. The solid line represents a non-linear least squares fit of Eq. 2 to the data points. Each data point represents the average of three experimental trials, with the exception of data points for the DB18C6 · H_4DFB^+ assembly, where each point represents one experimental trial. (▼) DC18C6 · H_4DFB^+ complex (monitored at 933 *m/z*), (△) 18C6 · H_4DFB^+ complex (monitored at 825 *m/z*), (◯) B18C6 · H_4DFB^+ complex (monitored at 873 *m/z*), (●) DB18C6 · H_4DFB^+ complex (monitored at 921 *m/z*) Conditions: [Crown Ether] = 6–600 μM, [H_4DFB^+] = 1 mM, [FeDFE] = 0.1 mM, or [tetra-*n*-butyl ammonium acetate] = 13 μM, T = 200 °C, Nebulizer = 15 psi, Dry Gas = 6 L/min

Fig. 8, which includes different peak intensities for the host–guest complexes of interest. This difference in signal intensities is related to both the concentration and the spray efficiencies of the host–guest complexes formed. We factored out the differences in spray efficiencies so that the signal was directly related to the concentration of host–guest assembly.

Differences in spray efficiencies have previously been accounted for in different ways [22–25]. Our approach uses a simple system of calibration plots. Calibration plots were made for each of the individual binary system host–guest complexes present in the complex mixture sample. A series

of samples was analyzed at a constant FeHDFB$^+$ concentration, and varying crown ether concentration. The relative host–guest complex intensities obtained from these samples were plotted as a function of host–guest complex concentration (Supplementary materials, Fig. S4). These concentrations were calculated from the association constants obtained using systems of binary mixtures, as described above. The host–guest complex intensities were converted to host–guest complex concentrations from these calibration plots. Using a series of equilibrium reactions for the DC18C6 · FeHDFB$^+$ and 18C6 · FeHDFB$^+$ systems (Eq. 4), and their respective equilibrium expressions, an
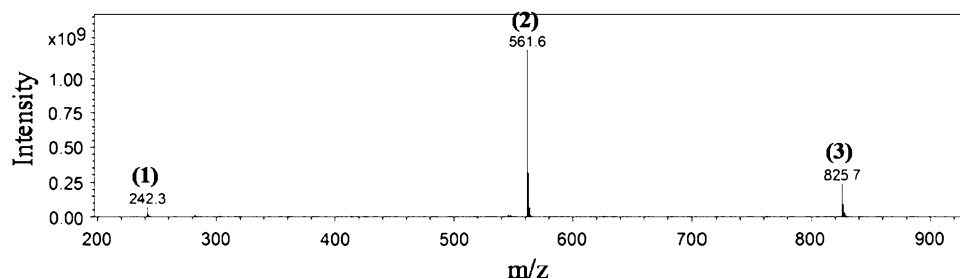

Fig. 3 ESI mass spectrum of the 18C6 · H_4DFB^+ host–guest system. (1) [Tetra-*n*-methyl ammonium acetate + H]$^+$, (2) H_4DFB^+, (3) [18C6 + H_4DFB]$^+$. Conditions: [18C6] = 150 μM, [H_4DFB^+] = 1 mM, [tetra-*n*-butyl ammonium acetate] = 13 μM, in MeOH, T = 200 °C, Nebulizer = 15 psi, Dry Gas = 6 L/min
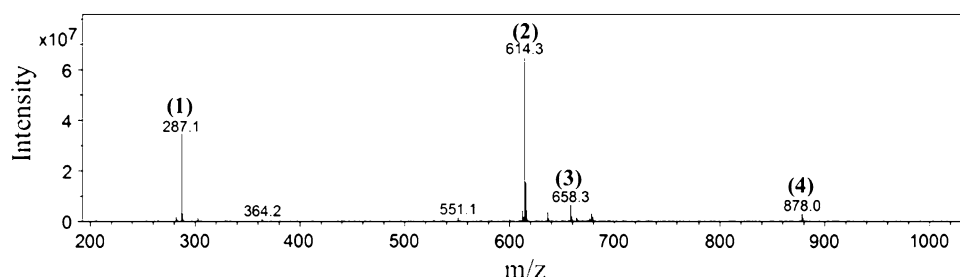
Fig. 5 ESI mass spectrum of the 18C6 · FeHDFB$^+$ host–guest system. (1) [18C6 + H]$^+$, (2) FeHDFB$^+$, (3) [FeDFE + H]$^+$, (4) [18C6 + FeHDFB]$^+$. Conditions: [18C6] = 0.3 mM, [FeHDFB$^+$] = 1 mM, [FeDFE] = 1 mM, in MeOH, T = 125 °C, Nebulizer = 15 psi, Dry Gas = 6 L/min
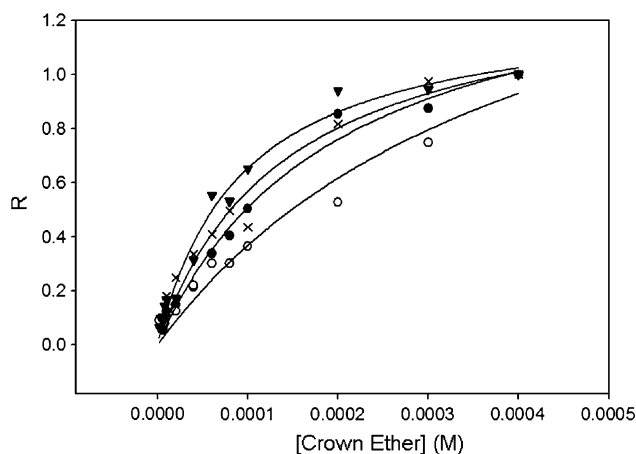
Fig. 6 Plot of the ESI mass spectrum peak intensity ratio (R) between host–FeHDFB$^+$ complex and standard peaks as a function of crown ether concentration. The *solid line* represents a non-linear least squares fit of Eq. 2 to the data points. Each data point represents the average of three experimental trials. (▼) 18C6 · FeHDFB$^+$ complex (monitored at 878 *m/z*), (×) DC18C6 · FeHDFB$^+$ complex (monitored at 986 *m/z*), (●) B18C6 · FeHDFB$^+$ complex (monitored at 927 *m/z*), (○) DB18C6 · FeHDFB$^+$ complex (monitored at 974 *m/z*). Conditions: [Crown Ether] = 6–400 μM, [FeHDFB$^+$] = 1 mM, [FeD-FE] = 0.1 mM, T = 125 °C, Nebulizer = 15 psi, Dry Gas = 6 L/min
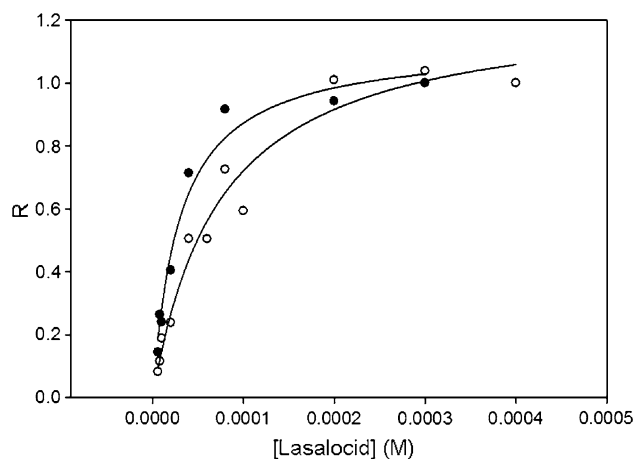
Fig. 7 Plot of the ESI mass spectrum peak intensity ratio (R) between lasalocid A–guest complex and standard peaks as a function of crown ether concentration. The *solid line* represents a non-linear least squares fit of Eq. 2 to the data points. Each data point represents the average of three experimental trials. (●) Lasalocid A · FeHDFB/Na$^+$ adduct complex (monitored at 1,226 *m/z*), (○) Lasalocid A · H$_4$DFB$^+$ complex (monitored at 1,151 *m/z*). Conditions: [Lasalocid A] = 6–400 μM, [FeHDFB$^+$] or [H$_4$DFB$^+$] = 1 mM, [FeDFE] = 0.1 mM, T = 125 °C, Nebulizer = 15 psi, Dry Gas = 6 L/min

equation for the binding selectivity was obtained, as shown in Eq. 5.

$$DC18C6 + FeHDFB^+ \overset{K_{DC18C6}^{mix}}{\rightleftharpoons} DC18C6 \cdot FeHDFB^+$$

$$18C6 + FeHDFB^+ \overset{K_{18C6}^{mix}}{\rightleftharpoons} 18C6 \cdot FeHDFB^+ \tag{4}$$

$$\left(\frac{K_{DC18C6}^{mix}}{K_{18C6}^{mix}} \right) = \frac{[DC18C6 \cdot FeHDFB^+][18C6]}{[18C6 \cdot FeHDFB^+][DC18C6]} \tag{5}$$

For the DC18C6 · FeHDFB$^+$ and 18C6 · FeHDFB$^+$ mixed system, the binding selectivity $K_{DC18C6}^{mix}/K_{18C6}^{mix}$ was found to be 2.0. This experimental value compares well with the binding selectivity ratio calculated from the individual association constants (Table 1) obtained through the system of binary mixtures, 2.13.

We have also used the complex mixture competition technique for other crown ether mixtures and FeHDFB$^+$;

e.g. 18C6 and B18C6. Here the host–guest binding selectivity $K_{18C6}^{mix}/K_{B18C6}^{mix}$ was found to be 2.06, in excellent agreement with that calculated from the individual association constants (Table 1) obtained for the system of binary mixtures, 2.0.

Summary and conclusions

We have presented a strategy by which ESI-MS has been extended to derive host–guest association constants and binding affinities for systems of binary and complex mixtures with applications to crown ether and linear ionophore hosts, and ammonium and alkyl ammonium guests, as well as to siderophore–ionophore supramolecular assemblies involving an apo and ferri-siderophore guest. It is significant that we are able to study in the gas phase second

Fig. 8 ESI mass spectrum of the complex mixture of 18C6/DC18C6 · FeHDFB$^+$.
A Extended view (0–1,000 m/z), (1) [18C6 + H]$^+$, (2) [DC18C6 + H]$^+$, (3) FeHDFB$^+$, (4) [FeDFE + H]$^+$, (5) [18C6 + FeHDFB]$^+$, (6) [DC18C6 + FeHDFB]$^+$.
B Focused view of spectrum (**A**) (850–1,000 m/z).
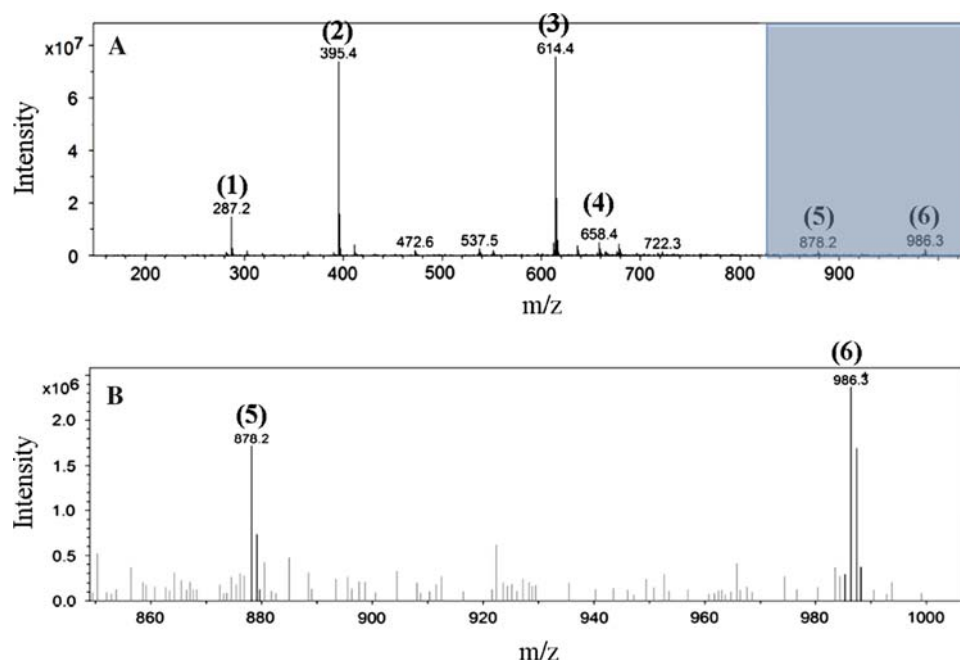Conditions: [18C6] = [DC18C6] = 6–600 μM, [FeHDFB$^+$] = 1 mM, [FeDFE] = 0.1 mM, in MeOH, T = 125 °C, Nebulizer = 15 psi, Dry Gas = 6 L/min

coordination shell host–guest complexes formed in solution.

Our results in MeOH solvent demonstrate a general lack of sensitivity to changes in host and guest structure for the systems investigated (Table 1). However, we find that the ferri-siderophore FeHDFB$^+$ exhibits slightly lower affinity for complexation by the various crown ethers than does the iron-free apo form, H$_4$DFB$^+$. This was also observed for DC18C6 in CHCl$_3$ using a liquid–liquid extraction method and may be due to a small steric effect [26]. We also note that the more flexible open chain lasalocid host forms slightly more stable host–guest complexes with H$_4$DFB$^+$ and FeHDFB$^+$ than the cyclic crown ethers, perhaps for steric reasons (Table 1).

Further, we have established that competitive host–guest association constants for complex mixtures may be obtained by ESI-MS. These data would be very difficult or impossible to obtain by other methods.

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