JOURNAL OF MASS SPECTROMETRY, VOL. 33, 212–220 (1998)

Electrospray Ionization Mass Spectrometry of Metalloporphyrins

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The magnesium, nickel, copper, zinc and vanadium metalloporphyrins from octaethylporphyrin, etioporphyrin I and tetraphenylporphyrin were characterized using electrospray ionization mass spectrometry (ESI-MS). The ion abundance of each of the porphyrins present in binary mixtures was monitored as a function of the porphyrin concentration and is dependent on the metalloporphyrin oxidation potential. It was found that, for binary mixtures of metalloporphyrins whose oxidation potentials differ by less than 0.1 V, the resulting ion abundance of each species is directly proportional to the concentration of each analyte in the mixture. For binary mixtures whose oxidation potentials differ by more than 0.1 V, relative abundances of the radical cations of each metalloporphyrin are determined by the oxidation potential and concentration of each metalloporphyrin with the analyte of lowest oxidation potential being ionized preferentially. The ability to ionize selectively one porphyrin over another in a binary mixture offers the potential to use ESI-MS for the qualitative analysis of porphyrins present in complex mixtures. © 1998 John Wiley & Sons, Ltd.

J. Mass Spectrom. **33**, 212–220 (1998)

KEYWORDS: electrospray ionization; mass spectrometry; metalloporphyrins; mixture analysis; electrochemical ionization

INTRODUCTION

Metalloporphyrins are of great importance in many processes involving biology, catalysis and geology, among others. Metalloporphyrins have been analyzed using a variety of mass spectrometric methods, including electron ionization (EI),[1,2] chemical ionization (CI),[3–8] fast atom bombardment,[9–12] plasma desorption,[13,14] thermospray,[15] laser desorption,[16–19] field desorption,[20] atmospheric pressure chemical ionization[21] and electrospray ionization (ESI).[22–24] Traditionally, EI and CI are useful for structural elucidation studies but complicate the analysis of mixtures as they produce mass spectra containing a large number of fragment ions. The remaining ionization methods generally produce a high abundance of molecular ions with little fragmentation, and are therefore useful for identification purposes.

Van Berkel *et al.*[22] were the first to demonstrate the use of ESI as a means to ionize porphyrins. In their original study, they showed that the porphyrin systems were ionized chemically via solution-phase protonation of the porphyrin, particularly for free base systems, or electrochemically via solution- or gas-phase ionization, particularly for the metalloporphyrins. Van Berkel and co-workers[23–25] showed that variables such as solvent polarity, needle voltage, flow rate, temperature, solvent dielectric constant and analyte oxidation potentials govern the capabilities of the electrospray source to effectively ionize a porphyrin system of interest. The addition of electrolytes, use of protic solvents and charge-transfer complexes have also been shown to contribute to the degree of ionization observed for various types of porphyrin systems.[23,25–28] These studies suggest that, under certain conditions, selective ionization of one porphyrin over another may be attainable in a solution containing an equimolar concentration of two different metalloporphyrins. Ultimately this type of analysis may prove useful for characterizing mixtures of metalloporphyrins.

Of particular interest to our group is the electrochemical ionization pathway and the possibilities of exploiting the variables which govern its mechanism for selective ionization of metalloporphyrin mixtures. Recent evidence suggests that ionization of metalloporphyrins in the positive ion mode is a direct function of their oxidation potentials.[29,30] Numerous studies have been performed on the electrochemical oxidation and reduction of porphyrins.[31–33] Selectivity in the mass spectrometric step, therefore, can be achieved potentially through an understanding of the oxidation potentials of metalloporphyrins and by adjusting the ESI operating parameters accordingly.

The goal of this research was to examine the applicability of electrochemical ionization for characterizing simple binary mixtures of metalloporphyrins. Three different families of metalloporphyrins were investigated (Fig. 1). The ion abundances for several porphyrins in

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Contract/grant sponsor: Louisiana State University.
Contract/grant sponsor: Louisiana State University Office of Research and Economic Development.
Contract/grant sponsor: Louisiana State University Center for Energy Studies.

CCC 1076–5174/98/030212–09 $17.50

Received 29 May 1997
Accepted 29 October 1997

binary mixtures were measured while varying the ionization source parameters. It was found that, under certain conditions, the ion abundance of one porphyrin relative to another could be enhanced as a function of the source parameters. In this study, we demonstrate that the selective ionization of metalloporphyrins in a mixture is feasible using ESI-MS. Further, limits on the selectivity of ESI-MS for metalloporphyrin mixture analysis were established.

EXPERIMENTAL

Chemicals

The following porphyrins were obtained from Midcentury Chemicals (Posen, IL, USA): copper etioporphyrin I, magnesium etioporphyrin I, vanadyl etioporphyrin I, zinc etioporphyrin I, nickel etioporphyrin I, copper(II) octaethylporphyrin, nickel(II) octaethylporphyrin, magnesium(II) octaethylporphyrin, zinc(II) octaethylporphyrin, vanadyl(IV) tetraphenylporphyrin, nickel(II) tetraphenylporphyrin and copper(II) tetraphenylporphyrin. The following porphyrins were obtained from Strem Chemical (Newburyport, MA, USA): *meso*-tetraphenylporphyrin, magnesium tetraphenylporphyrin, zinc tetraphenylporphyrin and vanadyl octaethylporphyrin. HPLC-grade dichloromethane was obtained from Curtis Matheson Scientific (Houston, TX, USA) and acetonitrile from Burdick and Jackson (Muskegon, MI, USA). All chemicals were used as received.

Mass spectrometry

All electrospray ionization mass spectra were acquired using a Finnigan-MAT 900 (San Jose, CA, USA) double-focusing mass spectrometer equipped with a first-generation Analytica electrospray ionization source utilizing a platinum tipped glass capillary (0.246 in o.d., 0.020 in i.d.) and a 33 gauge stainless-steel needle. Data acquisition was performed on a personal DECstation 5000 (Digital Equipment, Maynard, MA, USA). Samples were infused by using a Harvard Apparatus (South Natick, MA, USA) Model 22 syringe pump at flow rates of 2.0–3.0 $\mu l\ min^{-1}$ for all experiments, except where noted otherwise.

Stock solutions for all metalloporphyrins were prepared in dichloromethane. Solutions containing a single porphyrin were electrosprayed at a concentration of 25 pmol μl^{-1} in acetonitrile. Solutions containing two metalloporphyrins (binary mixtures) were electrosprayed at a final concentration of 5 pmol μl^{-1}–2.0 nmol μl^{-1} in acetonitrile or dichloromethane–acetonitrile (1:1). Twenty-five consecutive scans were collected for each sample at a scan rate of 10 s per decade with a scan range of 400–800 u.

Cyclic voltammetry

Cyclic voltammograms were recorded with a Model 273 Bi-Potentiostat (Princeton Applied Research, Princeton, NJ, USA) in potentiostat mode and a Yokogawa (Newnan, GA, USA) Model 3025 *x–y* recorder. Porphyrin solutions were prepared at a 1.0×10^{-3} M concentration in dichloromethane. The supporting electrolyte used was tetrabutylammonium fluoroborate (Sachem, Austin, TX, USA) at a concentration of 0.1 M. The experiments were run in a three-necked glass electrochemical cell with a platinum disk working electrode, a platinum auxiliary electrode and a saturated calomel reference electrode (SCE).

RESULTS AND DISCUSSION

Cyclic voltammetric determination of porphyrin oxidation potentials

Several groups have demonstrated a correlation between the electrochemical behavior observed in the electrospray source and the relative oxidation potentials of the analytes under investigation.[23,26,28–30] Because the electrospray ionization source is a controlled-current electrolytic cell, the relative mass spectral intensities of oxidized species in a mixture will be determined by the electrospray current and the relative oxidation potentials of the analytes. Therefore, in this study, the oxidation potentials of metalloporphyrins should serve as a reference point for comparison of measured ion abundances in a solution containing two metalloporphyrins. Table 1 lists the oxidation potentials measured for each of the porphyrins studied.

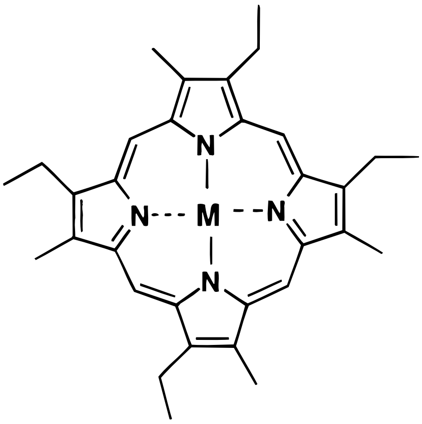
ESI-MS of solutions containing a single metalloporphyrin

Initially, 15 metalloporphyrins were characterized by ESI-MS. No supporting electrolytes[30] or charge-transfer reagents[22,25,27,34] were used in any of these experiments to enhance the ion current. Supporting electrolytes may result in ion signal suppression,[35] and the use of charge–transfer reagents could complicate

Table 1. Oxidation potentials of the metalloporphyrins studied, measured *vs.* SCE at a platinum working electrode in dichloromethane

Porphyrin	First oxidation potential $E_{1/2}$ (V)
Magnesium etioporphyrin	0.515
Vanadyl etioporphyrin	1.01
Nickel etioporphyrin	0.845
Copper etioporphyrin	0.830
Zinc etioporphyrin	0.690
Magnesium octaethylporphyrin	0.545
Vanadyl octaethylporphyrin	1.030
Nickel octaethylporphyrin	0.860
Copper octaethylporphyrin	0.840
Zinc octaethylporphyrin	0.745
Magnesium tetraphenylporphyrin	1.05
Nickel tetraphenylporphyrin	1.09
Vanadyl tetraphenylporphyrin	1.22
Copper tetraphenylporphyrin	1.07
Zinc tetraphenylporphyrin	0.870

our interpretation of the results obtained from the analysis of binary mixtures of metalloporphyrins. Further, in contrast to the previous results of Van Berkel *et al.*,[22] no trifluoroacetic acid was added to stabilize the radical cation in these experiments as aprotic solvents were used throughout. The major ions observed for each of the metalloporphyrins analyzed are shown in Fig. 1. Except for vanadyl octaethylporphyrin (VOOEP), which was detected as both the radical cation and the protonated molecular ion, the metalloporphyrins are detected exclusively as radical cations. Van Berkel *et al.*[22] found also that VOOEP can be



Etioporphyrin I

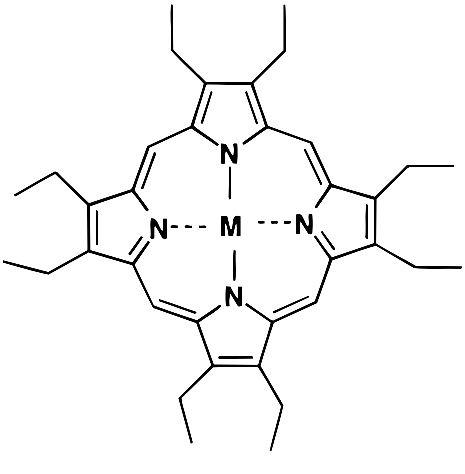
M= Mg 500 Da

M= Ni 534 Da

M= Cu 539 Da

M= Zn 540 Da

M= VaO 543 Da



Octaethylporphyrin

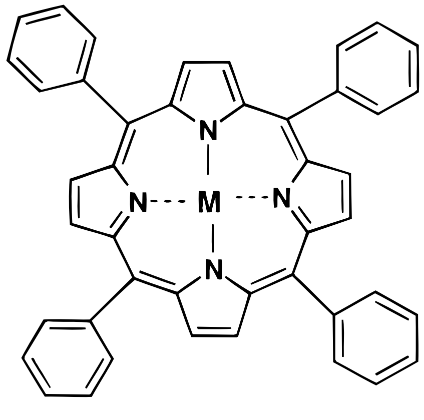
M= Mg 556 Da

M= Ni 590 Da

M= Cu 595 Da

M= Zn 596 Da

M= VaO 599 Da/ 600 Da



Tetraphenylporphyrin

M= Mg 636 Da

M= Ni 670 Da

M= Cu 675 Da

M= Zn 676 Da

M= VaO 679 Da

Figure 1. Selected metalloporphyrins studied by electrospray ionization mass spectrometry.

detected as either the radical cation or protonated molecular ion. In addition, the ESI source parameters were adjusted to reduce nozzle–skimmer fragmentation of the metalloporphyrins, resulting in almost exclusive production of the molecular ion for all metalloporphyrins studied.

Influence of oxidation potential on the ESI-MS of metalloporphyrin mixtures

The intent of this set of experiments was to determine the selectivity of the electrospray source relative to the oxidation potentials of the metalloporphyrins in a binary mixture (i.e. two different metalloporphyrins). A series of binary metalloporphyrin mixtures were prepared at different relative and absolute concentration values. In order to determine the effect of concentration on the ESI-MS results, other operating variables were held constant throughout. The optimum needle voltage was found for each metalloporphyrin mixture at equimolar concentrations, and this voltage was then utilized as the concentration ratios were varied.

Metalloporphyrin mixtures with nearly identical oxidation potentials. Nickel etioporphyrin (NiEtio)–nickel octaethylporphyrin (NiOEP) was the first mixture characterized. As can be seen in Table 1, the oxidation potentials for these two metalloporphyrins are nearly identical (NiEtio 0.845 V, NiOEP 0.860 V). Figure 2 is a plot of the ratio of the relative abundances of the molecular ions for these two metalloporphyrins *vs.* the ratio of the molar concentration of these two metalloporphyrins. According to the theory proposed by Van Berkel and Zhou,[29] when the oxidation potentials are nearly identical the resulting ion intensities are governed by the electrospray ion current, which is proportional to the analyte concentration. As can be seen in Fig. 2, the molecular ion abundances are indeed determined by the concentration of each metalloporphyrin in solution. When NiEtio and NiOEP are present in the mixture at equimolar concentrations, the ion abundances are nearly identical. As the concentration of NiEtio is increased relative to that of NiOEP, the ion abundance of NiEtio increases accordingly. Further, as one would predict, the response is linear.

We were interested in the effect that the absolute metalloporphyrin concentration would have on the electrochemical ionization process. Figure 2(a) shows the data obtained at a final metalloporphyrin concentration of 5 pmol μl^{-1} and Fig. 2(b) shows similar behavior when the final metalloporphyrin concentration was 110 pmol μl^{-1} In both cases, the relative ion abundances of the two metalloporphyrins in the mass spectrum are proportional to the relative concentration of each metalloporphyrin present. Below a maximum flow rate (necessary to ensure adequate mass transfer of each analyte to the electrode for ionization) there is no qualitative change in these data as a function of flow rate (data not shown). The larger error bars in Fig. 2(a) are due to the poorer reproducibility at the lower concentration, and are primarily a result of the poor sensitivity of our ESI source at these low concentrations. The use of an additional supporting electrolyte should improve the reproducibility at lower concentrations.[30]

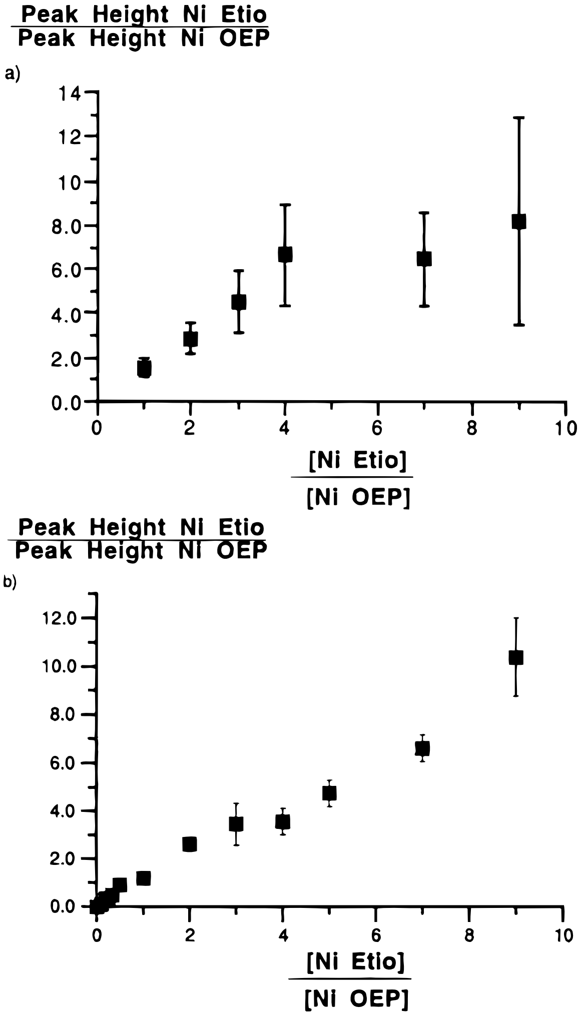


Figure 2. ESI-MS results from the characterization of a mixture of two metalloporphyrins whose ionization potentials are nearly identical. As the molar ratio of nickel etioporphyrin is increased relative to nickel octaethylporphyrin, the relative abundance of the radical cation of nickel etioporphyrin increases accordingly. Each data point is the average of five scans. Final metalloporphyrin concentration (a) 5 and (b) 110 pmol μl^{-1}.

Similar behavior (data not shown) was observed also for a mixture of magnesium etioporphyrin and magnesium octaethylporphyrin, whose oxidation potentials are also similar (cf. Table 1). These results suggest that electrochemical ionization may be useful for semiquantitative analysis of mixtures provided that the oxidation potentials of each analyte are nearly identical.

Metalloporphyrin mixtures with similar oxidation potentials

Our next investigations focused on metalloporphyrin mixtures whose oxidation potentials were similar but not (nearly) identical. As an example of such a situation, a mixture of NiOEP and zinc octaethylporphyrin (ZnOEP) was studied. The oxidation potentials for these two metalloporphyrins differ by ~0.1 V, with ZnOEP having the lower oxidation potential (ZnOEP

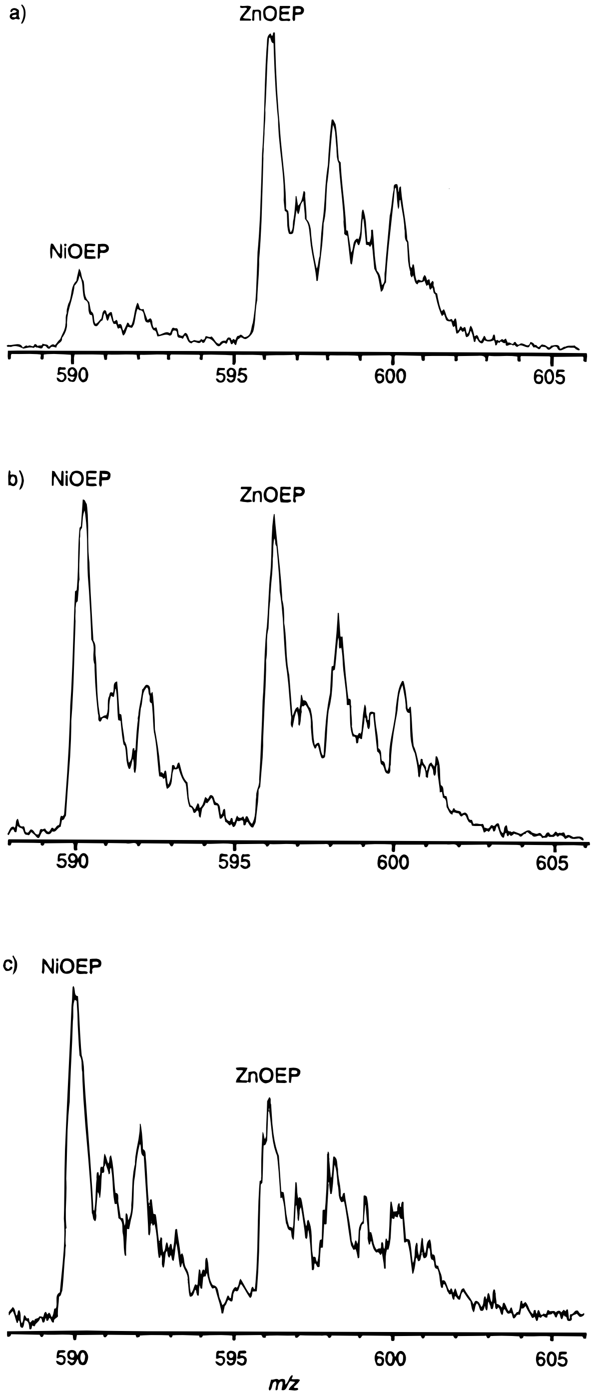


Figure 3. ESI-MS results from the characterization of a mixture of two metalloporphyrins (final metalloporphyrin concentration 5 pmol μl^{-1}) whose ionization potentials are within 0.1 V. As the molar ratio of the harder to oxidize metalloporphyrin is increased in the mixture, the relative abundance of its radical cation increases at a non-linear rate. (a) 1 : 1 nickel octaethylporphyrin–zinc octaethylporphyrin mixture in acetonitrile; (b) 2 : 1 nickel octaethylporphyrin–zinc octaethylporphyrin mixture in acetonitrile; (c) 9 : 1 nickel octaethylporphyrin–zinc octaethylporphyrin mixture in acetonitrile.

0.745 V, NiOEP 0.860 V). As can be seen in Fig. 3(a), at equimolar concentrations (final concentration of mixture 5 pmol μl^{-1}), the molecular ion abundance of ZnOEP ($M^{+\cdot}$ 596 u) is nearly five times that of NiOEP ($M^{+\cdot}$ 590 u). In this case, the ZnOEP is preferentially ionized because it has the lower oxidation potential of the two metalloporphyrins in the mixture.[29]

Once the available ZnOEP is oxidized, the remaining electrospray current will be due to the NiOEP in the mixture. What happens when one increases the relative concentration of the harder to oxidize analyte? Assuming that all of the easier to oxidize analyte can reach the electrode surface and is ionized, one would expect the abundance of the harder to oxidize analyte to increase until some maximum value, determined by the electrospray current, is reached.[29] As can be seen in Fig. 3(b), this indeed is the case for this binary mixture. Here the relative concentration of NiOEP is twice that of ZnOEP and the relative abundance of NiOEP has increased compared with Fig. 3(a). Further increases in the relative concentration of NiOEP result in an increase of the ion abundance of NiOEP [Fig. 3(c)] until the limit, established by the ESI source (i.e. the electrospray current), is reached.

As in the case of the mixture of NiEtio and NiOEP, we performed these experiments at various flow rates to ensure that each analyte present had a sufficient residence time in the capillary to undergo oxidation. Qualitatively, no change was seen in the relative abundances of the two metalloporphyrins as a function of flow rate (data not shown). The sensitivity and electrospray current were found to decrease dramatically at flow rates less than 1.5 μl min^{-1}, an effect we attribute to the loss of a stable spray from our ESI source.

An increase in the *absolute* concentration of each component of the mixture does not result in a qualitative change in the mass spectral results for these two metalloporphyrins. In Fig. 4(a), ZnOEP is twice as concentrated as NiOEP and the final metalloporphyrin concentration of this mixture is 2 nmol μl^{-1}. ZnOEP is oxidized almost exclusively under these conditions. In Fig. 4(b), NiOEP is now twice as concentrated as ZnOEP (final concentration unchanged) and, as seen in Fig. 3(b), the relative ion abundances are nearly identical. In this case, the ZnOEP is preferentially oxidized, owing to its lower oxidation potential, in spite of the excess of NiOEP in the mixture. As before, once all of the ZnOEP has been oxidized, the remaining electrospray current obtainable will be due to the oxidation of NiOEP.

At such high absolute concentrations, one might expect that the ion current would be due exclusively to the easier to oxidize analyte (ZnOEP). However, as seen in Fig. 4(b) there is sufficient electrospray current to oxidize the ZnOEP and the NiOEP even at these high concentrations. We have found that our electrospray source produces a stable spray when higher concentrations ($>$100 pmol μl^{-1}) are electrochemically ionized, presumably owing to the inherently poor sensitivity of the ESI source and the lack of additional supporting electrolyte in these studies. In addition, we noted a slight increase in the electrospray current when the higher concentration solutions (Fig. 4) were used compared with those concentrations used to obtain the results shown in Fig. 3.

To confirm that the results found for NiOEP and ZnOEP at various relative and absolute concentrations are typical of those seen for mixtures whose oxidation potentials are close but not identical, we studied also

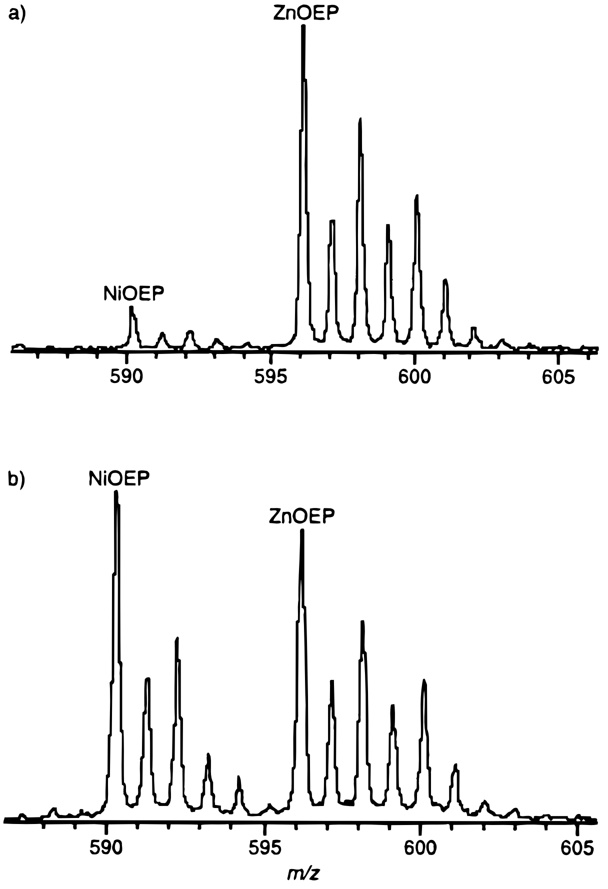


Figure 4. ESI-MS results from the characterization of a mixture of two metalloporphyrins (final metalloporphyrin concentration 2 nmol µl^{-1}) whose ionization potentials are within 0.1 V. Even at a higher absolute concentration, nickel octaethylporphyrin can be oxidized once all of the zinc octaethylporphyrin is oxidized. (a) 1 : 2 nickel octaethylporphyrin–zinc octaethylporphyrin mixture in acetonitrile. As zinc octaethylporphyrin has the lower oxidation potential, it is preferentially ionized in this mixture. (b) 2 : 1 nickel octaethylporphyrin–zinc octaethylporphyrin mixture in acetonitrile. Again, the lower oxidation potential of zinc octaethylporphyrin allows for its ionization even in the presence of a higher concentration of nickel octaethylporphyrin.

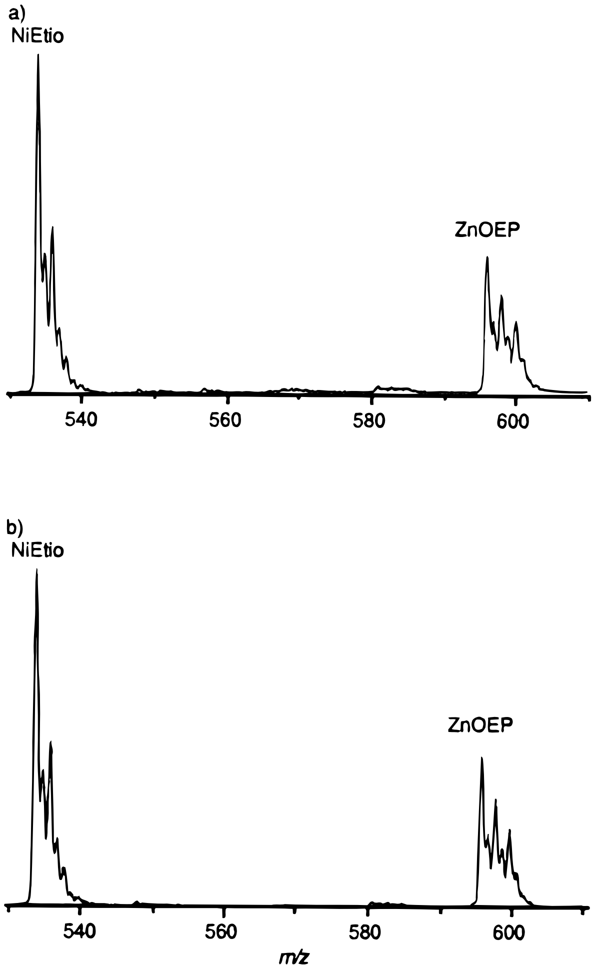


Figure 5. ESI-MS results from the characterization of a 9 : 1 mixture of nickel etioporphyrin and zinc octaethylporphyrin at (a) 10 pmol µl^{-1} and (b) 1 nmol µl^{-1}. The difference in oxidation potentials for these two metalloporphyrins is ~ 0.1 V and the mass spectral results are similar to those seen for other mixtures with oxidation potential differences in this range (Figs 3 and 4).

several other mixtures whose oxidation potentials differed by 0.1 V or less. As can be seen in Fig. 5(a) and (b), qualitatively similar results for a binary mixture of NiEtio and ZnOEP ($\Delta E_{1/2} = 0.1$ V) are obtained at 10 pmol µl^{-1} and 1 nmol µl^{-1} final concentrations, respectively. This trend was seen also during the analysis of a mixture of ZnOEP and CuOEP ($\Delta E_{1/2} = 0.095$ V; data not shown).

Metalloporphyrin mixtures with substantially different oxidation potentials. In order to determine the selectivity of electrochemical ionization for mixtures whose components have substantially different oxidation potentials, a mixture of ZnOEP and VOOEP, at a final concentration of 10 pmol µl^{-1}, was studied. The oxidation potentials for these two metalloporphyrins differ by 0.285 V (ZnOEP 0.745 V, VOOEP 1.03 V). ZnOEP has the lower oxidation potential, and one would predict that ZnOEP would be ionized preferentially over VOOEP. As can be seen in Fig. 6(a) and (b), this indeed is the case. Figure 6(a) is the mass spectrum resulting from the analysis of an equimolar solution of VOOEP and ZnOEP. Both metalloporphyrins are detected in this mass spectrum. The overlap of the $A + 3$ peak of the ZnOEP with the A peak for the VOOEP at m/z 599 complicates interpretation of the relative abundances of these two species detected under these conditions. The ^{67}Zn isotope is less than 10% as abundant as the ^{64}Zn isotope (4.11% *vs.* 48.89% natural abundance, respectively). It is clear from the relative abundances of the ions at m/z 596 and 599 that an additional component at m/z 599 must be present. The abundance of the m/z 599 peak relative to the m/z 596 peak increases as the relative concentration of VOOEP increases in the solution [Fig. 6(b)]. Hence, at these low absolute concentrations, both components of the metalloporphyrin mixture can be detected, with the analyte of lower oxidation potential being preferentially ionized as before.

However, dramatically different results are obtained when the absolute concentration of the metalloporphyrins in a mixture is increased. Exclusive ionization of one analyte over another is found when the analyte concentration is high and a large difference in the oxidation potentials of the analytes exists. For example, when ZnOEP and VOOEP are both present in solution at 1 nmol µl^{-1} each, only ZnOEP is detected [Fig.

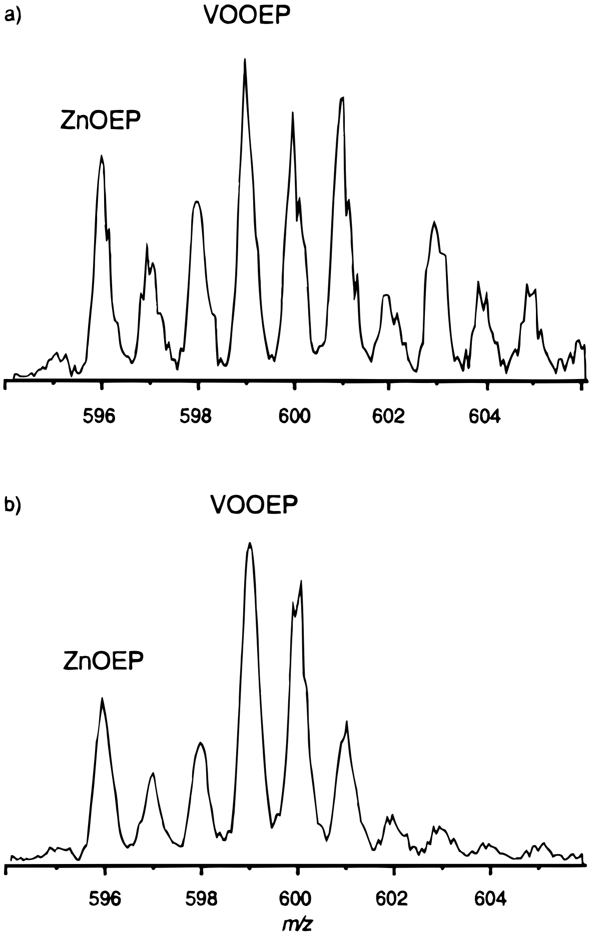


Figure 6. (a) ESI mass spectrum of an equimolar mixture (10 pmol μl^{-1} final metalloporphyrin concentration) of zinc octaethylporphyrin and vanadyl octaethylporphyrin in acetonitrile. Both components are detected in this mass spectrum (see text). (b) ESI mass spectrum of a 1 : 6 mixture of zinc octaethylporphyrin and vanadyl octaethylporphyrin in acetonitrile. As the relative concentration of vanadyl octaethylporphyrin is increased, the abundance of m/z 599 compared with m/z 596 increases accordingly.

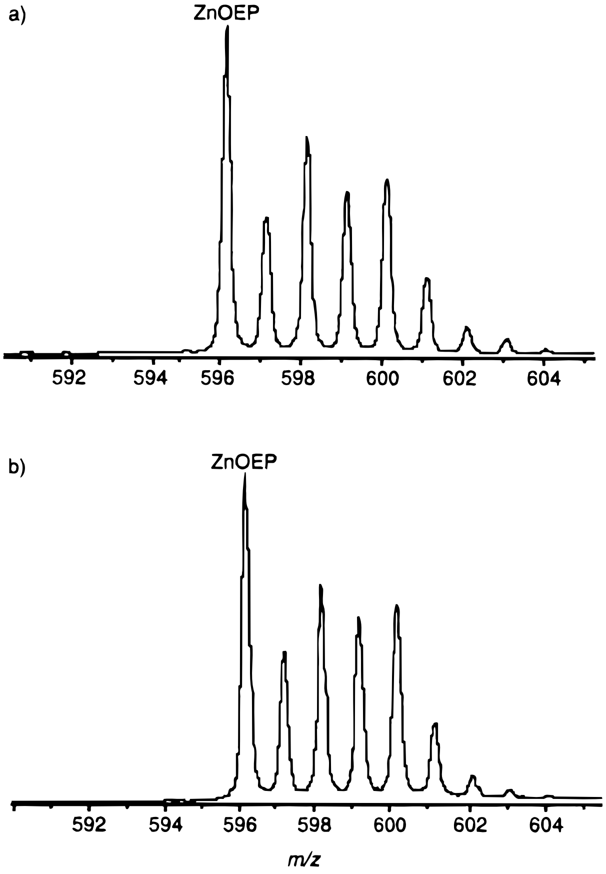


Figure 7. (a) ESI mass spectrum of an equimolar mixture (1 nmol μl^{-1} final metalloporphyrin concentration) of zinc octaethylporphyrin and vanadyl octaethylporphyrin in acetonitrile. As zinc octaethylporphyrin has a much lower oxidation potential than vanadyl octaethylporphyrin, zinc octaethylporphyrin is exclusively ionized in this solution. (b) ESI mass spectrum of a 1 : 10 mixture of zinc octaethylporphyrin and vanadyl octaethylporphyrin in acetonitrile. Again, the much lower oxidation potential of zinc octaethylporphyrin allows for its exclusive ionization even in the presence of a 10-fold molar excess of vanadyl octaethylporphyrin at these high absolute concentrations.

7(a)]. Further, even when the relative concentration of VOOEP is 10 times greater than that of ZnOEP, the radical cation of VOOEP was still not observed [Fig. 7(b)]. Under these conditions, the analyte with the lower oxidation potential serves as a 'redox buffer'[36] and is the sole source of the electrospray current. Similar results (data not shown) were also observed for binary mixtures of magnesium octaethylporphyrin (MgOEP) and CuOEP and of MgOEP and NiOEP. In all cases, the oxidation potentials differ by more than 0.2 V for the analytes in each mixture, and the analyte concentrations are relatively high (>100 pmol μl^{-1}).

Characterization of metalloporphyrins present in mixtures

As noted in Table 1, the metal and porphyrin both influence the oxidation potential of the metalloporphyrins. Magnesium containing metalloporphyrins have the lowest oxidation potentials and vanadium containing metalloporphyrins have the highest oxidation potentials for each of the three porphyrin families studied. The etioporphyrins and octaethylporphyrins, owing to their similar structures, generally have the same influence on the oxidation potential. The tetraphenylporphyrins have consistently higher oxidation potentials.

The use of ESI-MS for the selective ionization of metalloporphyrins can now be addressed. The results of these sets of experiments suggest that limits to the analysis of mixtures whose components have different oxidation potentials can be established relative to the concentration of the analyte sample. In the case of nearly identical oxidation potentials ($\Delta E_{1/2} < 0.1$ V), the electrospray response is directly proportional to the concentration ratio of the analytes. Analytes whose oxidation potentials differ by 0.1 to ~ 0.2 V can both be detected in ESI-MS if the relative concentration of the analyte of lower oxidation potential is not significantly greater than that of the analyte with a higher oxidation potential. If the concentration of the analyte with the lowest oxidation potential dramatically exceeds that of the analyte of higher oxidation potential, preferential ionization and detection of the analyte with the lower

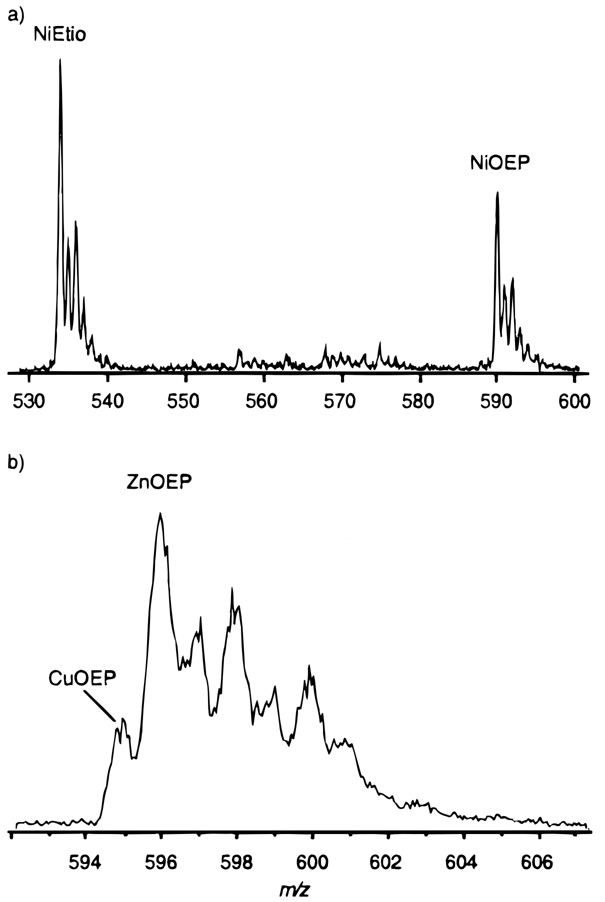


Figure 8. ESI mass spectra resulting from the electrochemical ionization of a mixture of (a) nickel etioporphyrin and nickel octaethylporphyrin (5 pmol μl^{-1} final metalloporphyrin concentration) and (b) copper octaethylporphyrin and zinc octaethylporphyrin (5 pmol μl^{-1} final metalloporphyrin concentration). ESI-MS offers the potential to characterize mixtures of metalloporphyrins based on metal ligand or porphyrin ring system if the oxidation potentials are within 0.1–0.2 V.

oxidation potential will be found. In the case where the oxidation potentials differ by 0.2 V or more, exclusive ionization of the analyte with the lower oxidation potential results at higher sample concentrations. At lower sample concentrations, however, both analytes can be ionized to a degree relative to the concentration ratio of the analytes in the mixture.

These results suggest that one can identify and distinguish between etioporphyrins and octaethylporphyrins when only one type of metal, e.g. nickel,[29] is present in both [Fig. 8(a)]. Mixtures of etioporphyrins or octaethylporphyrins with different metals [e.g. Fig. 8(b)] should also be amenable to analysis provided that the relative concentrations of each metalloporphyrin are similar. Qualitative analysis of mixtures of metalloporphyrins whose oxidation potentials are dramatically different (e.g. ZnOEP and VOTPP) is possible only if the concentration of the analyte of lowest oxidation potential is low in absolute terms ($\sim$1–10 pmol μl^{-1}) and relative to the analyte with the higher oxidation potential.

Electrochemical ionization could be used to ionize selectively, but not exclusively, etioporphyrins and/or octaethylporphyrins that are present in a large molar excess in a mixture with tetraphenylporphyrins. Except for zinc tetraphenylporphyrin, all of the other tetraphenylporphyrins have a higher oxidation potential than any of the other etioporphyrins or octaethylporphyrins listed in Table 1. Studies aimed at devising alternative strategies for ionizing tetraphenylporphyrins of low abundance in mixtures with other porphyrins of lower oxidation potentials are in progress.[37]

CONCLUSION

The behavior of the metalloporphyrin mixtures reported here should aid in gaining a better understanding of the use of ESI-MS for characterizing mixtures of analytes. These experiments have shown that the ability of the electrospray source to ionize one species relative to another is effectively a function of concentration when the oxidation potentials are nearly identical. When the oxidation potentials of two species differ by more than 0.1 V, the ability to ionize one species relative to another in the electrospray source becomes a function of the oxidation potentials of the species, with large differences ($>$0.2 V), resulting in the nearly exclusive production of the analyte of lower oxidation potential. This knowledge will aid in future experiments planned at utilizing the selectivity of ESI-MS for characterizing complex mixtures of analytes.

Acknowledgements

The authors thank Dr Robin L. McCarley at LSU for the use of the potentiostat and for helpful discussions. The authors also thank Dr Tracy D. McCarley and Dr Wenzhe Lu at LSU for their assistance with the Finnigan MAT 900. V.E.V. is supported by a Huel Perkins Fellowship from LSU. Financial support of this work was provided by Louisiana State University, the LSU Office of Research and Economic Development, and the LSU Center for Energy Studies.

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