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Electrospray photoionization (ESPI) liquid chromatography/mass spectrometry for the simultaneous analysis of cyclodextrin and pharmaceuticals and their binding interactions

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We report on the use of a multimode electrospray ionization/atmospheric pressure photoionization source (ESI/APPI or ESPI for short) with liquid chromatography/mass spectrometry (LC/MS) to measure all components of a mixed-polarity liquid sample containing: (1) low-polarity component (hormone, pharmaceutical or sterol), (2) polar component (cyclodextrin substrate), and (3) bound polar complex. The ESPI source has several advantages over both single ESI and multimode electrospray ionization/chemical ionization (ESCI) analysis, including an enhanced bound-complex detection and better performance at lower solvent flow rates. Relative binding constants are determined with (i) ESI mode, resulting in relative R(ESI-MS) values, and (ii) both ESI and APPI modes, providing relative K_D values. We find that low molecular-substitution (Ms) values of cyclodextrin, i.e., Ms = 0.4, preferentially bind to the low-polarity compounds tested. This investigation is intended to demonstrate the feasibility of ESPI as an additional tool for investigating mixed-polarity binding systems, providing mass-specific data for all solution components, both polar and non-polar. Copyright © 2008 John Wiley & Sons, Ltd.

The ability of the cyclic carbohydrate cyclodextrin (Cyd) to partially or fully encapsulate low-polarity compounds and thereby dramatically increase solution solubility has made Cyd an important *in vivo* delivery agent within the pharmaceutical[1] and food chemical industries.[2] Considerable attention has focused on calculating[3] or experimentally[4] determining the relative binding affinity of Cyd with a guest molecule. However, little experimental evidence has been shown to directly measure all three components of the system; namely, the unbound low-polarity molecule, the unbound polar Cyd and the bound polar complex. This is a consequence of the divergent chemical/physical properties of each of the non-polar and polar solution components. The recently developed multimode source technique of electrospray ionization (ESI) photoionization (PI) mass spectrometry (MS), or ESPI-MS, is capable of monitoring both polar (with ESI) and non-polar (with PI) compounds simultaneously.[5,6] The goal of this study is to present ESPI-MS as a new technique to measure the ratio of all three components simultaneously within a mixed-polarity binding system, thereby providing a diagnostic tool for studying biologically relevant binding interactions.

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APPI and detection of low- to non-polar compounds

Atmospheric pressure photoionization (APPI) is a relatively new ionization method that is used with liquid chromatography/mass spectrometry (LC/MS) analysis. The benefits of APPI relative to other ionization techniques, such as ESI or atmospheric pressure chemical ionization (APCI), include: efficient ionization of non-polar compounds, extended linear dynamic range,[7] enhanced sensitivity and less susceptibility to ion suppression.[8] Adding a dopant to the mobile phase in many cases can further increase sensitivity,[9] although the solvent itself can in many instances act as the dopant.[10,11] The benefits of APPI are most pronounced at low solvent flow rates (i.e., $\leq$200 μL/min), due to reduced light absorption by the solvent. However, good performance at moderate to high flow rates can be achieved using either dopants or photoionizable solvents, the latter a result of photo-induced chemical ionization, or PCI.[6,10,11]

Recently, we demonstrated the advantages of using a multimode ESPI source for the detection of several polar and low-polarity compounds.[6] The ESPI source has demonstrated enhanced sensitivity compared to an ESCI source at lower solvent flow rates and improved detection of unstable pharmaceutical compounds (e.g., the anti-inflammatory drug flurbiprofen). The combination of an ESI source for

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detecting polar species with an APPI source for detecting low- to non-polar species is an appealing combination due to the compatibility and orthogonality of the individual sources.

Properties of cyclodextrin

The form of Cyd is a large 'cup' that fits onto the low-polarity end of a compound, or, in the case of small molecules, surrounding them entirely. There are three common sizes of Cyd: six glucose subunits (α-Cyd), seven glucose subunits (β-Cyd) and eight glucose subunits (γ-Cyd). The β-Cyd molecule has been chosen for this study because it is often selected by the pharmaceutical industry due to its high binding affinity with several non-polar drugs and its lower relative toxicity. In particular, the modified version of β-Cyd containing hydroxypropyl (HP) groups will be studied here, termed HP-β-Cyd.

Cyclodextrin is used to form stable complexes with certain pharmaceutical compounds or other low-polarity molecules. The [Cyd] + [drug] → [drug-Cyd] complexation is a fast (ca. 1 s) and stable equilibrium,[12] with a specific dissociation constant, K_D, of the bound complex. The value for K_D can be determined by the ratio of the reverse-to-forward reaction rates of k_1 and k_2, or equivalently the ratio of unbound to bound species, $K_D = k_1/k_2 = [drug][Cyd]/[drug - Cyd]$. A typical binding constant of a drug-Cyd complex can range from 100 to 20 000 M^{-1}, but, even for a tightly bound drug, the 1:100 dilution that occurs after injection or ingestion can result in a complexation shift from 100% to 30%,[1] and thus an effective drug delivery system to the body. Since the ratio K_D cannot be determined by ESI alone (due to susceptibility to ion suppression and low ionization efficiency for non-polar drugs), another ratio, R(ESI-MS), is often calculated directly using ESI-MS, or R(ESI-MS) = [drug – Cyd]/[Cyd]. This value is determined from a single mass spectrum or average of mass spectra (i.e., over a single elution peak), but does not include any information concerning the unbound drug. It is important to mention that the analysis of bound complexes requires the use of flow injection analysis (FIA), not the use of a column for enhanced peak separation, as the equilibrium between bound-to-unbound states will shift by the time the effluent reaches the ion source. This emphasizes the importance of using an ion source that has minimal ion suppression, such as with a photoionization source.[13]

Drugs studied for binding with cyclodextrin

Five drugs were chosen for this study to demonstrate the use of ESPI-LC/MS for the simultaneous measurement of unbound and bound molecules. These molecules are all of medical interest, and include: cholesterol, progesterone and the non-steroidal, anti-inflammatory drugs: flurbiprofen, naproxen and nimesulide. All of the molecules have differing biological properties; however, they are all moderate to very hydrophobic in nature. Also, they are known to effectively bind with Cyd, although little to no evidence of direct measurement of all three components in a mixed-polarity solution has been performed.

Cholesterol, [CHOL] (MW = 386.65 g/mol). Cholesterol is either singly (1:1) or doubly (1:2) bound by cyclodextrin, depending on concentration, greatly enhancing the otherwise very low solubility of cholesterol in water of 2 μg/mL.[14] The stability constant for DOM-β-Cyd at a ratio of 1:1 has been found to be 109 M^{-1}, although when the cyclodextrin concentration is raised, the 1:2 formation is significantly favored, with a solubility constant of 56 800 M^{-1}.[15]

Flurbiprofen, [FLUR] (MW = 244.26 g/mol). Flurbiprofen has a low solubility in water of 14 μg/mL,[16] and when complexed with β-Cyd (1:1), the resulting effectiveness and stability of the drug are significantly increased.[17] Stability constants for the complex have been determined at 310 K for various β-Cyd compounds as 1430 M^{-1} (β-Cyd) and 2660 M^{-1} (HE-β-Cyd).[18]

Naproxen, [NAP] (MW = 230.26 g/mol). Naproxen has a very low water solubility (<7 μg/mL).[19] Complexation of naproxen with β-Cyd to enhance its solubility occurs with a ratio of 1:1, as measured by X-ray diffractometry, IR spectroscopy and differential scanning calorimetry, with solubility constants reported in literature of 568[20] and 1400 M^{-1}.[19]

Nimesulide, [NIM] (MW = 308.31 g/mol). Nimesulide has a low solubility in water (10 μg/mL),[21] and there have been attempts to improve solubility by complexation with Cyd.[22] When bound, this complexation occurs at a ratio of 1:1, measured by phase solubility, ESI-MS and NMR, while true inclusion occurs at a ratio of 1:2, measured by differential scanning calorimetry, X-ray diffractometry and scanning electron microscopy.[22] The solubility constant has been found to be 167 M^{-1} with β-Cyd.[22]

Progesterone, [PROG] (MW = 345.15 g/mol). Progesterone has a low water solubility of 13 μg/mL.[23] As a result, binding HP-β-Cyd is used to increase solubility, with a binding ratio of 1:1 to 1:2, but favors 1:2 at higher cyclodextrin concentrations, with high solubility constants reported as ranging from 11 200 to 16 500 M^{-1}.[23]

EXPERIMENTAL

Reagent solutions

Stock solutions were prepared by dissolving ca. 40 mg in 10 mL dichloromethane (DCM) or methanol (depending on solubility), resulting in a stock solution of 4000 ng/μL. This solution was then further diluted to reach the desired concentration in methanol. The drugs studied here are available from Sigma-Aldrich and are: 2-hydroxypropyl-β-cyclodextrin, or HP-β-Cyd (listed average molecular weight of 1380 g/mol, CAS# 128446-35-5), cholesterol (CAS# 57-88-5), flurbiprofen (CAS# 5104-49-4), naproxen (CAS# 22204-53-1), nimesulide (CAS# 51803-78-2) and progesterone (CAS# 57-83-0). A relatively high concentration of 1 mM was used for these binding experiments, favoring a forward reaction to a bound product, with equivalent mass concentrations of: HP-β-Cyd 1.4 mg/mL, cholesterol 0.39 mg/mL, flurbiprofen 0.24 mg/mL, naproxen 0.23 mg/mL, nimesulide 0.31 mg/mL, and progesterone 0.32 mg/mL.

Instrument, parameters and conditions

A Micromass ZQ LC/MS system (Waters Corporation, Milford, MA, USA) was used for these experiments. Low injection analysis was performed (2 μL sample loop) with a prototype ESPI source, with modifications described

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elsewhere.[6] The data acquisition software was MassLynx 4.0. After careful analysis using MassLynx acquisition software, we determined the ideal voltage settings for each ion species.

Temperature considerations

The stability of bound complexes at increased desolvation or source temperatures is a concern when operating in the APPI mode of ESPI-MS. This has not been found to be a problem at low temperatures (<150°C) using an ESI source (see above ESI references), due to short residency time within the sample capillary, minimizing any degradation prior to ionization and detection. For example, a total sample injection (5 μL) using a moderate flow rate (300 μL/min) takes 1 s to be delivered from the room-temperature port to the high-temperature ion source. The challenge in this current study is to determine if the increased desolvation temperature associated with APPI significantly degrades or otherwise affects the complexation binding ratio.

RESULTS AND DISCUSSION

Analysis of mixtures containing cyclodextrin and pharmaceuticals

Mass spectrometric analysis of a solution containing HP-β-Cyd indicates a distribution of ion masses, a result of the variation in actual hydroxypropyl-substitution number during the synthetic modification of β-Cyd. To identify an individual molecular type within this distribution, a ratio of the hydroxypropyl-substituted sites to the hydroxyl-substituted, i.e., non-substituted, sites is often used. This is termed the molecular-substitution ratio, Ms, and can range from a value of 0 (all hydroxyl groups) to 8 (all hydroxypropyl groups). For simplification, we will adopt here the standard notation of Cyd_{Ms} to indicate a particular Ms value of Cyd. The dominant species found here is $[Cyd_{0.91}+Na]^+$ at *m/z* 1448. Two mass/charge values are recorded using ESI, the Na_1 adduct with a 1+ charge, and the Na_2 adduct with a 2+ charge, which falls much lower on the mass/charge scale. The ideal sampling cone voltage within the ESI+ ion source was determined to be 50 V.

Analysis of mixed-polarity solutions

An example of a simultaneous analysis of a non-polar compound/polar substrate mixture is presented in Fig. 1. The example chosen is a 1:1 mixture of the pharmaceutical naproxen with the substrate (carrier molecule) cyclodextrin. Switching between the APPI and ESI sources is termed here 'mode switching' and was controlled by the native software on the LC/MS system (MassLynx), with some minor modifications to allow for ESPI operations using an ESCI software interface. As illustrated in Fig. 1, typical timing used was ESI (ON)/APPI (OFF) for 0.4 ms, followed by 0.2 ms both

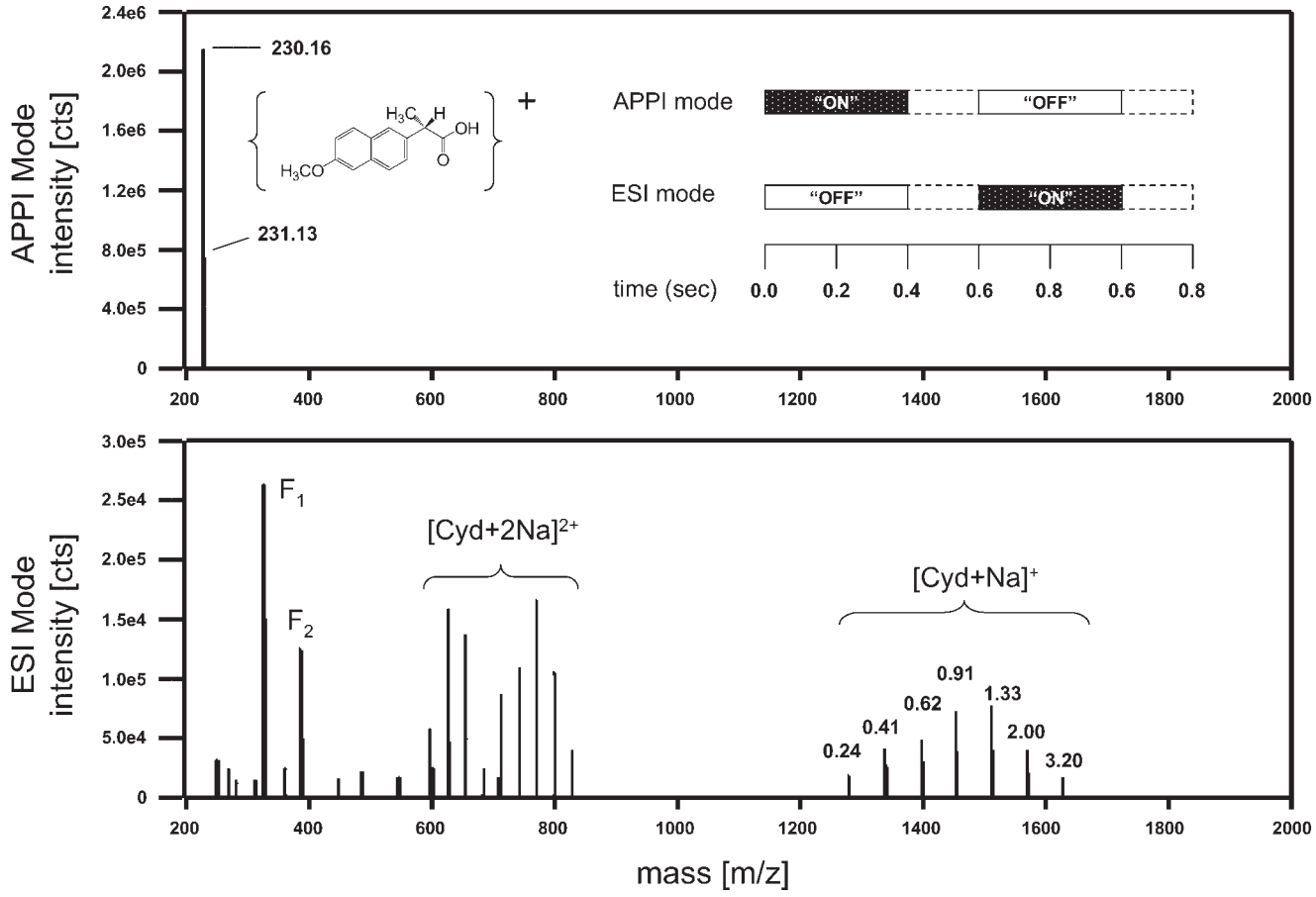

Figure 1. Mass spectra of a 1:1 mixture of naproxen and Cyd in methanol and 5% toluene dopant with an ESPI source. [Top insert] schematic showing timing sequence for the ESI and APPI modes: [top] The $[M]^+$ signal of naproxen is detected during the APPI mode, while [bottom] Cyd is measured as a Na^+ (with Ms values indicated) or Na_2^{2+} adducts. Fragments F_1 and F_2 originate from Cyd (possible impurities).

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OFF, and then ESI (OFF)/APPI (ON) for 0.4 ms, followed by 0.2 ms both OFF. FIA was performed in methanol at a flow rate of 100 μL/min. Analysis of the mixture demonstrates that the precursor ion, $[NAP]^+$ and $[NAP]H^+$, are the dominant ions in the APPI+ mode. Cyclodextrin is barely observable in APPI mode, even at a high concentration of 1 mM. The ESI+ mode performs in a complementary manner in that the polar cyclodextrin is measured efficiently (including a double sodium adduct and two peaks representing either undetermined fragments or impurities), while the naproxen is barely observed. Running multimode ESPI thus provides a clear separation of components, while still providing simultaneous analysis of non-polar and polar components within the solution.

One concern for the analysis of mixed-polarity solutions using LC/MS is the effect of ion suppression and resulting decrease in ion signal. This effect has been previously reported to be a concern for sources containing high concentrations of ions, such as seen with column bleeding, matrix ionization or multiple target ion species. This has been previously found to be an advantage for the single mode APPI source over APCI and ESI.[13,24]

To determine the relative analytical capabilities of ESPI and ESCI to measure a mixture of polar and non-polar components, the signal-to-noise (S/N) ratios of both the pharmaceutical (or drug) compound and cyclodextrin carrier molecule for five different mixed-polarity sample solutions were determined and the relative performance of ESPI vs. ESCI is presented in Fig. 2. Of note is that the relative performance of ESPI to ESCI is significantly increased for these complex solutions, in support of a previous study that presented only pure pharmaceutical solutions.[6]

Analysis of naproxen-cyclodextrin: resulting bound complex

Toluene was used in the above section to enhance detection of the pharmaceutical compound. However, when the bound complex is to be measured, one potential complication is that toluene may competitively bind with cyclodextrin. It is known that benzene forms a weak bond within the cyclodextrin core.[3] Although the binding of toluene within cyclodextrin is purely a van der Waals interaction, whereas the pharmaceuticals studied here have both van der Waals and the stronger polar and/or hydrogen bonding to cyclodextrin, most of the results to follow use acetone as the dopant in order to minimize any possible dopant interference while measuring the bound complex.

We find that there are specific Ms values associated with binding to naproxen (see Fig. 3), and further that these bound complexes are more prevalent for the lowest Ms values (fewer hydroxypropyl substitutions). This implies that the naproxen molecule requires more space to bind effectively within the cyclodextrin molecule. Thus, although the most abundant Ms value is 0.91, the dominant bound molecule with naproxen is at Ms = 0.24. This is in agreement with a previous study using ESI-MS to study the binding of HP-β-Cyd with barbiturates, concluding that the only significant influence on binding is the internal substitutions within the cavity, not the outer-cavity substitutions (i.e., external, electrostatic aggregation is not significant).[25]

Figure 3 (upper right) shows plots of the bound-to-unbound states of cyclodextrin as a function of naproxen concentration in a solution of 1 mM cyclodextrin. As expected for an equilibrium binding interaction, one observes first a rise in bound state of the $[NAP+Cyd_{0.24}]$ complex, followed by a saturation plateau as the naproxen concentration increases. Conversely, the signal of the unbound $Cyd_{0.24}$ falls off as the naproxen concentration increases.

In the left-hand plots in Fig. 3, it is observed that, under identical conditions, the bound-complex ion signal is more intense relative to the unbound Cyd when ESPI is used compared to ESCI. We speculate that the APCI component to the ESCI source, in particular the corona needle, interferes with detection of this weakly bound compound. This could be a similar effect as observed previously with easily fragmented molecules, such as flurbiprofen,[6] which is fragmented and not observed efficiently as a precursor ion

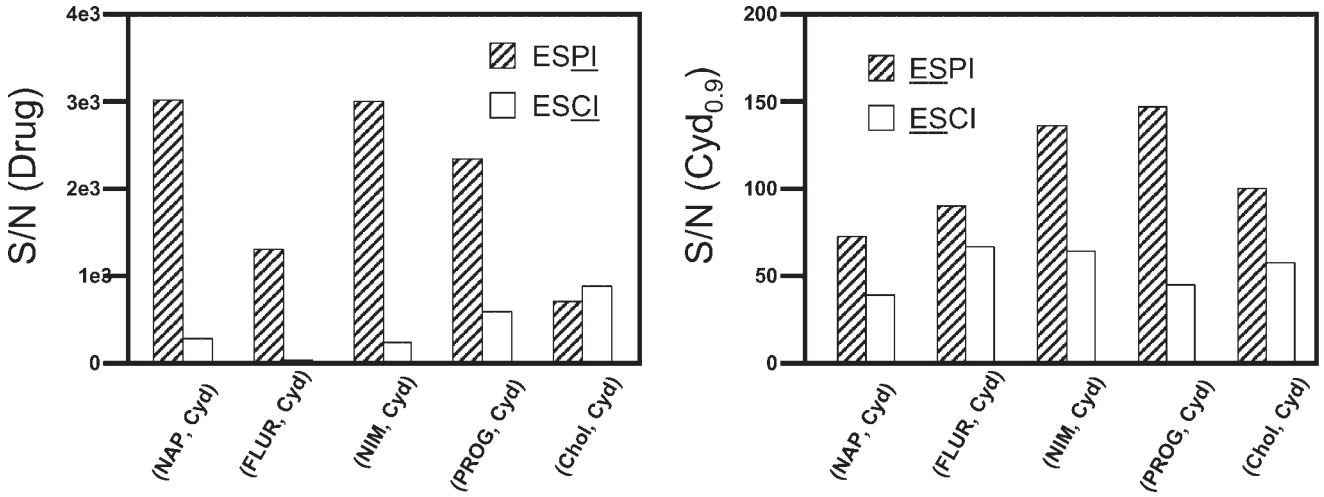

Figure 2. Comparative analysis of five solutions containing 1 mM cyclodextrin and one of the following: naproxen (NAP), flurbiprofen (FLUR), nimesulide (NIM), progesterone (PROG) and cholesterol (CHOL), using either ESPI or ESCI. Pharmaceuticals are measured using either APPI or APCI modes [left] and cyclodextrin is measured using ESI mode [right]. Samples include 5% toluene, using FIA in 100 μL/min methanol.

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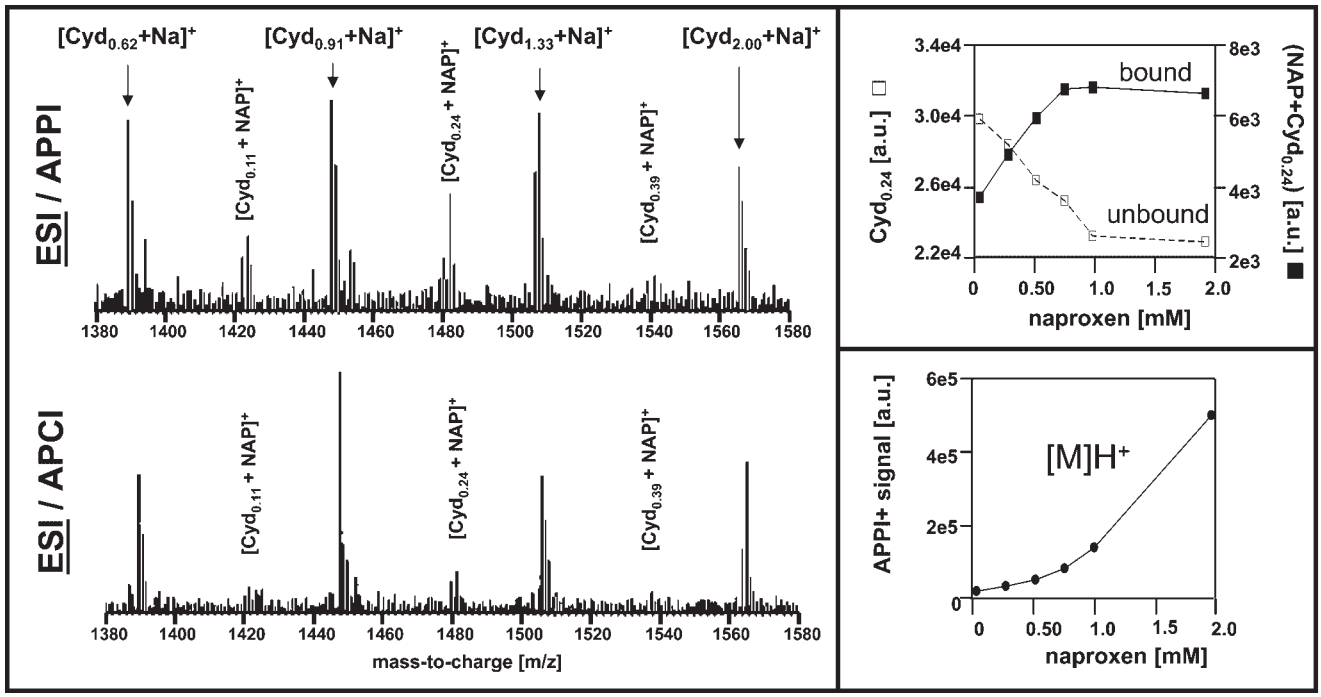

Figure 3. ESI mode mass spectra of a 1:1 mixture of naproxen and Cyd in methanol and 2% acetone dopant with an ESPI source [top left] and ESCI source [bottom left]. Bound and unbound signal intensity using ESI+ mode with increasing amounts of naproxen, saturating the bound state [top right], with APPI+ signal intensity of the drug naproxen using the APPI mode [bottom right].

with APCI. Another explanation is that the ESPI configuration allows a more optimum geometry and performance for the ESI source relative to the ESCI configuration as we showed previously.[6]

ESPI performance: vapor temperature and solvent flow

An often overlooked parameter in complexation analysis is the effect of ESI probe temperature on the resulting molecular stability. Often reported in literature and assumed to be the actual vapor temperature, the heater temperature does not accurately represent the vapor temperature. For example, at a heater temperature of 400°C, the temperature of the gas measured 2 mm downstream from the ESI probe tip is 150°C, or 124°C with a flow of 100 μL/min methanol. Higher solvent flows result in even lower vapor temperatures.

The significance of the vapor temperature can be demonstrated when monitoring the ion signals for a sample of (1:1) naproxen/cyclodextrin with 2% acetone dopant (Fig. 4, upper left). All ion signals noticeably increase above 300°C (corresponding to 80°C vapor), which is about 15°C above the boiling point of methanol at 64.7°C. It is reasonable to assume that this increase in ion signal corresponds to vaporization of solvent. As photoionization is much more sensitive to the 'dryness' of the vapor than electrospray, the enhancement in ionization is most noticeable with the APPI signal. In the current configuration, the ion signal is greatest at the highest temperature setting of 500°C (188°C vapor). This rise in ion intensity is also observed for the ESI signal, although to a lesser extent.

An additional factor tested here is the thermal stability of this bound complex. Although higher vaporization temperatures are typically best for APPI (Fig. 4, upper left), a higher temperature may not be suitable for weakly bound compounds. This can be observed in the upper right part of Fig. 4, indicating a temperature-dependent response of the bound signal. Bound complex stability and ion intensity rise with temperature up to a heater temperature of about 350°C (100°C vapor), but then noticeably falls off, presumably due to complex instability.

Finally, the influence of solvent flow is presented in Fig. 4 on both absolute signal intensity (lower left) and S/N ratio (lower right). Solvent flow affects several parameters in LC/MS, such as the vapor temperature, signal intensity and peak width, and also the amount of light that reaches the analyte during photoionization. In the case of low flow from 10 to 150 μL/min, there is no significant flow dependence on both ESI and APPI signals (Fig. 4, bottom left). However, when S/N ratios are determined, while the ESI signals are not affected by flow in this range, the APPI signal is significantly enhanced at the reduced flow of 50 μL/min (Fig. 4, bottom right). This is consistent with observations that APPI is often best at lower solvent flow.[26] Although this appears to limit the operating range of solvent flow rate when monitoring low-polarity compounds, this is only true for the use of the Kr lamp for photoionization. It has been recently demonstrated that a higher energy Ar lamp can extend this range to 1 mL/min with methanol, a result of PCI via the solvent monomer.[10]

ESPI as a tool to investigate pharmaceutical-substrate interactions

Table 1 compiles the observed signal intensities using both modes of the ESPI source (ESI and APPI). Five low-polarity compounds are studied here: naproxen, flurbiprofen, nimesulide, progesterone and cholesterol (each in a

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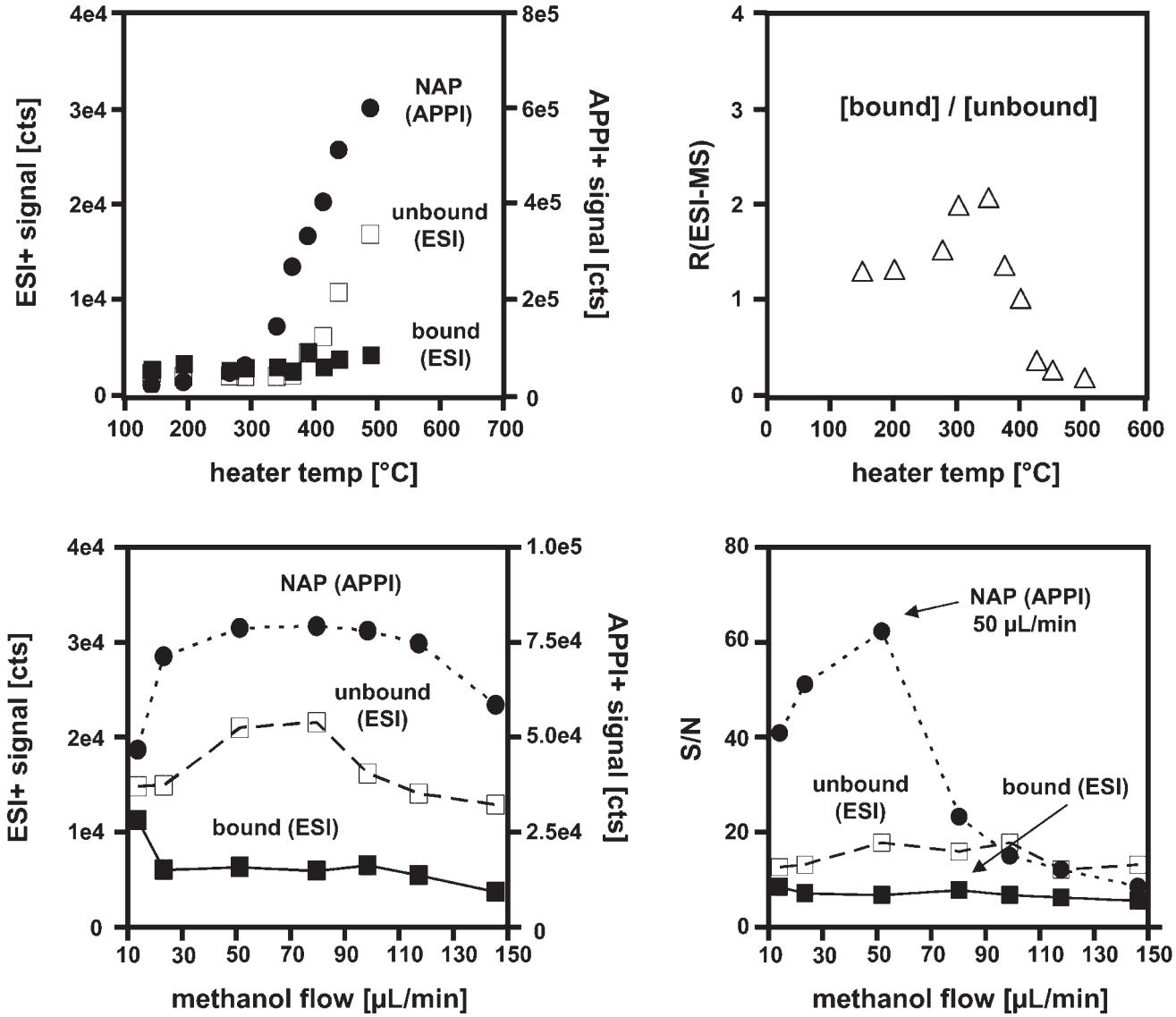

Figure 4. Analysis of a 1:1 mixture of 1 mM naproxen-cyclodextrin with 2% acetone dopant in 100 µL/min methanol using ESPI. Effect of heater temperature signal of naproxen (filled circles), unbound cyclodextrin (open squares) and bound complex (filled squares) [top left]. Indication of ideal heater temperature, with complex degradation at excessive heater temperatures [top right]. Effect of solvent flow on absolute ion intensity [bottom left] and S/N ratio [bottom right].

Table 1. Identified complexes formed with 1 mM drug and 1 mM HP-β-Cyd

Compound [*m*/*z*]	Complex (D = Drug)	Complex [*m*/*z*]	ESI R(ESI-MS)[a]	APPI Drug [a.u]	K_D
naproxen	$[Cyd_{0.11}+D]^+$	1424.7	3.6		4.9e5
[230.26]	$[Cyd_{0.17}+D]^+$	1453.1	1.2	1.77e6	1.5e6
	$[Cyd_{0.24}+D]^+$	1481.8	1.1		1.6e6
flurbiprofen	$[Cyd_{0.05}+D\text{-}H2]^+$	1406.4	3.6		5.6e4
[244.26]	$[Cyd_{0.11}+D\text{-}H2]^+$	1435.3	3.4	2.00e5	5.9e4
	$[Cyd_{0.17}+D\text{-}H2]^+$	1464.0	2.0		1.0e5
nimesulide	$[Cyd_{0.05}+D]^+$	1471.9	3.0		3.5e5
[308.31]	$[Cyd_{0.11}+D]^+$	1501.4	0.9	1.04e6	1.2e6
	$[Cyd_{0.17}+D]^+$	1529.6	2.2		4.7e5
progesterone	$[Cyd_{0.0}+D\text{-}H2]^+$	1446.7	9.8		1.6e5
[314.46]	$[Cyd_{0.05}+D]^+$	1477.6	4.3	1.56e6	3.3e5
	$[Cyd_{0.11}+D]^+$	1507.5	3.2		4.9e5
cholesterol[b]	$[Cyd_{0.11}+D]^+$	1580.0	2.3		1.5e5
[386.65][c]	$[Cyd_{0.17}+D]^+$	1608.7	2.4	3.55e5	1.5e5
	$[Cyd_{0.24}+D]^+$	1637.5	1.4		2.5e5

[a]R(ESI-MS) is the ratio of bound-to-unbound Cyd species, not absolute ion intensities.
[b]Extensive complexation observed as 2+ charge adducts, including Na_2 adducts.
[c]Complex formed is the precursor ion of cholesterol at *m*/*z* 386, not the dehydrated fragment at *m*/*z* 369.

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methanolic solution with an equal amount of cyclodextrin). All solutions were allowed to mix for at least 1 day. The ESI mode was used to determine the relative binding ratio, R(ESI-MS), while both the APPI and ESI modes were used to calculate the relative dissociation constant, K_D. It should be noted that these values do not incorporate ionization efficiencies, which are different from ESI and APPI, and therefore these values should be used qualitatively, not quantitatively.

The first observation is that the degree of molecular substitution (Ms value) of cyclodextrin is a significant factor in the binding with the compound tested. In all cases, the Ms value responsible for a bound species has a lower value than the dominant Cyd_{Ms} value of 0.91. Of note are the results for cholesterol-cyclodextrin binding, with the detection of extensive 2+ bound complexation with cholesterol. Unfortunately, the mass range of the instrument is *m/z* 2000, so that we could not absolutely identify the 1+ state of the Chol-Cyd_2 complexes. Also of note with cholesterol is that the dominant complexation ion is the precursor ion (*m/z* 387), not the dominant fragment signal *m/z* 369, observed with ESI when cholesterol is measured alone. This suggests that complexation with cyclodextrin protects the cholesterol from the ionization environment within the source and that the charge on the observed complex ion is most likely on the cyclodexterin moiety.

The gas-phase stability constant, R(ESI-MS), of the ions indicates that although the most abundant complexation ion is with Ms = 0.11 or 0.24, the stability R(ESI-MS) is greatest when the Ms value is lowest. Of all of the complexes studied, progesterone has the greatest bound-complex stability, while cholesterol has the lowest stability.

Determination of the ideal molecular-substitution value for binding

The final use of ESPI to study complexations is the determination of the maximum ratio of bound-to-unbound complex with respect to the molecular-substitution (Ms) value. Three examples are chosen to demonstrate this, all of which are 1:1 mixtures (1 mM) of the drug compound with cyclodextrin in methanol: (i) flurbiprofen, (ii) progesterone, and (iii) naproxen (see Fig. 5). These three complexes were chosen because they provide strong signals for both the ESI and APPI sources, and these compounds are of interest to the pharmaceutical industry.

In Fig. 5, we find that, for all three examples, the intensity of the unbound cyclodextrin species follows the expected Gaussian distribution associated with polymers. The dominant monomer of cyclodextrin is in this case Ms = 0.91. When the intensity of the bound species is correlated to the same Ms scale, then the maximum is observed to occur at a much lower value than the unbound cyclodextrin, ranging from Ms = 0.24 with flurbiprofen and naproxen, to Ms = 0.41 for progesterone. While the ratio of bound-to-unbound species indicates that the lowest Ms values are nearly always favored (Table 1), the absolute intensity of the bound complexes are shifted to higher values of Ms. This is due to the increasing abundance of higher Ms values of Cyd. Though the normalized results in Table 1 are more correct, the plots in Fig. 5 are interesting because they accentuate the subtle differences in drug binding interactions as a function of Ms value.

CONCLUSIONS

The simultaneous measurement of components within a mixed-polarity solution containing a pharmaceutical compound (non-polar), substrate (polar) and bound substrate (polar) using the ESPI-LC/MS technique is demonstrated. It is found that ESPI performs well versus ESCI at measuring bound complexes; specifically, lower bound-complex fragmentation, reduced ion suppression and better S/N ratios at low solvent flow rates. The example of naproxen-cyclodextrin is provided to demonstrate how ESPI can be used to monitor the binding behavior of naproxen to the substrate cyclodextrin. Two parameters of the source are tested, indicating that: (1) higher probe temperatures enhance APPI and ESI signals, but above a heater temperature of 350°C (100°C vapor temperature) there is a drop in bound-complex stability, and (2) lower solvent flow rates enhance the S/N ratio of the APPI mode, while the ESI mode is relatively unaffected in this flow range. Finally, relative binding constants R(ESI-MS) and K_D are presented for cyclodextrin with several pharmaceutical compounds.

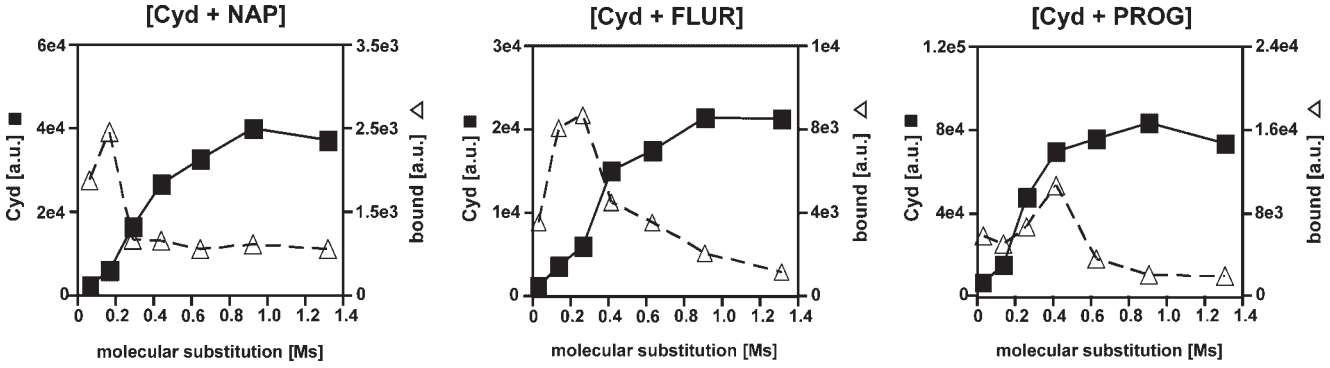

Figure 5. Intensity of ESI signal for three (1:1) mixtures containing cyclodextrin and naproxen (left), flurbiprofen (middle) and progesterone (right). Bound drug-cyclodextrin complex favors lower Ms values than the unbound cyclodextrin maximum at Ms = 0.91. Values represent averaged mass spectra using FIA in 100 μL/min methanol.

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The dissociation constants presented here are consistent with values reported in literature; however, ESPI provides a further level of detail, e.g., Ms-specific relative dissociation constants. This investigation is intended to demonstrate that ESPI-LC/MS can provide unique and supportive information in the investigation of non-polar/polar mixtures and bound complexes.

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