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Comparison of electrospray ionization, atmospheric pressure photoionization, and anion attachment atmospheric pressure photoionization for the analysis of hexabromocyclododecane enantiomers in environmental samples

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ABSTRACT

Anion attachment atmospheric pressure photoionization (AA-APPI) has been suggested as a means of expanding the range of compounds that may be analyzed by LC–MS, and has been found to enhance the ionization of some macromolecules (e.g., peptides, polymers) that were unable to be ionized by other techniques. In this study, AA-APPI was compared to APPI, using hexabromocyclododecane (HBCD) enantiomers as a model compound, to provide proof of principle of the use of AA-APPI for small molecule analysis. The use of AA-APPI, with 1,4-dibromobutane in toluene as a bromide source, offered increased sensitivity and lower limits of detection than APPI. Minimal matrix effects were found with AA-APPI in sediment extracts spiked with HBCD post-extraction, with less than a 6% enhancement in the ion signal. Furthermore, enantiomer fractions of HBCD enantiomers were racemic in spiked sediment extracts, in contrast to the more commonly used technique of electrospray ionization, for which matrix effects caused ion signal modification to cause non-racemic measurement artifacts. The use of AA-APPI offers a simple means of further extending the range of compounds ionizable by AA-APPI while maintaining minimal matrix effects.

1. Introduction

In recent years, liquid chromatography–mass spectrometry (LC–MS) has become increasingly popular for use in environmental analysis, and while a number of ionization techniques exist, electrospray ionization (ESI) remains the most widely used. However, while ESI is extremely sensitive for polar compounds, many environmental compounds are non-polar, and are therefore difficult to analyze using this technique. In 2000, atmospheric pressure photoionization (APPI) was developed as a complementary technique to ESI, and provides a means of ionizing low-polarity compounds [1]. For some analytes, APPI offers greater sensitivity [2,3] and larger dynamic ranges [4,5] than ESI. Furthermore, APPI may offer other advantages over ESI for environmental analyses. APPI ionization has been found to be less susceptible to matrix effects than ESI for a variety of analytes [4,6,7]. Matrix effects occur when the ionization efficiency of an analyte is either enhanced or suppressed, and is often attributed to matrix materials that co-elute with the analyte. This change in the analyte response can hamper quantification by leading to inaccurate and imprecise measurements of analyte concentration.

More recently, the use of anion attachment APPI (AA-APPI) has been suggested as a means of further expanding the range of compounds ionizable by APPI [8]. In general, the ionization of analytes by APPI relies on the presence of a photoionizable dopant, a low molecular weight compound with an ionization potential below that of the energies of the emitted photons. The dopant is introduced into the source, where it is photoionized to release a thermal electron. This, in turn, may initiate a series of gas phase reactions, subsequently yielding either a positively or negatively charged analyte ion [9]. In AA-APPI, analytes are ionized by the formation of a negatively charged adduct species within the source. The use of stable adducts to enhance the ionization of non-polar compounds in APPI, particularly large molecules such as peptides and polymers, has been demonstrated through the use of chlorinated solvents or chlorinated eluents [8,10,11]. However, to date this technique has not been shown to be applicable to small molecules, nor, to the best of our knowledge, do studies exist comparing the use of AA-APPI to other techniques, such as ESI or APPI.

Hexabromocyclododecane (HBCD) is a brominated flame retardant commonly added to consumer products, such as polystyrene

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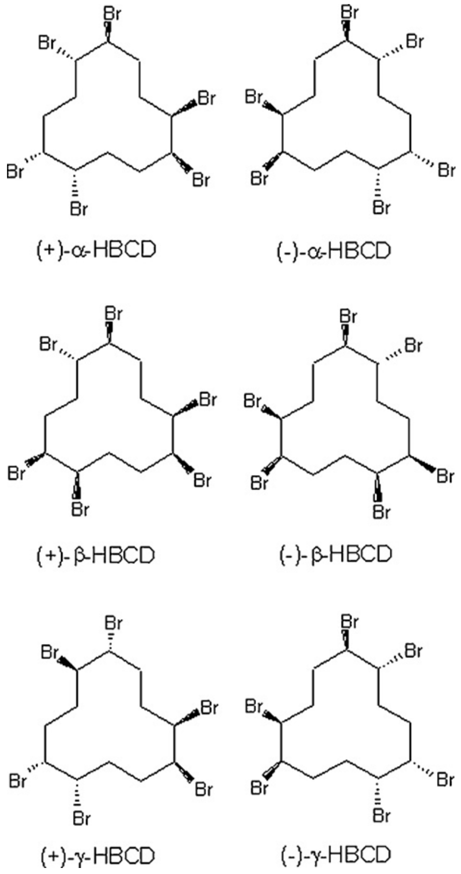

Fig. 1. Structures of major HBCD diastereomers and enantiomers.

foams and textiles, to reduce their flammability [12]. Within the last decade, increasing concentrations of HBCD have been found in air, sediment, biota, and human blood and milk [13]. Hexabromocyclododecane is present in the environment as a mixture of three major diastereomers: α, β, and γ (Fig. 1). Each diastereomer is chiral, and therefore each exists as a pair of enantiomers [14]. The chirality of a compound may have profound impacts on a compound's fate and behavior in the environment. For instance, individual enantiomers may vary in bioaccumulation, metabolism, and toxicology [15–19]. Therefore, the need exists to analyze chiral compounds on an enantiomer-specific basis in order to understand better the environmental fate of individual enantiomers. However, the accurate quantification of enantiomers is often hampered by matrix effects, as matrix effects may have a more detrimental effect on the quantification of enantiomers than on the sum mixture of enantiomers, as commonly measured by non-enantioselective chromatography as a single peak [20].

In this study, we compared the analytical characteristics and matrix effects of APPI and AA-APPI using HBCD as an environmentally relevant model compound. In doing so, we provide proof of principle evidence on the use of AA-APPI for the purposes of quantifying small molecules.

2. Experimental

2.1. Chemicals and reagents

Individual HBCD standards (α, β, and γ) were purchased from Accustandard (New Haven, CT, USA), all greater than 99% pure. Individual deuterated (d_{18}) HBCD isomers, of at least 98% chemical and isotopic purity, were purchased from Wellington Laboratories (Guelph, ON, Canada). HPLC grade methanol, acetonitrile, and toluene, as well as pesticide grade hexane and acetone were purchased from Fisher Scientific (Ottawa, ON, Canada), as was anhydrous sodium sulfate. Milli-Q water was obtained via a Millipore (Billerica, MA, USA) water filtration system. 1,4-Dibromobutane (99% purity) was purchased from VWR (Mississauga, ON, Canada). Silica gel (70–230 mesh) was purchased from Sigma–Aldrich (Oakville, ON, Canada). Certified reference material EC-5 (Lake Ontario sediment, certified for polyaromatic hydrocarbons, chlorobenzenes, and polychlorinated biphenyls) was obtained from Environment Canada.

2.2. Liquid chromatography/mass spectrometry

For all experiments, an Agilent 1100 HPLC system coupled to an Applied Biosystems QTrap 2000 (Foster City, CA) triple quadrupole mass spectrometer was used. Enantiomer separation was achieved on a Nucleodex β-PM enantioselective column (4.6 mm × 200 mm, 5 μm d_p, Macherey-Nagel, Bethlehem, PA) using an eluent of 49% acetonitrile/30% methanol/21% H_2O initially held for 2 min, then changed linearly to 59.5% acetonitrile/30% methanol/10.5% H_2O over 20 min at 500 μL min^{-1} [21]. This gradient was accomplished on a binary pump using 70:30 H_2O/methanol as the A solvent and 70:30 acetonitrile/methanol as the B solvent and changing the eluent composition from 30:70 A/B to 15:85 A/B over the course of the run [22]. Unless otherwise noted, an injection volume of 20 μL was used for all experiments. Chromatographic conditions were kept constant among source experiments.

Mass spectrometric experiments were carried out with a Photospray source (Applied Biosystems, Foster City, CA). Source parameters were optimized individually for all three sources in a similar manner. Optimization was done using a 1:1:1 mixture of all three native diastereomers (300 ng mL^{-1} each). An equal mixture of all three isomers was chosen in order not to place undue emphasis on one isomer over the others, as the mass spectral response may vary among isomers. This solution was introduced into the eluent flow via a tee connection at 20 μL min^{-1} using a syringe pump (Hamilton, Reno, NV). The eluent was flowed at 500 μL min^{-1}, and the composition was 77.5% A/22.5% B, which represents the midpoint in the gradient used for analysis. Optimization was carried out by systematically changing the user-adjustable source parameters to maximize the ion intensity of either the $[M-H]^-$ (APPI) or $[M+Br]^-$ (AA-APPI) ion (Table 1).

Unless otherwise noted, for all experiments and analyses, the carrier solvent or photoionization dopant was flowed into the source in conjunction with the auxiliary gas at 50 μL min^{-1} using either a syringe pump or a pneumatic delivery system [23]. For APPI experiments, toluene was used as the photoionization dopant.

2.3. Carrier solvent optimization

Prior to AA-APPI experiments and analyses, several experiments were carried out to determine the best source of bromide and the optimum carrier solvent (i.e., co-solvent being tested as a possible dopant) that would maximize the formation of the $[M+Br]^-$ ion. All experiments were carried out by injecting the analyte directly into the eluent flow, which was coupled to the mass spectrometer without any chromatographic column attached. For the first, 15 brominated aliphatic and alicyclic compounds of varying structures and degrees of bromination, were selected for screening due to their ready availability in our lab and their solubility in non-polar solvents. These compounds were characterized by their ability to fragment within the source to form bromide ions (m/z 79 and 81). This was done by injecting into the LC 1 μL of a 1% solution (v/v) of each compound in toluene. The LC eluent composition was held constant at 77.5% A/22.5% B at a rate of 500 μL min^{-1}. Full scan mass