Mass Spectrometry

Fundamental LC-MS

Atmospheric Pressure Chemical Ionisation (APCI)

Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.
Aims and Objectives

Aims

- Introduce Atmospheric Pressure Chemical Ionisation (APCI) concepts
- Explain the function of each major component of the APCI
- Indicate the major advantages of APCI and when it is used
- Present the most important elements that are currently present in an APCI interface

Objectives

_At the end of this Section you should be able to:_

- Describe the working principles associated with APCI
- List and describe the most important elements that are currently present in an APCI interface
- Explain the difference between positive and negative APCI, and when to use each mode
- Explain the most important APCI Ionisation Mechanisms
Content

Introduction 3
Interface Overview 3
Suitable Samples for APCI 4
APCI Interfacing Details 8
Nebuliser Types 9
APCI Pneumatic Nebulisers 10
Ion Sampling and Transfer in APCI Interfaces 10
APCI Analyte Ion Declustering 12
APCI Ionisation 13
  Positive Ion Mode 13
  Negative Ion Mode 14
Gas Phase Reactions 15
Reagent Gas Formation 17
Relative Proton Affinity 18
Effect of Eluent Additives on APCI 19
Signal Quenching 19
Other Ionisation Mechanisms in APCI 20
APCI Source Parameter Optimisation 22
Comparison of API Ionisation Techniques 23
References 24
Introduction

Atmospheric Pressure Chemical Ionisation relies upon the formation of a plasma of ions comprised, mainly, from the HPLC mobile phase components. The eluent molecules, which are in vast excess compared to the analyte, are ionised by the electron cloud around the corona discharge pin and act as the reagent gas in a chemical ionisation process. Analyte molecules are ionised by this reagent gas ‘plasma’ and are subsequently sampled into the nozzle-skimmer region of the mass spectrometer in a similar fashion as described for electrospray ionisation.

Unlike in ES, the solvent-evaporation and ion-formation processes are separated in APCI, this allows the use of low-polarity solvents that are unfavourable for ESI ion formation.

Another major difference between APCI and ES can be found in the LC flow-rates that are used. APCI is a technique that uses high flow-rates (1 mL/min and higher) whereas ES is limited to a few micro-litres per minute (or up to 200 μL/min with pneumatic assistance).

The region of applicability for the APCI technique is shown.\(^\text{[2]}\)

In general, APCI would be employed for analytes which are less polar and which have lower molecular weight than those suitable for electrospray ionization.

Interface Overview

In APCI the eluent is introduced into the interface using a capillary of similar design to the ESI source. However, no potential is applied to the capillary, instead the liquid emerges from the capillary surrounded by a flow of inert, nebulising gas into a heated (vaporising) region.
The combination of nebulising gas and heat forms an aerosol with the sprayed eluent that begins to rapidly evaporate. A pin is placed within or at the end of the heated region that has a high potential applied to it and produces an electrical discharge which ionizes molecules within the aerosol.

A combination of molecular collisions and charge transfer processes cause an ionized gas plasma to be formed, primarily from the eluent molecules that are in vast excess. Sample molecules that elute in the gas phase into this plasma may be ionized via transfer of protons to give either a positive or negative ion depending upon the proton affinity of the analyte species relative to the solvent gas plasma molecules.

This ionization mode is very ‘soft’ and rarely produces major fragmentation of the analyte ion – instead producing an intense molecular ion.

APCI interfacing for LC-MS is amongst the eldest approaches, initial work being carried out by Horning in 1974.\(^2\) However, due to the lack of commercially available instrumentation, the first wide-scale implementation of the technique didn’t take place until the early 1990’s.

**Suitable Samples for APCI**

The sensitivity, ruggedness and reliability of APCI put it ahead of electrospray ionisation techniques for many pharmaceutical and other applications. APCI appears to be much less sensitive to chemical interferences than electrospray-MS and the ionization process associated with APCI is one of the most efficient, approaching 100% under ideal conditions.

In the present example, the drug reserpine presents higher analyte response in APCI than in ESI at high ammonium acetate concentrations.\(^2\)
APCI finds most of its applications in molecular weights below 1000 Da for medium to low polarity molecules. The analytes will need some degree of volatility and should not be thermo labile. Examples of analytes suitable for APCI analysis include:

Polycyclic Aromatic Hydrocarbons (PAHs).\textsuperscript{[3]}

\textbf{Anthracene}

Phthalates.\textsuperscript{[4]}

\textbf{Di(2-ethylhexyl) phthalate (DEHP)}
Samples that contain hetero-atoms.\textsuperscript{[5]}

![Chemical structure of Zenarestat]

\textbf{Zenarestat}

Fatty acids.\textsuperscript{[6]}

![Chemical structure of Acid 9-hydroxi-10,12-octadecadiene]

\textbf{Acid 9-hydroxi-10,12-octadecadiene}

Triglycerides.\textsuperscript{[7,8]}

![Chemical structures of Oleic acid, Dodecanoic acid, and Trinolein]

\textbf{Trinolein}
Pesticides.\textsuperscript{[9]}

\begin{center}
\includegraphics[width=0.5\textwidth]{imidacloprid.png}
\end{center}

**Imidacloprid**

Samples that are thermally labile may decompose or react in the heated nebuliser of the source and should be avoided.

\begin{center}
\includegraphics[width=0.5\textwidth]{glutamine.png}
\end{center}

**Glutamine**

Samples that are typically charged in solution such as proteins, peptides, oligonucleotides or amino acids should also be avoided, as sensitivity will be much reduced.

\begin{center}
\includegraphics[width=0.5\textwidth]{pyroglutamylhistidilprolinamide.png}
\end{center}

**Pyroglutamylhistidilprolinamide (a tripeptide)**
APCI Interfacing Details

APCI interfaces usually consist of the following components:

- An HPLC system, delivering column eluent at flow rates between 0.5 and 2.0 mL/min
- A device for nebulising the column effluent and vaporisation of the produced droplets
- An ion source, containing a corona discharge electrode, which is capable of delivering the produced ions from the atmospheric into the high vacuum region of the mass spectrometer

In this respect, APCI ion sources differ from ESI ion sources in two major aspects:

- The inclusion of a heated region through which a gas is passed to vaporise eluent and sample components
- The inclusion of a corona discharge pin to produce a cloud of electrons that will initiate the chemical ionisation process in the vapour phase

Due to the essential similarity between ESI and APCI interfaces most modern LC-API-MS systems are equipped for both ionisation modes with a minimum of operator interaction or equipment modification. Although APCI is not as widely implemented as electrospray ionisation, a growing number of applications is reported within the literature.

**APCI elements**

This section will concentrate on the major differences between the APCI and the electrospray source which has already been discussed in detail.
Nebuliser Types

In contrast to electrospray ionisation, APCI ionisation occurs in the gas phase as a result of strong heating of the nebulised eluent. Several nebulising and vaporising devices are available from a variety of manufacturers.

The nebuliser described by Covey et al.\textsuperscript{[9]} is used extensively in modern instrument designs and consists of a concentric pneumatic nebuliser and a quartz vaporiser tube extending beyond the tip of the nebuliser capillary. In this system, flows of up to 1.5 mL/min. column eluent are nebulised with an inert gas (usually nitrogen) at pressures of approximately 0.8MPa. The aerosol created is swept through the heated nebuliser tube (usually 300 -500 °C), assisted by a make-up gas (nitrogen at 1 - 3 L/min., 0.5 MPa).\textsuperscript{[9,10]} This results in a vapour temperature of approximately 100°C, which achieves almost complete nebulisation of the aerosol components. The corona discharge electrode is positioned off axis, near the exit of the quartz vaporiser tube and this source type is available from Applied Biosystems (Foster City, Ca, USA).
APCI Pneumatic Nebulisers

Pneumatic nebulisers, comprising three concentric tubes (i.e. a liquid carrying capillary in the center, a nebuliser gas tube and an auxiliary gas tube), are used extensively. The nebuliser gas passes through a heater built into the tube and the pre-heated aerosol passes into a heated vaporiser region directly in front of the nebuliser to complete the vaporisation process. The corona electrode is placed axially and near to or within the heated vaporiser region of the source. This source design has been used by manufacturers such as Micromass (Wythenshawe, UK) and Thermo Finnigan (Riviera Beach, FL, USA).

In general the flow rates of gas used as nebulising, auxiliary, make-up and countercurrent flows in the APCI interface vary widely between instrument manufacturers. The flow rates of gas used will also vary depending upon the chemical nature of the eluent system. However, it should be noted that the required nitrogen supply could be as high as 600 L/h in some systems with a line pressure of 0.7 MPa, this will almost always require the Nitrogen supply to be directly pumped from a liquid nitrogen pressurised vessel or from a nitrogen generating unit supplied with high-pressure compressed air.

Ion Sampling and Transfer in APCI Interfaces

Ultimately the products of the APCI interface are gas phase ion products of eluent and analyte components, very similar to the products of the electrospray interface with the addition of eluent reagent ions such as large water clusters and heavily hydrated gas phase acids and bases.

Therefore, the hardware employed to de-cluster and transfer these gas phase ions to the high vacuum region of the mass spectrometer is essentially similar to that previously described for electrospray instruments.
The atmospheric pressure region terminates in an ion sampling opening (sampling orifice) or a transfer capillary (heated), which acts as a nozzle at the low-pressure face.

A region between the nozzle and a skimmer plate acts as a momentum jet separator and represents the first vacuum region of the instrument. Lenses may be employed in this region to promote the pre-concentration of analyte ions relative to neutral and low molecular weight ionic species arising from the eluent system.

An ion focusing device such as an RF only quadrupole, hexapole or octapole ion bridge is typically used to pre-concentrate analyte species and direct the ion beam onto an axial path prior to the mass analysing device. This region is usually pumped by an oil diffusion or turbo-molecular pump.

Some systems employ an extra skimmer to allow a second differentially pumped region of the instrument.
The effects of declustering analyte species require more attention with APCI than with ESI, as many of the gas phase analyte ions produced are clustered with water molecules and reagent ions derived from the eluent system. Declustering of analyte ions may be achieved using one, or a combination, of the following approaches:

- Using a counter current gas flow at the nozzle plate, also known as a curtain gas
- Using a heated transfer capillary between the API region and the nozzle-skimmer region

The charged species are desolvated due to the high temperature within the transfer tube.
• Using a drift voltage between the nozzle and skimmer plates, to promote intermolecular collisions between the ion clusters and background gas molecules (collision induced dissociation CID)

Solvent clusters formed without the analyte of interest have to be removed in order to prevent noisy baselines obscuring of the analyte signal. Increasing the drying gas flow rate and temperature in the main interface can reduce the formation of these clusters, however, too high drying gas flow rates will attenuate the signal from the analyte.\textsuperscript{[12]}

**Important:**

Ion declustering is important to prevent noisy baselines or obscured analyte signal. Increasing the curtain gas flow rate and increasing the temperature in the interface can reduce the presence of clusters, however very high values of these parameters may also attenuate the signal from the analyte of interest, due to analyte degradation or prevention from entering the mass analyser.

**APCI Ionisation**

**Positive Ion Mode**

APCI is based on chemical ionisation by ion-molecule or electron capture reactions carried out on gas phase products of the vaporised LC eluent at atmospheric pressure. APCI can occur in a number of ways, the following two mechanisms are perhaps the most prevalent:

• Proton transfer or charge exchange in the positive ion mode
• Proton abstraction or electron capture in the negative ion mode
Charge transfer with positive ions takes place between a reactant ion generated from a molecule possessing high ionisation potential, and a gas phase molecule with a lower ionisation potential. With a careful choice of the reactant ion, charge transfer can be a selective ionisation method. In positive ion mode benzene molecular ions have been used as reactant for selective ionisation of polychlorinated biphenyls. In negative ion mode the $\text{O}_2^-$ ion may be perfused into the API source to promote charge transfer reactions.

**Positive ion mode.**

If the analyte ($M$) has a larger proton affinity than the solvent ($S$), then the analyte will take a proton from the protonated solvent:

$$M + [S + H]^+ \rightarrow [M + H]^+ + S$$

If both species present similar proton affinities adducts are formed:

$$M + [S + H]^+ \rightarrow [M + S + H]^+$$

Ion formation by charge exchange:

$$M + S^+ \rightarrow M^{+*} + S$$

Remember:

The Proton affinity for the reaction:

$$M + H^+ \rightarrow MH^+$$

Is defined as the negative of the reaction enthalpy at 298.15K.

**Negative Ion Mode**

For the positive APCI mode, protic solvents work better than aprotic ones, while for the negative ion mode, solvents that capture electrons should be used. Volatile constituents (solvents, buffers, additives) in the mobile phase should be selected whenever possible (but this doesn't mean that non-volatile buffers can't be used).
Electron capture occurs if thermalised electrons are present in the API source, where suitable sample molecules are ionised by resonance electron capture, producing molecular anions, or by dissociative electron capture, producing negative fragment ions. Suitable analyte molecules present electronegative functional groups, such as carbonyl species, nitro groups, etc.

Gas Phase Reactions

The majority of ion-molecule reactions in APCI are gas phase acid-base type reactions. For positive ion operation the reactant ions are acids –protonated water, methanol and acetonitrile are all mild acids, and the ammonium ion is a weak selective acid. In negative ion mode, the reactant ions are bases –hydroxide ions are strong gas phase bases, CH₃O⁻ and NCCH₂⁻ are mild bases and CH₃COO⁻ and Cl⁻ are weak bases.

If reactant gases are mixed, protons are transferred to form the weakest possible reactant ion. If acetonitrile is added to water, H₂O⁺ transfers its proton to the acetonitrile gas phase ion. Similarly if ammonia is added to the methanol vapour, the initially formed protonated methanol transfers its proton to ammonia, giving NH₄⁺. It is not possible to convert a ‘weak’ reagent into a stronger one.
Gas phase acidities positive ion mode

Strong Acid

\( \text{H}_3^+ \) | \text{HYDROGEN} | \text{Weak Base}
--- | --- | ---
\( \text{CH}_5^+ \) | \text{METHANE} |
\( \text{N}_2\text{OH}^+ \) | \text{N}_2\text{O} |
\( \text{C}_2\text{H}_5^+ \) | \text{FORMALDEHYDE} |
\( \text{H}_3\text{O}^+ \) | \text{WATER} |
\( \text{HCO}_2\text{H}_2^+ \) | \text{FORMIC ACID} |
\( \text{CH}_3\text{OH}_2^+ \) | \text{METHANOL} |
\( \text{CH}_3\text{CNH}^+ \) | \text{ACETONITRILE} |
\( \text{t-C}_4\text{H}_9^+ \) | \text{ACETIC ACID / ACETONE} |
\( \text{NH}_4^+ \) | \text{PHENOL} |
\( \text{NH}_3 \) | \text{Iso - BUTANE} |
\( \text{VALINE, ANILINE} \) | \text{ETHYL ACETATE, DIETHYL ETHER} |
\( \text{METHYLAMINE} \) | \text{VALINE, ANILINE} |
\( \text{PYRIDINE, DIMETHYLAMINE} \) | \text{METHYLAMINE} |
\( \text{TRIMETHYLAMINE} \) | \text{VALINE, ANILINE} |
\( \text{TRIETHYLAMINE} \) | \text{METHYLAMINE} |
\( \text{TRIBUTYL AMINE} \) | \text{VALINE, ANILINE} |

Gas phase acidities negative ion mode

Strong Acid

\( \text{NH}_2^- \) | \text{NH}_3 |
\( \text{OH}^- \) | \text{WATER} |
\( \text{C}_6\text{H}_5\text{CH}_2^- \) | \text{TOLUENE} |
\( \text{CH}_3\text{O}^- \) | \text{METHANOL} |
\( -\text{CH}_2\text{CN} \) | \text{ETHANOL} |
\( -\text{CH}_2\text{CCH}_3 \) | \text{ACETONITRILE} |
\( -\text{CH}_2\text{CCH}_3 \) | \text{ACETONE} |
\( -\text{CH}_3\text{S}^- \) | \text{ACETONE} |
\( -\text{CH}_2\text{NO}_2^- \) | \text{ACETONE} |
\( \text{CN}^- \) | \text{ACETONE} |
\( \text{CH}_3\text{COO}^- \) | \text{ACETIC ACID} |
\( \text{CN}^- \) | \text{ACETIC ACID} |
\( \text{C}_6\text{H}_5\text{CO}_2^- \) | \text{ACETIC ACID} |
\( \text{HCl}^- \) | \text{ACETIC ACID} |

Weak Base

\( \text{Cl}^- \) | \text{HCl} |
\( \text{HCl}^- \) | \text{HCl} |
\( \text{HCN}^- \) | \text{HCl} |
\( \text{PHENOL} \) | \text{HCl} |
\( \text{ACETIC ACID} \) | \text{HCl} |

Weak Acid

\( \text{OH}^- \) | \text{HCl} |
\( \text{PHENOL} \) | \text{HCl} |
\( \text{ACETIC ACID} \) | \text{HCl} |
\( \text{ACETIC ACID} \) | \text{HCl} |
Reagent Gas Formation

The chemical ionisation reactions are initiated by means of electrons emitted from a corona discharge electrode kept at 5-10kV with a discharge current of 1-5μA. The energetic electrons released from the tip of the corona electrode, begin a series of reactions:

\[ N_2 + e^- \rightarrow N_2^+ + 2e^- \]

\[ N_2^+ + 2N_2 \rightarrow N_4^+ + N_2 \]

The major benefit to ionisation at atmospheric pressure is that all gas phase ions undergo significant collisions with surrounding gas phase molecules. This results in interactions of all surrounding gases (N\(_2\), H\(_2\), H\(_2\)O and air) and a cascade reaction is induced. The major resulting ions are N\(_2^+\), O\(_2^+\), H\(_2\)O\(^+\) and NO\(^+\) and in the presence of only trace amounts of water, cascade reactions are initiated that ultimately result in production of water cluster ions which predominate in the mass spectrum.
Relative Proton Affinity

In order to make some rudimentary predictions about the relative sensitivities of each analyte ion and any possible suppression effects attributable to eluent components such as volatile buffers, it is important to consider the relative proton affinity of the components.[16,17]

The optimum response region represented on the diagram is for analytes with greater proton affinity than water, but one must consider the proton affinity for ALL eluent components as the buffers and additives used may compete for protons with the analyte, potentially reducing sensitivity.[18]

Gas phase acid-base chemistry is of overriding importance in LC-APCI-MS where reactant gas mixtures are the rule rather than the exception.

Table 1. Proton affinity of selected organic molecules for APCI.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Proton affinity (kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>188</td>
</tr>
<tr>
<td>Acetone</td>
<td>202</td>
</tr>
<tr>
<td>2-Butanol</td>
<td>197</td>
</tr>
<tr>
<td>Dimethyl ether</td>
<td>190</td>
</tr>
<tr>
<td>Ethane</td>
<td>121</td>
</tr>
<tr>
<td>Ethyl formate</td>
<td>198</td>
</tr>
<tr>
<td>Formic acid</td>
<td>175</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>141</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>190</td>
</tr>
<tr>
<td>Methanol</td>
<td>182</td>
</tr>
<tr>
<td>Methyl amine</td>
<td>211</td>
</tr>
<tr>
<td>Methyl chloride</td>
<td>186</td>
</tr>
<tr>
<td>n-Propyl acetate</td>
<td>207</td>
</tr>
<tr>
<td>Toluene</td>
<td>187</td>
</tr>
<tr>
<td>Trifluoroacetic acid</td>
<td>167</td>
</tr>
<tr>
<td>Xylene</td>
<td>187</td>
</tr>
</tbody>
</table>

Table 2. Reagent Gas Proton Affinity

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Proton affinity (kcal/mole)</th>
<th>Reactant ion formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>100</td>
<td>$H_3^+$</td>
</tr>
<tr>
<td>Ethylene</td>
<td>160</td>
<td>$C_2H_5^+$</td>
</tr>
<tr>
<td>Water</td>
<td>165</td>
<td>$H_5O^+$</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>175</td>
<td>$CH_3CNH^+$</td>
</tr>
<tr>
<td>Methanol</td>
<td>182</td>
<td>$CH_3OH_2^+$</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>190</td>
<td>$t-C_3H_9^+$</td>
</tr>
<tr>
<td>Amonia</td>
<td>207</td>
<td>$NH_4^+$</td>
</tr>
</tbody>
</table>

The adequate selection of a suitable reagent gas permits in some cases the analysis of small polar compounds that are typically analysed by ESI.
Effect of Eluent Additives on APCI

Additives in the LC eluent used to improve separation may entirely change the chemical ionisation process. When eluents are used with additives and volatile buffers the APCI reactant ion spectrum is dominated by solvent-derived reactant ions and their clusters. For example, if ammonium acetate is added to the eluent, the reactant ions are ammonium and acetate ions, clustered with water, ammonia, acetic acid and other polar components in the eluent system.

Relative response of protonated ethylene C₂H₅⁺ (weakly basic sample) with the degree of hydration of the H₂O⁺ ion.

When water is the main component in the eluent system, the most abundant positive reactant ion is H₂O⁺ clustered with water molecules. It can be demonstrated that the gas-phase acidity of H₂O⁺(H₂O)ₙ decreases with increasing n. The ionization of weakly basic samples is less effective or even not possible if n is increased. As the vaporizer and interface temperature is increased, n decreases and the proton transfer efficiency will subsequently increase.

Signal Quenching

In APCI, due to possible charge exchange reactions methanol is usually a better choice than acetonitrile (which tends to reduce the sensitivity of the analysis). Samples with lower proton affinities than ammonia or triethylamine will often loose a proton, become neutralised, or form adducts in the gas phase and therefore their use should be carefully controlled.
One common problem with gas-phase acid base chemistry in negative APCI is signal quenching by acidic mobile-phase constituents. This effect is demonstrated opposite, in APCI negative ion mode, additions of 10 mmol/L formic acid or 10 to 100 mmol/L ammonium acetate to the LC eluent significantly decreased the response of the analyte of interest (nicotine in this case).[20]

The extent of the quenching (or signal suppression) is directly correlated to gas phase acidities and basicities. In general positive ion APCI is preferred over negative ion mode whenever this is possible.

Other Ionisation Mechanisms in APCI

A less well documented mechanism for APCI is derived via the triboelectric effect. As the mobile phase and analyte species exit the nebuliser, the sheer forces generated by the nebulising gas tear the liquid stream into droplets. The ‘friction’ generated by this process generates an electric charge (the triboelectric effect) at the liquid-gas interface. This can cause ion formation in some non-volatile analytes.
Triboelectric effect

Triboelectric APCI does not depend upon the electrons generated by the corona discharge electrode and ions may be generated (with their associated signals in the mass spectrum) when the corona discharge is very low or even turned off. Triboelectric APCI is most common for analyte molecules that are moderately polar and/or non-volatile.

Ion ejection is the mechanism that is responsible for most of the ion production in ESI-MS, but may also occur in APCI-MS. Some analyte molecules in the LC eluent will exist as ions in the solution before they reach the APCI ion source. After nebulisation the eluent droplets pass through the vaporiser tube or heated region and as they shrink due to solvent evaporation the pre-formed analyte ions concentrate at the droplet surface. From this point the mechanism of gas phase ion production closely matches that described in the ESI sections. This mechanism is common with analytes that are highly polar or ionic in solution and may be promoted or suppressed by adjusting the pH of the eluent solution.
In general APCI-MS interfaces are considered very simple to operate. Choosing the optimum settings for the more important interface parameters is in general less critical than for electrospray interfaces but choosing the wrong parameters could have a serious impact on your application. The case study presents an unrecognizable spectrum for the drug ibuprofen when the APCI parameters are not optimized.\(^{[21]}\)

Of all the parameters that may be optimised within the APCI, the vaporizing probe temperature has perhaps the most profound effect on signal intensity.\(^{[22]}\)

Some compounds that are normally charged in solution (such as penicillin and some dyestuffs) will be less volatile in typical reverse phase eluent systems, due to high intermolecular attractions between charged analyte species and polar eluent constituents. In these cases pH cannot be used to ensure that the analyte is neutral in solution, and the interface of choice will be ESI.
Higher molecular weight species are non volatile and therefore will not be efficiently ionised in the gas phase at the corona electrode. In these cases, the ionisation method of choice would be ESI. In general, higher molecular weight species such as peptides and proteins are not suitable for APCI analysis due to the extremely high temperatures required for vaporisation in the region immediately after the nebuliser.

**Comparison of API Ionisation Techniques**

**Table 3. ESI versus APCI**

<table>
<thead>
<tr>
<th>Samples that work well</th>
<th>ESI</th>
<th>APCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Samples that are multiply charged in solution such as proteins, peptides, and oligonucleotides</td>
<td>• Small, polar to non-polar molecules such as PAHs, PCBs, fatty acids, phthalates, and steroids</td>
<td></td>
</tr>
<tr>
<td>• Other samples that are ions in solution such as catecholamines, sulfate conjugates, and quaternary amines</td>
<td>• Samples that contain heteroatoms such as ureas, carbamates, and benzodiazepines</td>
<td></td>
</tr>
<tr>
<td>• Samples that contain heteroatoms such as carbamates and benzodiazepines</td>
<td>• Samples that can be analyzed by thermospray LC/MS or particle beam LC/MS</td>
<td></td>
</tr>
<tr>
<td>• Samples that can be analyzed by thermospray LC/MS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples to avoid</th>
<th>ESI</th>
<th>APCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Extremely non-polar compounds such as PAHs and PCBs</td>
<td>• Non-volatile compounds</td>
<td></td>
</tr>
<tr>
<td>• Samples that have preformed, multiply-charged ions in solution</td>
<td>• Samples that are thermally unstable</td>
<td></td>
</tr>
</tbody>
</table>

**Matrix and mobile phase effects.**

<table>
<thead>
<tr>
<th>ESI</th>
<th>APCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• More sensitive to sample matrix and mobile phase composition than APCI</td>
<td>• Sensitive to sample matrix and mobile phase composition but less sensitive than ESI</td>
</tr>
<tr>
<td>• Requires low concentrations of even very volatile buffers relative to APCI</td>
<td>• More tolerant of volatile buffers than ESI</td>
</tr>
<tr>
<td>• Forms adducts with cations (Na+, K+) and anions (Cl−, COO−)</td>
<td>• Organic solvents and additives affect ionization</td>
</tr>
</tbody>
</table>

**Solvent.**

<table>
<thead>
<tr>
<th>ESI</th>
<th>APCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Solvent pH has a major effect for analytes that are ions in solution</td>
<td>• Solvent choice is very important and will affect ionization processes</td>
</tr>
<tr>
<td>• Basic pH for negative ions</td>
<td>• Solvent pH has some effect on ionization efficiency</td>
</tr>
<tr>
<td>• Acidic pH for positive ions</td>
<td>• Basic pH for negative ions</td>
</tr>
<tr>
<td>• pH manipulation can enhance performance for analytes that are not normally ionized in solution</td>
<td>• Acidic pH for positive ions</td>
</tr>
</tbody>
</table>

**Flow rates.**

<table>
<thead>
<tr>
<th>ESI</th>
<th>APCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Works well at low flow rates (&lt;100 µL/min)</td>
<td>• Does not work well at low flow rates (&lt;100 µL/min)</td>
</tr>
<tr>
<td>• Does not work as well as APCI at high flow rates (&gt;750 µL/min)</td>
<td>• Works better than ESI at high flow rates (&gt;750 µL/min)</td>
</tr>
</tbody>
</table>
References

22. Data reproduced from Agilent Technologies 1100 LCMSD, (1998), Palo Alto, CA, USA.