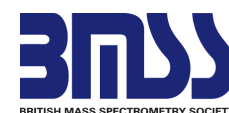


focus on Mass Spectrometry & Spectroscopy

Ambient Ionisation in Mass Spectrometry

Andy Ray and Jackie Mosely, www.BMSS.org.uk



Ambient ionisation mass spectrometry allows the rapid analysis of samples or objects in their native state in the open environment with no prior preparation. The ability of these techniques to provide selective analyte desorption and ionisation in combination with mass spectrometry (MS), has provided a growing number of powerful analytical alternatives across broad application areas, both quantitative and qualitative in nature, including pharmaceutical analysis, process chemistry, biological imaging, in vivo analysis, proteomics, metabolomics, forensics, and explosives detection. The commercial availability of DART, DESI and ASAP have enabled these techniques to be widely adapted within many industries. However with the emergence of new ambient ionisation methods, and the complementary nature of existing desorption and/or ionisation techniques, additional hyphenated methods have been devised, which pushes the total number of documented methods to almost thirty, these often combine separate desorption and ionisation processes in a single method.

The commercial availability of atmospheric pressure ionisation (API) sources, such as electrospray ionisation (ESI), has given greater flexibility within mass spectrometric analysis, and has overcome a number of issues in bridging the gap between the prepared sample and its introduction into the mass spectrometer vacuum system. The distinction between atmospheric pressure ionisation and ambient ionisation is vital in gaining clearer understanding of the impact of these new methods. Whilst ambient ionisation methods often have operational commonality with atmospheric pressure ionisation, techniques that fall strictly under the ambient ionisation banner display a number of basic characteristics; namely the direct analysis of untreated samples or objects in the open environment, whilst largely maintaining the native condition and spatial integrity of the sample. Here, analyte molecules derived from the sample, but not the whole sample itself, are transferred into the mass spectrometer. These rapid techniques offer unprecedented flexibility in sample analysis in the open environment, often remote from the mass spectrometer, and have made a significant impact on the world of analytical science. The term Direct Analysis has been used in relation to these techniques to distinguish from API techniques but the British Mass Spectrometry Society's (BMSS) Special Interest Group (SIG) (http://www.bmss.org.uk/SIG_ambient-ion.shtml) feels that Ambient Ionisation is the correct term to be used.

Boundaries between ambient ionisation techniques can be somewhat blurred, however to remain consistent with the initial disclosure of each technique, similar methods are classed as separate techniques, but can be discussed together under the same general mechanism. A broad classification based upon the technique(s) having the most influence on the resultant mass spectra (e.g. ESI type spectra for DESI) allows effective comparison of mechanisms.

ESI or Spray-Related Techniques (DESI, EESI, ND-EESI)

A simple assessment of the number of papers published since the invention of DESI by the Cooks group demonstrates the broad utilisation of this technique across a number of diverse application and analyte types, for both small (e.g. drugs) and large (e.g. proteins) molecule applications. This belies an inherent ease of incorporation, where often little modification of existing mass spectrometer hardware is required. Indeed, many early applications of DESI relied upon only slight modification to vendor-standard ESI sources. Commercial DESI sources are now available which helps make the technique amenable to incorporation in an industrial setting.

In the DESI process, a spray of charged micro-droplets from an pneumatically-assisted electrospray needle is directed towards the object or analyte of interest in the ambient environment and allowed to impact the surface, giving desorption of the analyte into the gas phase and subsequent ionisation, prior to being sampled by the mass spectrometer or ion collection device. The incident angle of the spray plume (relative to the sample) has been investigated primarily to maximise analyte response and selectivity, but also to help elucidate contributing or competing mechanisms for sample ionisation, often being optimised around 45 – 55° with shallow ion-collection angles (collector relative to the sample) of around 10°. Capillary-tip-to-sample and sample-to-collector distances, whilst largely dependent upon instrumental configuration, sample type and desired outcome (e.g. reduction of source fouling vs. maximised sensitivity), play a crucial part in devising a successful DESI experiment.

Variations on ESI spray-based techniques, have afforded a number of alternative methods, where subtle experimental changes have given shifts in analytical performance. For example, neutral desorption extractive electrospray (ND-EESI), a variant upon the extractive electrospray ionisation (EESI) method. ND-EESI relies upon initial desorption of sample molecules into a neutral gas stream, rather than a charged solvent stream used in EESI, which coincides with an ESI plume to give ESI-like sample spectra. The major benefits of ND-EESI are non-proximate

sample introduction, the removal of voltages from the ambient sampling process, and increased selectivity over conventional solution-based ESI, particularly for low molecular weight analytes. Similarly, the removal of spray voltages used in the DESI experiment have given rise to desorption extractive electrospray ionisation (DeESI), which is now called easy ambient sonic-spray ionisation (EASI). The use of a micro-junction interfaced to electrospray (LESA) has shown promise as a hyphenated method where the sampling and ionisation are separate processes and may overcome some of the issues associated with MALDI imaging.

Paperspray ionisation has recently been shown where ions are generated by applying a high voltage to a paper triangle wetted with a small volume of solution. Samples can be preloaded onto the paper, added with the wetting solution or transferred from surfaces using the paper as a wipe.

Spray-Based Photon/Energy Techniques (ELDI, MALDESI)

The disclosure of spray-based ionisation techniques that are combined with laser ablation sample desorption, have given rise to methods such as electrospray laser desorption ionisation (ELDI) and matrix-assisted laser desorption electrospray ionisation (MALDESI). Recent ELDI experiments have shown the use of femtosecond laser pulses combined with ESI post-sample desorption. These methods provide additional selectivity and scope, compared with conventional LDI experiments. Limitations in spatial resolution due to addition of matrix can be an issue for MALDESI and, technically, one could argue that addition of matrix (i.e. prior sample preparation) might preclude MALDESI from being classified as an ambient ionisation method. Recently, the advent of desorption atmospheric pressure photo-ionisation (DAPPI), has offered selectivity and soft ionisation, particularly for neutral, non-polar or highly-conjugated compounds by using dopants.

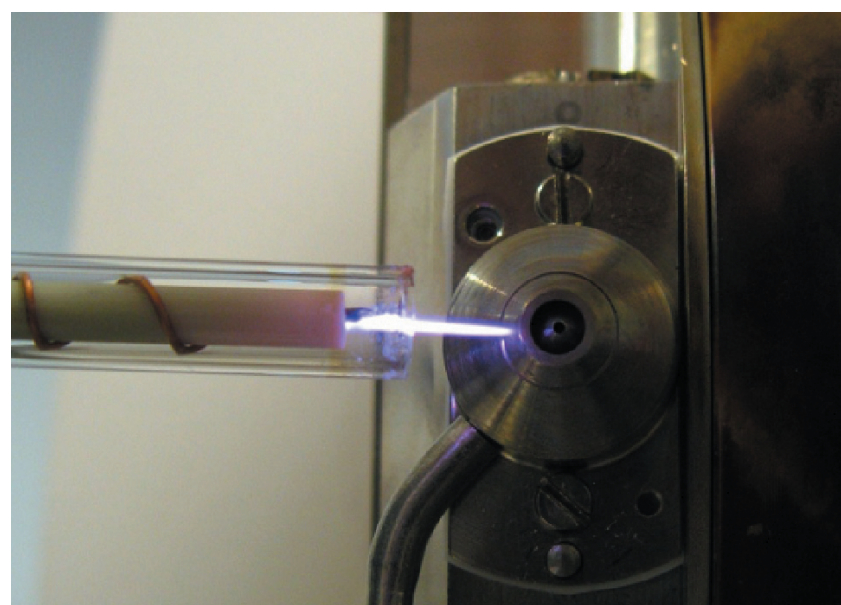


Figure 1. DART Ion Source (Direct Analysis in Real Time - Courtesy Peter Ryan, KR Analytical)

APCI-Related Techniques (DART, DAPCI, ASAP)

The increasing number of ambient ionisation techniques using an electrical discharge to effect ionisation, either before, during, or after sample desorption, can be broadly categorised as relying upon fundamental principles of atmospheric pressure chemical ionisation (APCI). These solvent-free systems include, for example, direct analysis in real time (DART), atmospheric solids analysis probe (ASAP), direct atmospheric pressure chemical ionisation (DAPCI), plasma-assisted desorption/ionisation (PADI), dielectric barrier discharge ionisation (DBDI), helium atmospheric pressure glow discharge ionisation (HAPGDI) or flowing afterglow flowing atmospheric-pressure afterglow (FAPA), surface activated chemical ionisation (SACI), and low temperature plasma (LTP). Typically, when used in LC-MS, APCI techniques are amenable to polar and non-polar, low molecular weight analytes and their transposition to ambient ionisation is no exception. As a general rule, these techniques do not usually extend to the analysis of larger (bio) molecules, much like the traditional APCI methods upon which they are based.

As with many APCI-based techniques, DART relies upon the formation of a (distal) plasma discharge in a heated helium gas stream. These mechanisms have been attributed to possible Penning ionisation or proton transfer from excited solvent (water) clusters, largely understood through early fundamental mass spectrometry, to give simple APCI-like spectra. It is proposed that a series of metastable helium atoms react with water molecules via chemical ionisation processes, and downstream ionisation of the sample occurs by thermal desorption into the hot gas stream, and subsequent sampling by the mass spectrometer.

In ASAP the effect of solvent conditions and source dryness can affect whether a conventional proton-transfer ionisation occurs or a preferential charge-transfer ionisation due to a reduction in available reagent species, such as residual water.

When considering applicability of the DART method, perhaps the main drawback is a lower effective mass range, which, although a general function of APCI-based ionisation mechanisms, is a clear disadvantage compared to DESI, where successful analysis of proteins up to 66 kDa have been reported. That said, DART mass spectra are often more simple to interpret, due to lack of gas-phase adduction from the solvent environment, as seen with ESI-based counterparts such as DESI.

The emergence in the last few years of a series of new plasma-based techniques, such as LTP, PADI and FA-APGD, have demonstrated a great deal of finesse, in particular for the potential removal of a number of issues often precluding a safe and elegant *in vivo* ambient ionisation experiment (e.g. elevated temperature, exposure to high voltages). These techniques rely upon ionisation at the sample surface using a proximate plasma source, rather than a distal plasma such as used in DART, and effect APCI-like ionisation. PADI uses a radio-frequency generated plasma for desorption and ionisation of target analytes. The temperature of the plasma approximated that of the ambient environment, which could be of use for thermally-labile samples, however the sample was still subjected to the actual discharge. A recently disclosed plasma-based method showing increasing applicability is the low temperature plasma (LTP) technique; here instead of the physical restrictions of placing the sample between two counter electrodes, as in conventional DBDI, the plasma is generated in a probe configuration allowing increased flexibility for nonproximate analysis of bulk samples or large objects, akin to a DESI experiment.

The field of ambient MS has progressed rapidly in the last few years, with key advances in both technological and mechanistic understanding creating new directions, which often seem limited only by the imagination of the scientist. Arguably, this area of science could be described as being in its formative years and recommendations have been made for closer investigation of sampling heterogeneity, ion suppression effects, and a critical evaluation of areas for improvement. It is for these reasons that the BMSS Ambient Ionisation SIG has been formed. The aims of the Ambient Ionisation SIG are to survey the level of use of ambient ionisation across BMSS, to support practitioners of ambient ionisation MS, demonstrate the applicability of the different techniques to different application areas, investigate sampling heterogeneity towards better quantitative analysis and understanding suppression/enhancement effects. The Ambient Ionisation SIG has surveyed BMSS members as to what techniques they are currently using and in what application areas. This information is currently being used to develop an Inter-lab comparison across as many techniques as possible in the most important application areas. The comparison will involve running a series of samples (which will be supplied) under test conditions which we would hope takes less than a day. The results of this will be fed back at a one day meeting to be held later in 2013.



Figure 2. PADI (Plasma assisted photon desorption/ionisation - Courtesy Dave Barrett, University of Nottingham)

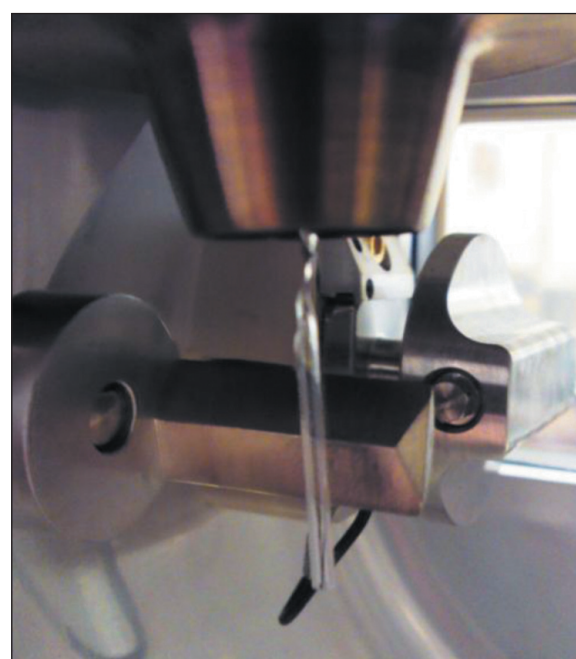


Figure 3. Courtesy of Peter Stokes, Durham University.

This article was originally published in the British Mass Spectrometry Society's Mass Matters, 67th Ed. April 2012, pp13-14.

Thanks to Dan Weston and the RSC for permission to use abstracts from The Analyst 2010 Apr. 135(4) 661.

ESI OR SPRAY RELATED TECHNIQUES	SPRAY-BASED PHOTON/ENERGY TECHNIQUES	APCI-RELATED TECHNIQUES
Desorption electrospray ionisation (DESI)	Electrospray Laser Desorption Ionisation (ELDI)	Direct Analysis in Real Time (DART)
Extractive Electrospray Ionisation (EESI)	Matrix-Assisted Laser Desorption Electrospray Ionisation (MALDESI)	Atmospheric Solids Analysis Probe (ASAP)
Neutral Desorption Extractive Electrospray Ionisation (ND-EESI)	Desorption Atmospheric Pressure Photo Ionisation (DAPPI)	Direct Atmospheric Pressure Chemical Ionisation (DAPCI)
Easy Ambient Sonic Spray Ionisation (EASI)	Laser Ablation Electrospray Ionisation (LAESI)	plasma-assisted desorption/ionisation (PADI)
Jet Desorption Electrospray Ionisation (JeDI)	Infra Red Laser Ablation Electrospray Ionisation (IR-LAESI)	dielectric barrier discharge ionisation (DBDI)
Liquid Extraction Surface Analysis (LESA)	Laser Desorption Atmospheric Pressure Chemical ionisation (LD-APCI)	helium atmospheric pressure glow discharge ionisation (HAPGDI)
Paperspray		flowing afterglow flowing atmospheric-pressure afterglow (FAPA)
		surface activated chemical ionisation (SACI)
		low temperature plasma (LTP)
		Laser Diode Thermal Desorption (LDTD)

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