ORIGINAL RESEARCH PAPER

Chemistry

ATMOSPHERIC PRESSURE PHOTOIONIZATION MASS SPECTROMETRY & ITS APPLICATION

KEY WORDS:

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ABSTRACT

Atmospheric pressure photoionization (APPI) is used as a novel ionization method for liquid chromatography-mass spectrometry (LC-MS), which was originally developed to widen the group of analytes that can be analyzed by LC-MS towards less polar compounds. APPI mass spectrometry is popular due to short analysis times, low solvent consumption and the lesser amounts of sample that are required for sensitive analysis. It is being used in combination with other mass spectrophotometer. It is more superior to ESI and APCI but still have some disadvantages. In this paper study is done regarding background, history, parameter and their optimization, application of APPI. Study depicts the needs research area in which still improvement is needed to widen its applicability and future development.

INTRODUCTION

Mass spectrometry has become the ubiquitous research tool. Mass spectrometry is used to determine atom and molecule, molecular weight which is useful in determining the identity of a species[1]. Four basic components which are standard in all mass spectrometers includes a sample inlet, an ionization source, a mass analyzer and an ion detector some with combination of ionization source, while others combine the mass analyzer and the detector. Sample molecules are introduced into the instrument through a sample inlet and converted to ions in the ionization source, then electrostatically propelled into the mass analyzer to separate them according to their m/z within the mass analyzer[2].

Fig 1- Basic instrument of Mass Spectrometer.

History And Comparison Of Different Type Of Mass Spectra

Development of mass Spectrometry: Thomson's protégé, Francis W. Aston from University of Cambridge, designed a mass spectrometer in which ions were dispersed by mass and focused by velocity--which improved MS resolving power by an order of magnitude over the resolution. **Dempster** developed the first electron impact source, which ionizes volatilized molecules with a beam of electrons from a hot wire filament[3]. In the 1940s Model 21-101 analytical mass spectrometer, manufactured by Engineering Corporation Pasadena, Calif. The magnetic sector type instrument was developed by Professor **Alfred O. C. Nier**. One type of double-focusing instrument was developed in the 1930s by professor **Josef Mattauch** from University of Vienna, Austria, and another Magnetic deflection instruments--both single-focusing and double-focusing was developed[4,5,6]. TOF MS was proposed in 1946 by **William E. Stephens** of the University of Pennsylvania. The cheaper time-of-flight (TOF), quadrupole, and ion trap mass spectrometers design was developed by **Nier** and physicist **E. G. Johnson,** of the University of Minnesota. TOF MS is fast, it is applicable to chromatographic detection, and it is used for the determination of large biomolecules. It is a time-lag focusing scheme that improved mass resolution by simultaneously correcting for the initial spatial and kinetic energy distributions of the ions[7]. Mass resolution was also greatly improved by the 1974 with invention by **Boris A. Mamyrin.** The direct coupling of gas chromatography (GC) and TOF MS was achieved in the mid-1950s by **Roland S. Gohlke and McLafferty.** Time of flight shows good scan and sensitivity, high resolution which provides high selectivity through exact mass measurement. Moreover, it is having good dynamic, mass range in TOF is 20,000 m/z. But It's sensitivity is lower than triple quadrupole. While Q-TOF shows good full scan sensitivity. It is having ability to get quantitation on multiple analytes during a single run. Resolution not affected by increased scan speed but have lower sensitivity when compared to a triple quadrupole running[8]. In 1974, **Melvin B. Comisarow and Alan G. Marshall** revolutionized ICR by developing Fourier transform ICR mass spectrometry (FT-ICR MS). The major advantage of FT-ICR MS is that it allows many different ions to be determined at once, instead of one at a time[9]. GC was coupled to a magnetic sector instrument by **Joseph C. Holmes and Frank A. Morrell**. Quadrupole mass spectrometers are not as accurate and precise as double-focusing instruments, they are fast, which is important for GC detection. Quadrupole ion trap was developed by **Paul** which can trap and mass-analyze ions using a three-dimensional quadrupolar radiofrequency electric field. GC detector design technology was developed by **Finnigan**. Tandem MS instrument is the triple quadrupole mass spectrometer was invented at Michigan State University by **Richard A. Yost**. Triple quadrupole shows good scan function sensitivity with MRM, also with matrix; its mass range is up to 3000 m/z[10]. Orbital trapping devices can have a limited dynamic range and having mass range of 4000 m/z[11].

Development in ionization techniques:

The classic methods of ionization are not used much with modern mass spectrometry except EI for environmental work using GC-MS. More modern techniques of atmospheric pressure chemical Ionization (APCI) , electrospray ionization (ESI), matrix assisted laser desorption ionization (MALDI) and other derivative methods have taken their place in the mass spectrometry laboratory[12].

Table 1- Conventional ionization methods[12]

Atmospheric Pressure Ionization

In atmospheric pressure ionization, sources are designed to operate at atmospheric pressure and high vacuum in the rest of the instrument. Ionization takes place outside vacuum region. Sample must be in solution. Series of skimmers and flow restrictors are placed between the source and the mass analyzer region allow ions to be efficiently transmitted to the high vacuum region while at the same time allow air, solvent vapors and other neutral volatile species to be pumped away[13]. (Fig 2)

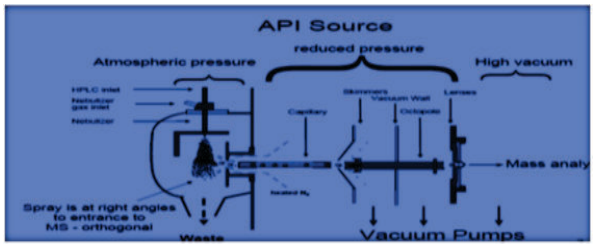


Fig 2- Instrument for Atmospheric Pressure Ionization

The type of atmospheric pressure ionization source are:

Electrospray ionization

Electrospray (ESI) –Assisted pneumatically in which flow rate is being controlled which include: High flow rate (100□L/min –1mL/min) , Capillary flow rate (2□L/min -100□L/min) , Low flow rate (<2□L/min) , Nano spray (200-500nL/min): In Electrospray ionization (ESI) sample in solution form passed through needle biased at several kV z . Charged droplets pass through capillary where solvent evaporates and molecules begin to inherit the droplets charge z Droplets shrink until their surface tension can no longer support the charge density—Rayleigh limit z Coulombic explosion occurs and the droplet bursts into smaller droplets that repel each other z Process repeats until all of the solvent in gone and analytes are multiply charged. The ions formed in ESI are typically protonated molecules ([M+H]+) or adducts (i.e. [M+NH4]+, [M+Na]+) in positive ion mode and deprotonated molecules ([M-H]-) in negative ion mode[14]. The sensitivity achieved by using ESI is excellent and, ionization takes place without heating, so large thermolabile molecules can be analyzed. However, because of the nature of ESI, it is best suited to ionization of polar compounds that can already be charged in the solution. Another disadvantage of ESI is signal suppression, which is caused by competition of the charge between the analytes and different solvent species. . This limits the use of buffers and demands careful sample preparation in case of complicated sample matrices[15].

Atmospheric pressure chemical ionization

The ion source for atmospheric pressure chemical ionization (APCI) was first developed by **Horning *et al*.** They introduced the sample either as gas in a solvent-free fashion or with solvent, which was vaporized together with the sample. The ionization took place by chemical ionization (CI) reactions in an atmospheric pressure reaction chamber external to the low-pressure region of the mass spectrometer. In the first design, the ionization reactions were initiated by electrons emitted by a 63Ni-foil. The 63Ni-later replaced by a corona discharge needle, which created a similar spectrum, but had a much higher reactant ion intensity and thus a larger dynamic response[16].

After the ionization takes place via gas-phase ion-molecule reactions, such as proton transfer and charge exchange. Proton transfer takes typically place when the analyte in question has high proton affinity (PA), whereas for charge exchange the analyte should possess low ionization energy (IE). Ionization through charge exchange can also utilized in the analysis of low PA and neutral compounds. Molecular ions are also very reactive and are therefore easily neutralized in ion-molecule reactions with other gas-phase components. Consequently, the ionization efficiency for non-polar analytes is usually poor in APCI. In addition, APCI often suffers from high background, because of efficient ionization of high PA gases, solvents and impurities. Because of the heat required in the vaporization process, only relatively small and stable compounds up to about 1000-1500 Da can be analyzed[17].

From the above study, it is determined that both ESI and APCI is that they can generate background ions from solvents. Additionally, ESI is especially susceptible to ion suppression effects, and APCI requires vaporization temperatures ranging from 350-500° C, which can cause thermal degradation. These cannot be used for low non-polar compound so there was rise in new technology called atmospheric pressure photoionization.

Atmospheric Pressure Photoionization Mass Spectrometer

Atmospheric pressure photoionization (APPI) is used as a novel ionization method for liquid chromatography-mass spectrometry (LC-MS), which was originally developed to widen the group of analytes that cannot be analyzed by ESI and APCI i.e. less polar compounds. Atmospheric pressure photoionization utilizes a discharge lamp that generates vacuum-ultraviolet (VUV) photons. Photons are absorbed by a species that has ionization energy (IE) below the energy of the photons, then single-photon ionization occur[18]: $M + h\nu \rightarrow M+. + e-$

An alternative reaction route may take place, if a carrier gas, such as nitrogen, is used, that strongly absorbs the VUV radiation:

$N2 + h\nu \rightarrow N2^*$ / $N2^* + M \rightarrow N2 + M+. + e-$

The photo ions formed by the two mechanisms generate a current, which flows through a collection electrode and forms the signal in the chromatogram. The lamp is usually selected so that the energy of the photons is below the IE of the carrier gas and above the IE of the analytes.

Thus, typical analytes usually possess IEs in the range of 7-10 eV, whereas the common GC carrier gases have higher IE values. A krypton discharge lamp that emits 10 eV and 10.6 eV photons is used. Using APPI, at an LC flow rate of 200 □L/min, it is possible to obtain analyte signal intensities 8 times as high as those obtainable with a commercially available corona discharge-atmospheric pressure chemical ionization source[19].

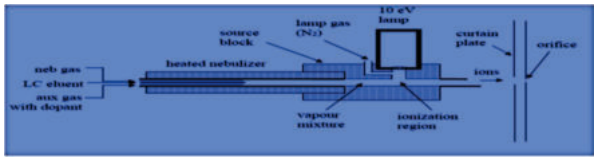


Fig 3: Atmospheric pressure photoionization mass spectra:

Principle:

The excitation of electrons in atoms and molecules by the absorption of one or more photons can be sufficient for the spatial separation of the electron and the atom or molecule. In the gas phase, this process is called photoionization[20]

*Ionization energy depicts amount of energy that needs to be absorbed by an atom or molecule in its electronic and vibrational ground states to form an ion that is also in its ground states by ejection of an electron (Range of IE is 7-15eV).

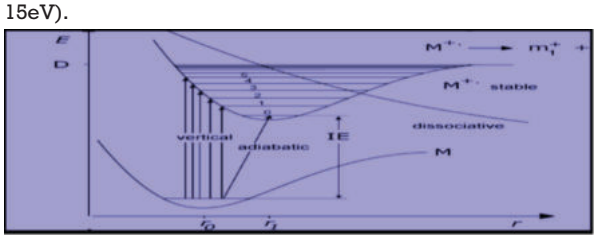


Fig 4: Graphs Depicting ionization and dissociation energy.

A diatomic molecule has only one vibrational motion, its bond stretching vibration, and therefore its potential energy can be represented by potential energy curves rather than potential energy surfaces. The minimum of the potential energy curve of the neutral, which is assumed to be in its vibrational ground state, is located at shorter bond length, r0, than the minimum of the radical ion in its ground state, r1.

The combined energy of the absorbed photons in this process must be above the ionization potential of the atom or molecule. A single photon has sufficient energy to overcome the ionization potential.

Background:

Photons were one of the first energy sources used to generate ions for mass spectrometry by **(Ditchburn and Arnot, 1929)**[21]. The APPI source was designed by **Bruins *et al*.** was

developed with the aim to broaden the range of compounds that cannot be analyzed by ESI and APCI like less polar compounds. Photoionization (APPI) sources were introduced for use on Liquid Chromatography-Mass Spectrometer (LCMS) instruments by **Robb and Syage in 2000. Revel's Skii** was first to connect mass spectrometry and APPI as the 63Ni source of a heated nebulizer was replaced with a photoionization lamp[22].

Instrumentation

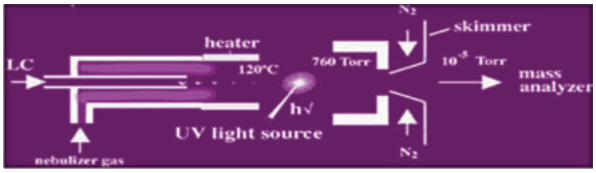


Fig 5: Instrument designed by Bruins *et al*

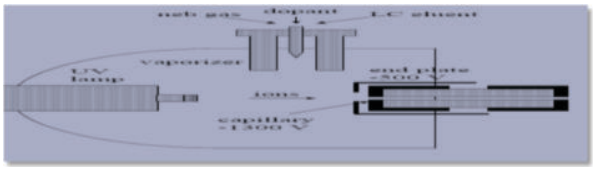


Fig 6: Syage *et al* also known as Photo Mate

Comparison between two instruments:
Both instrument work under same principle, the source used in both are similar except that the corona discharge needle is replaced by a krypton discharge lamp emitting 10 eV photons. Syagen source can achieve significant ionization also without the presence of dopant, due to greater lamp radiance output[23]. Because of a brighter lamp, direct photoionization of the analytes can be obtained, although the sensitivity is mostly improved when dopant is used. Syage's differ in geometry as it is orthogonal i.e. it is at right angle position)

Working of atmospheric pressure photoionization mass spectrophotometer:
In the instrument, UV light photons are used to ionize sample molecules. The technique works well with nonpolar or low-polarity compounds not efficiently ionized by other ionization sources. First the sample (analyte) is mixed with a solvent. Depending on the type used, the solvent could increase the number of ions that are formed. The liquid solution is then vaporized with the help of a nebulizing gas such as nitrogen, Helium, carbon dioxide, and compressed air were evaluated as discharge gases to generate photons then enters an ionization chamber at atmospheric pressure[24]. There, the mixture of solvent and sample molecules is exposed to ultraviolet light from a krypton lamp. The photons emitted from this lamp have a specific energy level (10 electron volts, or more) that is high enough to ionize the target molecules, but not high enough to ionize air and other unwanted molecules. So only the analyte molecules proceed to the mass spectrometer to be measured. The photons can ionize compounds that possess ionization energies (IEs) below their energy (10 eV), which includes; mostly larger molecules, possible analytes. Leaves out most of the typically used gases and solvents. Analytes can be ionized selectively, with minimum background interference. The ionization of the analytes is dependent on the IE of the analyte, rather than its proton affinity (PA) like with ESI and APCI, the ionization of molecules of low polarity is also possible[25].

Factors affecting Efficiency[25,26,27,28]:
Ionization Energy:
The Ionization energy of a compound is dependent on the size and the structure of the compound. Large compounds and compounds that possess a high degree of conjugation typically have lower IEs than small and aliphatic molecules i.e. 7-10 eV. Table depicting ionization energy given below:

Table 2: Ionization energy

IONIZATION ENERGY					
Toluene	8.83	Acetonitrile	12.20	n-Hexane	10.13
Benzene	9.24	Water	12.62	Methanol	10.84
Acetone	9.70	Nitrogen	15.58	Carbon dioxide	13.78
Ammonia	10.07	Oxygen	12.07		

Nebulization
The nebulizer consists of three concentric tubes, the eluent is pumped through the inner most tube and nebulizer gas and make-up gas through the outer tubes. The combination of the heat and gas flow desolates the nebulized droplets, producing dry vapor of solvent and analyte molecules. It depends on flow rate and temperature of nebulization gas. The signal loss was thought to be due to nebulization of the dopant molecular ions as the number of neutralizing species in the ion source increased

Desolvation :
When the droplet enters into desolvation zone, it will shrink due to evaporation. It is being aided by heated nitrogen.

Solvent choice:
The best ionization efficiency for all analytes was obtained in neutral and basic solvents, Solvents that formed low PA reactant ions (acidic solvents and ammonium acetate). Solvents of positive electron affinity (EA) deteriorated the ionization efficiency of the analytes significantly.

Additive Choice and Concentration:
Additive may change the ionization process due to cluster formation.

Flowrate:
Solvent flow rates that are too high may reduce the sensitivity due to neutralization of the dopant molecular ions and/or loss of photons. Best sensitivity is achieved at liquid flow rates around 200mL/min. Low flow rates, an enough the dopant must be present to ensure efficient ionization. Non-polar analytes, which are ionized through charge exchange, tend to be more dependent on the amount of dopant molecular ions than polar, high PA analytes and therefore require more careful optimization of the analysis conditions. Overall signal was often found to decrease due to increasing flow rate, in some cases the signal-to-noise ratio (S/N) of the analytes increased. So, optimization is needed.

Adduct formation:
Adduct ion. formed by the interaction of a precursor ion with one or more atoms or molecules to form an ion containing all the constituent atoms of the precursor ion as well as the additional atoms from the associated atoms or molecules. It can reduce the overall efficiency of APPI mass spectrometer

Ion suppression from matrix:
Ion suppression is one form of matrix effect when used with liquid chromatography–mass spectrometry (LC–MS) techniques suffer from, regardless of the sensitivity or selectivity of the mass analyzer used. Ion suppression negatively affects several analytical qualities such as detection capability, precision, and accuracy.

Dopant addition:
Dopants of very low IE could perhaps increase the number of thermal electrons in the system and thus lead to increase in the overall ionization efficiency in negative ion mode. Different dopants might also affect the ionization routes, such as the formation of substitution products. To increase the likelihood of analyte ionization, a chemical can be doped into the reaction chamber to act as a charge carrier. Variety of discharge gases used for photoionization lowers the cost of construction and improves the ruggedness of photoionization

ionization sources.

Effect of a micro-APPI source to overcome flow effect- Efficient ionization of the analytes was achieved in positive and negative ion modes Development of the micro-APPI source, which can be used at very low flow rates makes possible the combination of micro/nano-LC or microfluidic devices to MS. Furthermore, the manufacturing costs of micro-APPI are much lower. The long-term stability of the micro-APPI source, as well as the capability of analyzing polar and non-polar analytes from very small sample volumes are advantages over present ESI chips and make the micro-APPI a considerable alternative for interfacing low flow rate separation systems

Optimization of factors are required which includes optimizing flow rate, dopant concentration, type of solvent used and temperature, current Ionization is most efficient at flow rate of 1-5 µl/min, the ionization efficiency in APPI decreases as the solvent flow rate is increased. A flow rate of the dopant is about 1/10 of the solvent flow rate, which in turn is typically 100-300 µl/min. Current inside the ion source decrease as the solvent flow rate was increased, at constant dopant concentration.

Types Of Ionization In Appi Mass Spectra:

Direct APPI: two types of ions (M^+ and $[M + H]^+$) from one compound

A minority of sample get ionized directly by the UV light (photoionization)[28]. As depicted in the equation below, the photons (hν) will excite the analyte molecule (M) enough to cause the loss of an electron (e^-), creating a radical cation (M^+).

$M + h\nu \rightarrow M+.+ e$

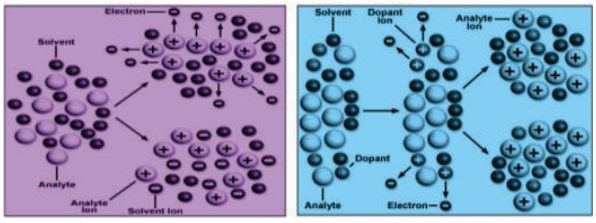


Fig 7: Mechanism of ion formation

Solvent-assisted- Some of the analyte molecules can be indirectly ionized with the help of the solvent molecules. The photons also excite the solvent molecules which far more than the analyte molecules due to high collisions per second at atmospheric pressure, in which the solvent molecule donates a proton to the analyte molecule (protonation)[28,29].

$M + S + h\nu \Rightarrow [M+H]^+ + [S-H]^-$ (solvent dependent)

- Dopant-Assisted APPI- yields two types of analyte ions

Here a third compound called a dopant is added, to increase the percentage of analyte molecules that get ionized, Different compounds can be used; toluene, acetone, anisole is a commonly used as dopant.

- When the dopant molecules are ionized directly by the UV photons. Because the dopant molecules far more than the analyte molecules, there can be many more collisions that result in formation of an ion. But it may produce unwanted signal
- Then the dopant ion can donate a proton to the analyte molecule
- $D^+ + M \Rightarrow [M+H]^+ + [D-H]$
- The dopant ion receives an electron from the analyte molecule:
- $D^+ + M \Rightarrow M^+ + D$

The ionization efficiency is usually 1–2 orders of magnitude higher with dopant than without dopant. In positive ion mode, the analytes were ionized either by charge exchange or by proton transfer[29,30].

APPLICATION

APPI is being used in combination with other mass spectra (Variant) which includes:

- **APPI - LC/MS:**

Atmospheric Pressure Photoionization (APPI) sources were introduced for use on Liquid Chromatography-Mass Spectrometer (LCMS) instruments by Robb and Syage in 2000). With LC, Atmospheric pressure photoionization (APPI) provide high sensitivity to analysis of compounds[31].

LC → APPI → INTERFACE → MASS ANALYZER → DETECTOR

(Ion Source) (Orifice/ Capillary) (Quadrupole, Ion trap, TOF) (CEM, CCD, Photomultiplier)

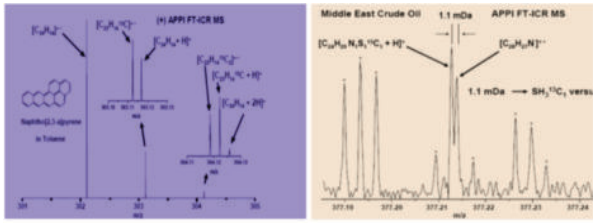
UNDER VACCUM

The mixture of samples and solvent eluting from an HPLC is fully evaporated prior to introduction into the photoionization region and use of ionizable dopant allowing for a great abundance of dopant photo ions to be produced. In LC flow rate of 200 µL/min is maintained to obtain analyte signal intensities 8 times as high as compare to other ionization techniques used. APPI- being remarkably tolerant of matrix components of HPLC additives makes it more applicable to research area use like quantitative, multiplexed assays of plasma, biological samples, vitamins, steroids, lipophilic compounds.

APPI generated more reproducible signals and was less susceptible to ion suppression than APCI. APPI generated higher peak area and lower baseline noise, and therefore much higher S/N ratios. APPI sensitivity (i.e., S/N ratio) was approximately 2-130 times higher than APCI by FIA and was approximately 2.6-530 times higher than APCI by on-column analysis depending on specific compounds[32]. **The APPI sensitivity is better as compared to APCI**. Comparison of atmospheric pressure photoionization and atmospheric pressure chemical ionization for normal-phase LC/MS chiral analysis of pharmaceuticals was Studied by **Cai SS *et al*** in 2007 in which APPI offers no concern of explosion hazard relative to APCI corona needle discharge or ESI high voltage discharge when flammable solvents (Hexane) which provide self-doping effect.

APPI- FTICR MS

Atmospheric pressure photoionization (APPI) coupled to a home-built 9.4 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer for the analysis of naphtho [2,3-a]pyrene and crude oil mass spectra by Jeremiah Michael Purcell in 2007 reveals that protonated molecules, deprotonated molecules and radical molecular ions are formed simultaneously in the ion source, the ultrahigh mass resolving power and mass accuracy of FT-ICR MS enabled definitive elemental composition assignments, even for doublets . **APPI efficiently ionizes nonpolar compounds that are unobservable by electrospray** and allows nonpolar sulfur speciation of petrochemical mixtures as shown fig below[33].This coupling is necessary for analysis of complex mixtures by APPI mass spectrometry. APPI can produce both even and odd ions as shown below (left), Spectrum of high Sulphur middle ease crude oil (complex mixture) as analyzed by APPI-FT-ICR MS shown below on right[34].



LA-APPI

Laser ablation atmospheric pressure photoionization (LAAPPI), a novel atmospheric pressure ion source for mass spectrometry. In LAAPPI the analytes are ablated from water-rich solid samples or from aqueous solutions with an infrared

(IR) laser running at 2.94 μm wavelength studied by **Anu Vaikkinen** *et al* (2012). In the study, above the sample surface, the ablation plume is intercepted with an orthogonal hot solvent (e.g., toluene or anisole) jet, which is generated by a heated nebulizer microchip and directed toward the mass spectrometer inlet. The ablated analyte is desolvated and ionized in the gas-phase by atmospheric pressure photoionization using a 10 eV vacuum ultraviolet krypton discharge lamp. The effect of operational parameters and spray solvent on the performance of LAAPPI need to be study more but it **shows high sensitivity and selectivity**[35] in terms of performance.

APPI-Qq-TOF MS

Quantitative determination of Polyisobutylene Derivatives by APPI Quadrupole Time-of-Flight Mass Spectrometric measurements were performed in the positive and negative ion modes with a MicroTOF-Q type Qq-TOF MS instrument equipped with an APPI source. So, APPI can be used with combination of Quadrupole Time-of-Flight Mass Spectrometric for better results[36].

Application And Advatages Over Esi And Apci

APPI is having wide range of applicability in analysis of compounds given below

Henion *et al*. in turn compared APPI and APCI in the analysis of idoxifene and its two metabolites in human plasma. Especially for the neutral metabolite SB20, APPI proved to give better sensitivity than APCI. **Henion *et al*.** also found that the flow rate of the solvent had a significant effect on the ionization efficiency of the analytes[37]. **Hakala *et al*.** compared the suitabilities of APPI and ESI in the analysis of pharmaceuticals used in permeability studies of Caco-2 cell lines. Studies revealed that **chemical noise in APPI was a lot lower than in APCI, which resulted in improved selectivity for the analytes**. Both methods gave sufficiently low limits of detection, but APPI provided a **broader linear range than ESI**[38]

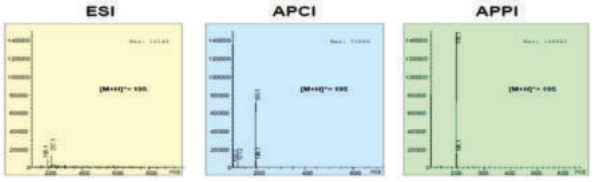
Cortisol and cortisone in plasma were analyzed using APPI. In this method, Cortisol and cortisone were extracted with methyl-*tert*-butyl ether from 100 μl of serum or plasma. The extract was evaporated, reconstituted with mobile phase, and analyzed by tandem mass spectrometry using a photoionization interface. The transitions monitored were: *m/z* 363 to 121 and 363 to 97 for cortisol, 361 to 163 and 361 to 105 for cortisone[39].With APPI the results obtained were significant.

The structures of some non-polar hydrocarbons which were successfully analyzed by APPI-MS eg: Carbamazepine, Caffeine, Idoxifene, polycyclic aromatic hydrocarbons (PAHs) like and Naphthalene, Benzo[a]pyrene. For investigation of these compounds with very low limits of detection were obtained with APPC in combination of normal phase LC, while results were also achieved with reversed-phase LC and addition of a dopant. Many of the PAHs are known to be carcinogenic, which makes their analysis important in view of both health and environment. Because the PAHs lack polar groups, they are difficult to ionize using ESI or APCI. Both reversed phase- (RP) as well as normal phase-liquid chromatographic (NP-LC) conditions have been tested for the analysis of **PAHs by APPI and better ionization efficiency has usually been achieved by using NP solvents. Takino *et al*.** have studied compounds of environmental and toxicological interest (pentafluorooctane sulfonates, patulin and chloramphenicol) in challenging matrices, such as citrus fruits, apple juice and fish meat by using APPI-MS. **In their comparisons with APCI-MS, APPI was found to be less susceptible to matrix effects**[40].

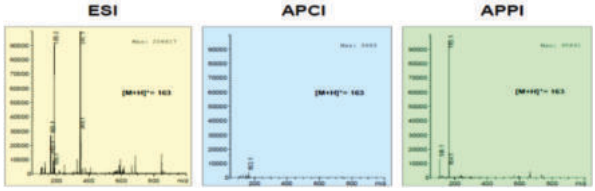
APPI technique to the analysis of different groups of compounds, including flavonoids, steroids and drug metabolites[41]. **Leinonen *et al*.** compared ESI, APCI and APPI in the detection of free anabolic steroid fraction in human urine. Eluent composition, ion source parameters and fragmentation were optimized for ESI, APCI and APPI, after which the methods were compared with respect to specificity and detection limit. The use of dopant was essential for ionization in APPI, but its flow rate within a range of 5-25 μl/min did not affect the results. Toluene and acetone were compared as dopants and with toluene approximately 20-50 % higher sensitivity was achieved. According to **Delobel *et al*., APPI would work best as a complementary technique to ESI**; ESI giving stable multiply protonated ions and APPI sequence information[42].

Dorcier *et al*. observed a significant increase in the signal-to-noise ratio of coordination and organometallic compounds when a photon source was added to an ESI source. Also, the **combination of APPI with APCI has extended the range of compounds that can be analyzed in a single run and increased the analyte signal levels**. APPI when used in drug discovery and development, generate higher peak area and lower baseline noise, and therefore much higher S/N ratios. **APPI sensitivity (i.e., S/N ratio) was approximately 2-130 times higher than for the analysis of normal-phase LC/MS chiral analysis of pharmaceuticals compounds**[43]. APPI interface with respect to the ionization of drugs under varying conditions (nature and concentration of dopants, flow rates, temperatures and mobile phase composition) in comparison to other techniques like APCI and a "turbo ionspray" (modified ESI) interface. Vitamin A and vitamin E were investigated by APPI where Vitamin A and vitamin E directly ionized in the APPI source without the need for a dopant[44].

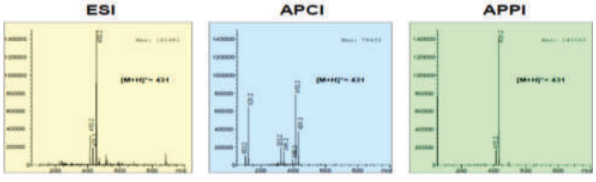
- Comparison of APPI, APCI, ESI based on results obtained for analysis compounds like Caffeine (Peak of ion formed at m/z 195)



Methomyl (Peak of ion formed at m/z 163)



Budesonide (Peak of ion formed at m/z 431)



From all above graphs, it is being concluded that Peaks obtain with APPI with all three compounds sharp and specific peaks were seen with APPI than APCI, ESI. Results with APPI shows less background, matrix effect than APCI, ESI. No overlapping of peaks obtained with APPI.

From the above application and analysis, it is concluded that APPI has various advantages over APCI and ESI, which includes:

Lower Chemical noise, Improved selectivity of analytes, Better Sensitivity than APCI, broader linear range of detection limit than APCI, its suitability for flammable solvents because

photons instead of a discharge gas are used for the ionization. It is Less susceptible to matrix effects than ESI or APCI. Compounds can be analyzed in a single run with increased the analyte signal levels in APPI. Act as suitable interface for other mass spectrophotometers. Minimum background interference It Works good with buffers as compare to ESI. Act as great tool for qualitative and quantitative determination. Provide accuracy.

However, despite of various advantages it also suffers from disadvantages which includes:

Some typical LC solvents (acetonitrile, methanol and/or water) deplete much of the photon flux resulting in poor analyte ionization efficiency. Poor ionization efficiency by direct photoionization leads to formation of unwanted signal by using solvent and dopant along analyte. Mass range is less than ESI. Optimization of parameter is required.

Future/ Recent Developments In Appi

In Recent developments, new instrumental developments have resulted in combined APPI/ESI (PA ESI) and APPI/APCI sources and a microfabricated APPI source, and its combination with different separation techniques, novel instrumental developments – like LA-APPI, gas chromatography and ambient mass spectrometry. More work requires understanding mechanism of ionization. Testing of new dopants in positive and negative ion modes could open new perspectives and can lead to significant improvement in sensitivity and selectivity. Future challenge will be the combination of APPI and micro-APPI with matrix-assisted laser desorption ionization (MALDI).

DISCUSSION:

API techniques have the advantages of ready coupling with Liquid Chromatography (LC), can efficiently ionize polar species and to some extent less polar species, and are robust. However, non-polar compounds are inaccessible by ESI and can be problematic for APCI. Atmospheric Pressure Photoionization (APPI) was initially introduced as a soft ionization method through direct photoionization and later with dopant-assisted ionization coupled to LC, and can produce ions of low-polarity and even non-polar species not efficiently ionized by ESI and APCI An APPI ion source typically uses a vacuum ultraviolet (VUV) gas discharge lamp) and can produce radical molecular ions from species with first ionization energies (IE) below the photon energy. However, some typical LC solvents (acetonitrile, methanol and/or water) deplete much of the photon flux resulting in poor analyte ionization efficiency is problematic. Poor ionization efficiency by direct photoionization sensitivity by promoting proton transfer reactions and charge exchange

CONCLUSION:

APPI is a suitable alternative method found out to analyze low polar compounds which cannot be done by ESI and APCI with high accuracy. In this article, we have seen that APPI can be used alone or as an interface with other mass spectra. It provides various advantages over ESI, APCI as discussed above. It is having wide applicability in life science, clinical diagnosis, pharmaceutical industries and it is used in analysis of protein, pharmaceutical in biological fluid. It is used for analysis of petroleum products and environmental toxin. Great tool for qualitative and quantitative determination. More work need to be done to widen its applicability and to overcome its drawback. Its mechanism of ionization need to be more thorough.

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