Negative electrospray ionization mode in mass spectrometry: A new perspective via modeling

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Christos I. Gioumouxouzis, Maria G. Kouskoura, Catherine K. Markopoulou *

Laboratory of Pharmaceutical Analysis, Department of Pharmaceutical Technology, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

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ABSTRACT

Electrospray ionization technique is used for production of gas phase ions without fragmentation and is considered as one of the most sensitive analytical methods for structural characterization of molecules. Nonetheless, the determination of some parameters (physicochemical properties or structural features) that may enhance the signal response especially in the negative ion mode has not yet been clarified. The present work is an attempt to correlate the signal response behavior of 110 compounds used as probes, with their characteristics (molecular descriptors, X variables). In order to quantify this phenomenon, Partial Least Squares which is a software capable of performing linear multivariate analysis was applied. The models derived explore the positive or negative effect of 49 X variables on the signal response of each analyte, expressed as Y variable. The process of gas phase ions formation was verified by both flow injection and column analysis. The models derived are proven reliable for the study of such mechanisms, with small number of components and good linearity ($R^2 > 83\%$, $Q^2 > 70\%$). The present study showed that parameters as pK_a, ionization percentage of the analyte, PSA, HBA, —COOH, water solubility and surface tension of a solid are affecting ion formation. At the same time, slight differentiations of the influence of certain parameters were observed on column injection analysis due to the chromatographic delay of some analytes.

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1. Introduction

Electrospray (ES) was a widespread process for the electrostatic dispersion of liquids and the creation of aerosols long before its application on mass spectrometry. Electrospray ionization (ESI) is a soft ionization technique extensively used for production of gas phase ions (without fragmentation) of thermally labile large molecules. This method was known for more than a hundred years and was initially applied by Dole in the production of gas phase polystyrene ions [1,2]. In 1989, Fenn et al. characterized large biomolecules in the gas phase by electrospray ionization mass spectrometry [3] and created a new dimension in the field of proteomics. ESI has been recently used for studies of non-covalent solution and gas phase association of species, particularly in the realm of molecular recognition, metal chelation and complex formation [4].

After the development of ESI-MS, different assumptions and hypotheses were made in the early 1990's to interpret multiple charging of an analyte by the ES process [5–8]. Until then, it was thought that the distribution of the charge states in the ESI-MS spectra actually reflects the degree of charging of an analyte in a neutral solution [9–11]. Much later, based on a report by Kelly et al. [6], it seems that the correlation between solution and electrospray charging is not that clear. Their results proved that the charging process in ESI, might occur in an entirely different manner than the one previously implied. In general, when an analyte is transferred as a solution to the gas phase via ESI, it undergoes three major processes. These are: (a) production of the charged droplets from the high voltage capillary tip where the analyte solution is injected, (b) repeated solvent evaporation (from the charged droplet) and droplet disintegration, resulting in a very small charged droplet which is able to produce the charged analyte, and finally (c) a mechanism by which the gas phase ion is formed.

The first two processes were thoroughly studied in the past and are nowadays well understood [12,13]. However the latter, which is the mechanism of gas phase ion formation from a very small highly charged droplet, is still under investigation and the exact process is not known. According to the literature, there were several experimental evidences that triggered discussion and new hypotheses over this issue [14–16]. It was shown that the gas phase ion formation is achieved in three stages: (a) production of

Descriptors (X variables)

Constitutional

Molecular weight

S, N, O, F, Cl, Br, halogens

benzene, benzene like, nonaromatic, heterocyclic (N), condensed, all rings

number of side chains, C atoms on side chains

-NH$_2$, -NH, N tertiary, -NO$_2$, -OH, -CO-, -COO-, -COOH, -SO$_2$-, -CH$_3$ on rings, -OCH$_3$ or -OR

Lipophilicity
logP and logD
water solubility

Surface tension
Enthalpy of
vaporization

Electronic
Refractivity
Polarizability
H bond acceptor
H bond donor

pK$_a$
Ionization %

Topological
Free rotatable bonds

Geometrical
Polar Surface Area
Solvent Accessible Area
Molar Volume
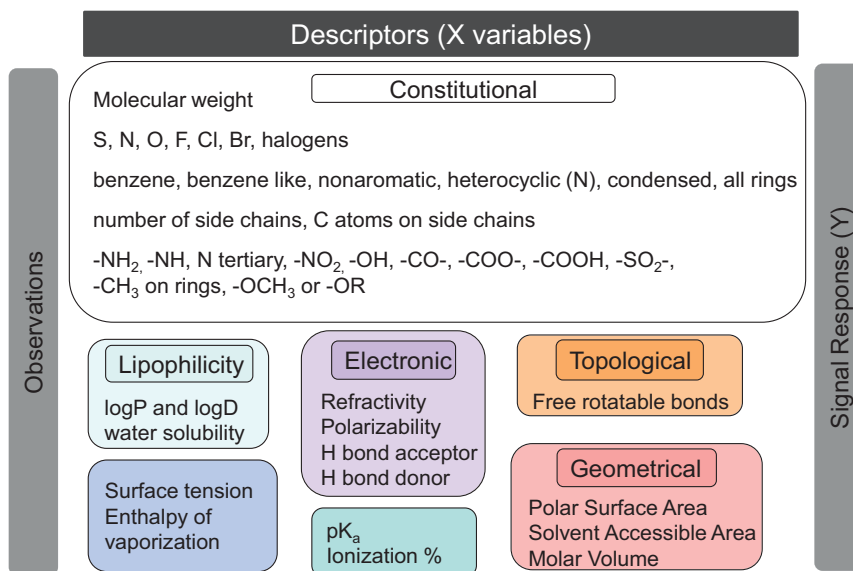
Observations

Signal Response (Y)

Fig. 1. Descriptors of the probes (X variables).

charged droplets [17–19], (b) Coulomb explosion and disintegration of charged droplets [20–22] and (c) formation of gas-phase analyte ions from charged droplets.

In order to describe this phenomenon accurately, researchers focus on either positive or negative mode, while the choice of the mobile phase and the appropriate probes might be the key point for the determination of behavior models [23,24]. However, studies on ESI negative mode showed that volatile bases, such as ammonium hydroxide, resulted in a poor detection limit and lower stability in methanolic or aqueous solutions [25]. Confirming this assumption, Ferrer et al. [26] used an acidic mobile phase and an octadecyl column for trace analysis of oxanilic and sulfonic acids in environmental analysis of chloroacetanilide- herbicide metabolites in surface and groundwater. Schug and McNair focused on negative adduct ions using benzoic acid derivatives and showed that log$P$ and pK_a values may be associated with the relative efficiency of formation of [M–H]$^-$ and [2M–2H+Na]$^-$ ions [27].

Nevertheless, although there is major development in this area, questions still arise concerning the correlation between observed gas phase ions, their nature and their response signal on negative mode. Further clarifications are needed, not only in terms of the physicochemical properties of the analytes, but also in terms of their structural characteristics. Such information would be helpful to explain why the signal is increased or decreased, as well as to quantify this effect.

Given the fact that there is not only one mechanism for ion formation of the probes, one may wonder whether there are any parameters which either favor or impede the mechanism of gas phase ion formation. The present work attempts to offer some answers to such problems through a detailed study of several characteristics of the analytes (physicochemical properties and structural features) which affect the response signal in negative ion mode. Additionally, the effect of different solvents on the analytes' signal is clarified using different mobile phases.

An overall view of this phenomenon is given using multivariate analysis and more specifically Partial Least Squares (PLS). Application of PLS may evaluate as well as quantify the effect of a number of descriptors. This research was applied to a large number of analytes used as probes in order to interpret in a more reliable way the effect of their characteristics on the ESI mechanism using both column and flow injection analysis (FIA).

2. Experimental

2.1. Mass spectrometry

The instrument used was a Shimadzu LCMS-2020 (Asteriadis, Athens, Greece) mass spectrometer. Standard LC-20AD dual pistons, pumps with high pressure mixing, provided flow (0.2 mL/min) for interface and detector. Samples were injected (100 μL volume) by a Shimadzu SIL-20AC HT autosampler which operated for both flow and column injection analysis. Flow injection analysis of the compounds is used as a quick and reliable way to study ion formation, while the same experiments were repeated on a column (Discovery HS C$_{18}$, 25 cm × 4.6 mm, 5 μm) to confirm the results. The mobile phase used in flow injection analysis was MeOH:H$_2$O 80:20 v/v, since this was the mobile phase that could give the more intense signal. When the measurements were carried out on the chromatographic column, the mobile phase mixture was MeCN:H$_2$O 80:20 v/v in order to ensure faster elution.

The LC–MS-2020 operates in the negative ionization SCAN and the selected ion monitoring (SIM) modes. Scans were made from 50 to 500 at 0.5-s intervals (scan speed = 1500 amu/s). Initially, the two main (−) m/z ions for each analyte in the scan method were chosen and then the analysis was repeated at the SIM mode and the response of these ions was measured. The results were verified by triplicate measurements.

The sample solution is drawn into a capillary pipe with a high voltage of −3.5 kV applied. Nebulizer gas is blown out around the outside of the capillary pipe, spraying the solution and generating fine droplets electrostatically charged with the same sign as the applied voltage. After being sprayed and ionized by the ionization probe, the sample passes through the sample introduction line (Desolvation Line – DL) oriented at 90° to the spray into the first stage primary vacuum chamber (lens system). Excess solvent is expelled through the drainage port.

The temperature and voltage of the curved desolvation line (the inlet for the high vacuum region) were set at 250 °C and 0 V, while the nitrogen nebulizer gas flow remained constant at 1.5 L min^{-1} respectively. The reliability of the signal response of the instrument was controlled by measuring the response surface area of a standard solution at regular time intervals (warfarin 10 ng/mL on FIA and 200 ng/mL on column injection analysis).