JOURNAL OF CHEMICAL EDUCATION

Commentary

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Clarifying Misconceptions about Mass and Concentration Sensitivity

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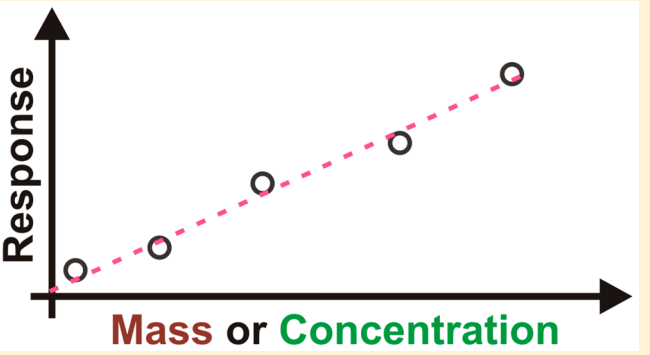
ABSTRACT: This commentary discusses differences between the so-called "mass sensitivity" and "concentration sensitivity". These terms are freely used in analytical chemistry literature to characterize operation of analytical techniques and methods. The type of sensitivity in an analytical method delimits the method's applications (e.g., in analysis of volume-limited and concentrated, or large-volume but dilute samples). It is helpful to instruct students how to distinguish between mass-sensitive and concentration-sensitive methods. Introduction of mass and concentration sensitivity can be included in graduate courses related to instrumental analysis, and, if time is available, also in the upper-level undergraduate courses.



KEYWORDS: *Graduate Education/Research, Analytical Chemistry, Laboratory Instruction, Misconceptions/Discrepant Events, Bioanalytical Chemistry, Calibration*

WAYS TO EXPRESS ANALYTICAL PERFORMANCE

An important goal of academic training in analytical chemistry is to familiarize the students with the main characteristics of various analytical methods. One of the relevant topics in modern analytical chemistry is sensitivity. In this commentary, I aim to clarify misconceptions about the terms "mass sensitivity" and "concentration sensitivity", used to characterize analytical methods. I would also like to encourage academic teachers to explain these terms to students to build a conceptual framework for the introduction of advanced analytical strategies.

Sensitivity is generally defined as "the slope of the calibration curve"[1] relating signal intensity to absolute quantity (mass or number of moles) or concentration. On the other hand, the limit of detection (LOD, or detection limit) characterizes the ability of a method to detect very small amounts or concentrations of substances that can be differentiated from the blank (for in-depth discussions, see, for example, refs 2 and 3). According to Miller and Miller[4] "...there is no single generally accepted English word synonymous with 'having a low limit of detection'. The word 'sensitive' is generally used for this purpose, giving rise to much ambiguity." Thus, in common parlance, the two terms "sensitivity" and "LOD" are often incorrectly used as synonyms. Analytical performance reports often include LOD, limit of quantification (LOQ), and a measure of sensitivity. Sensitivity[1] *sensu stricto* (often referred to as "calibration sensitivity")[5] does not substitute the "true" performance indicators because it does not consider the influence of noise.[2,5,6] LOQ as well as sensitivity are important for quantitative analysis. Despite the "official" definition[1] of sensitivity and LOD, other dissimilar and specific definitions of sensitivity are occasionally admitted, for example, in atomic absorption spectroscopy, sensitivity is defined as concentration of analyte that produces an absorbance of 0.0044.[7]

From the practical point of view, it makes a big difference whether an analytical method is mass-sensitive or concentration-sensitive. Different methods are utilized to analyze volume-limited samples with relatively high concentrations of metabolites (e.g., nucleotides in single cells), or large samples with very low concentrations (e.g., traces of pesticides in lake water). Because mass- and concentration-sensitivity determines the range of applications of analytical methods, chemists need to be trained how to distinguish between the two types of sensitivity. For example, the ultraviolet absorption detection provides concentration sensitivity according to the Lambert–Beer's law.[8] If mass-sensitive techniques are used to analyze dilute large-volume samples, preconcentration steps are often required.

One should also realize that various units are used to describe performances of analytical methods. They include the following: absolute quantity units and concentration units. Absolute quantity units are typically moles and grams, while concentration units are moles per liter, grams per liter, and percents. The selection of units to express the figures of merit such as LOD, even without noting the exact values, may give a hint about characteristics of an analytical method. A non-mandatory but widespread determinant of the units (concentration or absolute quantity) to report LOD is the way of introducing samples to analytical systems, continuously (undefined volume) or as a plug (finite volume; see also ref 2). Moreover, whether we define LOD and LOQ by mass/quantity or concentration can be influenced by the anticipated applications. However, it is often dictated by the character of

Received: December 8, 2015
Revised: March 28, 2016

DOI: 10.1021/acs.jchemed.5b00986
J. Chem. Educ. XXXX, XXX, XXX−XXX

Table 1. Comparison of Mass-Sensitive and Concentration-Sensitive Analytical Platforms[a,b]

Characteristics	Mass-Sensitive	Concentration-Sensitive
Response to −	absolute quantity, amount (e.g., mass)	relative quantity (e.g., mass per unit volume)
Techniques	gravimetry	
	gasometry	
	fluorescence microscopy	UV−vis absorption spectroscopy
	flow cytometry	optical atomic spectroscopy
	EI-MS	fluorescence spectroscopy
	CI-MS	
	nanoESI-MS	potentiometry
	APCI-MS	ESI-MS
	APPI-MS	LC-ESI-MS
	MALDI-MS	CE-ESI-MS
	nanoLC-nanoESI-MS	nuclear magnetic resonance
	CE-nanoESI-MS	
Samples	single nanoparticles	
	single cells	environmental samples of water, soil, and air
	forensic specimens	clinical specimens (e.g., urine, blood)
	biomedical specimens (e.g., biopsy tissue, blood)	
Areas of application	analysis of main components in volume and mass-limited specimens related to biochemistry, medicine, and forensics	analysis of low-concentration analytes in large-volume samples related to environmental science
Typical units (e.g., to report LOD)	(sub)moles	(sub)moles per liter
	(sub)grams	(sub)grams per liter
		percent

[a]Examples of techniques (used under common operational regimes), samples, applications, and units, are provided. Thus, the proposed classification may not hold in all applications. [b]Acronyms: EI, electron ionization; MS, mass spectrometry; CI, chemical ionization; nanoESI, nanospray electrospray ionization; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; MALDI, matrix-assisted laser desorption/ionization; nanoLC, nanoflow liquid chromatography; CE, capillary electrophoresis; ESI, electrospray ionization; LC, liquid chromatography.

the analytical method: whether it is *mass-sensitive* or *concentration-sensitive*.

MASS AND CONCENTRATION-SENSITIVE DETECTORS

Detector is one of several important elements that influence characteristics of analytical methods. The criteria used to distinguish between mass- and concentration-sensitive detectors were well-defined in chemical literature more than 30 years ago.[9] In the realm of separation science, the concentration-sensitive detectors respond to the relative amounts of analytes eluted within a solvent volume. Conversely, the mass-sensitive detectors respond to the total amounts of analytes or to the rates of analyte introduction regardless of the concentration[8,9] (cf. Table 1). In the case of nondestructive mass-sensitive detectors, the response is proportional to the number of moles of the analyte. On the other hand, in nondestructive concentration-sensitive detectors, the response is proportional to the mole fraction of the analyte in the effluent of chromatographic column (e.g., mole ratio of solute to effluent). In destructive mass-sensitive detectors, the response depends on the number of analyte moles eluting in a given time interval.[8]

Various detectors have different sensitivity characteristics. For example, the ultraviolet absorption and conductivity detectors, often coupled with liquid chromatography columns, are concentration-sensitive.[8,10] In gas chromatography, thermal conductivity detector is regarded to be concentration-sensitive, while flame ionization detector is regarded as mass-sensitive.[11] Thus, LODs of such detectors can be stated in grams per milliliter (g mL^{-1}) or grams per second (g s^{-1}), respectively. Switching from macroscale chromatographic columns to capillary columns can increase mass sensitivity, and extend applications of a method toward volume-limited samples.[10] Analytes eluted from columns with smaller diameter are more concentrated than the analytes eluted from columns with larger diameter (under standard operating conditions for each one).[8] On the basis of this characteristic, we can see that not only the detector, but also other components of analytical instrumentation, contribute to the sensitivity regime. However, as explained below, the type of sensitivity can also be affected by certain parameters of analytical methods using very similar instruments. Classification of detectors, techniques and methods according to the type of sensitivity holds for other cases, also where no separation is involved. It even concerns individual steps of complex analysis workflows.

CASE STUDY: SENSITIVITY OF ELECTROSPRAY MASS SPECTROMETRY

Considering the importance of electrospray ionization (ESI) mass spectrometry (MS) in modern chemical analysis, it is helpful to discuss the difference between mass and concentration sensitivities using this technique as an example. In fact, ESI-MS can operate in two flow regimes: (i) at high flow rates, or (ii) at low flow rates. These flow regimes lead to high concentration- or mass-sensitivities, respectively. The conventional ESI interfaces operate at high flow rates: typically, 1−1000 μL min^{-1}. Within this range, increasing the flow rate does not normally increase the signal.[8,12] Therefore, ESI-MS is a typical concentration-sensitive technique. NanoESI-MS, which operates at low flow rates (typically, <100 nL min^{-1}), exhibits superior mass sensitivity. The high mass sensitivity of nanoESI is especially evident when this ion source is coupled with nanoflow liquid chromatography, and utilized to analyze very

DOI: 10.1021/acs.jchemed.5b00986
J. Chem. Educ. XXXX, XXX, XXX−XXX

small volumes of samples (nanoliters). Such volume-limited secondary samples are often prepared from biomedical specimens.

The high perceived sensitivity of nanoESI-MS is related to the fact that very small (nanoscale) droplets are produced that may only contain few analyte molecules. There is a high probability that these analyte molecules will acquire electric charge following desolvation. Therefore, ionization efficiencies observed in nanoESI are also very high,[13] theoretically approaching 100%.[14] Naturally, not all the gas-phase ions produced near the electrospray can enter the vacuum compartment of mass spectrometer, and contribute to spectral signals. Some ions are inevitably lost, although the relative losses should be smaller in nanoESI than ESI due to a better match of spray cross section and MS inlet diameter. Nevertheless, when the overall size of the electrospray is smaller (as in nanoESI), relatively few ions are lost near the inlet of the mass spectrometer, and many ions survive their route toward the mass analyzer.

Ionization efficiencies in ESI and nanoESI depend on the selection of analytes and analysis conditions. They span over wide ranges.[13,15] In a rudimentary calculation of ion yields in ESI and nanoESI, we assumed ionization efficiencies of 0.1% and 10%, while the flow rates were 10 μL min^{-1} and 10 nL min^{-1}, respectively (Table 2). For a 1-μM sample, these ionization conditions lead to formation of ~10^{-17} and ~10^{-18} mol of gas-phase ions, respectively (in a 0.1-s interval). Thus, in this particular case, the signal in nanoESI-MS analysis should be 10× lower than that in ESI-MS analysis, at least when using an ion-trap mass analyzer. Please note that using a nonaccumulating mass analyzer, such as time-of-flight, could lead to a different result, with high influence of the ion sensor placed at the end of the flight tube. On the basis of the above estimation, we realize that despite the superior ionization efficiency of nanoESI, due to the low flow rate, the number of ions can be much lower in nanoESI than in ESI. Thus, for analysis of large-volume samples, it is reasonable to select a concentration-sensitive technique such as ESI-MS. On the other hand, if the initial sample volume is <1 μL, it is more proper to select a mass-sensitive technique such as nanoESI-MS. Such volume-limited samples cannot readily be introduced to the sample tubing of an ESI interface. If we introduce a nanoliter-range-volume sample plug to the ESI interface, it can easily be dispersed due to turbulence, Taylor dispersion, and diffusion, inside the tubing, thus leading to low ion currents. However, in general, nanoESI can be regarded as "less wasteful" than ESI. In nanoESI, the whole sample plug can be transferred to the ionization zone with little dispersion due to low Reynolds number of the flow line upstream from the ion source and low dead volume of the spray emitter. The ions can be formed with high efficiency due to formation of nanodroplets. They can be delivered to the MS inlet with fewer losses, and transported to a trapping mass analyzer in a short period of time, leading to high ion currents. Nanoflow liquid chromatography and capillary electrophoresis (CE) operate at very low flow rates (typically measured in nanoliters per minute). They are particularly compatible with nanoESI. In this case, "sensitivity" is not compromised by dilution effects. Hence, due to the limited delivery of charge-carrying species, capillary-based systems such as CE-(nano)ESI-MS are characterized as mass-sensitive.[14] Please note that the above discussion does not take into account other benefits of using ESI or nanoESI, which become apparent in certain application areas. For further coverage of the sensitivity issues relevant to (nano)ESI-MS, the readers are encouraged to consult the specialized publications.[16−19]

Table 2. Calculation of the Hypothetical Amounts of Ions Delivered to an Ion-Trap Mass Analyzer Using ESI and NanoESI Sources[a]

Characteristics	ESI	nanoESI
Assumed ionization efficiency	0.1%	10%
Analyte concentration in the sample	1 μM	1 μM
Preset sample flow rate	10 μL min^{-1}	10 nL min^{-1}
Predicted ion flux	10^{-14} mol min^{-1}	10^{-15} mol min^{-1}
Preset ion accumulation time in ion trap	0.1 s	0.1 s
Predicted amount of ions entering ion trap	1.7×10^{-17} mol	1.7×10^{-18} mol

[a]It was assumed that the ionization efficiency of nanoESI is 100× greater than the one of ESI. In this particular case, the amount of ions produced by nanoESI was 10× lower than that produced by ESI. Ion losses during the transfer to the mass analyzer have been neglected. For a more accurate comparison of the two ionization schemes and discussion, see the review by Smith et al.[14]

The high suitability of nanoESI-MS for the analysis of volume-limited samples, especially in conjunction with micro-scale separations, imposes the classification of nanoESI-MS as a mass-sensitive technique (as opposed to conventional ESI-MS). The mixed response of ESI/nanoESI to samples has sometimes been perceived as a disadvantage. Interestingly, technical developments have been made to extend the mass-sensitive flow regimes of ESI toward higher flow rates.[20] Apart from nanoESI, other popular ionization techniques used in MS, such as electron ionization, chemical ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization are mass-sensitive.[8,21]

CONCLUDING REMARKS

Whether an assay is mass- or concentration-sensitive depends on the choice of the instrumental technique as well as analysis conditions. Therefore, in some cases, it is necessary to refer to "methods" (rather than "detectors" or "techniques") as either mass- or concentration-sensitive. The brief treatment of sensitivity and related terms in various analytical chemistry textbooks[5,7,22] is generally regarded as appropriate for the undergraduate-level audience. However, the individuals who continue their education at the graduate level might lack thorough understanding of this important concept. Thus, I suggest to include a more detailed discussion of different types of sensitivity in the syllabi of graduate courses related to instrumental analysis, and, if time is available, also in the upper-level undergraduate courses. I also recommend emphasizing the differences between various methods regarding sensitivity in the discussions following students' presentation in graduate seminar classes. These clarifications will equip students with better skills to evaluate analytical chemistry literature, specifications of instruments, and realize applicability of various approaches. The additional instruction will also help students to avoid errors when reporting analytical results. It is especially useful to exemplify the problem of concentration and mass sensitivity when introducing electrospray ionization mass spectrometry. Subsequently, students can be challenged with questions about types of sensitivity when discussing other analytical schemes.

DOI: 10.1021/acs.jchemed.5b00986
J. Chem. Educ. XXXX, XXX, XXX−XXX

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Notes

The author declares no competing financial interest.

ACKNOWLEDGMENTS

I thank the Ministry of Science and Technology of Taiwan (MOST 104-2628-M-009-003-MY4) for the financial support.

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DOI: 10.1021/acs.jchemed.5b00986
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