The "Beer's Law" Of Mass Spectrometry, Again.

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Introduction

A few years ago one paper stated that there was no "Beer's Law" for mass spectrometry (MS) (1). In another recent editorial comment on MS research, it was forwarded that mass spectrometry was "not inherently quantitative (2)." Also, MS has been blamed for proteomics "lack of success at clinical biomarker implementation (3)." These statements are misunderstandings of the technology that must be corrected.

Here, for the first time for EI, CI, CID, ESI, MALDI and new techniques like DART, we show that when ionization processes can be described as first ordered or pseudo-first ordered and Dalton's Law is obeyed, in ion transmission MS systems for analyte, internal standard pairs, that equations for relative ion current (and sometimes absolute ion current measurements) are of a form that is analogous to Beer's Law for spectrophotometry. This analogy is used to explain why we observe linear calibration curves across techniques.

The objective of this poster is to show that, in fact, across many sample introduction, ionization, ion transmission and ion detection approaches that mass spectrometry is ALWAYS both quantitative and qualitative. In fact, for mass spectrometry, **qualitative and quantitative analysis are simply different parts of the same equations.**

Experimental

Selected conditions are given below. Please see the original publications for more detail.

EI data acquired with comm. available GC/QMS or GC/QQQ/MS. 70 eV/EI current was employed with ca. 1 sec scan times (45 to 450 amu) using 30 m using He carrier @ 1 ml/min ca. 30 m, 0.32 mm, 30- 260C @ min FSCC. Interlab papers used up to 10 different GC/QMS systems.

For CI, i.e., PPINIC GC/MS Finnigan 4023 data. Scan time was for negative ion 0.333 sec from 30-435 amu and 0.333 sec form 45-450 amu. Methane CI gas at 0.2 torr with a 240C source temperature. Internal standard was anthracene-d10 for HCB with FSCC 30 m SE 30, splitless inj.,He @ 1 mL/min, 35C, 2 min-265C @ 10/min, 265C 10 min.

For CID, Todd and McLafferty's data, a Hitachi RMH-2 high resolution MS-I followed by a special collision region and an electrostatic analyzer, MS-II. A 9.8 keV ion accelerating voltage was used which had optional acceleration up to 25 keV. See the publication for more details.

For our ESI/LC/MS work cited, we employed a 250 x 2.1mm id 3 um 120AYMC ODS-AQ column with A = 400 mM HFIP pH 7.0, B = 50% MeOH 400 mM HFIP pH 7.0, 30 to 60 B/30 min @o.2mL/Min with column temp at 50C using an HPQMS from 400-1000 amu/sec.

For T. Tu's MALDI work, an ABI 4700 TOF with a Nd:YAG laser (355 nm, 3 to 7 ns pulses) operated in the positive ion reflectron mode with Vac at 20 kV with the laser operated at fixed fluence ca. 5% above ionization threshold at 200 Hz, set to uniform. Each spot was obtained from 1000 laser shots (40 subspec in positions, 25 shots/subspec.) and averaged for one smoothed spec. nL spots were made using a nL Cool Wave System I nanoliter. Bradykinin CHCA and the ILM of Bu-CHCA were employed in this work for nL and uL depositions.

DART TOF/MS data: 60-900 amu/sec, RF Ion guide 600v, 30v,5v,5v for orifice1,2 and ring lens. nL droplets from a Cool wave device were shot directly into the 400 u orifice with the systems resolving power set at 6000, (FWHM).

The 1960's

In Kiser's classic book entitled: An Introduction To Mass Spectrometry And It's Applications (4), the partial, core of the "Beer's Law" for mass spectrometry for electron impact (EI) ionization in transmission MS systems was given. Kiser, provided basic relationships for EI mass spectrometry for BMS systems, as he cited the importance of Dalton's Law.

Kiser wrote that for the pseudo-first ordered process below that for the jth component, of a primary species in the source at m/z, i, the ion current (Iij) was directly related to pressure.

$$I_{ij} \propto p_i = r_i s_j p_j$$

assuming the process and constant 70 eV, EI source conditions for

$$M + e^- \rightarrow M^+ + 2e^-$$

where r is the relative abundance of the peak, I, in question, which we refer to later in this poster as F, the fractional ion abundance, the percentage of ion current of one m/z value of the total ion current.

Without saying such, the assumption was that ionization was described by a pseudo-first ordered process, implying the core relationship that "sensitivity" is in this case formally **current per unit pressure,** an often forgotten core tenant of MS for linear systems.

In the book; however, Kiser's representation was incomplete. For example, as to ionization cross section estimates, the "additivity approach" of Otvos and Stevenson was cited (5), but the book reference only addressed compounds containing carbon and hydrogen. There were other short comings and no interlaboratory data to support this approach. Nevertheless, the excellent book reflected the state of the art in 1965 and it remains a recommended read to this date. It can be purchased from ASMS.

1. X. Zhang, et al, An Automated Method for the Analysis of Stable Isotope Labeling Data in Proteomics, . *Journal of the American Society of Mass Spectroscopy* 2005, 16, 1181-1191.

2. A. Doerr, Quantitative Mass Spectroscopy, Nature Methods, Vol. 6, No. 1, 2009.

3. J. R. Dasch, K.R. Solomon, M. Hincapie and D. Sarracino," Standardization In Biomaarrker Discovery," Genetic Engineering News, Vol. 29, No. 9, 32-33, May 2009.

4. Robert Kiser, Introduction to Mass Specrtometry And Its Applications, Prentise Hall, Inc, 1965. Now in print through ASMS.

5. J. W. Otvos and D.P. Journal of the American Chemical Society, 78, 546 (1956).

EI

70 eV/Electron Impact GC/MS Relative Ion Current Measurements

In attempting to understand and use 70 eV, EI response factors (which are relative ion current measurements for an analyte and an internal standard at equal injected weights) in the national implementation of GC/MS for the multivariate environmental monitoring of GC/MS data quality, we recognized this shortcoming. Since few Q values were available at that time, we derived a way to calculate them. It has been shown that the following equation is valid for many molecules for a wide range of conditions.

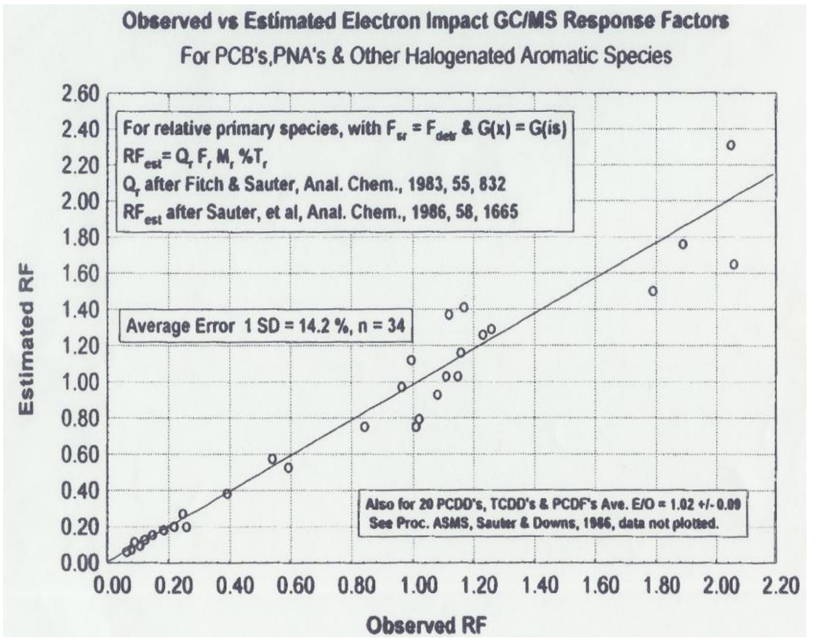
$$I_t = QI_e dN$$

where I_t is the total ion current, I_e is the ionizing electron current, d is the ionizing path length and N is the concentration of molecules in the source. In 1983, we demonstrated that the additivity approach of Otvos and Stevenson (5) could be applied to estimate relative EI cross section of compounds containing C,H,N,O,N, S, F, Cl Br, I and D atoms to 4.69% at one SD for 179 small molecules using the following equation given below (6).

$$\sum_{i=1}^{11} a_i n_i + 0.943$$

Then in 1986, using this approach for ion creation, we derived from first principles and showed for the first time that one specific approach that could estimate relative RF values for molecules under going 70eV, EI with GC sample introduction for QMS. The approach was shown to be accurate to ca. 9 to 14 percent at 1 SD (7,8) for two groups of aromatic analytes, as plotted in the graph below.

It was also shown in this period that relative response for ca. 100 small molecules undergoing internal standard quantification on instruments with different Q analyzers and between as many as ten different labs that the relative response for 100 analytes referred to as "semivolatile" were statistically identical or numerically "similar" (9,10,11,12).



Beer's Law Analogy

After some time we came to realize that our model to estimate EI response values was similar to Beer's Law where there was an energy term, a path length or transmission term and a molar or concentration term, multiplied by a fraction. That is…….

Beer's Law, Spectrophotometry, $A = ebc$

70eV/EI GC/QMS MS RF Analogy $I_{r(m/z)} = F_r Q_r T_r N_r$

where ε is an energy term, as is Q the relative ionization cross section for 70 eV/EI/QMS,

where b is the path length and % T in QMS, depends on the path length of ion traveling, l, in an analyzer of freq f, at velocity, v, and

where c is the solution concentration and N is the amount of moles in the source often a dynamic process in FIA, GC or LC that hopefully mirrors, c,

and the fractional ion abundance is a non-dimensional constant.

$F = I_x / \Sigma$ I total Σ F's = the spectral pattern or the qualitative term/s

Hence, the Beer's Law analogy, and since the qualitative and quantitative term are different parts of the same equation, it is impossible to do one without the other.

Estimating the concentration of unknowns from the analogy.

As an important aside, we have also shown that one can estimate the concentration of an analyte when standards are not available using this scheme. For example, observing the ion current ratio of some "unknown" analyte, the spectral F value, can be observed. Then by calculating T_r and Q_r (assuming an empirical formula) one can solve for the amount of analyte. Hence, we can estimate the amount of an unknown analyte in a sample without having a standard. Since our agreement between predicted and observed RF values for 53 compounds shown above was ca. 10 percent, we forwarded that one could estimate the concentration of a compound that was not a part of the standardization regime to about 10 to 15 percent at one standard deviation using this approach.

Finally, we speculated that a similar approach to the estimation of relative response was "general" and could be applied to "other" ionization types and MS systems, (6). The ability to estimate the concentration of "unknown" analytes or analytes for which there are no available standards, could have great value across proteomics, genomics and in other areas of ESI, MALDI and other mass spectrometry techniques.

6. W.L. Fitch and A.D. Sauter, "Calculation of Relative Electron Impact Total Ionization Cross Sections for Organic Molecules," Anal. Chem. 1983, 55, 832.

7. A.D. Sauter and J.J. Downs, "Model for the Estimation of Electron Impact GC/MS Response Factors for Quadrupole Mass Spectrometers," presented at the 1985 Pittsburgh Conference, Anal. Chem. 1986, 58, 1665.

8. A.D. Sauter, J.J. Downs, et al, "The Quantitation of Chlorinated Biphenyls, Dibenzodioxins, and Dibenzofurans Using Response Factor Estimation," presented at the 1986 ASMS meeting, Cincinnati, OH, June, 1986.

9. A.D. Sauter, L.D. Betowski, B.N. Colby, T.R. Smith, and R.G. Beimer, Journal of High Resolution Chromatography and Chromatography Communications, August, 1981, 366-384.

10. A.D. Sauter, P.M. Mills, W.L. Fitch, and V. Lopez-Avila, Journal of High Resolution Chromatography and Chromatography Communications, January, 1982, 27-30.

11. A.D. Sauter, L.D. Betowski, and J.M. Ballard, Anal. Chem. 1983, 55, 116.

12. A.D. Sauter, "The Quantitative & Qualitative Effects of Ion Abundance Tuning Criteria on Modern Quadrupole Mass Spectrometer Systems, Results from an Interlaboratory (Ten Lab) Study," presented at the 1989 ASMS meeting, Miami Beach, FL, May, 1989.

CI & CID

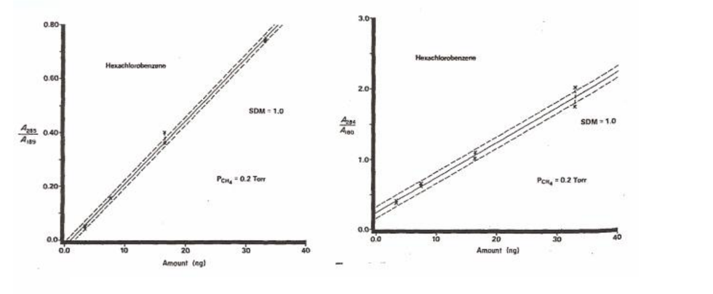
Chemical Ionization (CI) GC/QMS Relative Simultaneous Positive and Negative Ion Current Measurements

Similarly, we, as have many others, published linear calibration curves for small molecules undergoing the classic technique of pulsed positive ion, negative ion chemical ionization (PPINICI) in internal standard analysis (13). In one paper, linear calibration curves for molecules were observed for both the protonated positive ions (M+H)+ and M- ions of the same compound in the source at the same time. Hence, for resonance capture and for protonation by methane where M was hexachlorobenzene, linear calibration curves shown below were acquired for simultaneous, but different processes, although not without limits, as discussed in that paper.

$$M + e^- \rightarrow M^-$$

$$CH_5^+ + M \rightarrow (M+H)^+ + CH_4$$

In that effort, we addressed fundamentals and employed a Langevin treatment which neglects the polarity of the molecule and quadrupole contributions of the molecule and where the electron is treated as a point charge, to estimate relative sensitivities of negative to positive ions. This simple treatment yielded an estimated negative ion to positive ion sensitivities or up to 160 where in practice for HCB it is was observed as roughly a factor of 20.



Relative ion current for HCB, (M+H)+ (left) and M- (right) relative to anthracene-d10 measured simultaneously, per experimental conditions.

So given positive ion and negative in CI, ***simultaneously*** occurring in the source, with m as the slope and assuming a small or zero intercept we can write…

$I_{r+} = m_+ N_r$ $I_{r-} = m_- N_r$

As m depends directly on F and T per our EI analogy, the relative ion current can be written as…

$I_{r+} = F_{+r} k_{+r} T_{+r} N_{+r}$ $I_{r-} = F_{-r} k_{-r} T_{-r} N_{-r}$

k_r can be determined experimentally.

In Kiser's classic book, the qualitative and quantitative powers of MS to study the reaction rates, was shown along with instrument issues e.g., ion source residence time (τ) for magnetic sector mass spectrometers of that time, where

$\tau = (2dm_i/eE)^{½}$

and where d is the distance from the electron beam to the exit slit for an ion of mass m_i, and e the electronic charge with E the field applied to the source. Kiser gives the rate constant for ion molecule reactions that, of course, have no activation energy as

$K = g_i Q_{cir}$

where g_i is the speed of the ion and Q_{cir} is the chemical ionization reaction cross section.

As such, instrumental and fundamentals such as CI reaction rates can be studied as Kiser showed for even bimolecular reactions, clearly a qualitative and a quantitative MS task.

Collision Induced Dissociation

The total collision cross section, σ_c, had been given in 1981 by Todd and McLafferty (14).

$$I/Io = \exp(-\sigma_c nl) \text{ or } \ln(Io/I) = \sigma_c kP$$

where n is the number density, k is a constant and P is the target gas pressure where I and Io are the intensities of the ion beam with and without collisions, the Beer's Law analogy being obvious.

Interestingly, for cross sections, in 1995, Roussis showed that our previously cited approach for the estimation of relative collision cross section of electrons and molecules (6), could be employed to correlate the kV collision of ions with gas molecules. He then derived a similar, but improved expression, for the collision of ions and molecules in the kV range on a double focusing sector MS system (15). Here, the structure of the ion is ignored and the additivity principle is applied to the van der Waals radii, ρ, where Σ ρi is the summation of the atomic van der Waals radii (ave. error = 5.2%, n= 53 ions)

$$\sigma_c = 0.097 + 0.849\ \Sigma\ \rho i$$

To summarize Roussis and our work whose form is "similar" for 70 eV/EI electrons with molecules and for kV collisions for molecules with ions under constant ionization conditions in the energy ranges and instruments reported where scattering is minimal…………. the larger the molecule, the larger the collision cross section and hence the more product ions. This is observed in direct relationship to the number of constituent atoms which correlates with their bond lengths, or roughly by the size of the molecule in agreement with hard sphere collision theory.

We note here; however, that for macromolecules that have extensive higher ordered structure, alternate, more complicated cross section expressions may be needed. Also, here for a B/E instrument, equations for ion production in an absolute sense show a Beer's Law analogy too.

Hard Sphere Collision Model Cross Section

$\sigma_c = \int_0^d 2\pi\ b\ db = \pi\ d^2$ d= radii of two spheres b is the distance of closest approach.

13. L.D. Betowski, H. Webb, and A.D. Sauter" Biomedical Mass Spectrometry, 1983, Vol. 10, No. 6, 369.

14. Todd, P. J.; McLafferty, F. W., Int. J. Mass Spectrom. Ion Physics. 1981, 38,371.

15. Roussis, S. G., J. Am. Soc. Mass Spectrom. 1995, 6, 803-811.

ESI

Electrospray Ionization

In 2009, Kebarle and Verkerk published an updated version of an old paper, "Electrospray: From Ions In Solution To Ions In the Gas Phase, What We Know Now." Old ideas are presented as are newer ones and we quote heavily from it below (16).

The fundamental papers on the charge residue model of Dole from 1968 (17) and the 1967 Ion evaporation model (IEM) of Iribane and Thomson (18) have been employed to describe the mechanism of formation of gas phase ions from charged drops in light of much work including that of Enke (19).

According to the Kebarle and Verkerk, the state of discussion here is that the IEM is supported for organic ions, but the CRM is more plausible for macromolecular species (16). That stated, Gross (20) favors a sequential IEM to CRM model and de la Mora (21), Samamnikova and Grandori (22) have established position as well, some being questioned still (23). It is not our intent of this presentation to resolve the intricacies of ESI. Rather, we present data that further supports our analogy and that hopefully informs on the power and the limitations of ESI.

So fundamental ionization issues notwithstanding, linear ESI calibration curves can be routinely observed in practice for many systems. That stated, no technique and especially ESI can be linear over the 14 orders of magnitude of potential interest in discovery proteomics. Moreover, we note that despite the power of ESI, it is a 20 year old technique, that utilizes only a small amount of the sample. ESI is conductive and dispersive.

Nevertheless, in the cited reference, Kebarle re-published a linear calibration curve for Morphine HCl's (M+H)+ corrected for ion transmission, for a system with one analyte showing that ion current can be proportional to concentration and ion transmission and namely that.

$$I_{(M+H)+} = k\ T_m\ N_m$$

Our EI analogy is directly supported by Kebarle, as the protonated parent is the only ion, hence F= 1.0 and k is the proportionality constant.

In another ESI example, for a solution with equal concentrations of tetrabutylalkyl ammonium halide and cocaine, near identical sensitivities were observed for their protonated molecular ions until high concentrations. Hence, for ESI for simple molecules, Kebale and Verkerk showed linear calibration curves where ion currents and rate constants were related as given below.

$I_{(Bu4N)+} = k_{tb}\ T_{(Bu4N)+}\ N_{(Bu4N)+} = k_{CodH}\ T_{(CodH)+}\ N_{(CodH)+}$ and where $k_{tb} = k_{CodH}$

So, in **simple** ESI systems the literature offers direct support to our Beer's Law analogy.

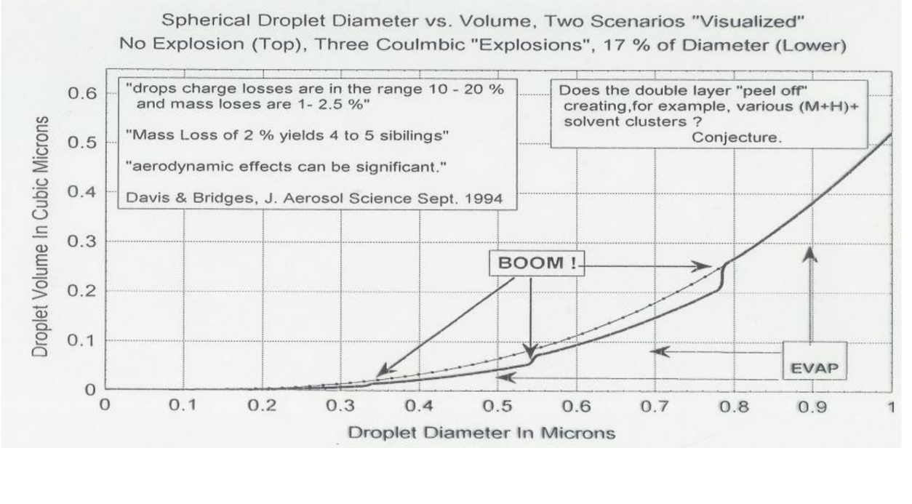
In other applications, Kebarle also showed that ESI yields powerful quantitative and qualitative information. As for protein substrate reactions, many studied reactions shown below for protein (P) substrate (S) non covalent complexes yield,

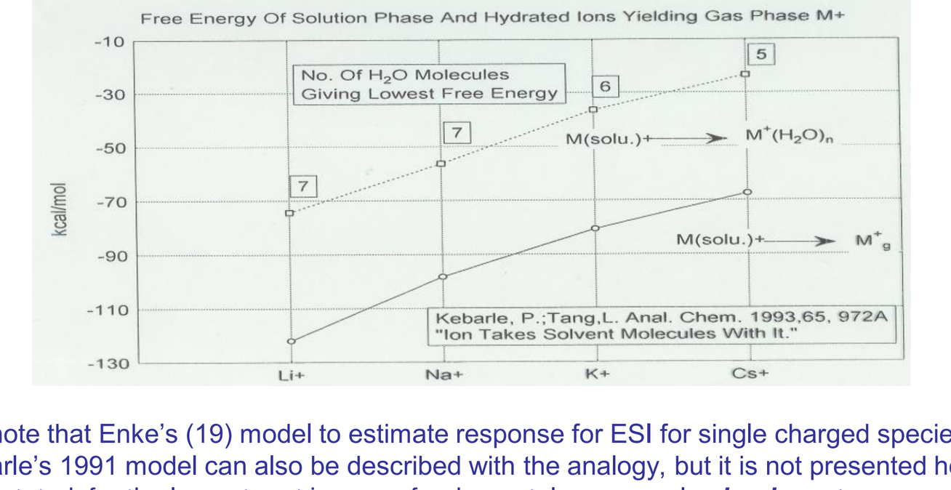
P + S = PS and an equilibrium constant, K_{as} K_{as} = (PS)/((P)(S)) = (I ps)/(Ip Is)

which can be observed and calculated by the comparison of relative ion current ratios as given above. Kebarle asserted that the "titration method" where measured ion currents of S, P and PS are used as given above and observed at different concentrations "have often been in agreement with the requirements" of the equilibrium equation. Therefore, while there are countless ways to produce nonlinear relationships in ESI, ion current can be directly related to concentration, in a manner that mirrors our analogy.

So we propose that for ESI, for simple systems where for example the molecular envelope is composed of isotopes, whose given percentage for an m/z value compared to the total ion current is F, we could include rewrite the above relationship as given below which is, of course, of the exact form of our EI model and hence analogous to Beer's Law where the ability to study ion creation and other complicated processes may be possible (24).

$$I_{esi} = F_m\ k_{esi}\ T_m\ C_m$$





We note that Enke's (19) model to estimate response for ESI for single charged species Kebarle's 1991 model can also be described with the analogy, but it is not presented here. That stated, for the largest part in many fundamental papers only ***simple*** systems are addressed. It is very important that we come to understand ESI of more complicated analytes in actual analysis systems, if we are to knowledgably identify disease or health state biomarkers.

16. Kebarle, P., Verkerk, U.H., Mass Spectrometry Reviews, 2009, 28,898-917.

17. Dole M, Mack LL, Hines RI, Mobley RC, Ferguson LD, Alice MB, 1968, J Chem Phys 49:2240-2249.

18. Iribrane JV, Thompson BA, 1976, J Chem Phys 64:2287-2294.

19. Enke CG, 1997, Analytical Chemistry, 69, 4885-4893.

20. Hogan CJ, Carroll JA, Rohrs HW, Biswas P, Gross ML, 2008, J AM Chem Soc 130:6926-6927.

21. Loscertales IG, F. de la mora, J Chem Phys 103:5041-5060.

22. Samalnikova M, Grandori R, 2005 J Mass Spec 40:503-510.

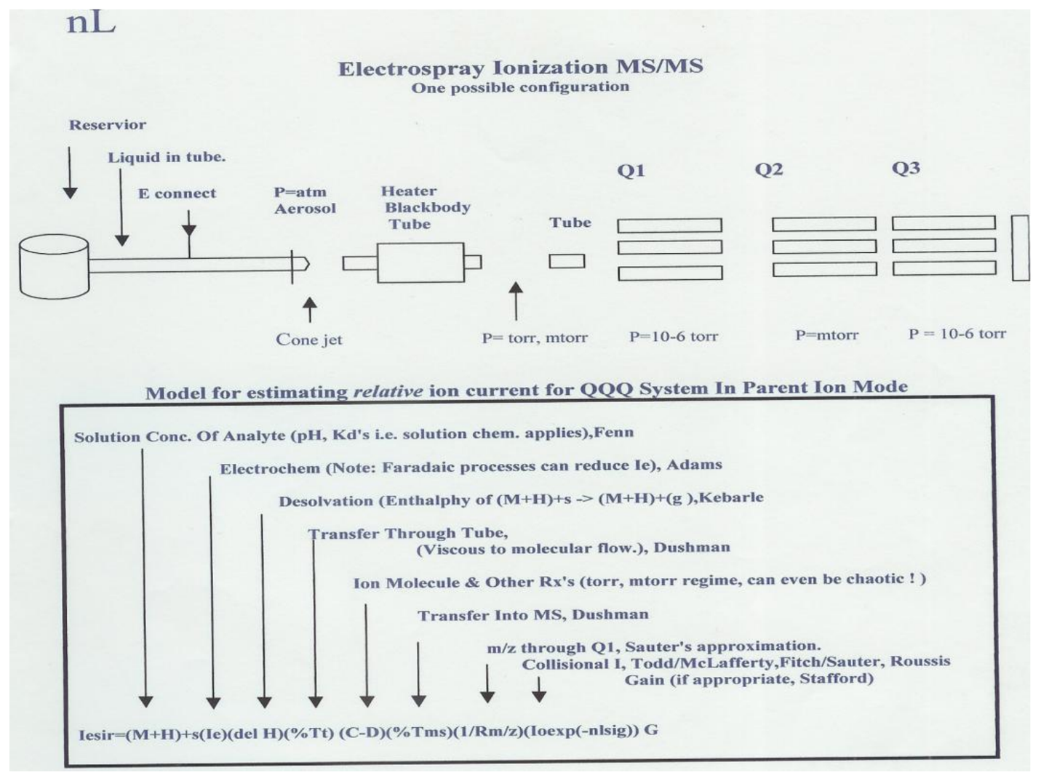
23. Nesatty, VJ, Suter MJF, 2004, J. Mass Spec 39:93-97.

24. A. D. Sauter, W. Fitch, J. Chakel, A. Affel, R. Willoughby and E. Sheenan, "Approaches For Estimating ESI/MS/MS Relative Response," Pittcon 97, New Orleans, LA, March 1997.

ESI

ESI of Complicated Analytes and Samples

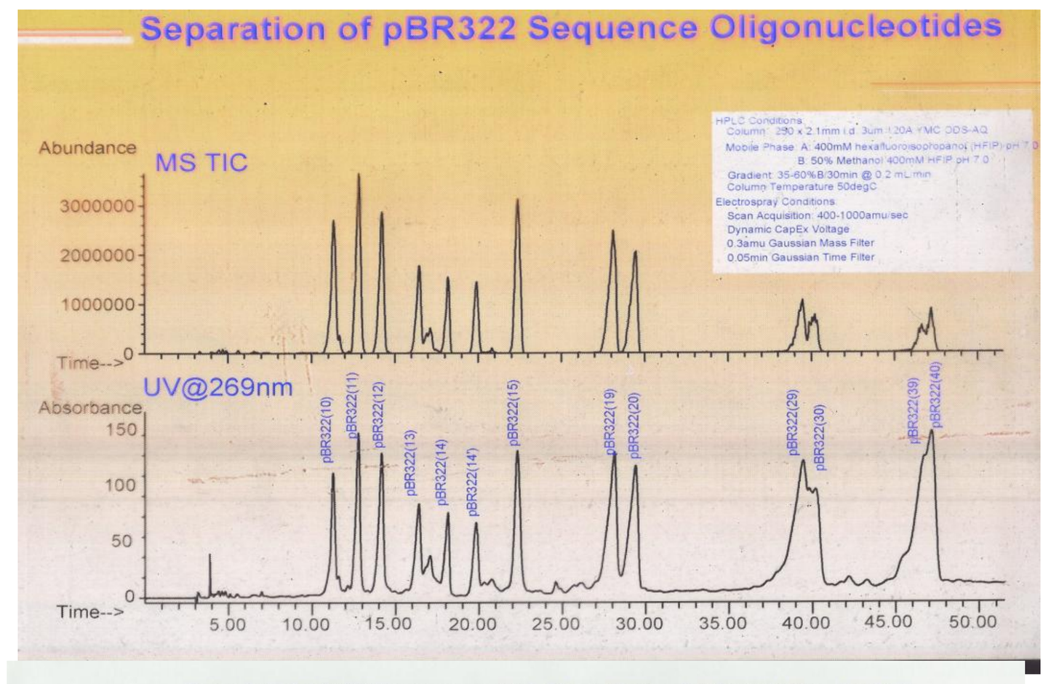
In an informal collaboration with Affymax, Agilent and Chem Space, we reported in March of 1997 the first model to attempt to estimate ion current produced in simple and complex situations (24). The model for one configuration of ESI/LC/MS/MS assumed that ESI was a combination of seven discrete processes citing work from Fenn, Adams, Kebarle, Dushman, Sauter, Todd and McLafferty, Rousis and Stafford. We proposed a relative response approach outlined below, but do not explain it here due to space considerations.

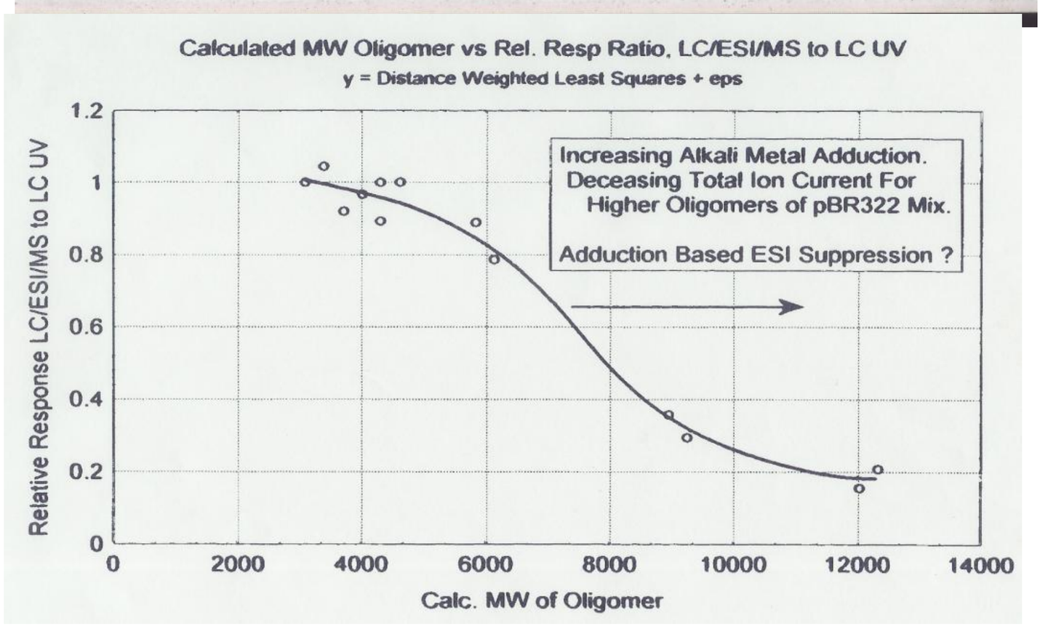


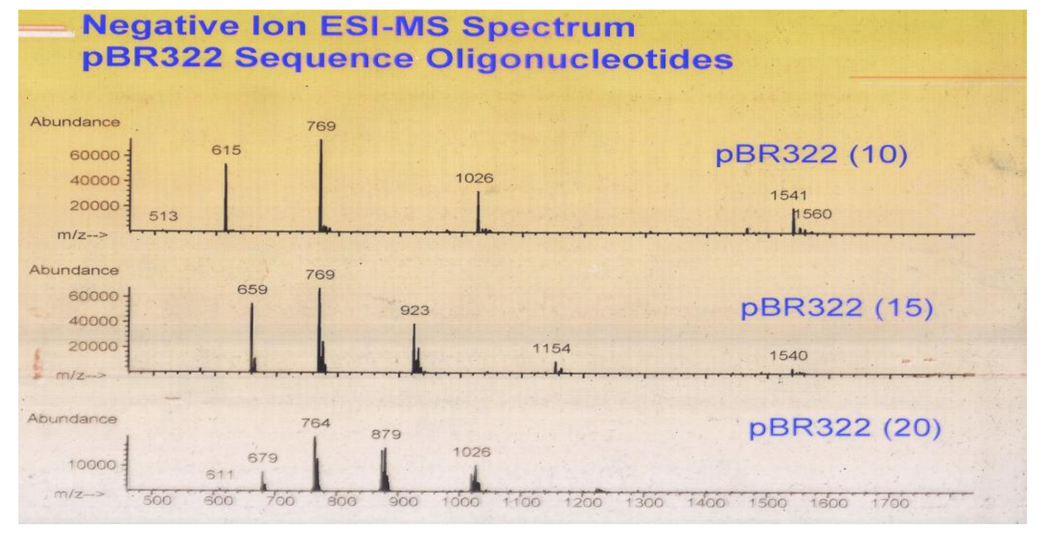
Below, we present an example of a more complicated analyte, the ESI analysis of pBR322. We show TIC for the separation of the various oligonucleotides by ESI/LC/QMS and LC/UV. Note the higher oligomers on the ESI data are much less intense than in the LC/UV data. Clearly, some process ***altered*** the relative molar relationships in this sample when it was analyzed by ESI as compared to analysis by LC/UV. The spectrum of the 20 mer has many highly adducted species including species like ($M+3K^+Na^+$).

We note here that if one is studying complicated ions in complex samples of biological origin, at low levels, as we often are in discovery proteomics, there is no doubt that the ESI/LC/MS/MS methods for complicated analytes could be challenged. In certain situations ion formation may be impacted by the concentration of alkali metals and other molecules or solution parameters in the sample. Ionization variations due to solution concentrations and other variables (e.g., solvent programming) could be extremely problematic in looking for low level disease biomarkers for complicated analytes; hence quality control checks are required to understand ion current production in samples.

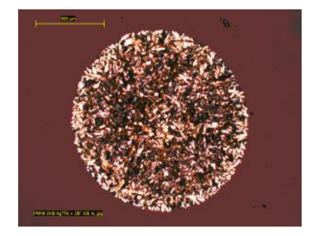
One potential value of our Beer's Law analogy is that one could test and compare results for different oligomers to standards (internally or externally) or through the running of sample dilutions. Such comparisons knowledgably applied, should prove that a system as linear or alert one to analytical problems that compromise ESI LC/MS/MS sample results in proteomics and elsewhere just as they can in spectrophotometry.







MALDI



Nanoliter- IBF Deposition
(Cross polars courtesy of Prof. Harmon et al, USF. *Polymer*, Volume 50, Issue 10, 8 May 2009, Page 2334.)

MALDI TOF MS and derivatives such as LC/MALDI are also extremely complicated experiments where samples are deposited with or without separation, evaporated, blasted with a laser without ion containment and subsequently mass analyzed. An incomplete list of authors publishing on MALDI fundamentals would include Knochenmuss (25, 26), Dreisewerd (27), Karas (28) and Hillenkamp (29) and others to whom we apologize. All show MALDI to be an extremely complicated process. Recently, Knochenmuss concluded that the "dynamic aspects of MALDI cannot be neglected" (26). The excellent fast photography poster presented at ASMS 2009 by Xing Fan and K. Murray (30), MALDI ablation can produce a mushroom cloud of analyte/matrix whose heterogeneity is visually apparent in the plume. Tissue MALDI and other MALDI experiments are more complicated from a sample preparation and ionization perspective than traditional MALDI experiments.

Moreover, the MALDI experiment is complicated by what is termed the "dried droplet method". Because it is impossible to accurately deposit low µL volumes of volatiles liquid using traditional devices without significant volumetric and spatial placement error "coffee drop rings" or heterogeneous sample spots often result andn complications exist. Using McCombie and Knochenmuss's estimation of laser surface heating shown below (31), we can consider what this means.

$$\Delta T = ILPEo\ (1\text{-}fr)/Cp d\rho A\delta$$

Where ΔT is the temperature between the surface and some depth δ and where ILPEo is the incident laser pulse energy, fr is the fraction reflected, with Cp the heat capacity, and ρ the sample density with A the irradiated area, we note that heterogeneous samples **must** yield different ion populations from different MALDI laser shots when the sample spot is irregular as ΔT is different. Such extremely simple, crucial aspects of sample preparation, dispensing and reproducible spatial sample placement have historically not been considered in fundamental papers. That stated, as shown by Tingting Tu, M. Gross et al, linear calibration curves can be obtained for (M+H)+ signals of analytes like Bradykinin when careful preparation methods are used in positive ion reflectron mode for TOF/MS analysis using nL Bu CHCA(32).

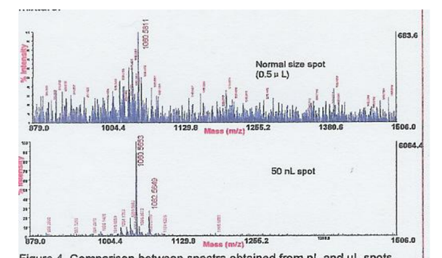
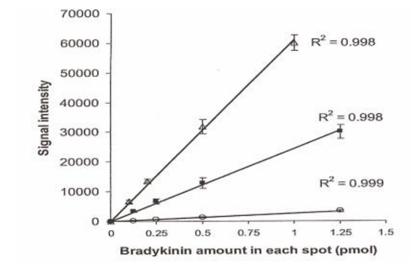


Figure 4. Comparison between spectra obtained from nL and uL spots.



Above, a 50 nanoliter deposit yields much greater signal/noise ratio than a 0.5 uL deposit. In fact quantitatively, for positive ion reflectron mode, the calibration curve shows the open circle calibration for Bradykinin with the Eppendorf pipettor (0.5 µL) is the least sensitive and those made with our nL IBF pipettor gave the best sensitivity for the solid squares 50 nL and the 20 nL using Bu-ChCA as the matrix an extremely counter intuitive result.

We believe that the reported nL-IBF depositions gave greater sensitivity than uL depositions due to the increased spatial concentration of the analyte, the excellent IBF based morphology which may be due to the fact that crystallization can start from the outside, move towards the center, a flow that is opposite the evaporative flow of a drop which may (not proven) aid mixing and hence signal intensity. (The graphs are used here from JASMS with permission requested from Elsevier.)

These results and others reported last year at ASMS proposed (33) that the ion current proportionality constant could be treated as we did in our EI work or as Kebarle did for ESI. We wrote that per our Bradykinin JASMS reference for (a = absolute) that

$$I_a = k_a\ N_a$$

If we used an internal standard, analyte pair, for relative ion current we could write……

$$I_r = k_r\ N_r \text{ or } I_r = F_r\ f(I_0) T_r\ N_r\ \varepsilon_r^*$$

εr is an optional detector analyte, internal standard correction term.

The sensitivity term was collected in one "constant" or function where the specifics of the laser energy, wavelength, power and other variables that influence the processes on the surface or in the plume, can be studied by studying k_r. This is stated as we recognize that the use of relative ion current can mask insights into fundamental processes, as it improves the data analytically.

Similarly, excellent, very useful MALDI results can be obtained for the analysis and accurate characterization of polymers as shown by Harmon (34, 35) where the crystallization of PMMA (Top) shows that the sample forms large crystals from outside to the center. Even more recently, the exciting ability to identify bacteria by NIST has been demonstrated (36). So despite complications of sample preparation and ionization, excellent progress has been and is being made in making MALDI and *LDI techniques in both qualitative and quantitative terms.

In that vein, Wallace et al have recently published a general method for quantitative measurement of molecular mass distributions as applied to C60 fullerenes functionalized with ethylpyrrolidine and measured by MALDI TOF (37). In their derivation, it was proposed that in the "linear range of target concentration vs signal intensity for each oligomer (designated i = 1, 2 , 3)" then the intensity, Ii, is related as shown, where ki converts number of oligomers in the sample n_i into a signal intensity.

$$I_i = k_i\ n_i$$

The authors who assume linear response noted that accurate molecular mass distributions (MMD) in positive ion mode gave an "accurate measured MMD which required only a small correction." They observed that "mass spectra taken in the negative ion mode did not provide as accurate MMD and could not be correlated." This anomaly does not agree with our previously cited PPINICI observations where simultaneous, but different ionization mechanisms produced linear results. These data may be indicative of a higher ordered ionization process or may be caused by ionization suppression. They are being studied.

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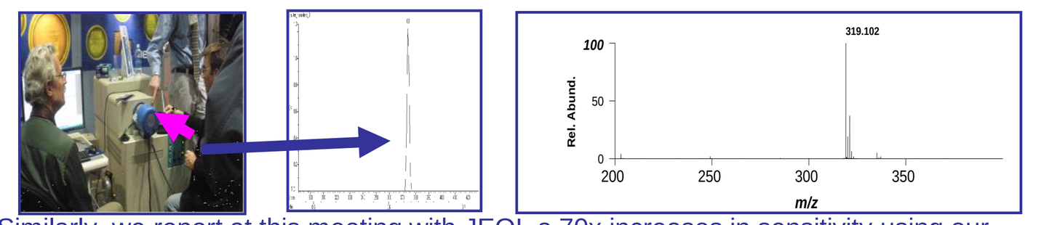
DART & New MS Techniques

100% Non-Spray MS Sample Introduction With Enhanced Sensitivity.

We've working on new ways to introduce samples in to a DART TOF/MS with Drs. Cody and Dane of JEOL. We literally shot 100 percent of nL aliquots of liquids into MS systems using nanoliter dispensers without spraying, detecting chlorpromazine below using an nL dispenser. This was done on the floor of Pittcon 2009 for the first time. See below.



Similarly, we report at this meeting with JEOL a 70x increases in sensitivity using our nanoLiter pipette with 50 nL urine samples containing oxazepam, as compared to 2.0 uL where suppression for larger volumes was apparent. We are working on maximizing parameters, but we note that by using a **non spray sample introduction like IBF** one might be able to increase sample introduction efficiency by factors approaching 100 to 1000. This should result in a major increase in the ability to detect drugs, explosives, agents, etc. The would result from much higher analyte transfer efficiency into the source with a concurrent reduction in ionization suppression. Similar enhanced results have been observed with the US Army at Edgewood, but they not cleared as of yet as we reported at LabAutomation 2010.

That stated, given the integral of the work here, we propose that the DART system with IBF sample introduction will follow the equation below if the ionization process is first or pseudo first ordered which in fact is the case. At a minimum, the "Beer's Law" formula is a useful starting point for studying 100% sample introduction that could dramatically increase MS sensitivity. (See, also Short Answer poster.)

$$I_r = F_r\ k_r\ T_r\ N_r$$

MS Data Processing Topics.

Signal/Noise, One Approach For Linear Shift Invariant Systems

Digital signal processing is extraordinarily well defined and understood (38). That stated, confusion often arises in chemical measurement applications for the definition of signal and noise given what has been called "chemical noise" in GC, LC MS and MS/MS systems. Here we only address linear shift invariant systems, not MS signals that are periodic in nature such as those common in FT/MS.

Historically, in continuous or discrete data distributions, the barrier between signal and noise is often defined as the mean current value of the ion current distribution plus three standard deviations. This assumes that the background signal is "flat" which is rare and the data is normally distributed. This problem was solved when we, inspired by Tukey's classic text Exploratory Data Analysis (39), applied a frequency filter to background distributions in examining 20,000 data files acquired in a national 17 different systems in the 70's (40). In practice, one can use spline function, f(ε), based background correction. So, for adjacent ion current sequence midpoints, ε, a joining function can be evaluated as

$$f(\varepsilon) = 1 - 3\varepsilon^2 + 2\varepsilon^3 \quad \text{with } \varepsilon = (t-t_{midi})/(t_{mid(i+1)} - t_{mid(i)})$$

Then the background ion current is defined as

$$B(t) = f(\varepsilon)\ Pi(t) + [1 - f(\varepsilon)]\ Pi{+}1$$

where the P values are the linear least square regression results for adjacent ion current sequences where mimima are employed to evaluate P. Using this approach, in spline function corrected binned ion current profiles, we found that if one collects those binned intensity values that occur more than once, that distribution was associated with noise. That is, after spline function background correction to "flatten" ion current chemical noise we found that………. noise are those intensity values that occur frequently!

We then iteratively found that the 99th percentile of the spline function corrected, frequency filtered distribution provided a boundary that agreed with human judgment on peak integration. The beauty of this approach was if the noise was Gaussian it was exactly at 3 sigma above the mean noise intensity, alternatively if the data was distributed otherwise, we were able to integrate the peaks producing "areas" in agreement with human judgment with **ONE** a data adaptive approach irrespective of whether the noise was distributed in Gaussian, non-Gaussian manner or in mixtures thereof. Therefore,

Signal intensities ≥ I(mean) + 3SD for gaussian data or more universally

Signal intensities ≥ 99th percentile BG corrected, frequency filtered ion current values.

Accurate Peak Profiles, Peak Picking

After background subtraction, one can then apply peak pickers to the date to detect peaks, the simplest being shown below with typically n, co-maximizing required for a "detection."

$$I\,i \le I\,i{+}1 \ge Ii{+}2$$

Finally, a very important point. We assume here that our data acquisition rates are adequate to accurately reflect the solution concentration of dynamically changing column effluents. We assume that we sample at greater than the Nyquist frequency (ca. ½ the peak width) and we acquire enough points across the elution of an analyte that these dynamic MS measurements accurately reflect the changes in moles vs time (dn/dt) into the source and ultimately to the static solution sample concentration.

In early, hyphenated/MS work, this was almost never the case for too many reasons to list. Today, many attempt a similar situation can arise when too many MRM's are attempted per eluting peak which can result in poor statistics and very significant quantitative error.

Ion Current Detection

Most of what we have discussed here we have presumed that an internal standard or labeled analogue (a special case of an internal standard) was present which would correct for differences on the ion current detected for an analyte and an internal or other (labeled) standard. Two papers by Stafford and Smith can help us understand how current produced vs m/z varies for continuous dynode electron multipliers and for microchannel plate detectors (41, 42). It is interesting to note that for example, for EI GC/QMS systems reported here, as the molecule gets larger, we anticipate more ion current/mole. However, ion transmission (7) and (40, 41) detector sensitivity decreases with larger, slower ions. Hence, these competing factors confound attempts to understand ion current production as it related to first principles. Also, the fact that devices can be contaminated in use can reduce the effective potentials and hence the amount of an ion packet sampled and ultimately detected always complicates MS analysis.

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Conclusions

Using MS references across the last six decades, we have shown that for EI, CI, CID, ESI, MALDI, DART and new techniques that mass spectrometers can produce linear data and always provide **both** qualitative and quantitative information, simultaneously.

We have shown that for EI for transmission systems (not traps) that when ion current is directly related to the pressure (or injected weight) and Dalton's Law is obeyed that a Beer's Law analogy can be seen as shown below..

Beer's Law, Spectrophotometry

$$A = ebc$$

EI Relative Response Beer's Law Analogy

$$I_{r(m/z)} = F_r Q_r T_r N_r$$

Here ε is an energy term, as is Q the relative ionization cross section for 70 eV/EI/QMS.

where b is the path length and % T in QMS, depends on the path length of ion traveling, l, in an analyzer of freq f, at velocity, v.

where c is the solution concentration and N is the amount of moles in the source often a dynamic process that hopefully mirrors, c of our sample.

and where the fractional ion abundance, a non-dimensional term encodes the mass spectral pattern, the qualitative term.

F = Ix/ Σ I total Σ F's = the spectral pattern or the qualitative term/s

Hence, for EI mass spectrometry qualitative and quantitative analysis are simply different parts of the same equation!

In fact, we have proposed that across MS techniques as given below, quantitative and qualitative analysis are ***different parts of equations of similar form to Beer's Law*** when the aforementioned criteria are met. We summarize.

Technique	MS Response Analogy
EI	$I_{r(m/z)} = F_r Q_r T_r N_r$
CI	$I_{r(m/z)} = F_r (g_i\ Q_{cir}) T_r N_r$
CID	$I/Io = \exp(-\sigma ln)$ or $\ln(Io/I) = \sigma ln$
ESI	$I_{esi} = F_m\ k_{esi}\ T_m\ C_m$ ideally $k_{esi,}$pure energy term
MALDI	$I_{rtof(m/z)} = F_r\ f(I_0) T_r N_r \varepsilon_r$ $f(I_0)$ = f(surface, plume, source, post source) ε_r = detector correction term, optional
New MS Techniques*	$I_r = F_r\ k_r\ T_r\ N_r$

* where the ionization is first ordered, pseudo first ordered for transmission ion systems (not traps) for an analyte, internal standard pairs in an linear analysis regime and where Dalton's Law is obeyed.

Summary

A "Beer's Law" analogy has been proposed for MS, across application spaces.

It is proposed that the analogy can be employed to verify the multivariate data quality of complex MS analysis in complex samples acquired in the m/ z, tr, R, Q, F, T, and N space of interest. Data so qualified, can then be applied to questions of larger medical, biomedical or other significance, knowing that a linear system was applied to the question at hand.

The analogy can be employed to estimate the concentration of unknowns, in a proper quality control context.

The analogy can be employed to address the accuracy of and problems with sample preparation (43).

Finally, most of this has been said before. Given (1,2,3), it is obviously worth restating some fundamentals of MS. That noted, this is the first time to our knowledge, that this Beer's Law analogy has been proposed and discussed across EI, CI, CID, ESI, MALDI and new techniques like DART for Q, QQQ, BE, TOF and yet to be invented MS ionization and analyzing systems.

We continue to evolve this work and to articulate these relationships. We look to collaborate with interested parties..

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