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Use of Liquid Reagents for Positive Chemical Ionization on the 5973 MSD

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Introduction

Two powerful features of the technique of positive chemical ionization (PCI) mass spectrometry are selectivity and control over the extent of fragmentation of the analytes of interest. This is made possible by selecting reagent gases that have different proton affinities (PAs). The potential advantages in using liquids as PCI reagents are their availability and purity, ease of handling and introduction into the mass spectrometer, low consumption, and low cost. For example, the common organic solvents—methanol, acetone, and acetonitrile—have high vapor pressures and a range of PAs that make them attractive as potential PCI reagents (Table 1). Intense adducts of the molecular ion (M^+) are possible for additional confidence in molecular identity. This is helpful in situations in which dehydration or abstraction mechanisms may convolute establishing molecular identity. Also these additional intense confirming adduct ions can improve quantitative confidence. For example, methane, the most common PCI reagent, usually produces a protonated molecular ion $(M+H)^+$ as the most abundant ion for an analyte in PCI spectra with the $(M+C_2H_5)^+$ and $(M+C_3H_5)^+$ adducts being less than approximately 10% and 5%, respectively. These ratios are very stable and are reproducible on the Agilent Technologies 5973 MSD and, by adjusting the source parameters, can be easily controlled in such a way as to be favorable for confirmation work.

The situation in PCI is very compound-specific; however, to generally explore liquid reagents and adduct formation, benzophenone (BZP) was chosen as a model compound (Figure 1).
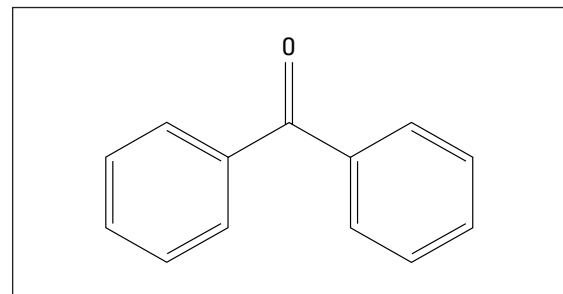

Figure 1. Benzophenone: $C_{13}H_{10}O$, molecular weight 182 g/mole.

Table 1. Compound Physical and Thermochemical Properties.

Compound	Molecular Weight	Vapor Pressure (at °C)	Proton Affinity (kcal/mole)[1]
Methane	16	–	131.6, 162.6
Methanol	32	125 torr (25°C)	181.9
Acetonitrile	41	89 torr (25°C)	188.2
Acetone	58	184 torr (20°C)	196.7
Ammonia	17	–	204.0

Experimental

The Agilent Technologies 6890 GC Plus with the 5973 Mass Selective Detector with CI option was used for all experiments. A PCI autotune with methane reagent gas was used for the initial source tuning parameters and lens voltages.

Liquids were introduced into the CI manifold through a short length of Teflon tubing (0.317 cm o.d. tubing with 0.159 cm wall) with a 1/8-inch Swagelok connector. Liquids were contained in either a standard 1-liter HPLC bottle (Figure 2) or in a custom glass tube of 100 ml capacity (Figure 3) and positioned below the manifold to avoid siphoning of liquid into the instrument. The end of the Teflon tubing was suspended just above the surface of the liquid, and the vessel was purged with argon or helium prior to use. The liquid's vapor displaces air or purge gas in the vessels and generates an atmosphere that consists primarily of the chemical of interest. Placing the vessel in an ultrasonic cleaner assists in generating vapor.

The flow into the source, which is directly proportional to source pressure, was controlled by the standard 5973 CI manifold. Although this reagent gas flow is continuously adjustable, experiments with each liquid were conducted at four flow increments: 5%, 10%, 15% and 20% of the total flow of 5 ml/min. In each experiment, benzophenone was injected and spectra were recorded in full scan with a 200-amu mass range. The mass range was adjusted to avoid collecting reagent ions.

Results

The most critical parameters affecting the intensities of reagent ions in the source and the abundances of the $[BZP+H]^+$ and $[BZP+Adduct]^+$ products were source temperature and pressure. As expected, the lower the source temperature, the higher the abundance of the adducts. A source temperature of 150°C was selected. The results for each liquid are presented as the intensities of the reagent ions in the source versus flow setting. These reagent ion intensities are normalized to the most abundant reagent monomer ion ($[Reagent+H]^+$) intensity. Similarly, abundances of the $[BZP+H]^+$ and the $[BZP+Reagent+H]^+$ products versus source pressure are presented relative to the most abundant $[BZP+H]^+$ produced at any flow setting.

Figure 2. HPLC Bottle as liquid CI reagent container.

Figure 3. 100 ml liquid CI reagent tube.

Methanol Results

Methanol (MeOH) easily generated dimers and trimers in the source (Figure 4). A flow setting of 10% generated the greatest abundance of the $[MeOH+H]^+$ reagent ion. As flow into the source increased, the degree of "solvation" of the proton increased $[H^++(MeOH)_n]$, and at the highest setting used there was 4 times more dimer and 3 times more trimer than monomer.

Despite the high abundances of dimers and trimers, relatively small amounts of adduct were formed with benzophenone (Figure 5). The highest flow setting applied generated the highest amounts of total reagent ions, as well as product $[BZP+H]^+$ and $[BZP+MeOH+H]^+$ ions.
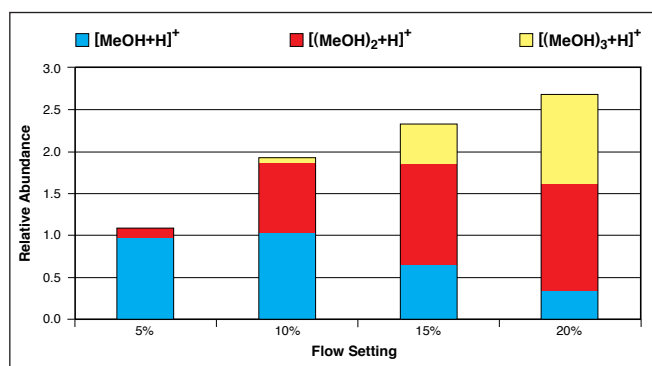

Figure 4. Abundance of methanol PCI reagent ions as a function of flow setting.
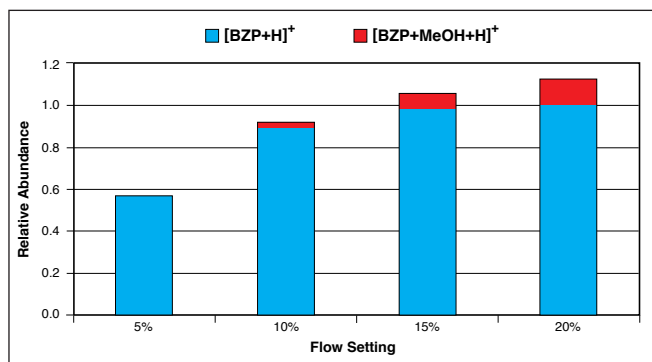

Figure 5. Benzophenone product on abundances as a function of methanol reagent gas flow setting.

Acetonitrile Results

Acetonitrile (ACN) readily formed dimers in the source (Figure 6). The most abundant monomer was formed at the lowest source pressure studied, the 5% flow setting, and the abundance of dimers was approximately 50% that of monomers. The next higher increment in flow to the source, the 10% flow setting, equivalent to doubling the source pressure of ACN, generated the largest total ion intensity in the source (sum of monomers and dimers) and the largest amount of $[BZP+H]^+$ product ion (Figure 7). As the source pressure was further increased from 10% to 20%, the abundance of $[(ACN)_2+H]^+$ reagent ion remained relatively constant, but the intensity of $[ACN+H]^+$ reagent decreased. Simultaneously the percentage of adduct ion increased until at the highest source pressure studied, the $[BZP+ACN+H]^+$ adduct ion intensity was nearly 75% that of the $[BZP+H]^+$ ion. However, the product ion signal reached a maximum near the 10% flow setting.
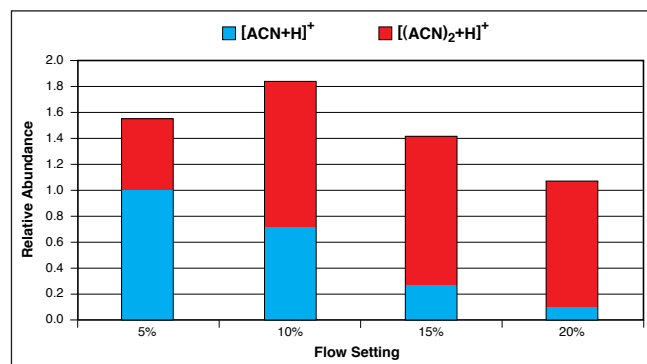

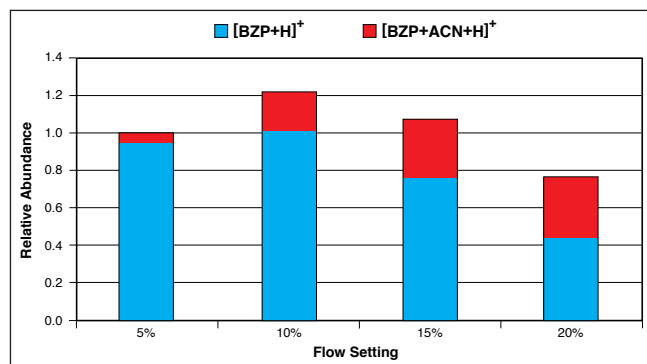**Figure 6. Acetonitrile PCI reagent ions as a function of flow setting.**

Figure 7. Benzophenone product ion abundances as a function of acetonitrile reagent gas flow setting.

Acetone Results

The trends for acetone were similar to those for acetonitrile. Protonated acetone reagent dimers dominated monomers in the source at every flow setting studied, most likely because of the large dipole moment and molecular size (Figure 8). The absolute dimer abundance was essentially constant at the four flow settings, but monomer abundance decreased as the source pressure increased, similar to the findings for acetonitrile. At the 20% flow setting, dimer abundance was more than 40 times that of the monomers.

Product ions were similarly dominated by adduct formation over protonated BZP at all settings, and $[BZP+H]^+$ decreased as flow to the source increased (Figure 9). The 5% and 10% flow settings produced the maximum total signal and relative adduct intensities of 65% and 190% of $[BZP+H]^+$. This implies that a situation that is optimum for quantitation may be produced where the intensity of the quantitation ion and confirming ion are of equal intensity; that is, when $[BZP+H]^+ = [BZP+Acetone+H]^+$.

Deuterated Liquid Reagents: CD_3CN

Deuterated organic solvents are readily available and inexpensive. In PCI they can produce $[M+D]^+$, as well as identifying adducts. A mixture of 50:50 (w/w) of CH_3CN and CD_3CN was used as the CI reagent at a flow setting of 20%. Using this reagent, a variety of species is possible in the source. Table 2 shows the source ion composition and relative intensity in the source at this setting.

The resulting PCI mass spectrum for benzophenone shows intense BZP+D and BZP+H ions, as well as a variety of adducts (Figure 10). Table 3 lists the product ion compositions, mass-to-charge ratios, and relative abundances.
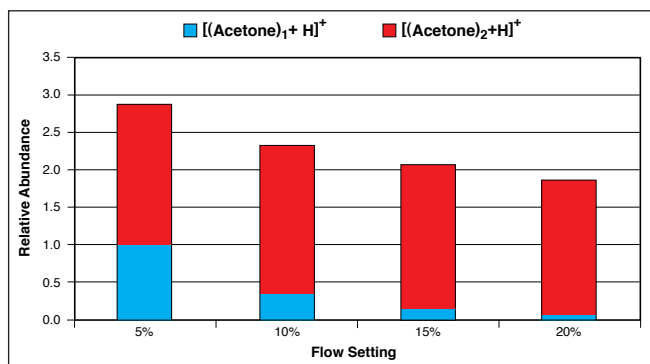

Figure 8. Acetone PCI reagent ions as a function of flow setting.
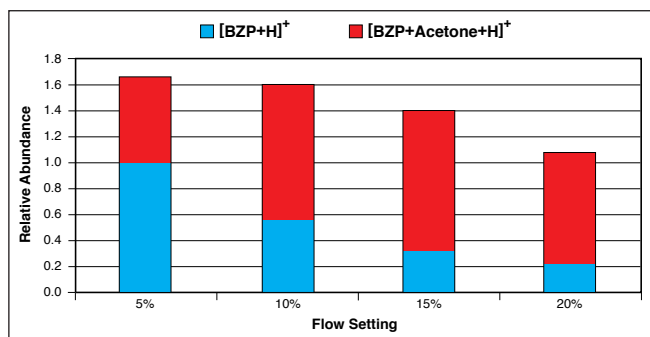
Figure 9. Benzophenone product ion abundances as function of acetone reagent gas flow setting.

Table 2. Reagent Ion Composition, Mass-To-Charge Ratio and Relative Abundance generated by a 50:50 mixture of CH_3CN and CD_3CN.

Reagent Ion Formula	m/z (Relative Abundance)
$[CH_3CN+CD_3CN+H]^+$	86 (100%)
$[CH_3CN+CD_3CN+D]^+$	87 (80%)
$[(CH_3CN)_2+H]^+$	83 (62%)
$[(CH_3CN)_2+D]^+$	84 (62%)
$[(CD_3CN)_2+H]^+$	89 (44%)
$[(CD_3CN)_2+D]^+$	90 (40%)
$[(CH_3CN)+H]^+$	42 (5%)
$[(CH_3CN)+D]^+$	43 (4%)

Table 3. Product Ion Composition, Mass-To-Charge Ratio and Relative Abundance generated by a 50:50 mixture of CH_3CN and CD_3CN.

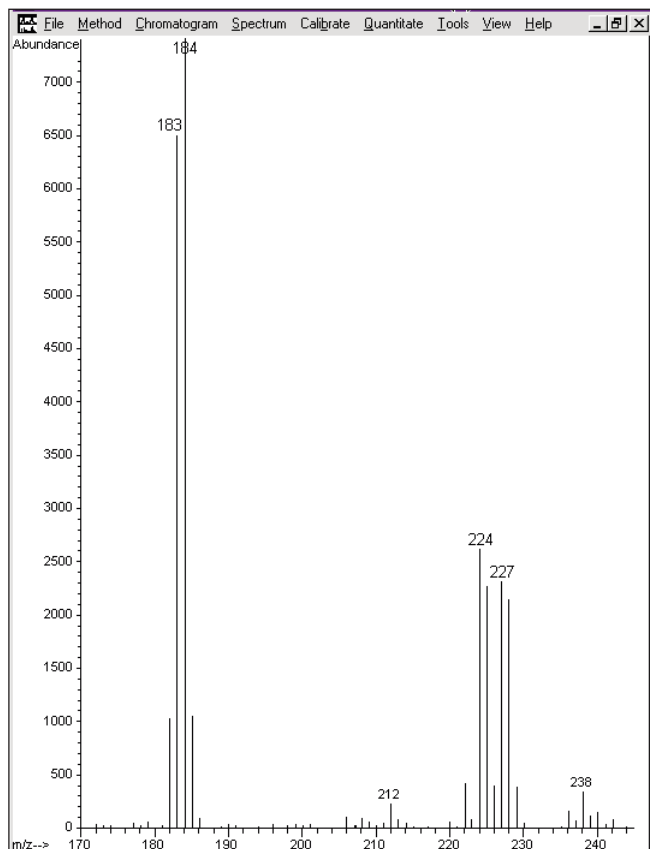| Product Ion	m/z (Relative Abundance)
$[BZP+D]^+$	184 (100%)
$[BZP+H]^+$	183 (88%)
$[BZP+CH_3CN+H]^+$	224 (35%)
$[BZP+CH_3CN+D]^+$	225 (31%)
$[BZP+CD_3CN+H]^+$	227 (32%)
$[BZP+CD_3CN+D]^+$	228 (30%)

Figure 10. Benzophenone PCI spectra with acetonitrile/acetonitrile-d$_3$ (50:50 w/w) PCI reagent gas.

Conclusions

Liquid reagents are *easily* implemented on the 5973 MSD-CI for use in PCI. The instrument parameters provide a great deal of flexibility, which allows the analyst to optimize PCI performance for the compounds of interest. The most important parameters for any specific reagent gas or analyte are source temperature and reagent pressure. By adjusting the source parameters of temperature and reagent flow setting, the intensity of the protonated molecular ion can be optimized or the abundance of adducts or both. Just as with the traditional CI gases (methane, ammonia, etc.), these adducts can be utilized to confirm the analyte molecular weight since they will result in a known m/z value and relative abundance of M+Adduct ion. For example, the results for PCI of

benzophenone with acetone show that the highest intensity of protonated benzophenone occurs at the lowest setting (Figure 9). However, increasing the flow slightly allows the adduct to increase with little loss in total intensity; a situation useful in quantitation where an intense confirming ion is useful. The results for acetonitrile show that equal intensities of the protonated benzophenone and benzophenone-adduct are not achieved until the flow setting has been raised to 20% (Figure 7).

Similarly, mixtures of native and deuterated liquid reagents can generate a variety of confirming adduct ions in addition to M+1 and M+2. This feature may be helpful in distinguishing ions formed by abstraction $(M–1)^+$ or dehydration $(M–H_2O)^+$ from those molecular ions expected via molecular protonation which can confound the determination of molecular weights. In other words, if one expects to see M+2 or M+Adduct in the mass spectrum of a particular compound but they are not observed, it suggests that another mechanism of ionization is taking place such as loss of hydrogen, $[M–H]^+$, or water, $[M–H_2O]^+$. This in turn provides some information about chemical structure and functional groups. The ability of the 5973 MSD-CI to conveniently manipulate the reagent source pressure allows the analyst to manipulate the ratios of adduct formation and protonation to aid in confirmation or quantitation.

However, it is *highly* recommended that the classic gaseous reagents be explored before resorting to liquid reagents. Methane and ammonia span a greater range of proton affinities than the liquid reagents and are well characterized in their behavior and operation. All the aforementioned conclusions about source parameters and adduct formation, etc., are equally valid for the gaseous PCI reagents. The most efficient and straight-forward approach for confirming identity, studying functional groups, and arranging favorable quantitation ratios and one that is extremely easy to implement on the 5973 MSD-CI, is to first apply methane and then ammonia. Further, perdeuterated methane and ammonia are both readily available and relatively inexpensive and, since CI gas use is only of the order of 0.5–1 ml/min, their use is economically feasible.

References

1. Harrison, A.G.: *Chemical Ionization Mass Spectrometry,* Second Edition, CRC Press [1992]

Method Precautions

NEVER HEAT flammable solvents such as those used in this study! Use of halogenated solvents and carbon disulfide should be avoided as these may affect the electron capture negative ionization sensitivity.

Acknowledgments