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GASEOUS ACIDITY AND BASICITY SCALES AS GUIDES TO CHEMICAL IONIZATION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY WITH DIRECT LIQUID INTRODUCTION

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SUMMARY

During direct liquid introduction (DLI) liquid chromatography–mass spectrometry (LC–MS), the detectability of acetone is shown to be predictable, based on consideration of the gas-phase acidities and basicities of solvents methanol and acetonitrile and modifiers formic acid and ammonium formate. Consequently, ion formation in DLI LC–MS resembles gas-phase chemical ionization processes; since the order of acidities is altered in solution, solution ionization must be much less important than gas-phase. Ion populations could be predicted on the basis of gas-phase proton affinities and acidities: acetone could be detected in all solvent mixture by positive ions, but it was not readily detected by negative ions when acid was also present. In solvents without additives it was always detected.

INTRODUCTION

Current techniques for the coupling of liquid chromatography (LC) and mass spectrometry (MS) may be classified according to whether the chromatographic solvent is fully removed before introduction of the LC effluent into the MS ion source. In those procedures where a proportion of the solvent is retained, ion production may involve both condensed-phase and gas-phase interactions, with the chemical nature of the solvent being of key importance. Consideration of the direct liquid introduction (DLI) technique (in which analyte ion production is promoted by the generation of an ion plasma in a chemical ionization MS source) has included nebulizer design^{1–8} and vacuum system considerations^{2,9}. This DLI reagent ion plasma has been modified through the introduction of ammonia^{10,11} or dichlorodifluoromethane¹² into the ion source. Solvent selection for DLI LC–MS, however, has usually been based mainly on chromatographic considerations, with the additional mass

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spectrometric requirement that if buffers are used, they should be of fairly low molecular weight and high volatility. Solvent selection for DLI based on mass spectrometric criteria has thus far been limited to the addition of chlorinated solvent modifiers for LC-chloride-attachment negative chemical ionization MS¹³.

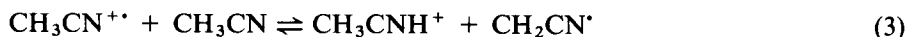
Selecting a solvent should also be based on its properties in chemical ionization MS. The most common solvents for reversed-phase LC are water, acetonitrile and methanol. We propose that their role in controlling the mass spectra produced by direct liquid introduction of the eluate can be understood by reviewing¹⁴⁻¹⁷ ionic equilibria in the gas phase. Water and methanol can function as reagent gases in chemical ionization MS¹⁸, effecting proton transfers to sufficiently basic solutes (M) as in reaction 1.



On the other hand, MH⁺ ions observed in the LC-MS analysis of acetonitrile solutions are not expected to arise from reaction 2.



The protonated acetonitrile ion is usually not abundant in spectra when pure acetonitrile is solvent, for even though the reaction of self-protonation of acetonitrile (reaction 3) is exothermic by 327 kJ mol⁻¹ (refs. 16 and 19) it is expected to be a slow process because a C-H bond breaks²⁰.



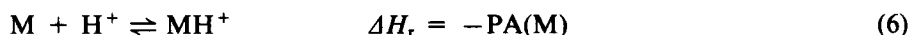
As no CH₃CNH⁺ is available for the formation of MH⁺, it must be formed by a self-protonation (reaction 4) of M.



When solvent mixtures are used, the situation can become more complicated. For example, many Lewis bases can be protonated by both CH₃OH₂⁺ and H₃O⁺ in a gaseous water-methanol solvent, and the product ion MH⁺ has a different average internal energy depending on whether it was formed by H₃O⁺ or CH₃OH₂⁺. Ion internal energy arises from the exothermic nature of the proton transfer (reaction 5)



and is determined by the difference in proton affinities (PAs, defined in eqn. 6)¹⁶ of the two bases A and B.



Thus for reaction 5, ΔH_r is equal to [PA(A) - PA(B)]. Protonation by H₃O⁺ is 49 kJ mol⁻¹ more exothermic than protonation by CH₃OH₂⁺, because the difference in their proton affinities is 49 kJ mol⁻¹ (refs. 16 and 19). (The formation of a CH₃OH₂⁺ ion from H₃O⁺, being exothermic by 49 kJ mol⁻¹), is efficient. Therefore,

CH_3OH_2^+ is more abundant, and MH^+ is formed from CH_3OH_2^+ in greater proportion than predicted by the solvent ratio). In acetonitrile-water, CH_3CNH^+ would be formed from H_3O^+ and CH_3CN , because the reaction is exothermic^{16,19}, and so, with this new source of CH_3CNH^+ , reaction 2 could proceed.

The situation is also more complex in principle when an acid or a buffer is added. The presence of formic acid in the solvent means that protonation by HCOOH_2^+ could compete with protonation by protonated solvent; the presence of ammonium formate requires the consideration of protonation by NH_4^+ .

Quantitative prediction of proton-donating ability in the gaseous phase can be made from Table I, which lists proton affinities of the reversed-phase liquid chromatography solvents and of acetone, the model solute used in this study.

TABLE I
PROTON AFFINITIES OF REVERSED-PHASE LC SOLVENTS IN GAS AND CONDENSED PHASES

<i>Solvent</i>	<i>PA¹⁶ of gaseous M (kJ mol⁻¹)</i>	<i>pK_a of MH⁺ (solution)</i>
Ammonia	858	+9.24 (ref. 21)
Acetone	825	-7.2 (ref. 22)
Isobutane	820	
Acetic acid	798	-6.1 (ref. 23)
Acetonitrile	797	-10 (ref. 21)
Methanol	773	-2 (ref. 21)
Formic acid	765	< -6.1 (refs. 24 and 25)
Water	724	-1.74 (ref. 21)
Methane	552	

Thermochemical predictions of negative chemical ionization results in LC-MS can be generated from Table II, which lists the ΔH_{acid} values (eqn. 7)²⁶ of the same species.



TABLE II
 ΔH_{acid} VALUES OF REVERSED-PHASE LC SOLVENTS IN GAS AND CONDENSED PHASES

<i>Solvent</i>	<i>ΔH_{acid} (gaseous)²⁶ (kJ mol⁻¹)</i>	<i>pK_a of M (solution)</i>
Ammonia	1671	34 (ref. 21)
Water	1635	16 (ref. 21)
Methanol	1587	16-17 (refs. 21 and 27)
Acetonitrile	1557	25 (ref. 21)
Acetone	1543	20 (ref. 28)
Acetic acid	1458	4.75 (ref. 29)
Formic acid	1444	3.75 (ref. 29)
Hydrogen chloride	1394	

The ΔH_{acid} measures the endothermicity of dissociation into gaseous ions of the species listed. The stronger the acid, the lower the endothermicity of dissociation. So, for example, in gaseous water–methanol, the major ion is expected to be CH_3O^- , since its formation from any hydroxide initially formed and CH_3OH molecules is 48 kJ mol^{-1} exothermic^{19,26}. Similarly, CH_2CN^- should be the principal ion in the gaseous water–acetonitrile mixture, for its formation from any original hydroxide present and CH_2CN^- is 78 kJ mol^{-1} exothermic^{19,26}. (It is important to note that experimental confirmation of these proposals would establish the dominance of gas-phase ionic processes over condensed-phase processes in DLI, at least in this system, since the order of acidities reverses between solution and gas phase.) The presence of formic acid in the solvent would deplete the spectra of methoxide and CH_2CN^- , because the production of formate from the acid and methoxide and CH_2CN^- is exothermic, by 143 and 113 kJ mol^{-1} , respectively^{19,26}.

The detection of a solute in these systems depends upon the addition of a proton to the solute molecule for positive ion spectra or the removal of a proton for negative ion spectra. (We defer discussions of examples where electron capture or anion attachment predominates.) According to this model of gas-phase reactivity, because the test solute acetone has a higher PA than any of the common solvent components except ammonia, it would accept a proton from any protonated solvent component except ammonia, and so it can be easily detected. Conversely, because its ΔH_{acid} is lower than those of water, methanol and acetonitrile, its conjugate base $\text{CH}_3\text{COCH}_2^-$ is formed exothermically from their conjugate bases (by 92, 44, and 14 kJ mol^{-1} , respectively)^{19,26} and can be detected in negative ionization mass spectra. On the other hand, $\text{CH}_3\text{COCH}_2^-$ readily accepts a proton from CH_3COOH and HCOOH , since their ΔH_{acid} values are lower (*i.e.*, dissociation into ions is less endothermic for them) by 85 and 99 kJ mol^{-1} . The presence of acetic and formic acids in the solvent system should make the detection of acetone difficult by negative ion MS.

Substantial collections of data are available only for protonation and deprotonation thermochemistry. The prediction of equilibria of cluster species (reaction 8)³⁰ is, for the most part, beyond the scope of this paper, simply because the positions of equilibria involving two molecules bound to a proton have not been broadly established.



EXPERIMENTAL

The liquid chromatograph–mass spectrometer has been previously described^{31,32}. It consists of a Waters chromatograph (Waters Assoc., Milford, MA, U.S.A.) and a Finnigan Instruments 3300 quadrupole mass spectrometer interfaced via a DLI apparatus (Finnigan-MAT, San Jose, CA, U.S.A.). A C_{18} reversed-phase column (Brownlee Labs., Santa Clara, CA, U.S.A.) was used, but only as a guard column; single solutes were introduced via repeated injection. Solvents were HPLC grade (Fisher Scientific, Fair Lawn, NJ, U.S.A.); solutes were purchased from Aldrich (Milwaukee, WI, U.S.A.). The flow-rate used was 1 ml/min, with approximately 20–30 $\mu\text{l/min}$ of the flow being directed into the source of the mass spectrometer.

Acetone was injected in quantities sufficient to cause a decrease in the reagent ion signals; details are given in the tables.

RESULTS AND DISCUSSION

Our results confirm the predictions of the hypothesis above qualitatively, but not quantitatively. The intensity ratios of ion related by a proton transfer are predicted to be greater or less than unit by consideration of proton transfer equilibria, but the ratios calculated by the Boltzmann equation are not reached.

Positive ions

Water (Table III). The behavior of acetone in 100% water is unremarkable, and the spectrum is included for completeness. The ions due to water are H_3O^+ and its solvate, $\text{H}_3\text{O}^+ \cdot \text{H}_2\text{O}$. When acetone is added, proton transfer to form $\text{CH}_3\text{COHCH}_3^+$ from H_3O^+ , a process exothermic by 101 kJ mol^{-1} , occurs as expected. There is also substitution in $(\text{H}_2\text{O})_2\text{H}^+$ to give $(\text{CH}_3\text{COCH}_3)(\text{H}_2\text{O})\text{H}^+$, which we write as a water solvate of protonated acetone, $\text{CH}_3\text{COHCH}_3^+ \cdot \text{H}_2\text{O}$, to reflect the greater proton affinity of acetone in the competition of the bases in the cluster for the proton. A tiny amount of the doubly substituted species $(\text{CH}_3\text{COCH}_3)_2\text{H}^+$ also appears.

When 0.1 M ammonium formate is added as a buffer, the most intense peak is due to NH_4^+ , but, since practically all the ammonia present is protonated, competition between other neutral Brönsted bases for the protons generated in the first chemical-ionization ion-molecule reaction can occur. Formic acid, more basic than water, produces an m/z 47 ion much larger than its concentration alone would suggest, but the intensity of the remaining H_3O^+ peak suggests either that HCOOH neutrals are depleted or that the reaction is far from equilibrium. Both of the protonated species, HCOOH_2^+ and H_3O^+ , protonate acetone in strongly exothermic processes.

Aqueous formic acid (1%) yields HCOOH_2^+ as the dominant solute ion, and when acetone is introduced, the intensity of the protonated formic acid is transferred to protonated acetone. Again, protonated acetone should be the ultimate product of proton transfer in the system because of its thermochemical stability.

Methanol and aqueous methanol (Table IV). When acetone is injected, the increase in the m/z 59 ion is accompanied by a decrease in m/z 65, consistent with the formation of protonated acetone ultimately from protonated methanol. Little changes when water is added to the solvent (water-methanol, 50:50). Protonation is expected to be mostly from CH_3OH_2^+ , since H_3O^+ will have been consumed efficiently by collision with CH_3OH to produce CH_3OH_2^+ as noted in the introduction. The H_3O^+ ion is not totally consumed because m/z 19 is still present, even with a difference of 49 kJ between the proton affinities of water and methanol. For a Boltzmann distribution, the fraction of H_3O^+ expected is less than $1 \cdot 10^{-8}$. This is typical behavior for ions from mixed solvents in our instrument: commensurate quantities of the conjugate acids of each component appear. This is a vivid demonstration that the equations are not a quantitative guide to observed behavior. After the ranking of acidities of gaseous alcohols was found to be reversed from their ranking in solution³³, it was discovered that the acidities of singly solvated alcohols were an in-

TABLE III

RELATIVE ABUNDANCES OF SAMPLE AND SOLVENT IONS (WATER AND ACETONITRILE, POSITIVE IONS)

A, 100% acetonitrile; B, acetonitrile-water (50:50); C, 100% water; D, water containing 0.1 M ammonium formate; E, water-acetonitrile (50:50) containing 0.1 M ammonium formate; F, water containing 1% formic acid; G, water-acetonitrile (50:50) containing 1% formic acid.

Ions (<i>m/z</i>)	A		B		C		D		E		F		G	
	-*	+**	-*	+**	-*	+**	-*	+**	-*	+**	-*	+**	-*	+**
<i>Solvent</i>														
108	1	1	-	-	-	-	-	-	-	-	-	-	-	-
95	11	22	-	-	-	-	-	-	-	-	-	-	-	-
83	100	18	100	1	-	-	-	-	100	12	-	-	16	-
65	-	-	-	-	-	-	6	2	3	3	8	3	-	-
61	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	4	11	1	3	-	-	-	-	-	-	-	-	-	-
55	1	2	-	1	6	2	3	-	4	3	5	1	-	1
54	4	7	1	1	-	-	-	-	-	-	-	-	-	-
47	-	-	-	-	-	-	-	32	-	-	-	-	5	-
42	11	15	12	4	-	-	-	-	59	33	-	-	100	5
40	1	2	1	1	-	-	-	-	-	-	-	-	-	-
37	-	-	-	-	71	38	26	-	-	-	52	19	-	-
36	-	-	-	-	-	-	4	-	-	-	-	-	-	-
19	-	-	1	1	100	71	73	6	4	6	100	58	4	7
18	-	-	-	-	-	-	100	30	36	69	-	-	1	1
<i>Sample</i>														
117	-	17	-	100	-	1	-	15	-	10	-	1	-	28
100	-	100	-	32	-	-	-	-	-	34	-	-	-	-
77	-	-	-	1	-	3	-	1	-	-	-	2	-	-
76	-	-	-	-	-	-	-	3	-	1	-	-	-	-
73	-	-	-	1	-	-	-	1	-	-	-	-	-	1
59	-	17	-	56	-	100	-	100	-	100	-	100	-	100
43	-	4	-	6	-	1	-	7	-	-	-	1	-	17
15	-	1	-	-	-	-	-	-	-	-	-	-	-	-

* No acetone injected.

** 10 μ l acetone injected.*** 50 μ l acetone injected.§ 100 μ l acetone injected.§§ 30 μ l acetone injected.

intermediate case between solution and unsolvated gas-phase ions³⁴. Thus our results may reflect proton transfers occurring between species solvated by one or a few molecules. A less attractive explanation is that equilibrium is not achieved. In any case, the gaseous thermochemistry of the system is only a qualitative guide to ion behavior.

There are two peaks of more complex origin. The methylation of acetone to give m/z 73 is related to the methylation of methanol to give m/z 47, protonated methyl ether, a process well known from the earliest days of studies of ion-molecule reactions of functional-group compounds³⁵. Methyl ion transfer from m/z 47 to acetone to produce m/z 73 is not definitely known to be exothermic, but most likely is, because studies of methyl cation affinities³⁶ have shown that most of the time if a transfer of a proton from A to B is exothermic, so also is transfer of a CH_3^+ .

Table IV illustrates the appearance of the spectrum upon addition of 0.1 *M* ammonium formate to the water-methanol mixture. As with water, the largest peak

TABLE IV

RELATIVE ABUNDANCES OF SAMPLE AND SOLVENT IONS (WATER AND METHANOL, POSITIVE IONS)

A, 100% methanol; B, methanol-water (50:50); C, methanol-water (50:50) containing 0.1 *M* ammonium formate; D, water-methanol (50:50) containing 1% formic acid.

Ions (m/z)	Tentative ID	A		B		C		D	
		-*	+**	-*	+**	-*	+**	-*	+**
<i>Solvent</i>									
97	$(\text{CH}_3\text{OH})_3\text{H}^+$	8	-	7	5	-	-	1	-
83	$(\text{CH}_3\text{OH})_2(\text{H}_2\text{O})\text{H}^+$	-	-	2	2	-	-	1	-
79	$(\text{CH}_3\text{OH})_2\text{CH}_3^+$	10	-	10	9	1	-	14	1
65	$(\text{CH}_3\text{OH})_2\text{H}^+$	100	2	100	100	22	1	100	1
51	$(\text{CH}_3\text{OH})\text{H}_3\text{O}^+$	-	-	3	3	1	-	7	-
50	$(\text{CH}_3\text{OH})\text{NH}_4^+$	-	-	-	-	4	1	-	-
47	$(\text{CH}_3\text{OH})\text{CH}_3^+$ or HCOOH_2^+	10	3	10	11	10	1	45	1
46	HCOOH^+	-	-	-	-	-	-	5	1
36	$(\text{H}_2\text{O})\text{NH}_4^+$	-	-	-	-	2	1	-	-
33	CH_3OH_2^+	22	25	38	37	6	9	68	5
31	CH_2OH^+	2	5	3	3	-	1	2	1
19	H_3O^+	-	-	9	9	1	3	7	1
18	NH_4^+	-	-	-	-	100	97	2	-
15	CH_3^+	3	7	4	3	1	1	-	1
<i>Sample</i>									
117	$(\text{CH}_3\text{COCH}_3)_2\text{H}^+$	-	100	-	1	-	11	-	14
91	$(\text{CH}_3\text{COCH}_3)\text{CH}_3\text{OH}_2^+$	-	17	-	8	-	2	-	5
76	$(\text{CH}_3\text{COCH}_3)\text{NH}_4^+$	-	-	-	-	-	4	-	-
73	$(\text{CH}_3\text{COCH}_3)\text{CH}_3^+$	-	6	-	-	-	5	-	3
59	$(\text{CH}_3\text{COCH}_3)\text{H}^+$	-	66	-	8	-	100	-	100
43	CH_3CO^+	-	10	-	1	-	6	-	6
15	CH_3^+	-	7	-	3	-	1	-	1

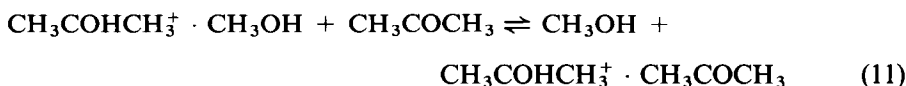
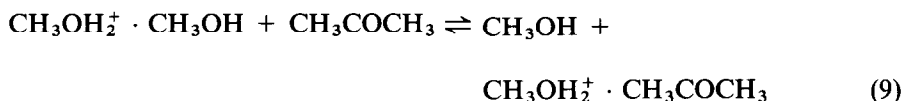
* No acetone injected.

** 100 μl acetone injected.

in the spectrum is the ammonium ion. Addition of acetone again produces a large protonated acetone peak. The origin of the protonated acetone peak cannot be from ammonium ion, but instead from protonated solvent species. If virtually all the ammonia present is already protonated, then the conjugate acids of both water and methanol will protonate the second most basic species present, acetone, efficiently.

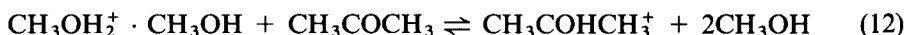
Table IV also illustrates the behavior upon addition of 1% formic acid. In the absence of acetone, a substantial m/z 47 peak is observed. Its intensity is in excess of that seen previously for protonated methyl ether from the methanol, and so in large part it is due to protonated formic acid. Both methanol and formic acid are of similar proton affinity, and achievement of equilibrium between their protonated and unprotonated forms would be less efficient than strongly exothermic processes according to studies of rates of only slightly exothermic proton transfers³⁷. However, when acetone is added as a solute, each of these species can transfer a proton efficiently to the much more basic acetone, and the major protonated species once again is the m/z 59, protonated acetone.

Many peaks are due to ions with masses considerably above those of individual molecules. These cluster ions result from solvation of the protonated species by other Brönsted bases: $\text{CH}_3\text{OH}_2^+ \cdot \text{H}_2\text{O}$, $\text{NH}_4^+ \cdot \text{CH}_3\text{OH}$, $\text{NH}_4^+ \cdot \text{H}_2\text{O}$, $\text{CH}_3\text{OH}_2^+ \cdot \text{H}_2\text{O}$, $\text{CH}_3\text{OH}_2^+ \cdot \text{CH}_3\text{OH}$, $\text{CH}_3\text{COHCH}_3^+ \cdot \text{CH}_3\text{OH}$, $\text{NH}_4^+ \cdot \text{CH}_3\text{COCH}_3$, $\text{CH}_3\text{COHCH}_3^+ \cdot \text{CH}_3\text{COCH}_3$; and the multiply solvated $\text{CH}_3\text{OH}_2^+ \cdot 2\text{CH}_3\text{OH}$ and $\text{CH}_3\text{OH}_2^+ \cdot \text{CH}_3\text{OH} \cdot \text{H}_2\text{O}$. A hydrogen bond to the first solvating molecule in the gas phase is considerably stronger, 80–150 kJ mol^{-1} , than in solution³⁸, and such species must be stabilized by collision to carry off excess internal energy. Very little is known about thermochemistry of these cluster ions, for no studies in mixed water–methanol–ammonia–acetone systems have been carried out, and very little in simpler systems either^{30,39,40}. It is impossible to point to an adequate literature basis, then, for interpreting behavior of these ions. On the other hand, introduction of acetone reduces the intensity of the proton-bound dimer of methanol, $\text{CH}_3\text{OH}_2^+ \cdot \text{CH}_3\text{OH}$, so that a displacement mechanism such as reactions 9–11 is plausible.



The mechanism suggests that methanol and acetone compete for solvated protons with the same result as they have for unsolvated protons: acetone provides the more stable product.

On the other hand, recognition of so large a bond strength of the hydrogen bond in cluster species makes it implausible that a thermalized cluster species is ever the precursor of protonated solute ion. Consider the proposed reaction 12.



While 48 kJ mol^{-1} is gained on transferring the proton from methanol to acetone, much more than that is lost in breaking the hydrogen bond, and the process is endothermic.

Acetonitrile and aqueous acetonitrile (Table III). Analogous behavior is noted in the acetonitrile–water solvent system, but we will deal with complications by a large number of peaks to reactions of ions from acetonitrile with neutral acetonitrile molecules first. Thus, in 100% acetonitrile solvent, there are ions at m/z 108, 95, 56, 55 and 54, due to the condensation of acetonitrile species. These have all been observed in ion cyclotron resonance, high-resolution, or collisional activation studies^{41–43} and are not subjects of speculation. The principal ions are protonated acetonitrile and its solvate by another acetonitrile molecule. Addition of acetone provides m/z 59, from protonation by CH_3CNH^+ , exothermic by 28 kJ mol^{-1} , and m/z 100 and 117, presumably by stepwise displacement of acetonitrile by acetone from the cluster ($\text{CH}_3\text{CNH}^+ \cdot \text{CH}_3\text{CN}$), analogously to the proposal for the methanol system.

Addition of 50% water accelerates the rate of protonation of acetonitrile, so that the condensation products are much lower in intensity. Acetone is efficiently protonated, as expected, and in the cluster ion, acetonitrile solvates protonated acetone more effectively than does water. Thus once again the order of affinities for solvated protons parallels that for unsolvated protons.

The presence of 0.1 *M* ammonium formate in water–acetonitrile (50:50) alters the spectrum as expected. The dominant ammonium ion derives from the additive, and remaining protons generated in the chemical ionization process are ultimately attached to acetonitrile, the strongest base present out of the solvent molecules water, formic acid, and acetonitrile. Clustering with acetonitrile as the second molecule is also more common.

Addition of 1% formic acid to water–acetonitrile (50:50) also alters the spectrum as expected, with protonated acetonitrile and the cluster ($\text{CH}_3\text{CNH}^+ \cdot \text{CH}_3\text{CN}$) dominant; and when acetone is added as solute, the major peaks are protonated acetone, the proton-bound dimer ($\text{CH}_3\text{COHCH}_3^+ \cdot \text{CH}_3\text{COCH}_3$) and the acetonitrile solvate ($\text{CH}_3\text{COHCH}_3^+ \cdot \text{CH}_3\text{CN}$). Again, all the major aspects of the spectra are predicted qualitatively by thermochemical considerations.

Most of the aspects of reconstructed ion chromatograms can be reconciled with the preceding arguments. For example, the behavior of major ions when acetone is eluted in acetonitrile–water (50:50) is shown in Fig. 1. The reactant ion, m/z 42, is depleted as acetone is eluted, and the decrease in its concentration depletes m/z 83, the protonated dimer ion from acetonitrile. Either of these ions would thus be a candidate for reactant ion monitoring⁴⁴ of compounds within the appropriate range of basicities. The intensities of protonated acetone, m/z 59, and its solvate by acetonitrile, m/z 100, rise as those of the reactant ions decrease. That of the protonated dimer of acetone, m/z 117, does also, but the peak has a narrower width at half height than the rest; this narrowing is a consequence of its dependence on the square of the acetone concentration, while the intensities of all the rest of the peaks are proportional to the first power of the acetone concentration. In a sample of ions taken from acetone eluting with 100% acetonitrile (Fig. 2), most ions behave similarly to those in Fig. 1, but the m/z 100 in this run has an inverted top. Since the m/z 117 ion reaches an even greater intensity without inverting, a change in extraction efficiency

TABLE V
RELATIVE ABUNDANCES OF SAMPLE AND SOLVENT IONS (WATER AND ACETONITRILE, NEGATIVE IONS)

A, 100% acetonitrile; B, acetonitrile-water (50:50); C, 100% water; D, water containing 0.1 M ammonium formate; E, acetonitrile-water (50:50) containing 0.1 M ammonium formate; F, water containing 1% formic acid; G, acetonitrile-water (50:50) containing 1% formic acid.

Ions (<i>m/z</i>)	A		B		C		D		E		F		G		
	-*	+***	-*	+**	-*	+**	+***	-*	+***	-*	+***	-*	+***	-*	+***
<i>Solvent</i>															
91	-	-	-	-	-	-	-	12	17	87	100	97	93	100	100
64	33	15	2	-	-	-	2	100	100	60	65	100	100	36	46
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
40	13	3	100	5	-	-	-	-	-	3	-	-	-	1	1
38	17	5	-	-	-	-	-	-	-	-	-	-	-	-	-
35	6	6	9	7	25	11	-	-	-	-	-	-	-	-	-
26	100	100	22	9	-	-	-	-	-	-	-	-	-	-	-
17	-	-	2	-	100	56	-	-	-	-	1	1	1	-	-
<i>Sample</i>															
57	-	14	-	100	-	100	-	2	-	-	3	-	1	-	1
55	-	10	-	-	-	18	-	-	-	-	-	-	-	-	-
41	-	-	-	3	-	15	-	-	-	-	-	-	-	-	-

* No acetone injected.

** 50 μ l acetone injected.

*** 100 μ l acetone injected.

("defocussing") because of a significant change in the dielectric in the source is unrealistic, and the possibility must be considered that the acetone peak is so large that acetonitrile is seriously depleted when the maximum of the acetone peak is eluted.

In general, then, when acetone is the test solute, the major aspects of positive ion spectra are in accord (but only qualitatively) with the predictions of gaseous ion thermochemistry that are currently available. The behavior of cluster ions seems in reasonable accord with extrapolations from thermochemistry of simple protonated molecules, even though the numbers that are truly appropriate are not available. In general, the proton affinities of most solutes will be greater than those of any component of or additive to the solvent (except for ammonia, which is almost completely protonated when added anyway), so that protons will be transferred to solute eventually, and therefore the system will almost always be sensitive for solute.

Negative ions

Water (Table V). The principal ions from water are, as expected, OH^- and $\text{OH}^-(\text{H}_2\text{O})$. When acetone competes with water, their intensities are reduced, and $\text{CH}_3\text{COCH}_2^-$ is formed, for the proton transfer from CH_3COCH_3 is exothermic [in the gas phase (by 92 kJ mol^{-1}) but not in solution!]^{19,26}. The proton transfer is so exothermic that further loss of H_2 or CH_4 can occur.

Acetonitrile and aqueous acetonitrile (Table V). The negative ions from acetonitrile are predominantly CN^- , a previously unreported m/z 64, and CH_2CN^- , which can either lose H_2 or react with a molecule of acetonitrile to give m/z 64, $\text{H}_2\text{C}_4\text{N}^-$, after loss of ammonia. The ions observed do not agree closely with those

TABLE VI

RELATIVE ABUNDANCES OF SAMPLE AND SOLVENT IONS (WATER AND METHANOL, NEGATIVE IONS)

A, 100% methanol; B, methanol-water (50:50); C, methanol-water (50:50) containing 0.1 M ammonium formate; D, water-methanol (50:50) containing 1% formic acid.

Ions (<i>m/z</i>)	Tentative ID	A		B		C		D	
		-*	+**	-*	+***	-*	+**	-*	+§
<i>Solvent</i>									
91	(HCOOH)HCOO ⁻	—	—	—	—	20	22	55	61
81	(H ₂ O) ₂ HCOO ⁻	—	—	—	—	—	—	2	1
63	(CH ₃ OH)CH ₃ O ⁻	57	—	100	—	—	—	—	—
49	(H ₂ O)CH ₃ O ⁻	—	—	25	—	—	—	—	—
45	HCOO ⁻	—	—	—	—	100	100	100	100
31	CH ₃ O ⁻	100	—	5	—	3	1	—	—
17	OH ⁻	—	—	1	—	—	—	—	—
<i>Sample</i>									
72	C ₄ H ₈ O ⁻	—	2	—	—	—	—	—	—
57	CH ₃ COCH ₂ ⁻	—	100	—	100	—	5	—	1

* No acetone injected.

** 100 μl acetone injected.

*** 10 μl acetone injected.

§ 30 μl acetone injected.

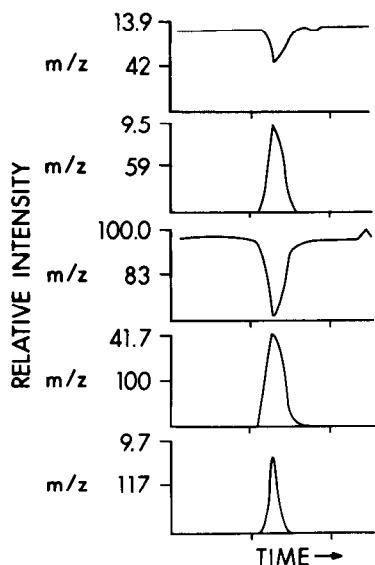


Fig. 1. Reconstructed ion chromatograms for acetone injected into acetonitrile-water (50:50) mobile phase, positive DLI.

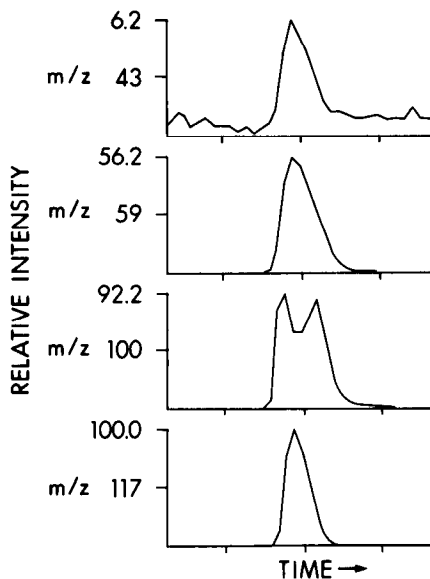


Fig. 2. Reconstructed ion chromatograms for acetone injected into 100% acetonitrile mobile phase, positive DLI.

observed under ion cyclotron resonance conditions^{41,42}; the difference could result at least partially from the difference in energy content of the reactant ions. Addition of acetone produces the $\text{CH}_3\text{COCH}_2^-$ ion, as predicted from thermochemistry, and the $\text{C}_3\text{H}_3\text{O}^-$ ion by subsequent loss of H_2 . Cyanide ion would not affect the $\text{CH}_3\text{COCH}_2^-$, because the ΔH_{acid} value of HCN is 1461 kJ mol^{-1} , and proton transfer from acetone to CN^- would be 82 kJ mol^{-1} endothermic.

Adding 50% water to acetonitrile apparently provides a more effective route for formation of CH_2CN^- , proton abstraction by OH^- . Hence in water-acetonitrile, CH_2CN^- is the dominant ion. When acetone is present, it transfers a proton to CH_2CN^- efficiently enough to consume most of the CH_2CN^- , even though the reaction is only 12 kJ mol^{-1} exothermic. If one considers that acetone is predominantly in the keto form in the gas phase, so that the reaction must be a proton transfer between two carbon acids, the rate is surprisingly high.

Almost all ions present after either ammonium formate or formic acid is added to either water or acetonitrile-water are derived from formic acid. Any solvent molecule deprotonates formic acid in the gas phase to give HCOO^- ; energetics to compare solvates of HCOO^- are not available, however. Most significantly, no significant change in the spectrum occurs when acetone is admitted, for the protonation of HCOO^- by acetone, to produce a peak for the acetone, is 99 kJ mol^{-1} endothermic. Apparently both the swamping of the rest of the spectrum by preformed formate (in the case of added ammonium formate) and the production of formate by proton transfer from added formic acid to every other anionic species formed prevent detection of the ions due to the conjugate base of acetone. Hence the presence of for-

mate interferes with the analysis of negative ions from solutes of lower acidity in practice as well as in theory.

Methanol and aqueous methanol (Table VI). In methanol solvent, the only ions observed are methoxide and methoxide solvated by one methanol molecule. Addition of acetone produces its conjugate base from the methoxide, as expected from gaseous (but not solution) thermochemistry; the reaction is 44 kJ mol^{-1} exothermic.

Addition of ammonium formate to 50% aqueous methanol nearly swamps other ions. Added acetone does not compete effectively in donating a proton to methoxide; this suggests that enough formic acid exists in equilibrium with the formate that it is this formic acid, not acetone, that is deprotonated. Since acetone is a carbon acid, it should be deprotonated more slowly than formic acid as well. Both of these effects would work against deprotonation of acetone and give the observed results.

The direct addition of 1% formic acid to 50% methanol totally depletes the spectrum of methoxide, as thermochemistry demands: the reaction is exothermic by 143 kJ mol^{-1} . Acetone cannot compete effectively with the added formic acid, for any $\text{CH}_3\text{COCH}_2^-$ formed would deprotonate HCOOH in a reaction 99 kJ mol^{-1} exothermic.

In general, for acetone as test solute, the major aspects of DLI LC-MS spectra are again in qualitative accord with the predictions of thermochemistry of gaseous negative ions. There are strong restrictions on the solutes that may also be present in the solvent along with sample. In agreement with prediction, acids inhibit detection of acetone solute, and as we have seen, large quantities of formate also swamp the negative ion spectrum so that acetone is not readily detected.

We caution that these remarks are not necessarily applicable to thermospray ionization. Studies of thermospray LC-MS are in progress.

CONCLUSIONS

The acid-base reactions of solvents with solutes in DLI LC-MS resemble acid-base reactions in the gas phase, not the condensed phase. The ion-molecule reactions not involving clusters are predictably determined by the acid-base properties of the solvent, which serves as a reagent gas. Thus, from the known directions of gaseous equilibria one can predict correctly that positive ions from a typical solute like acetone can be detected in the common reversed-phase solvents and in the presence of added acid and buffer, but that negative ions cannot be efficiently detected if acid is present.

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