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ELUENT pH AND THERMOSPRAY MASS SPECTRA: DOES THE CHARGE ON THE ION IN SOLUTION INFLUENCE THE MASS SPECTRUM?

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SUMMARY

The thermospray (with buffer, without filament) mass spectra of the nine compounds studied in this paper exhibit little change as the charge on the predominant analyte ion in solution changes. In addition, the reagent ions observed in positive and negative ion thermospray are seen to decrease as the sample elutes. These observations indicate that, under the conditions employed in this study, gas-phase ionization predominates. This conclusion is further supported by the difference between the thermospray and fast atom bombardment mass spectra of those compounds that exhibit poor sensitivity in thermospray. The poor sensitivity of certain solutes in thermospray can be explained in terms of the inability of the ammonium ion to protonate those solutes in positive ion thermospray. In negative ion thermospray, those solutes with higher gas-phase proton affinities than the acetate ion will not be detected.

INTRODUCTION

It has been reported[1–4] that, for neutral species in solution, the ions observed in positive and negative ion thermospray (without filament, with buffer) can be predicted on the basis of gas-phase acid–base reactivities. In this paper, we report the behavior of species that are ionic in solution. The compounds selected for study were glycine (GLY), histidine (HIS), N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), L-canavanine (LCAN), L-α-hydroxyglutaric acid (LHGA), 5′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP), L-glutamic acid (GLU), and L-aspartic acid (ASP). Fig. 1 shows the molecular weights and structures of these compounds. Compounds were selected on the basis that the pK_a value were within the pH range accessible with the conventional thermospray (TSP)

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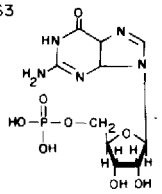
L-Aspartic Acid MW 133
$HO_2CCH_2CH(NH_2)CO_2H$

L-Canavanine MW 176
$NH{=}C(NH_2)NHOCH_2CH_2CH(NH_2)CO_2H$

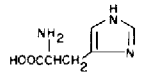
L-Glutamic Acid MW 147
$HO_2CCH_2CH_2CH(NH_2)CO_2H$

Glycine MW 75
$H_2NCH_2CO_2H$

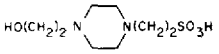
Guanosine 5'-monophosphate (5'-GMP)
MW 363



Histidine MW 155



N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic Acid (HEPES)
MW 238



L-α-Hydroxyglutaric Acid MW 148
$HO_2CCH_2CH_2CH(OH)CO_2H$

Inosine 5'-monophosphate (5'-IMP) MW 348

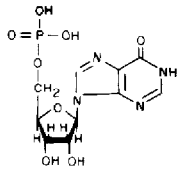


Fig. 1. Structures and molecular weights of the compounds studied without regard to zwitterionic forms.

buffer systems used. For these compounds, the dominant solute ionic species could be changed by varying the pH of the buffer system.

It is possible to propose two methods of ion formation from compounds that exhibit different ionic charges at different pH values: first, direct desolvation of solution-phase ions[6-14] such as in electrospray and ion evaporation; second, formation of gas-phase ions from acid-base reactions[1-4]. A combination of the two mechanisms is also possible.

If the ions observed in "filament-off" thermospray liquid chromatography–mass spectrometry (TSP LC–MS) are formed directly from solution, then adjustments of pH through the pK_a value should affect ion yield and appearance of the TSP mass spectrum. As the solution pH is varied through the pK_a value, the dominant ionic species changes (for example, eqns. 1 and 2):

$$H_2A^+ \longrightarrow HA + H^+ \quad (1)$$

$$HA \rightarrow H^+ + A^- \quad (2)$$

—— increasing pH →

If the primary sample TSP ions are formed directly from solution, the TSP mass spectra should change as the ionic species change. Thus, for solution-phase ionization, a plot of ion intensity *versus* pH would resemble a titration curve. Conversely, if gas-phase ionization dominates, changes in the pH of the mobile phase should have little effect on the TSP mass spectra, and a plot of ion intensity *versus* pH would be a straight line. Moreover, in the gas phase, decreases in gas-phase reagent ion intensities should be observed when the sample peak elutes[1,3].

For the nine compounds selected for study, the charge on the dominant solution-phase solute species can change from negative to neutral to positive across the range of pH values studied (pH 4.4 to pH 11). These nine compounds and their pK_a values are shown in Table I.

TABLE I

pK_a VALUES OF COMPOUNDS STUDIED

Compound	pK_{a1}*	pK_{a2}*	pK_{a3}*
Aspartic acid	2.09	3.86	9.82
L-Canavanine	2.50	6.60	9.25
L-Glutamic acid	2.19	4.25	9.67
Glycine	2.34		9.60
5'-GMP	2.40	6.10	9.40
Histidine	1.82	6.00	9.17
HEPES	7.55		
L-α-Hydroxyglutaric acid**	3.80	4.70	
5'-IMP	1.54	6.04	8.90

* By convention, pK_{a1} here refers to ionization of $RCH(NH_3^+)COOH$; pK_{a2} refers to ionization of remote acid functions; and pK_{a3} refers to ionization of $RCH(NH_3^+)COO^-$.

** Estimated from the pK_a of glutaric acid and the ΔpK for butanedioic acid and 2-hydroxybutanedioic acid[5].

It has been reported[16] that pK_a values can be determined from the fast atom bombardment (FAB) mass spectra of weak acids in solutions. One of the compounds studied here, HEPES, has previously been studied by FAB-MS; its FAB spectrum is dependent on the solution pH[16]. Dramatic changes in the organic secondary ionization mass spectrum (SIMS) of tris(hydroxymethyl)methylaminopropanesulfonate as a function of pH have also been reported[17]. A comparison between TSP mass spectra and FAB mass spectra should yield further evidence as to whether TSP ion formation is similar to FAB and organic SIMS ionization processes.

EXPERIMENTAL

A VG 12-250 quadrupole mass spectrometer (VG Masslab, Altrincham, U.K.) was used in the thermospray mode: vaporizer temperature, 290°C; desolvation chamber temperature, 250°C; source temperature, 220°C; VG "focus 4" electrode, 200 V. A Gilson HPLC system consisting of two Model 302 pumps and a Model 802B manometric module (Gilson Medical Electronics, Middleton, WI, U.S.A.) was used. Samples were injected into a Rheodyne Model 7010 injector (Rheodyne, Cotati, CA, U.S.A.) fitted with a 50-μl loop. The pH measurements were made with a Fisher Digital 1, Model 107 pH meter (Fisher, Fair Lawn, NJ, U.S.A.).

The water (HPLC grade), ammonium acetate, glacial acetic acid (HPLC grade), and ammonium hydroxide were purchased from Fisher. The histidine used was from Aldrich (Milwaukee, WI, U.S.A.). All other compounds studied were obtained from Sigma (St. Louis, MO, U.S.A.).

The compounds were dissolved in, and then injected into, a mobile phase which consited of 0.1 *m* aqueous ammonium acetate, adjusted to the desired pH with either acetic acid or ammonium hydroxide. Experiments were performed at pH 4.4, 7, 8, 10 and 11. The flow-rate was 0.9 ml/min. For each experiment, duplicate 20-μl injections of a 1 μg/μl solution were made.

The FAB-MS experiments were performed with a VG ZAB-E double focusing mass spectrometer (VG Analytical). Positive ion mass spectra were obtained with

glycerol containing 1% oxalic acid as the matrix, the sample having been first dissolved in methanol containing 1% trifluoroacetic acid (TFA). Negative ion experiments were performed with both glycerol and triethanolamine (TEA) as matrices. Xenon was the bombarding species, with an energy of 8 keV.

RESULTS AND DISCUSSION

Positive ion TSP mass spectra

At every pH, the positive ion TSP spectra of GLY, HIS, GLU, and ASP show intense base peaks corresponding to $[M+H]^+$. In addition, a lower intensity ion characterized as the $[2M+H]^+$ species was also observed, but at a relative intensity lower than 35% in all cases. In the case of GLY, a small peak at *m/z* 93 is observed, and is characterized as the $[M+NH_4]^+$ ion.

The TSP spectrum of LHGA showed an $[M+NH_4]^+$ ion. It also contained a peak corresponding to $[M]^+$, instead of the expected $[M+H]^+$. The so-called $[M]^+$ ion may be rationalized as the $[M+NH_4]^+$ ion losing a water molecule. The base peak in the spectrum of LHGA is seen to be $[M+60]^+$ at all pH values studied, with the $[M+H]^+$ ion being no more than 55% of base peak (at pH 10). The identity of the $[M+60]^+$ ion is not known, but may correspond to loss of water from an adduct of the molecule with protonated ammonium acetate. The remaining compounds (HEPES, 5′-IMP, and 5′-GMP) yield very weak spectra. For HEPES, the $[M+H]^+$ ion is the only ion observed. None of the ions observed from 5′-IMP is readily explicable in terms of its structure; in the case of 5′-GMP, only a weak ion at *m/z* 152 can be explained, the $[\text{guanine}+H]^+$ ion.

In spectra where the $[NH_4]^+$ ion is not saturated, a definite decrease in the $[NH_4]^+$ signal is observed as the sample enters the ion source. Fig. 2 shows the reconstructed ion chromatograms (RIC) for GLY (a) and HIS (b) injected into pH 8 mobile phase. Ammonium ion does not protonate GLY and HIS in solution[5], but it does in the gas phase. The decrease in the ammonium ion intensity thus indicates that a gas-phase process is occurring.

It is not easy to say whether the poor sensitivity for HEPES, 5′-IMP, and 5′-GMP is due to the inability of the ammonium ion to protonate these compounds, since their proton affinities are not known. Even if they cannot be protonated by the ammonium ion, it should still be possible to observe desorption of these ions directly from solution, since all three of these compounds should be ionic under the conditions of the experiment (pH 4.4–11; see Table I). These ions, however, are not observed to an appreciable extent.

As the pH was changed, the TSP spectra did not change appreciably. Straight-line plots of ion intensities *versus* pH were obtained for all of the compounds studied in positive ion TSP. No changes in slope were observed, either for relative or absolute intensities, as the solution pH was varied through the pK_a values. Typical results are presented in Fig. 3, which shows a plot of the intensities of the predominant ions from GLY (a) and ASP (b) as a function of pH. These results are in sharp contrast not only to those from parallel FAB and organic SIMS experiments[16,17], but also to recent experiments in thermospray without buffer[6], where major changes in the appearance of spectra occur when the major ionic form of the analyte changes as the pH of the solution crosses the pK_a.

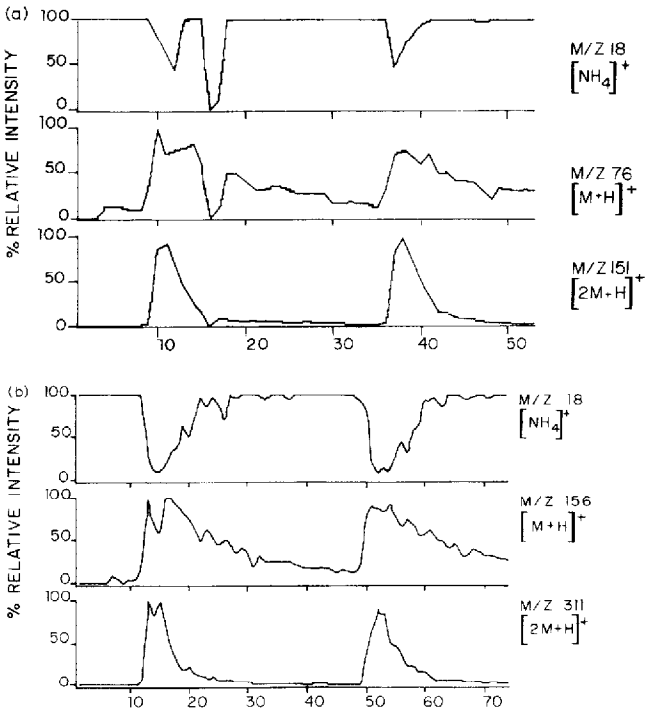


Fig. 2. Positive ion TSP reconstructed ion chromatograms for GLY (a) and HIS (b) injected into pH 8 mobile phase.

Whatever the process by which the buffer and unionized solutes enter the gas phase, the ultimate process that produces the ions actually observed in the spectrum is gas-phase chemical ionization process by NH_4^+. If no buffer is present in TSP, then this chemical ionization does not occur, and there is no inconsistency between our results and those previously observed in the absence of buffer.

Failure to produce ions from samples ionized in solution has been observed previously for TSP[19] and ion evaporation[20]. The poor ion yield was proposed to be an effect of the high concentration of buffer salt which could cause the droplets to solidify during the desolvation process at an earlier stage than might be optimal for good ion yield. Another hypothesis was that competition was occurring between buffer ions and solute ions for surface sites as the droplet diameter was decreasing.

Postive ion FAB mass spectra

The positive ion FAB spectra of LCAN, LHGA, HEPES, 5'-IMP, and 5'-GMP all show intense $[M + H]^+$ peaks, indicating that protonation is favorable under the conditions employed. Solution-phase ionization is an important process in FAB, since pK_a values can be determined from FAB spectra[16,17]; thus different ionization mechanisms may be occurring in TSP and FAB. It was also noted that when these compounds were studied by FAB with glycerol plus oxalic acid as the matrix, poorer sensitivity was achieved than when TFA was also used. This difference may be ex-

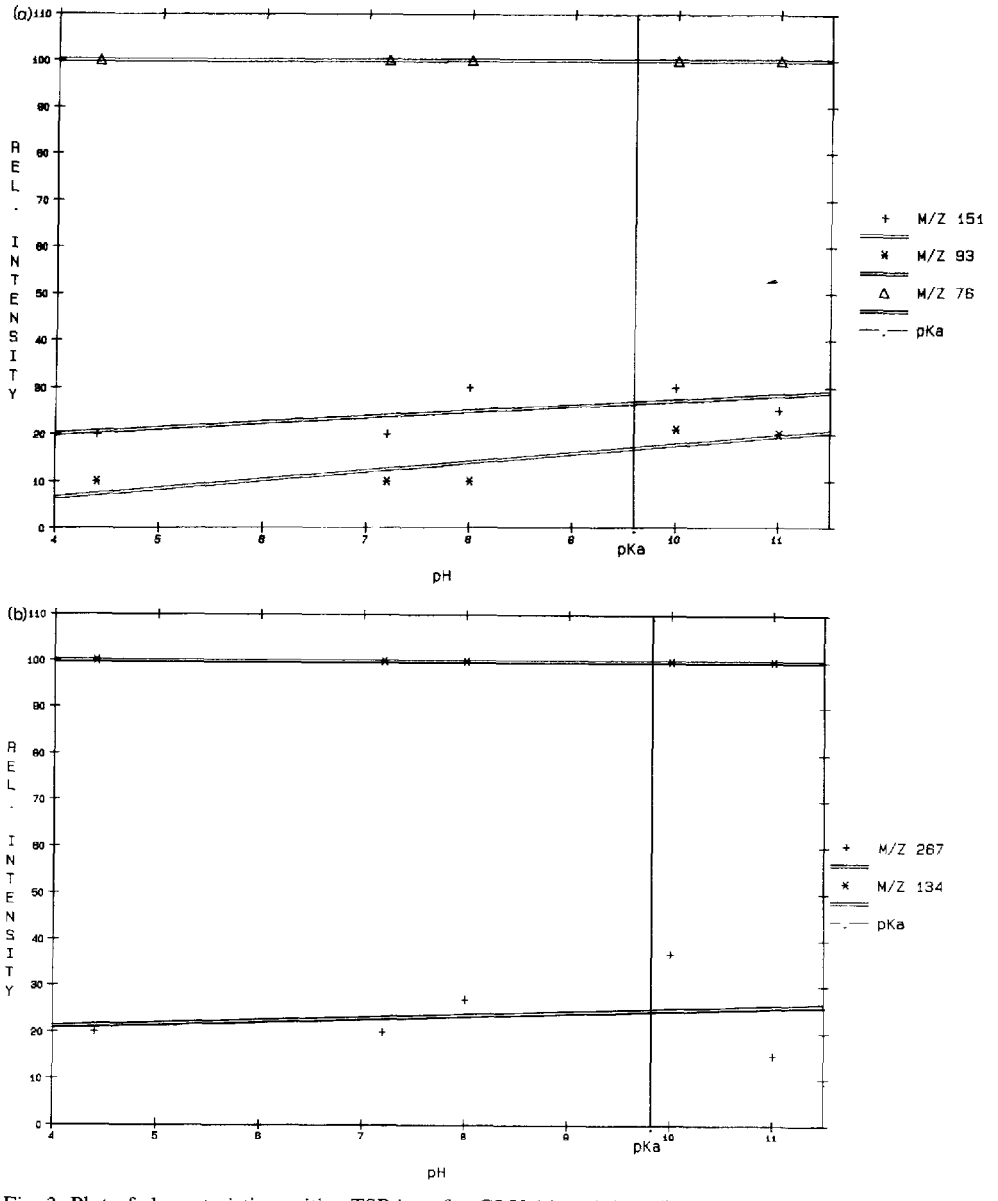


Fig. 3. Plot of characteristic positive TSP ions for GLY (a) and ASP (b) *versus* pH.

plained in terms of the lower acidity of oxalic acid $pK_a = 4.19$[5] compared to TFA, which has a pK_a of 0.23[5].

Negative ion TSP mass spectra

The negative ion TSP mass spectra of ASP is simple, in comparison with the other eight compounds studied. It yields, as base peak, an ion at m/z 132, charac-

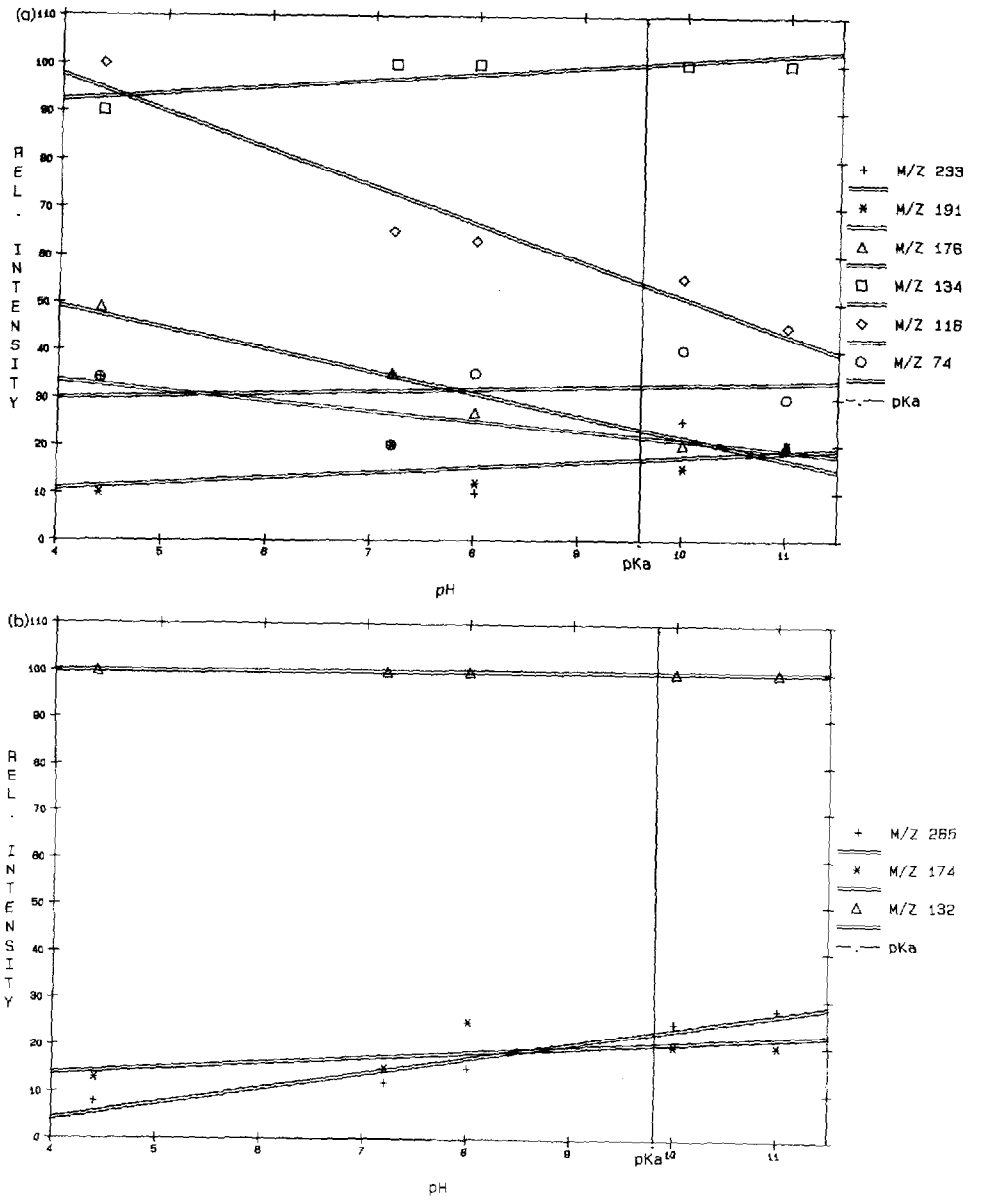


Fig. 4. Plot of characteristic negative TSP ions from GLY (a) and ASP (b) *versus* pH.

terized as $[M-H]^-$. In addition, the deprotonated dimer, $[2M-H]^-$, is observed with a relative intensity of 5%. There is also an ion at *m/z* 174 (12% relative intensity), tentatively identified as $[M+CH_3COO-H_2O]^-$. This type of ion is also observed in the TSP spectra of GLY, at *m/z* 134, where it is the base peak at pH 4.4, HIS (relative intensity 8%), LHGA (10–20%), and GLU (4%). All compounds except 5'-IMP and

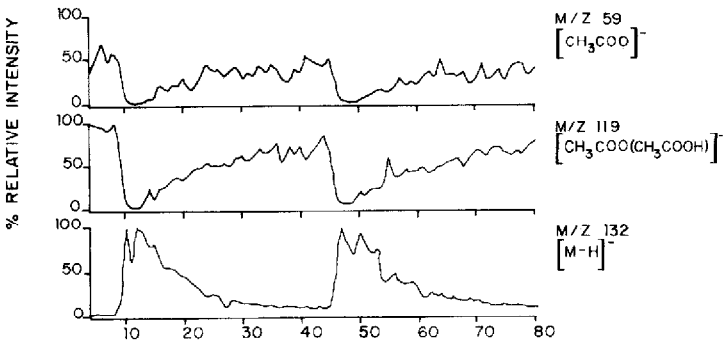


Fig. 5. Negative ion TSP reconstructed ion chromatograms for ASP injected into pH 7 mobile phase.

5′-GMP yield an ion characterized as the $[M-H]^-$ species; in most cases, however, (GLY, HIS, LCAN, and LHGA) it is not the base peak. The $[M-H]^-$ ion is the base peak in the very weak spectrum of HEPES, and in the spectra of GLY and ASP. The $[2M-H]^-$ ion is observed for HIS, LCAN, GLU, and ASP. This ion is of low intensity (less than 10%) in every case except for HIS, where it is the base peak at all pH values studied, with the exception of pH 11, where it is at 55% relative intensity. Some of the remaining ions are not easily rationalized in terms of expected fragmentations; in the case of GLU, however, losses of one and two water molecules are observed from the $[2M-H]^-$ species, each fragmentation being 10% relative intensity. There is also a loss of H_2O from the $[M-H]^-$ ion of LHGA. The picture for GLY is more complex. In addition to the ions described above ($[M+CH_3COO-H_2O]^-$ is at *m/z* 116), there was a cluster ion tentatively identified as $[M+CH_3COO(CH_3COOH)-H_2O]^-$ at *m/z* 176, and a $[2M+CH_3COO-H_2O]^-$ ion (*m/z* 191), whose analogue is also observed for HIS.

The sensitivity of negative ion TSP for GLY, HIS, LCAN, GLU, and ASP is much greater than that for HEPES, 5′-IMP, and 5′-GMP. HEPES yields a very weak ion at *m/z* 237, $[M-H]^-$; 5′-IMP shows low-intensity ions that were characterized as $[base-H]^-$ and $[base+CH_3COO]^-$. No structurally significant ions could be identified from 5′-GMP.

In all of the experiments performed, no dramatic changes in ion intensities (relative or absolute) were observed when the pH was varied from pH 4.4 to pH 11 for any of the compounds studied by negative ion TSP. Representative plots are shown in Fig. 4, which shows plots of ion intensity *versus* pH for the major ions observed from GLY (a) and ASP (b). As was the case in positive ion TSP, the reagent ions were seen to decrease as the sample eluted. These reagent ions, $[CH_3COO]^-$ (*m/z* 59) and $[CH_3COO(CH_3COOH)]^-$ (*m/z* 119) in negative ion TSP are shown in Fig. 5 when ASP elutes at pH 7. Thus, as was the case in positive ion TSP, gas-phase ionization again predominates, with little contribution from ion evaporation-type mechanisms.

Negative ion FAB mass spectra

This conclusion is further supported by the results obtained when these compounds were studied by negative ion FAB. In contrast to the negative ion TSP results,

good sensitivity was observed for HEPES, ASP, HIS, LCAN, 5′-GMP, and 5′-IMP in negative ion FAB with both glycerol and triethanolamine as matrices. All of the compounds yield $[M-H]^-$ ions; HEPES and ASP also give $[2M-H]^-$ ions of lower relative intensity. When triethanolamine is used as the matrix, $[M-H+TEA]^-$ adduct ions were also observed for HEPES, LCAN, and 5′-IMP. Compared with TEA, glycerol gave poorer sensitivity for HEPES, ASP, HIS, and LCAN, with the $[M-H]^-$ ion predominating. No structurally significant fragment ions were observed for 5′-IMP and 5′-GMP. The change in sensitivity presumably reflects the more basic nature of the TEA matrix, promoting greater deprotonation of the samples than did the less basic glycerol.

CONCLUSIONS

The pH of the solution has little effect on the ion yield and nature of the ions observed in TSP without filament, with buffer. It has previously been observed[16,17] that for FAB and organic SIMS, the ion intensities are relatively constant until the pH of the solution nears the pK_a, when there is a rapid decrease in certain ion intensities and increase in others. The lack of this spectral change in TSP suggests that the ionization mechanism of TSP differs from the mechanisms of these other techniques. In thermospray, gas-phase ionization appears to predominate, even when the solutes are ionized in solution. Thus, even for these compounds, where ion evaporation could be an important mechanism for producing ions, the predominant mechanism still appears to be a gas-phase process.

Gas-phase ionization, by protonation (by NH_4^+ in positive ion TSP) or deprotonation (by either CH_3COO^- or $[CH_3COOH]CH_3COO^-$ in negative ion TSP) seems to predominate when ammonium acetate buffer is present. Ion evaporation seems to be suppressed under these "filament-off" TSP conditions. Thus, it should be possible to rationalize the positive and negative ion TSP mass spectra of species that are ionic in solution in terms of gaseous acid–base reactivity, as is the case for neutrals in solution[1,2].

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