

EMITTER CHEMICAL IONIZATION
MASS SPECTROMETRY OF
KANAMYCINS

Sir:

Emitter chemical ionization mass spectrometry (Emitter CIMS) developed by HUNT *et al.* is a new technique for analysis of involatile and thermally unstable compounds¹. This method has been performed by use of activated field desorption (FD) emitter as a solid probe under CI condition. However, no systematic investigation of this technique has been reported, whereas, interest in the mass spectrometric analysis of aminoglycoside antibiotics is enhanced considerably due to the fact that amino sugar sequencing of this type of antibiotics except for gentamicins has not reproducibly been determined by EI, CI and even FDMS^{2,3}. The present investigation was undertaken to explore the utility of this technique for structural characterization of aminoglycosides, particularly kanamycins, which afforded no reproducible sequence ions in the FD mass spectra⁴.

All emitter CI mass spectra were recorded on a Shimadzu LKB 9000A mass spectrometer, equipped with a CI source. 2~10 μ g of sample dissolved in water was placed on the carbon emitter consisted of tungsten wire (10 μ m). The emitter current was increased at a rate of 0.2 mAs⁻¹. Optimum emitter currents were 24~35 mA. The source temperature was 250°C. The emission current was 120 μ A and electron energy was 150 eV. Both isobutane and ammonia reagent gas pressures were maintained at 0.1 Torr.

Isobutane and ammonia emitter CI mass

spectra of intact kanamycin A (**1**) are compared in Fig. 1. Both emitter CI mass spectra afforded unequivocally protonated molecular ion (MH⁺) at m/z 485. Besides, molecular ion-cluster in the isobutane emitter CI mass spectrum is weakly observed at m/z 523 (M·C₃H₃⁺), 525 (M·C₃H₅⁺), 527 (M·C₃H₇⁺) and 541 (M·C₄H₉⁺). These ions indicate precisely the molecular weight of **1**. In both isobutane and ammonia emitter CI mass spectra, major fragmentations are virtually identical. Cleavage of the glycosidic linkages results in the formation of five series of sequence ions (*a~e*) marked on Fig. 1. The ions *a* and/or *d* at m/z 162 arise from protonation of one of the glycosidic oxygen atoms, followed by elimination of pseudodisaccharide portion. The losses of kanosamine and/or 6'-amino-6'-deoxyglucose unit from MH⁺ give ions *b* and/or *c* at m/z 324. The ion *e* at m/z 163 represents a further loss of monosaccharide unit from the ions *b* and/or *c*.

Then, emitter CI mass spectra of kanamycin B (**2**) and C (**3**) were determined. In addition to MH⁺ ions, sequence ions (*a~e*) are also observed with both isobutane and ammonia as the reagent gases. The principal fragmentation pattern and the m/z values of the above ions are summarized in Fig. 2. These are the ions formed by reproducible fragmentation process at glycosidic linkages. Moreover, the shifting technique using ammonia-*d*₃ as the reagent gas which is characteristic of CIMS could be successfully employed to characterize the diagnostic ions⁵.

The preliminary results indicate that Emitter CIMS can be adopted for the structural analysis of other involatile and thermally unstable compounds. These results will be reported elsewhere.

Fig. 1. Emitter CI mass spectra of kanamycin A (**1**) using (A) isobutane and (B) ammonia.

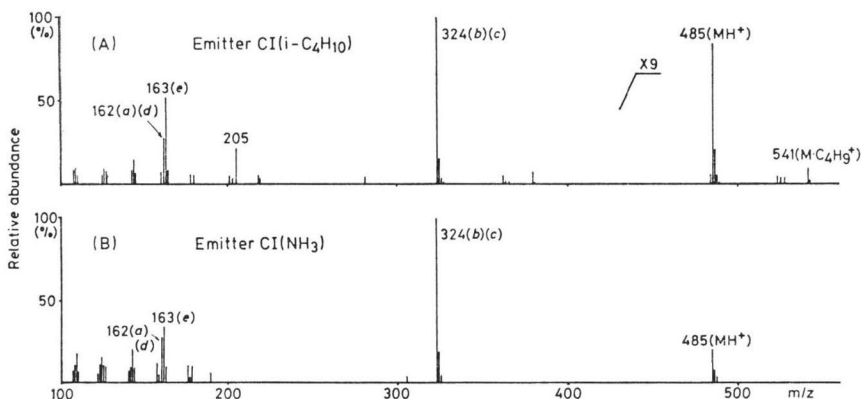
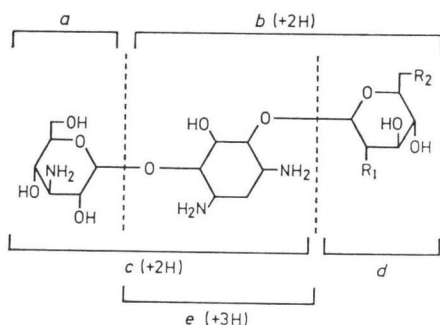


Fig. 2. Major fragmentations and the resulting sequence ions observed in the emitter $CI(i-C_4H_{10}/NH_3)$ mass spectra of **1**, **2** and **3**.



	MH ⁺	a	b	c	d	e
Kanamycin A (1) R ₁ =OH, R ₂ =NH ₂	485	162	324	324	162	163
Kanamycin B (2) R ₁ =NH ₂ , R ₂ =NH ₂	484	162	323	324	161	163
Kanamycin C (3) R ₁ =NH ₂ , R ₂ =OH	485	162	324	324	162	163

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