



ELSEVIER

# Detection of accelerated large water cluster ions and electrosprayed biomolecules with passivated solid state detectors

Y.K. Bae<sup>1</sup>, R.J. Beuhler<sup>\*</sup>, Y.Y. Chu, G. Friedlander, Y. Xu<sup>2</sup>, L. Friedman*Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973, USA*

Received 4 October 1995; revised form received 12 December 1995

## Abstract

The passivated solid state detector with a 500 Å boron doped window was observed to be sensitive to impacts of large  $(\text{H}_2\text{O})_n\text{H}^+$  ( $100 < n < 1500$ ) ions and multiply charged macro biomolecular ions accelerated in a 600 kV Cockcroft–Walton accelerator, although the ions have isolated atomic ranges smaller than the thickness of the detector window. For biomolecular ions generated by an electrospray ion source the detector pulse height spectra showed clearly resolved peaks that are consistent with the existence of aggregate ions of composition  $M^z$ ,  $2M^{2z}$ ,  $\dots$ ,  $5M^{5z}$  ions.

## 1. Introduction

Silicon surface barrier detectors have been extensively used for measuring energy deposition by impacts of energetic atomic or nuclear particles. They have been mainly used to detect particles with sufficient velocities to penetrate the detector window. In an effort to measure the stopping power of small water cluster ions  $(\text{H}_2\text{O})_n\text{H}^+$  ( $n < 10$ ) [1], we have discovered that the passivated solid state (PSS) detector, with a  $500 \pm 50$  Å boron doped window, is sensitive to impacts of large  $(\text{H}_2\text{O})_n\text{H}^+$  ions with  $n$  up to 1500.

A surprising aspect of this observation is that the isolated atomic ranges of these ions are much smaller than the thickness of the detector window which is too thick for the penetration of small particles at these energies. For example, the range of an isolated O atom with 444 eV energy [using, for example,  $(\text{H}_2\text{O})_{1000}\text{H}^+$  ions at 500 keV] is calculated to be  $\sim 20$  Å [2], which is  $\sim 25$  times smaller than the thickness of the window. Clearly, the energy deposition by the coherent collective collision of many particles is much different from that of isolated particles. Subsequently, we applied this detection technique for measuring the stopping power of multiply charged large molecular ions generated by an electrospray source (ES), such as albumin ions ( $MW = 66\,431$ ) with 47 charges,

by coating thin aluminum films of varying thicknesses directly on detectors [3]. The result showed evidence of collective hydrodynamic-shock-like interaction and the pressures estimated from the stopping power were hundreds of megabars.

In the present paper, we report further measurements of the response of the PSS detector to singly charged water cluster ions and compare this response to the impact of multiply charged ions generated by an ES ion source. In particular, the PSS detector appears to not only make possible the detection of large multicharged polymer ions but also allows the discrimination between events resulting from impacts of different mass polymer ions having the same mass to charge ratio.

## 2. Experimental

The experiments were carried out in a Cockcroft–Walton accelerator [1] coupled with two types of ion sources shown in Fig. 1. The detailed description of the apparatus can be found in Ref. [1]. The  $(\text{H}_2\text{O})_n\text{H}^+$  ions were generated by supersonic expansion of weakly ionized mixtures of water in He carrier gas. Further details of this cluster ion source can be found in Ref. [4]. Multiply charged biomolecular ions were generated in an Analytica ES source modified with additional differential pumping [5]. Ions generated in the ion source were subjected to mass analysis with a quadrupole mass analyzer, followed by post acceleration in the Cockcroft–Walton accelerator and detection with the off axis PSS detector. The PSS detectors used in the present studies were ORTEC Ultra solid state detectors with 500 Å boron doped windows.

<sup>\*</sup> Corresponding author. Tel +1 516 344 4326, fax +1 516 344 5815.

<sup>1</sup> Present address: Phillips Laboratory/RKFE, 10 E Saturn Blvd., Edwards AFB, CA 93524-7680.

<sup>2</sup> Present address: Uniroyal Chemical Company, Inc., Chemistry Section, Benson Road, Middlebury, Conn. 06749.

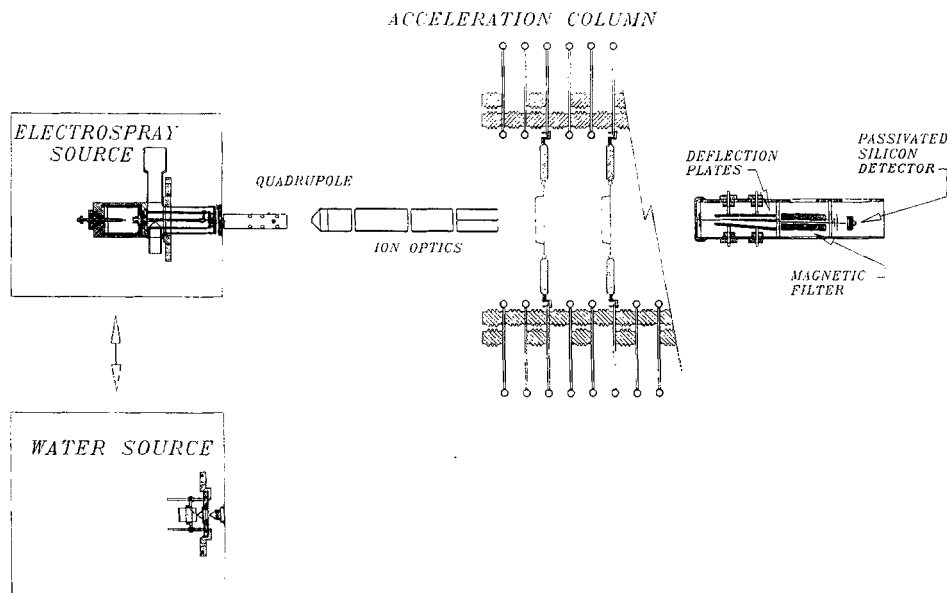


Fig. 1. Schematic diagram of the experimental apparatus that combines a Cockcroft–Walton accelerator with the water cluster ion source and the electro spray source. The particle detector system consists of the off-axis electrostatic and magnetic filter system and the silicon detectors.

The electro spray mass spectra typically show resolved peaks for ions differing by unit charges so that the mass and charge of the ions can be identified [5].

In addition to the PSS detectors, “ruggedized” OR-TEC silicon detectors with  $50 \mu\text{g}/\text{cm}^2$  of Al layer (equivalent to 2300 Å of Si) were used. The accelerated ions were filtered through electrostatic deflection plates and a magnetic filter [1]. This filtering system decreases the transmission of atomic and small molecular impurity ions generated in the acceleration column by several orders of magnitude. Fast neutrals were eliminated by deflecting the ion beam off axis with the electrostatic plates. Pulse height spectra obtained with a detector placed on the beam axis without the filtering system were completely dominated by high velocity light fragment ion signals so that no peaks corresponding to the signal pulses due to the large ion impact were observed. This observation is a necessary condition for ensuring that the signals result from the primary ions rather than from the fragment ions.

### 3. Results and discussion

Pulse height data as a function of cluster size obtained with impacts of 500 keV  $(\text{H}_2\text{O})_n\text{H}^+$  ions on the respective silicon detectors are presented in Fig. 2. For  $n < 50$  the output pulse height of the PSS detectors (open circles) decreases monotonically with increasing cluster size, for  $50 < n < 300$  it is nearly constant, and for  $n > 300$  it gradually drops with increasing cluster size. On the other hand, the results obtained with the “ruggedized” detector

(open squares) in Fig. 2 show that the detector is insensitive to the ions with  $n > 7$  in agreement with the calculated ranges [2]. This observation also supports the conclusion that observed signals for  $n > 50$  with the PSS detector are not generated by fragment ions in the cluster ion beam.

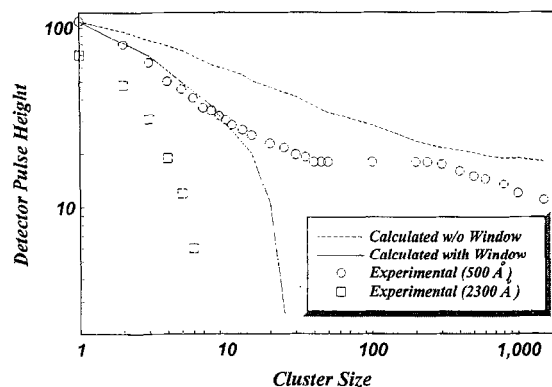


Fig. 2. The measured and calculated detector pulse height of singly charged water cluster ions as a function of cluster size. The impact energy of the cluster ions is 500 keV. The open circle data represent the measured pulse height with the passivated detector with the boron-doped 500-Å window. The open square data represent measured pulse height with the ruggedized detector with the 2300-Å Al window. The solid line represents the results of calculation for the passivated detector with the energy losses through the window. The dashed line represents the calculated results without the energy losses through the window.

We first attempted to simulate the observed signals with the assumption that most of the cluster kinetic energy is carried by oxygen atoms, and energy losses of the clusters in the detector can be calculated from losses expected for isolated constituent atoms moving with the cluster velocity. Here, the electronic energy available when the cluster reaches the sensitive area of the detector is considered as a dominant factor in determining the magnitude of observed output pulse heights. The calculated solid line in Fig. 2 reflects the available electronic energy of the clusters in the sensitive region of the detector beyond the 500 Å window as a function of cluster size. The experimental data (open circles) are in good agreement with the calculated curve for  $1 < n < 10$ , but they start to deviate from the calculated curve for  $n > 10$ . Thus, it seems that the signals for  $n > 10$  cannot be accounted for by the energy deposition via isolated atomic stopping.

The dashed line in Fig. 2 shows results of a calculation which assumes no loss of energy in the 500 Å window of the PSS detector. The drop in the calculated pulse height with increasing  $n$  reflects the decreasing fraction of electronic energy available with the slower heavier clusters having the same kinetic energy. At  $n \sim 300$  the measured detector sensitivity approaches  $\sim 70\%$  of the sensitivity without a window loss. The mechanism for such high detector sensitivity to the impact of large clusters whose range is much smaller than the window thickness is not understood now. One possibility is that each cluster impact produces an intense shower of secondary electrons, some of which penetrate into the active region of the detector. In this case, the detector pulse height should be affected by a change in the window thickness, because the change will alter the transport characteristics of the low energy electrons generated by the cluster impact. This possibility was tested by tilting the detector by  $45^\circ$ , thereby increasing the window thickness by a factor of 1.41. Because the mechanism due to the intense electron shower should involve transport properties of the slow electrons through the window, the pulse height spectra should be affected by this 41% increase in the window thickness. No change was observed in the detector pulse height. Thus, it seems that the extraordinary detector sensitivity does not result from intense electron showers.

Another possibility is that the window of the PSS detector becomes partially active when there is strong shock compression. The evidence for strong shock generation induced by large biomolecules was obtained in our recent experiment [3]. If we assume that  $n = 500$  water clusters accelerated at 600 keV can generate shock, the shock pressure can be estimated by

$$P_s \sim (4/3)\rho_p v_0^2, \quad (1)$$

where  $\rho_p$  is the density of water,  $10^3 \text{ kg/m}^3$ , and  $v_0$  is the velocity of the water cluster ions at 600 keV,  $1.13 \times 10^5 \text{ m/s}$  [3]. The estimated pressure is  $1.7 \times 10^{13} \text{ Pa}$ , 170 Mbar. Also the transient temperature of the impact area is

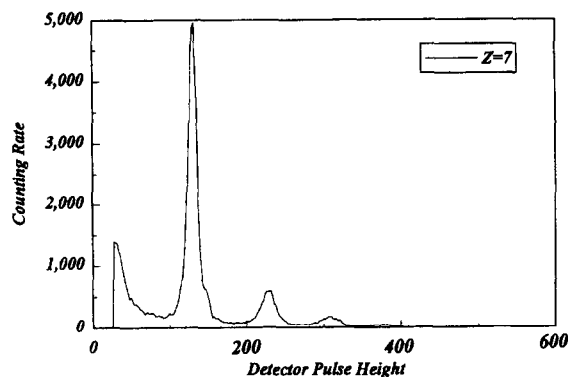


Fig. 3. A passivated detector output spectrum obtained with 3.5 MeV cytochrome-c (MW = 12400). The mass and charge states of the ions correspond to  $M^{+7}$ .

expected to be extremely high. At this high pressure and temperature silicon will be metallic, thus a good conductor. Thus, we speculate that the characteristics of the detector window around the impact area may be significantly modified which may possibly lead to the generation of detectable signals. The connection between the characteristics of the impact area and the generation of the detectable signal is not clear and remains a subject of further studies. Perhaps the measurement of time characteristics of the detector pulses will provide further information.

We have extended the impact energy of the particles on the detectors from the upper limit 600 keV obtained with singly charged water clusters to  $\sim 30 \text{ MeV}$  with the use of multiply charged biopolymer ions generated with an ES ion source. Results obtained with cytochrome-c (MW = 12400) with a charge state of +7 and accelerated at 500 kV, i.e. the equivalent impact energy of 3.5 MeV, are shown in Fig. 3. A primary peak lies at a channel number  $\sim 120$  and has an FWHM of 10%. The truncated peak near channel 30 is ascribed to noise counts which decrease rapidly with increasing channel number. This low energy peak is truncated by the discriminator setting in the detector amplifier and is interpreted to result from impacts of fragment ions and neutrals generated in the acceleration column. This interpretation was supported by the observation that the low energy peak intensity was roughly proportional to the background pressure in the accelerator.

The minor peaks at higher channel numbers appear at impact energies approximately in integral multiples of the deposited energy of the primary peak, though there is an indication that the spacing decreases between increasing peaks. We interpret these minor peaks as resulting from impacts of fully accelerated aggregates, i.e. dimers and trimers, etc., of cytochrome-c molecules with the same mass to charge ratio as the monomer. This interpretation is consistent with the following observation on the change of aggregate size with ions of different mass to charge ratio.

Output pulse height spectra of cytochrome-c ions accelerated at 500 kV with charge states of 7, 11, and 18 are shown in Fig. 4. The output peak intensities are plotted on a semilog scale and the plots of the respective spectra have been shifted vertically in the graph to facilitate comparison of relative peak positions. For the charge state 18 which is close to the maximum charge that can be attached to the monomer ions, the curve shows only a primary peak and a barely perceptible secondary peak. As the charge state decreases, peaks corresponding to polymers of multi-charged ions become more prominent. The peak corresponding to the pentamer is clearly observable in the spectrum of the 7 charge state cytochrome-c ions. Because the ions with higher charge states are anticipated to have a smaller critical size for coulomb explosion, the above observation is consistent with the interpretation that the minor peaks indeed result from the impact of aggregate ions with  $m/z$  equivalent to the monomer ions.

A summary of the data obtained with a variety of water cluster and biomolecular ions on the PSS detector is shown in Fig. 5. The data are presented in reduced form to show the relation between output pulse height per amu and energy input on the same basis. The nonlinearity of the relation between output pulse height and energy input for albumin and cytochrome-c is greatest in the lower energy range of the results. The efficiency of the detector, i.e. the ratio of output pulse energy to input energy, is lower for albumin ions than for all singly charged water cluster ions studied; for cytochrome-c ions of charge +18 the efficiency decreases from the high values characteristic for water cluster detection to values less than the albumin values at energies per amu less than 0.1 keV. The results indicate that the PSS detector should be calibrated for each molecular species.

The data in Fig. 5 can also be used to explain qualitatively why the positions of the peaks in Fig. 4 are not precisely linear with aggregate size. For example, the

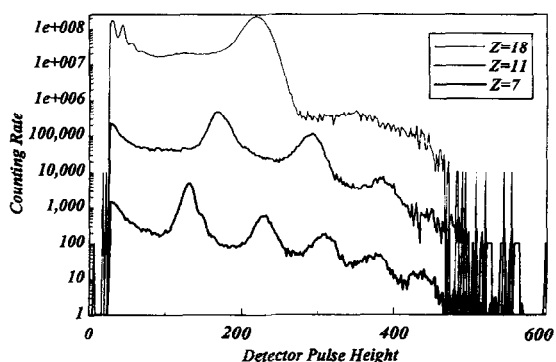


Fig. 4. Spectra of cytochrome-c ions with nominal charge states of 7, 11, and 18, accelerated at 500 kV. Note that the output peak intensities are plotted on a semilog scale and the plots for the respective spectra have been systematically shifted for comparison.

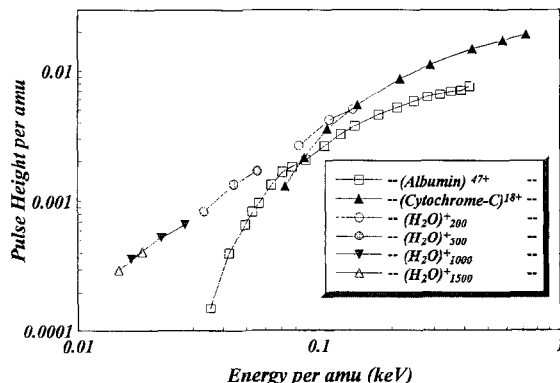


Fig. 5. Reduced detector pulse height (pulse height per amu) of various water cluster ions and biopolymer ions as a function of reduced energy per amu.

detector pulse height spacing for  $z/M = +7$  ion between peaks 1M and 2M in Fig. 4 is 100, and decreases to 60 between 4M and 5M. The corresponding values of pulse height per amu for each peak 1M, ..., 5M are 0.011, 0.0092, 0.0084, 0.0076, and 0.0071, respectively. These data were taken at a value of energy per amu of 0.28 keV. At this value of energy per amu in Fig. 5 the pulse height per amu for +18 cytochrome-c is 0.012, and for +47 albumin the value is 0.0065. Cytochrome-c and albumin differ in mass by approximately a factor of 5, so it is reassuring that the albumin values of pulse height per amu decrease by about the same ratio as the pentamer of cytochrome-c. Further work will have to be done to better characterize the detector response as a function of ion energy and complexity.

There appear to be higher threshold energies for detector response with albumin and cytochrome-c than with the water clusters of comparable mass in Fig. 5. The higher specific threshold energies may reflect the higher threshold energies to trigger the necessary shock behavior because the highly charged biomolecular ions are less dense than water cluster ions. The density of water cluster ions has been shown to be comparable to liquid water based on measured attenuation cross sections of large water cluster ions in inert gases [6]. Volumes of albumin ions and cytochrome-c ions can be estimated from collision cross sections measured by Covey and Douglas [7] if one assumes that the ions have spherical symmetry and that the projected area corresponds to the measured cross section. Respective values of cross section for +18 charged cytochrome-c and +47 charged albumin from Ref. [7] are  $4120 \text{ \AA}^2$  and  $14500 \text{ \AA}^2$ , the latter value obtained by extrapolation from measurements on lower charged states. The resulting calculated particle densities for the various cluster projectiles are shown in Table 1; they are presented as mass densities to compare ions with somewhat different constituent atomic composition. The mass densities of multiply charged albumin and cytochrome-c ions with

Table 1

Mass densities of various particles. The volumes of cytochrome-c and albumin ions were estimated from the collision cross sections measured by Covey and Douglas [7]

	Mass density [amu/A <sup>3</sup> ]
H <sub>2</sub> O clusters	0.60
(Cytochrome-c) <sup>18+</sup>	0.061
(Albumin) <sup>47+</sup>	0.051

charge states of 47 and 18 are 12 and 10 times smaller respectively than of water clusters, and this can be qualitatively understood by assuming that the high charge state causes these ions to spread out compared to the tight geometry of a spherical droplet. With much lower density, the impact properties of the biomolecular clusters are expected to approach those of isolated atoms with a reduction in range and correspondingly lower specific threshold energies for detection. At sufficiently high velocity the cytochrome-c data and the water cluster data in Fig. 5 appear to converge to a qualitative linear relationship between output pulse height and input energy plotted on a per amu basis.

There is evidence that dimers and higher polymers of electrosprayed ions have been observed previously. For example in the work of Axelsson, Reimann and Sundqvist [8] the measured distribution of secondary electrons ejected by +7 charged cytochrome-c molecules peaks at 22 electrons and has a broadened hump on the high electron side which they attributed to the dimer ion. This observation led to the use of the electron distribution to give information on the mass of the ions detected. Also in their mass spectra there were peaks which appear to be dimer ions with odd numbers of charges. In the work of Winger et al. [9] there were peaks in the mass spectrum of electrosprayed cytochrome-c which were identified as multimer peaks up to 5M<sup>11+</sup>. However, in none of these experiments is there a clear separation of detection events caused by different polymers with the same mass to charge ratio. The present technique combined with a mass spectrometer, such as a quadrupole mass spectrometer, makes possible the selective detection of a particular polymer species with a specific charge number by choosing only the pulses of a particular height. For example, by accepting only events with pulse heights between 250 and 350 in Fig. 4 it would be possible to selectively detect the 2M<sup>+22</sup> species of cytochrome-c.

A current topic under active investigation is the determination of conformation of gas phase macro-ions. Gas phase exchange reactions in a Fourier-transform mass spectrometer [10] have shown that cytochrome-c ions formed in an electrospray ion source can exist in four different stable conformations. In the present experiments the response of the PSS detector to bombardment with accelerated cytochrome-c ions from the ES source does not

appear to be sensitive to the conformation of the ion, because no structure was observed in the monomer peak of the charge states where different conformers were found to coexist. The use of a PSS detector with somewhat higher resolution might show different responses to ions with different conformations. However the present experiments point out a potential problem that can exist in any experiment designed to determine ion conformation. The problem is what effect dimers and higher *n*-mers would have on the interpretation of the experiment. In the experiments of Quist et al. [11] the morphology of craters or hillocks caused by accelerated macroions striking a target was imaged with an atomic force microscope. Damage from a dimer ion or higher polymer ion could be expected to be larger compared to damage from the monomer ion, just as damage was found to increase with macroion size for constant impact velocity. In our experiments with the +7 charge state of cytochrome-c the intensities of dimer and trimer ions were respectively 12% and 4% of the monomer ion intensity. Measurements of both the intensity and composition (presence of dimer or higher polymer ions) of the beam are necessary to interpret the number of impacts and the morphology of damage in imaging experiments.

Although the mechanism for the unexpected sensitivity to the macro molecules of the detector to produce a signal above ambient noise levels is not currently understood, a reliable correlation between detector output pulse height and projectile kinetic energy can be established with water clusters and a variety of other projectiles over a range of energies. Finally, we note that the detector can be used for studying macro biomolecules and clusters with acceleration voltages less than 100 keV. Further improvement of the detector amplification system will allow detection of the multiply charged ions at acceleration voltages of a few tens of keV. Then, the detection system including an accelerator with the PSS detector can be very compact and more versatile compared with other techniques, such as secondary electron detection [4,8].

### Acknowledgements

The invaluable technical contributions of V. Chiampou, S. Howell, J. Alessi, and V. LoDestro are gratefully acknowledged. This research was carried out at Brookhaven National Laboratory under Contract No. DE-AC0276CH00016 with the US Department of Energy and supported by its Advanced Energy Projects and Division of Chemical Sciences, Office of Basic Energy Sciences.

### References

- [1] Y.K. Bae, R.J. Beuhler, Y.Y. Chu, G. Friedlander and L. Friedman, *Phys. Rev. A* 48 (1993) 4461.
- [2] J.F. Ziegler, J.P. Biersack and U. Littmark, *The Stopping and Ranges of Ions in Matter*, Vol. 1 (Pergamon).

- [3] Y.K. Bae, Y.Y. Chu and L. Friedman, *Phys. Rev. A* 51 (1995) R1742.
- [4] R.J. Beuhler and L. Friedman, *J. Chem. Phys.* 77 (1982) 2549.
- [5] Y. Xu, Y.K. Bae, R.J. Beuhler and L. Friedman, *J. Phys. Chem.* 97 (1993) 11883.
- [6] H. Udseth, H. Zmora, R.J. Beuhler and L. Friedman, *J. Phys. Chem.* 86 (1982) 612.
- [7] T. Covey and D.J. Douglas, *J. Am. Soc. Mass Spectrom.* 4 (1993) 616.
- [8] J. Axelsson, C.T. Reimann and B.U.R. Sundqvist, *Int. J. Mass. Spectrom. Ion Processes* 133 (1994) 141.
- [9] B.E. Winger, K.J. Light-Wahl, R.R. Ogorzalek Loo, H.R. Volseth and R.D. Smith, *J. Am. Soc. Mass Spectrom.* 4 (1993) 536.
- [10] D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler and F.W. McLafferty, *Proc. Natl. Acad. Sci. USA* 90 (1993) 790.
- [11] A.P. Quist, J. Ahlbom, C.T. Reimann and B.U.R. Sundqvist, *Nucl. Instr. and Meth. B* 88 (1994) 164.