

**METHOD OF PRODUCING MULTIPLY
CHARGED IONS AND FOR DETERMINING
MOLECULAR WEIGHTS OF MOLECULES BY
USE OF THE MULTIPLY CHARGED IONS OF
MOLECULES**

RIGHTS STATEMENT

The U.S. Government has a paid-up license to this invention and the right in limited circumstances to re-
quire the patent owner to license others on reasonable
terms as provided for by the terms of Grant No. 2RO1
GM31660-04A1 awarded by the National Institutes of
Health.

This is a file wrapper continuation application of
application Ser. No. 07/683,105, filed Apr. 10, 1991,
which is a file wrapper continuation application of Ser.
No. 07/354,393 filed May 19, 1989, both now aban-
doned.

TECHNICAL FIELD

This invention relates to improvements in a method
for mass spectrometric analysis of chemical compounds
in solution. In particular it is concerned with deter-
mining the mass or molecular weight of large fragile solute
species with greater speed, convenience and accuracy
than has been possible by previous methods. The inven-
tion also relates to new compositions of matter compris-
ing populations of ions having a multiplicity of charges.

BACKGROUND ART

Mass spectrometry consists in "weighing" individual
molecules by transforming them intact into ions in
vacuo and then measuring the response of their trajec-
tories to various combinations of electric and/or mag-
netic fields. Attempts to extend the application of mass spec-
trometric methods to the analysis of very large polar
organic and bio-organic molecules have long been frus-
trated by the difficulties of transforming such molecules
into ions. The analytical advantages of mass spectrom-
etry for such parameters as detection sensitivity, accu-
racy of mass measurement and abundance determina-
tions cannot be realized if the prerequisite ions cannot
be formed. Large polar molecules generally cannot be
vaporized, even in vacuo, without extensive, even
catastrophic, decomposition. Consequently, one cannot
apply the classical methods of ionization based on gas
phase encounters of the molecule to be ionized with
electrons as in Electron Ionization (EI), photons as in
Photo Ionization (PI), other ions as in Chemical Ioniza-
tion (CI), or excited atoms or molecules as in Penning
Ionization (PeI) encounters can form ions from a neu-
tral molecule by a variety of mechanisms including
removal or attachment of an electron and removal or
attachment of a positively charged entity, typically a
proton.

In recent years a number of so-called "soft" ioniza-
tion methods have been developed which with varying
degrees of success have been able to produce intact ions
from molecular species of ever increasing size. One
class of such methods is based on very rapid deposition
of energy on a surface over which the species to be
analyzed (analyte) has been dispersed. The idea is, as
first suggested by Beuhler et al, *Journal of American
Chemical Society*, 96,3990 (1974), that if the heat re-
quired is applied rapidly enough, vaporization may
occur before decomposition has a chance to take place.
The rapid heating methods now in use include Plasma

Desorption (PD), in which disintegration of a radioac-
tive isotope, usually Californium-252, produces a small
blob of plasma on the surface from which a few intact
ions of analyte emerge; Secondary Ionization Mass
Spectrometry (SIMS), hereafter referred to as Fast Ion
Bombardment (FIB), in which the analyte-containing
surface is bombarded by ions, e.g. Cs⁺, accelerated to
energies in the tens of kilovolts; Fast Atom Bombard-
ment (FAB) in which the accelerated ions are neutral-
ized by charge exchange before they strike the surface;
Lase Desorption (LD) in which photons comprise the
vehicle for depositing energy on the surface. These
methods have been able to produce intact ions from
remarkably large analyte species even though, except
for LD, they are highly irreversible and characterized
by brute force. To date, intact ions have been produced
from bio-organic compounds with molecular weights
on the order of 210,000 with LD (M. Karas and F.
Hillenkamp, paper presented at 11th International Mass
Spectrometry Conference, Bordeaux, France 1988; cf.
Analytical Chemistry (1988) 60,2299), 24,000 with FAB
(or FIB) (M. Barber and B. N. Green, *Rapid Communi-
cations in Mass Spectrometry*, (1987) 1, 80) and 45,000
with PD (G. Jonsson, P. Hakansson, A. Hedin, D.
Fenyó, B. Sundqvist, H. Bennich and P. Roepstorff,
Rapid Commun. Mass Spectrom. in press). The ion cur-
rents in these methods have been very small and except
for LD decrease rapidly with increasing molecular
weight. When the ions get very large their detection
with multipliers requires post-acceleration voltages that
are often awkwardly high. Except possibly with LD,
the ions produced often have high levels of internal
excitation which can result in substantial peak broaden-
ing due to predissociation.

Quite different in practice and principle from these
"violent" ionization methods are techniques that use
very strong electrostatic fields to extract ions from a
substrate. In so called Field Desorption (FD) ionization
the analyte molecules are applied to a fine wire on
whose surface is disposed an array of sharp pointed
needles or "whiskers." When the wire is placed in a
vacuum system and a high voltage is applied while it is
carefully heated, the analyte molecules will desorb as
ions from the tips of the needles where the effective
field strength is very high. Even though it can trans-
form very involatile analytes into ions in vacuo FD has
not become widely used, in part because sample prepa-
ration is tedious, in part because of difficulties in adjust-
ing the wire to just the right temperature and voltage,
and in part because the desorbed ions have such high
energies that relatively expensive magnetic sector ana-
lyzers must be used for mass determination. In so-called
Electrohydrodynamic Ionization (EH) analyte is dis-
solved in a non-volatile liquid (e.g. glycerol) and in-
jected into an evacuated chamber through a small capil-
lary tube maintained at high voltage. The solvent liquid
must have a low vapor pressure so that it won't "freeze-
dry" from rapid evaporation into vacuum. Solute ions,
along with molecules and clusters of solvent, are des-
orbed from the emerging liquid by the high field at its
surface and can be mass analyzed. EH has not been
widely practiced, in part because few liquids that have
low vapor pressure are good solvents for large polar
bio-organic compounds, in part because the desorbed
ions are usually solvated with one or more molecules of
the solvent, and in part because they often have a wide
distribution of energies. Moreover, as in the case of FD,