International Journal of Mass Spectrometry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

High energy gas cluster ions for organic and biological analysis by time-of-flight secondary ion mass spectrometry

Tina B. Angerer [a], Paul Blenkinsopp [b], John S. Fletcher [a,*]

[a] Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg SE-412 96, Sweden
[b] Ionoptika Ltd., Chandlers Ford, Southampton, UK

ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 9 May 2014
Accepted 21 May 2014
Available online xxx

Keywords:
Time-of-flight secondary ion mass spectrometry (ToF-SIMS)
Imaging
Gas cluster ion beams
GCIB
High energy
Mouse brain
Irganox
Cholesterol

ABSTRACT

There is considerable excitement surrounding the application of gas cluster ion beams (GCIBs) for SIMS analysis in order to study organic materials and biological samples such as cells and tissues. These ion beams, that often comprise several thousand argon atoms in the primary ion, have been used mainly for the etching of organic materials to remove damage from the surface allowing molecular depth profiling experiments to be performed. The energy of the ion beam is normally 2–20 keV. There have been relatively few studies reported on the use of GCIB as analysis beams, due to difficulties related to fast pulsing and focusing of the beam along with the sometimes low ionisation efficiency. In this study, we report on the use of a new higher energy (40 keV) GCIB operated in a continuous mode. When compared to lower energies depth profiles on thin films of Irganox 1010 show an increase in sputter yield signal while fragmentation, damage accumulation and ionisation efficiency remains unchanged. Experiments on brain tissues show increased signal levels especially for higher mass secondary ions (m/z 500+) in comparison to C_{60}^+ at 40 keV and Ar_{4000}^+ at 20 keV impact energy. The use of higher energies facilitates better focusing of the primary ion beam as demonstrated here on a human hair sample where we achieve a spatial resolution of <3 μm. Even with this small spot size, we can detect enough signal from and high mass species for clear localisation. All results indicate that higher energies are beneficial for most aspects of ToF-SIMS applications in biology.

Introduction

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been successfully applied to a wide range of samples. The technique finds application in materials science, defect/failure analysis, semi-conductor analysis and increasingly biological analysis [1]. In recent years, the ability to generate higher mass, more chemically distinct species during the desorption ionisation process, has been greatly aided by the introduction of cluster ion beams such as Au_n and Bi_n (where n is most commonly three) [2,3]. Polyatomic ion beams such as C_{60}^+ provide the additional benefit as sub-surface damage accumulation is greatly reduced [4]. For the detection of molecular type ions, this damage build up limited the analysis to approximately 1% of the sample surface when conventional atomic and even cluster species were used. The use of C_{60} ion beams has allowed this limit to be relaxed and in some cases completely abandoned such that the sample can be analysed with very high primary ion beam dose density. In some cases, this can be used to increase sensitivity and in other applications allows molecular depth profiling [5–8] and 3D molecular imaging to be performed [9–13]. An expanding area of research using ToF-SIMS is biological imaging. Cell and tissue samples have been analysed in many studies in laboratories around the world to great effect [14–16]. Despite the improvements that have been realised due to the introduction of polyatomic ion beams, lateral resolution is normally limited by the available signal and not the ability to focus the ion beam. Also, the signal from higher mass species is much lower than that achievable using MALDI imaging. The latest approach to overcome some of these issues is the application of gas cluster ion beams (GCIBs) for SIMS.

GCIBs were initially employed for semi-conductor surface processing but have since been adapted and used as ion beams for SIMS [17,18]. The beams are produced by the expansion of pressurized gas as it enters a vacuum chamber through a small (10 s of μm diameter) aperture/nozzle. The cooling of the gas results in cluster formation, and these clusters are then ionised by electron impact and accelerated. The desired cluster size is selected either by time-of-flight or by combination of electric and magnetic

* Corresponding author. Tel.: +46 731414466.
E-mail address: john.fletcher@chem.gu.se (J.S. Fletcher).

http://dx.doi.org/10.1016/j.ijms.2014.05.015
1387-3806/

fields (a Wien filter). There has been great interest in such ion beams recently as they have shown a range of benefits over C_{60} for depth profiling challenging molecular systems such as organic electronic related materials [19].

The uptake of GCIB technology for depth profiling of organic materials has been significant over the last 2–3 years. Almost all new ToF-SIMS instruments are equipped with a GCIB. There has been considerable interest for use as sputtering beams for depth profiling in the XPS also [20]. Several laboratories have reported ToF-SIMS studies detailing the sputter rate of such beams though different types of materials. Seah and co-workers have studied the sputter rate of different argon clusters at different energies through gold [21]. Irganox based test samples constructed by vapour deposition of Irganox 1010 and Irganox 3114 have been used as standard test materials for inter-laboratory studies of organic depth profiling using conventional, polyatomic [22] and most recently GCIBs [23].

Several groups have reported considerable benefits for the use of GCIBs in the analysis of biological samples. Fletcher et al. showed increased signal persistence during depth profiles of tissue samples using Ar clusters for etching and analysis [24] while in a recent report Brunelle and co-workers used interleaved Bi_3^+ analysis and Ar GCIB etching to sputter all the way through an area of an initially 14 μm thick brain tissue section while still detecting intact lipid signals the authors calculate potential signal increases of 100× compared to standard analysis [25]. There are clearly advantages to the use of GCIBs, but the full potential of these ion beams has not yet been realised, and the optimum operational parameters of the ion beams have not been verified. Existing data is predominantly from experiments where the GCIB was employed solely for etching of the sample and not as an analysis beam. When, GCIBs have been used as analysis beams several general observations have been reported. Fragmentation, particularly fragments below m/z 200, is reduced while an increase in the signal from higher mass species is observed [26]. In the analysis of polystyrene, changing the energy per atom in the cluster has been shown to dramatically change which pseudo-molecular ions [27], and characteristic fragments [28] are formed suggesting the beams may be employed to add a spectroscopic element to the mass spectrometric analysis. Overall secondary ion yields can be low as ionisation is often less efficient.

Several research groups have very recently reported studies aimed at utilising, and improving the capabilities of GCIBs as analysis beams for the interrogation of biological samples. The aims being to maintain the benefits of the GCIBs, low sub-surface damage and reduced fragmentation, while improving the ionisation efficiency of the beams. Tian and co-workers have recently reported the use of mixing CH_4 with the Ar gas to provide a proton donor that might improve $[M+H]^+$ formation efficiency [29]. A 4-fold increase in the molecular ion signal intensity from trehalose was observed using 3% CH_4 in Ar compared to only a 50% increase in signal from the $[M+Na]^+$ ion indicating that the increase in signal was a result of the improved proton availability. A more radical step is the development of a water cluster ion source by Vickerman and co-workers where 10–20 fold signal increases have been reported for lipids and pharmaceutical reference samples [30]. So far, all results have indicated that with argon clusters higher energies result in increased secondary ion yield.

In this paper, we use a prototype 40 kV GCIB system to assess the potential benefits, and any potential drawbacks, of using higher energy clusters as analysis beams for ToF-SIMS. We use a range of test samples to investigate sub-surface damage accumulation, secondary ion yields from biological samples and achievable spatial resolution.

Materials and methods

ToF-SIMS

ToF-SIMS analysis was performed using a J105-3D Chemical Imager (Ionoptika Ltd.) [31]. The instrument uses a continuous primary ion beam to generate a stream of secondary ions that fill a buncher. The buncher compresses the secondary ion stream producing a time focus at the entrance to a quadratic field reflectron-ToF analyser. The instrument was installed with a 40 kV C_{60} ion gun and has recently been fitted with a prototype 40 kV GCIB system a schematic of which is shown in Fig. 1. The use of a continuous primary ion beam removes many of the difficulties associated with the operation of GCIBs as analysis beams on conventional ToF-SIMS instruments.

Initial cluster formation is dependent on the pressure of the gas entering the expansion chamber. Higher pressures result in larger

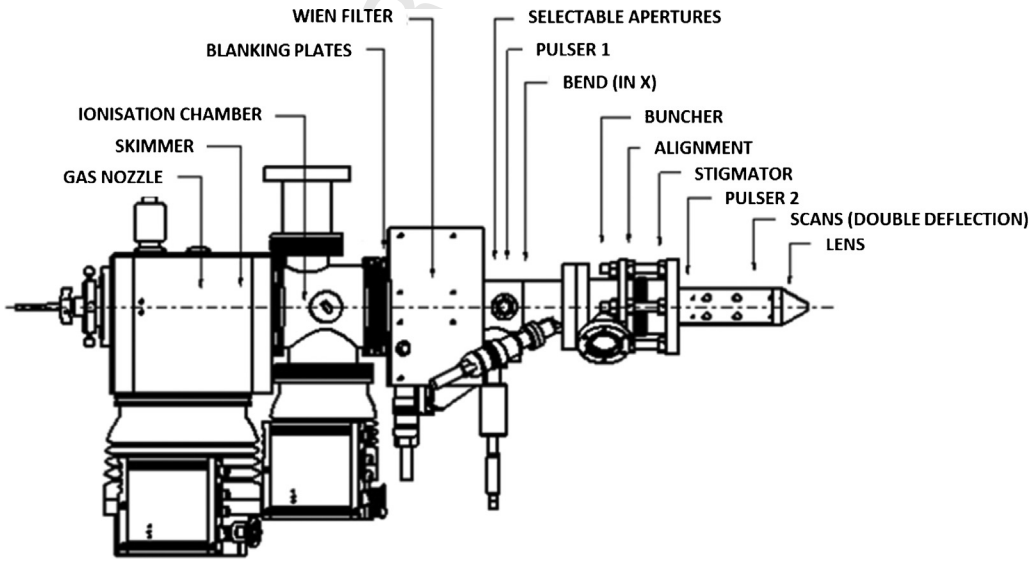

Fig. 1. Schematic of the 40 kV gas cluster ion beam system. Gas passess through a nozzle into the expansion chamber and cools, forming clusters. The cluster pass through a skimmer and enter an ionisation chamber where electron bombardment takes place. Ionised clusers are extracted, size selected using a Wien filter and focused onto the sample.

Please cite this article in press as: T.B. Angerer, et al., High energy gas cluster ions for organic and biological analysis by time-of-flight secondary ion mass spectrometry, Int. J. Mass Spectrom. (2014), http://dx.doi.org/10.1016/j.ijms.2014.05.015