ANALYSING NATURE WITHOUT COSTING THE EARTH

KRATOS ANALYTICAL
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STRUCTURAL DETERMINATION IN MALDI BY EXAMINATION OF THE NEUTRAL SPECTRUM

KOMPACT

APPLICATION NOTE

STRUCTURAL DETERMINATION IN MALDI BY EXAMINATION OF THE NEUTRAL SPECTRUM

INTRODUCTION

The MALDI technique is finding increased use in structural determination by the use of post source decay (PSD)[i]. Ions that are metastable tend to decay in the field free region between the source and the entrance to the reflectron. The charged fragments from the decay process are used to give post source decay spectra. These fragments provide valuable structural information. What is often overlooked is the equally useful information that is available by a consideration of the neutral spectrum, a component from the PSD process that is usually ignored.

EXPERIMENTAL

Experiments were performed on a Kratos Kompact MALDI 4. This is a bench-top, combined linear and reflectron instrument featuring pulsed extraction[ii] for time-lag-focusing. All spectra were acquired in positive ion and are typically the sum of 100 laser shots. The system has been described in more detail elsewhere[iii].

The instrument has several modes of operation. There is normal linear mode of operation which is used for rapid mass analysis. Alternatively, the ions can be directed into the curved field reflectron (CFR)[iv] which allows the acquisition of either normal reflectron spectra or finally, by gating a single species and inducing fragmentation, of producing seamless PSD spectra.

In addition, by switching the signal from the linear detector through a separate transient recorder during reflectron operation, it is possible to acquire a spectrum from the neutral particles. This simultaneous acquisition of linear mode neutral spectrum and reflectron/s-PSD mode ion spectra is a very powerful tool for gaining additional information.

These four main modes of operation are summarised below-

(i) Linear Mode - ions (and neutrals) are collected at the linear detector,
(ii) Reflectron mode - ions are collected at the reflectron detector,
(iii) s-PSD mode - parent and all fragment ions are collected at the reflectron detector in a single acquisition,
(iv) Neutral mode - whilst ions are being collected at the reflectron detector (i.e. in modes (ii) or (iii)) the neutrals are collected at the linear detector.

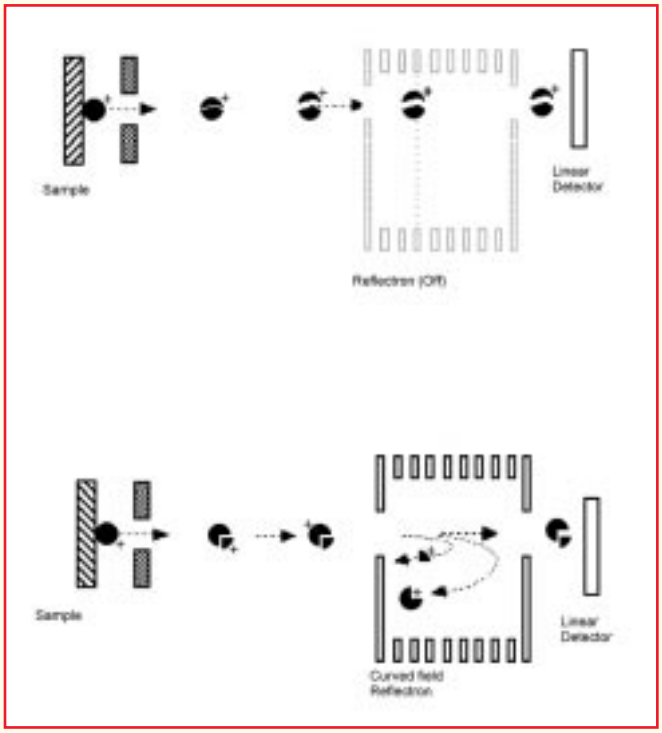

Figure 1: Schematic of modes of operation in the Kratos Kompact MALDI 4 showing (top) linear mode and (bottom) reflectron/neutral mode

The samples used in this study were commercially available polyethylene glycols (PEG1000 & PEG 1500) which were analysed using 2,5-dihydroxybenzoic acid (DHB) as a matrix.

Cationisation was accomplished using a range of alkali metal salts (LiCl, NaCl, KCl, RbCl & CsCl). The solutions were mixed together in a molar ratio of sample:matrix:salt = 1:100:10 and then air-dried on to a stainless steel sample slide.

RESULTS

MALDI spectra were acquired for PEG1500 both in linear mode and reflectron mode with different alkali metal ions as cationising agents. These are shown in figure 2. There is an obvious difference in the relative yield of the polymer with the different cationising species. In linear mode the larger cations (Rb & Cs) give the most intense signals whereas in reflectron mode it is the smaller cations (Li & Na) that give the best response. The implication is that the polymers with the larger cations are not being transmitted by the reflectron. This may be because they are undergoing metastable decay before they have time to enter the reflectron analyser. This can be confirmed by an analysis of the neutral spectrum.

Figure 3 shows three spectra of PEG1000 with both sodium and potassium present as cations. The spectra were acquired in different modes as follows-

Fig. 3a) Linear mode - ions and neutrals collected
Fig. 3b) Reflectron mode - only stable ions collected
Fig. 3c) Neutrals mode - only neutrals collected

In both linear and reflectron mode the $[M+Na]^+$ species dominate over the $[M+K]^+$ whereas in the neutral spectrum the reverse is true with the $[M+K]^+$ dominating. This implies that the $[M+K]^+$ is much less stable than the $[M+Na]^+$ and is much more likely to undergo post source decay.

Neutral spectra were then acquired for the PEG1500 samples with the five different cationising species. The results (figure 4) show the expected trends, with there being very little fragmentation of the $[M+Li]^+$, slightly more for the $[M+Na]^+$, rising again for the $[M+K]^+$ and then increasing dramatically for the $[M+Rb]^+$ and $[M+Cs]^+$.

An explanation of this can be surmised from a consideration of the relative ionic radii of the cations involved.

Ion	Li	Na	K	Rb	Cs
Radius/Å	0.90	1.16	1.52	1.66	1.81

It is known the PEG oligomers tend to form helices of comparable dimensions to these ion sizes. It is likely that the smaller cations will be able to enter the helix to form a stable oligomer/cation complex. In the larger cations this will not be possible and the cation will be forced to remain outside of the helix where the resulting complex will be much less stable. This is shown schematically in figure 5.

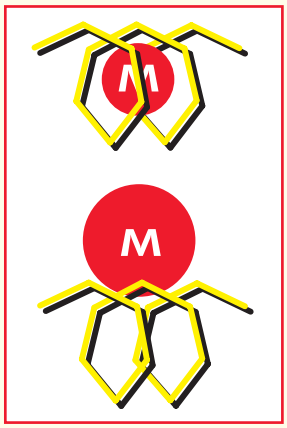

Figure 5: Schematic of helical PEG oligomers with cations of differing sizes

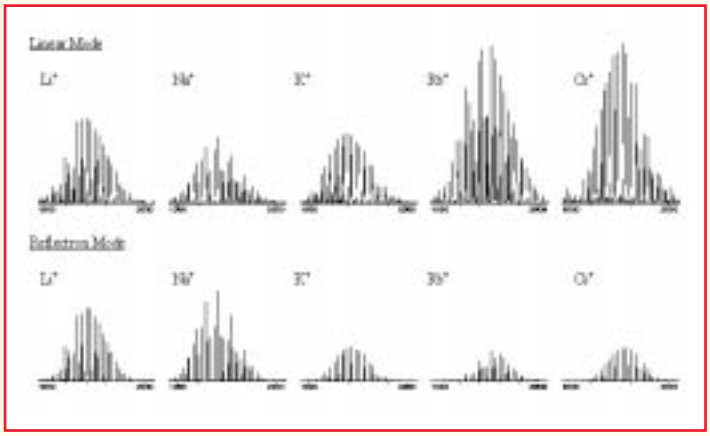

Figure 2: MALDI spectra of PEG1500 acquired in linear and reflectron modes with differing cations

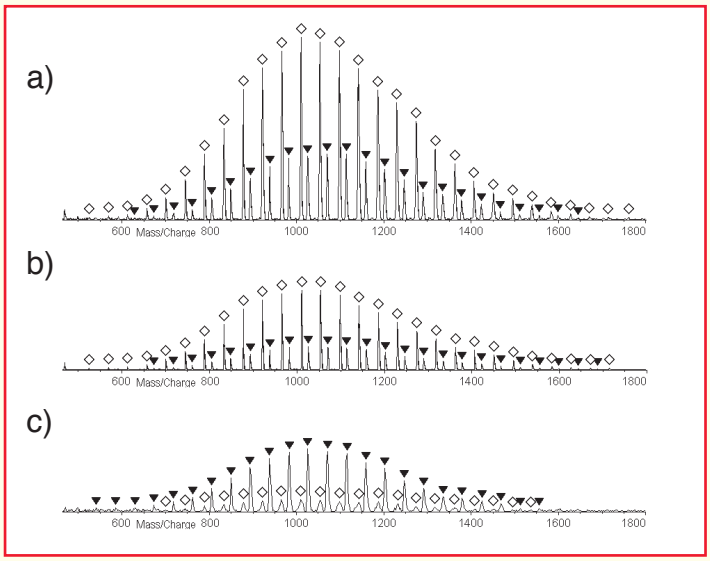

Figure 3: MALDI spectrum of PEG1500 acquired in a) linear, b) reflectron and c) neutral modes

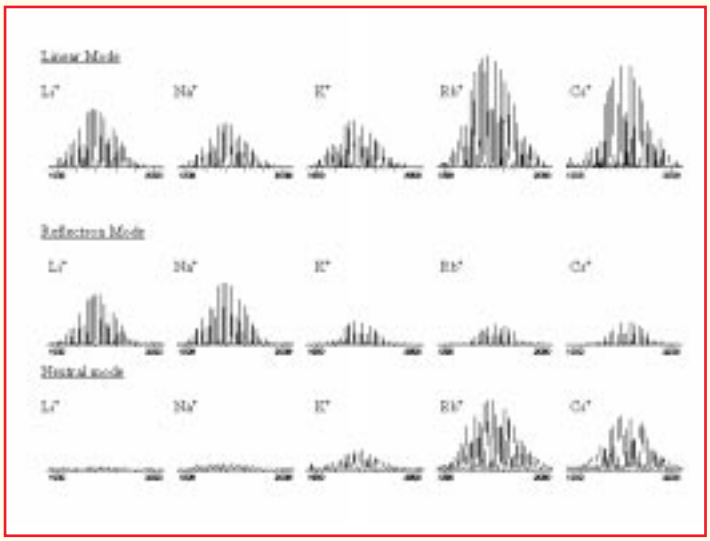

Figure 4: MALDI spectra of PEG1500 acquired in linear, reflectron and neutral modes with differing cations

CONCLUSIONS

The Kompact MALDI 4 provides a wealth of different types of information by the full use of all the modes available -

(i) Linear Mode - molecular weight
(ii) Reflectron mode - more precise molecular weight
(iii) s-PSD mode - structural information
(iv) Neutral mode - ion stability information

The results show clear differences between neutral and ion spectra that can be explained in terms of relative molecular stabilities. Structural explanations can be suggested for different species that are consistent with the results seen.

REFERENCES

i B Spengler, D Kirsh, R Kaufmann & E Jaeger; Rapid Comm. Mass Spec. 6 (1992) 105

ii WC Wiley & IH MacLaren; Rev. Sci. Instrum. 26 (1953) 1150-1157.

iii AR Bowdler, DC Dingley & P Humphrey; Proceedings of 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, Oregon, USA, May 1996, p1342.

iv TJ Cornish & RJ Cotter; Rapid Commun. Mass Spectrometry, 8, 781, (1994).

AUTHORS:

Shin-Ichirou Kawabata[1] and Koichi Tanaka[2]

1 Shimadzu Corporation, 3 Kanda-Nishikicho, 1-Chome, Chiyoda-ku, Tokyo, Japan
2 SRL (Europe) Ltd., Trafford Wharf Road, Manchester M17 1GP, UK

KOMPACT

UK/Europe Wharfside, Trafford Wharf Road, Manchester, M17 1GP, UK
Telephone: +44 (0)161 888 4400 Facsimile: +44 (0)161 888 4402 e-mail: maldi.sales@kratos.co.uk

North America 100 Red Schoolhouse Road, Building A, Chestnut Ridge, New York 10977, USA
Telephone: +1 (914) 426 6700 Facsimile: +1 (914) 426 6192 e-mail: maldi@kratos.com

Japan Shimadzu Corporation, 380-1 Horiyamashita, Hadano-City, Kanagawa 259-1304, Japan
Telephone: +81 463 88 8660 Facsimile: +81 463 88 8670 e-mail: kawabata@shimadzu.co.jp

Germany Shimadzu Deutschland GmbH, Albert-Hahn Strasse 6-10, D-47269 Duisburg, Germany
Telephone: +49 (203) 768 70 Facsimile: +49 (203) 711 734 e-mail: mr@shimadzu.de

Australia Shimadzu (Oceania) PTY Ltd, Unit T, 10-16 South Street, Rydalmere, NSW 2116, Australia
Telephone: +61 (2) 684 4200 Facsimile: +61 (2) 684 4055 e-mail: john.hewetson@shimadzu.com.au

Pacific Rim Shimadzu (Asia Pacific) PTE Ltd, 16 Science Park Drive#01-01, The Pasteur, Singapore Science Park, Singapore 0511
Telephone: +65 778 6280 Facsimile: +65 779 2935 e-mail: regie@shimadzu.com.sg

Website http://www.kratos.com

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