**Introduction**

DCTB\(^1\) is a nonpolar, aprotic matrix, and may be used in the analysis of a variety of compounds by MALDI-TOFMS. The classes of compounds include coordination compounds, organometallics, conjugated non-polar organic compounds, and polar organic compounds. Comparisons are made with spectra acquired with the use of 1,8,9-trihydroxyanthracene (dithranol), 2,5-dihydroxybenzoic acid (DHB) matrices. DCTB is a charge-transfer matrix, therefore, radical ions are observed. Exceptions to this are salts, when constituent positive and negative ions, usually singly charged, are observed, and polar samples, when metal adduct formation is promoted. Certain limitations of DCTB are noted and good working practices for the use of the matrix are also outlined.

**Experimental methodology**

**Chemicals**

All samples were submitted for analysis to the EPSRC National Mass Spectrometry Service Centre (NMSSC) as part of the normal operation of the Centre. DCTB, dithranol, and DHB, matrices (highest purity available) were all purchased from Fluka (Dorset, UK). HPLC grade dichloromethane (DCM), acetonitrile (MeCN) and methanol (MeOH) solvents were purchased from Fischer Scientific (Loughborough, UK). Sodium iodide (NaI), lithium chloride (LiCl), and potassium acetate (KOAc) salts were purchased from Sigma-Aldrich (Dorset, UK).

**MALDI Sample Preparation**

DCTB and dithranol matrix solutions were made to a concentration of 20 mg mL\(^{-1}\) in DCM. DHB matrix solution was made to a concentration of 10 mg mL\(^{-1}\) in 1:1 (v/v) DCM/MeCN. All salt solutions were made to a concentration of 10 mg mL\(^{-1}\) in MeOH. Sample solutions were made to an approximate concentration of 1 mg mL\(^{-1}\) in customer-specified solvent, generally DCM. In a plastic, snap-top lid sample vial, 1 \(\mu\)L of sample solution was vortex-mixed with 49 \(\mu\)L of matrix solution, and with 0.5 \(\mu\)L of salt solution as required. 0.5 \(\mu\)L of the final mixture was spotted onto the sample plate (gold-plated, deep-welled plates are advantageous for organic solvent based mixtures) and allowed to dry, leaving an opaque crystal layer.

**Tip!** DCM tends to leak out from pipette tips, so quick transfer from vial to plate is essential. It is possible to maintain the surface tension of the droplet between the plate and the pipette tip, with minimal or no contact between plate and tip, such that the rate of evaporation and leakage are roughly equal. The tip can be removed when empty, leaving the remaining DCM to evaporate fairly evenly across the sample well.

**Mass Spectrometry**

MALDI-TOFMS spectra were acquired using an Applied Biosystems Voyager DE-STR spectrometer (Framingham, MA, USA), which is equipped with a nitrogen laser (\(\lambda = 337\) nm). The instrument was operated in positive ion, reflectron mode. The accelerating voltage was 20 kV, while the grid voltage was maintained at 66 %. The delay time and laser fluence were optimised for each sample. The laser was fired at a frequency of 3 Hz and spectra were accumulated in multiples of 25 laser shots, with 100 shots in total.

**Results and discussion**

DCTB is a softer matrix than dithranol and DHB, requiring less laser fluence to ionise an analyte. This is an advantage when analysing non-covalent coordination complexes, which may fragment more easily. MALDI-TOFMS using DCTB gains a similar advantage over the use of electrospray (ESI), fast-atom bombardment (FAB), liquid secondary ion mass spectrometry (LSIMS) techniques in analysing such compounds.

Being aprotic, DCTB cannot protonate analytes, therefore only radical ions are observed\(^2\), whereas, with acidic matrices, a mixture of radical and protonated ions is observed. This is illustrated for a substituted porphyrin in Figure 1. The DCTB data matches very closely to the theoretical molecular ion isotope pattern with the \(^{12}\)C isotope at 100 % relative intensity at m/z = 762. With dithranol, a weak acidic matrix, the relative intensity of the m/z = 762 peak drops to 60 %, and the overall pattern match becomes poor. With DHB, a strong acidic matrix, the relative intensity is only 10 %, which may lead an analyst to the incorrect interpretation that the analyte contains boron.
Compounds in the form of salts are analysed commonly by ESI, but several different charge-state species and fragments complicate the spectra, the pseudo-molecular ion may be of very low intensity. Figure 2 shows DCTB MALDI-TOFMS data for an erbium salt. A single species at m/z = 1432 is observed, which corresponds to [M – NO₃]⁺ species, making data interpretation unproblematic. For salts with multiple anions, low intensity singly and doubly charged [M – 2 anions] species may be observed.

DCTB may also be used to characterise polar organic compounds, but with less conjugation, there is less chance of observing the radical molecular ion. For these compounds, cationisation or metal adduct formation is promoted by the addition of a Group I salt. MALDI-TOFMS data acquired with DCTB and LiCl for an alkylated carbohydrate is given in Figure 3. Four species are observed, which initially may indicate that the sample is a mixture. However, on closer inspection, the species correspond to [M + Li/Na/K/Cu]⁺.

Figure 1. Comparison of (a) the theoretical molecular isotope pattern with data acquired with (b) DCTB, (c) dithranol, and (d) DHB.

Figure 2. MALDI-TOFMS data for an erbium salt showing the [M – NO₃]⁺ species.

Figure 3. MALDI-TOFMS data for (a) a carbohydrate and (b) showing various cationised species.

DCTB can also be used in negative ion mode when analysing acidic compounds, the anion component of salts, and fullerenes. However, DCTB is not water-soluble and so unsuited to the analysis of peptides and proteins.

All the points contained herein are discussed in greater detail in the literature.

Conclusions

It has been shown that DCTB is a very affective matrix in the characterisation of a wide variety of compounds by MALDI-TOFMS. Clear, easily interpretable, spectra are obtained, generally offering unambiguous determination of analyte identification. DCTB has also been shown to be comparable, if not better, in performance than traditional polar acidic matrices, and much lower laser fluence is required to achieve desorption/ionisation. However, DCTB is not well suited for the analysis of aqueous samples.

References


Acknowledgements

We wish to thank Dr A. N. Cammidge and Prof. R. A. Field, University of East Anglia, UK, and Dr A. W. G. Platt, Staffordshire University for permission to reproduce data pertaining to their compounds.

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NMSSC Doc. No. 1 2nd November 2006