Sublimation properties of *x,y*-dihydroxybenzoic acid isomers as model matrix assisted laser desorption ionisation (MALDI) matrices

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Abstract

The enthalpies of sublimation of the six isomers of dihydroxybenzoic acid were determined by thermogravimetry as part of a study to investigate their performance as model matrices in matrix assisted laser desorption/ionisation-mass spectrometry. The enthalpy of sublimation could be determined to within an error of ±7%. In the MALDI mechanism, sublimation is thought to play an important part, however in the present study no positive correlation between this parameter and the performance of the material as a matrix was found. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Matrix-assisted laser desorption/ionisation (MALDI) has been used to analyse peptides and proteins [1] oligonucleotides [2], natural products, such as Catechin [3], carbohydrates [4] and synthetic polymers [5]. The ease by which MALDI has been utilised for peptide-sequencing [6], end-group determination [7] and molecular weight characterisation of polymers [8] has made it the technique of choice in many instances. The analyte is usually incorporated within a matrix which is then subjected to an intense laser pulse. This causes the analyte to be brought into the gas phase as an ionised species whose molecular mass can then be determined by, for instance, a time-of-flight mass spectrometer.

The choice of matrix is an important experimental parameter in MALDI analysis. One 'standard' matrix is 2,5-dihydroxybenzoic acid (2,5-DHB) [9]. It has been determined that closely related compounds can have very different properties as matrices. For example, 2,5-DHB is a 'good' matrix while the corresponding 2,4 isomer is not [10].[1] It was initially believed that

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[1] The ability of a substance to act as a good matrix, depends upon its solid state UV absorption coefficient, at a specified wavelength. In MALDI 337 nm is implied unless otherwise specifically stated, since most UV-MALDI instrumentation utilises a nitrogen laser, which operates at this wavelength. As is the case in laser desorption a substance can be a 'good' absorber at one wavelength and a 'bad' absorber at another. In the present study this refers to 337 nm. A 'good' matrix was defined as one which gave analyte signal (S/N~3) with the minimum laser power. Low laser power gives rise to less matrix clusters and less analyte fragmentation. The sequence was (2,3-DHB, 2,5-DHB), 2,6-DHB, (3,5-DHB, 3,4-DHB) and 2,4-DHB, where (2,3-DHB, 2,5-DHB) gave a substance P signal at threshold laser power, in which the laser power used, was considerably lower than the corresponding case for 2,4-DHB as the test matrix.

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presence of carboxylic groups was a prerequisite for a good matrix, but it is now known that this is not the case [11]. The matrix is thought to undergo vaporisation at the laser threshold, since the energy has to be higher than the sublimation temperature of the matrix [12], or loss of functional groups [13]. Laser threshold can be defined as the laser power at which the analyte signal peak is three times that of background (S/N~3). At threshold a number of peaks are seen, amongst them the matrix peak $[M^+]$, also seen are other matrix-fragment peaks, for example $[M-H_2O^+]$ can be seen. The matrix is thought to isolate the analyte molecules from each other and on laser irradiation, the sudden input of energy ('thermal spike') causes the matrix to undergo sublimation [14]. The time scale is of the order of nanoseconds in which thermal degradation is avoided. Bulk matrix sublimation from the surface due to local heating is seen. Energy transfer from the matrix to the analyte follows from which analyte ejection occurs. The analyte molecules attain translational energy and some internal energy due to poor vibrational coupling. This 'bottle-neck' in the MALDI process allows effective desorption yet minimises fragmentation [15]. The physio-chemical characteristics of the matrix are important parameters that may distinguish a 'good' matrix from a 'bad' matrix. In this study, we investigate the sublimation properties of the isomers of dihydroxybenzoic acid which may play an important part in the MALD process.

2. Experimental

The six isomers of dihydroxybenzoic acid were obtained from the Aldrich (>95% pure), benzoic acid (99.999% thermochemical grade) was obtained from British Drug House (BDH) Lutterworth, UK.

The melting temperatures of the compounds were determined by differential scanning calorimetry (DSC) using a TA Instruments 2910 DSC. Samples were encapsulated in hermetically sealed aluminium pans and heated from ambient to 250°C at 10°C/min under nitrogen (flow rate: 10 ml/min). The instrument was calibrated for temperature and heat flow according to the melting point and enthalpy of fusion of indium. Instability of the baseline above the melting point and visual inspection of the samples post-run indicated that the compounds decomposed to some extent on melting.

Thermogravimetry was performed using a TA Instruments 951 TGA. The thermobalance was calibrated for temperature according to the method of Stewart [16] using indium, tin, bismuth and lead. The magnitude and linearity of the balance response were checked with standard milligram masses. Samples were placed in tared aluminium sample cups (internal diameter: 12.5 mm, depth: 3 mm) of the type used for DSC measurements. The cup was filled completely with compacted sample so as to obtain a uniform surface. The thermocouple was kept as close as possible to the surface of the specimen in order to record accurately its temperature without interfering with the operation of the balance. The furnace was purged with dry, oxygen-free nitrogen (flow rate: 100 ml/min). Measurements were carried out under stepwise-isothermal conditions of 15 min dwell time at 5°C increments from 30° to 300°C. Rates of mass loss down to ca. 2.5 mg min^{-1} could be resolved without difficulty.

3. Results and discussion

Analysis of the thermogravimetric data is based on the Langmuir equation for evaporation *in vacuo* [17]:

$$\frac{dm}{dt} = p\alpha\sqrt{\frac{M}{2\pi RT}} \tag{1}$$

where $-dm/dt$ is the rate of mass loss per unit area, p the vapour pressure, M the molecular weight of the effusing vapour, R the gas constant, T the absolute temperature and α the vaporisation coefficient (usually assumed to be 1).

In the case of a material volatilising into a flowing gas stream at 1 atm rather than *in vacuo*, α can no longer be assumed to be unity. Rearranging Eq. (1) gives:

$$p = k\frac{dm}{dt}\sqrt{T/M} \tag{2}$$

where $k = \sqrt{2\pi R}/\alpha$. Price and Hawkins [18] have shown that k is sample-independent and can be evaluated by calibration with substances of known vapour pressures.

The temperature dependence of the vapour pressure can be described by the Clausius–Clapeyron equation:

$$\frac{d(\ln p)}{dT} = \frac{\Delta H}{RT^2} \tag{3}$$

where ΔH is the molar heat of sublimation (ΔH_{sub}) for the solid or heat of vaporisation (ΔH_{vap}) for the liquid.

For most practical purposes, and over limited temperature range, ΔH is considered constant; thus Eq. (3) may be written in the form:

$$\ln p = B - \frac{\Delta H}{RT} \tag{4}$$

where ΔH is the molar enthalpy of sublimation (ΔH_{sub}) in the case of a solid or enthalpy of vaporisation (ΔH_{vap}) in the case of a liquid.

Combining Eq. (4) with Eq. (2) gives:

$$\ln\left(\frac{dm}{dt}\sqrt{T/M}\right) = B' - \frac{\Delta H}{Rt} \tag{5}$$

and $B' = B - \ln k$

Ashcroft [19] has shown that enthalpies of sublimation (and vaporisation) can be found directly from the rate of mass loss obtained by thermogravimetry providing the exposed surface area is constant. This can be achieved by melting the substance to obtain a specimen with a flat surface [18]. The dihydroxybenzoic acid isomers could not be fused without decom-

position and therefore the surface areas of the samples could not be controlled precisely. For a zero-order process such as sublimation or evaporation, the rate of mass loss ($-dm/dt$) should be constant. However, at high temperatures, $-dm/dt$ was a weak function of time due to the changing exposed surface area of the sample. In order to accommodate for this, the strategy of Flynn and Dickens [20] was adopted whereby the rates of mass loss were extrapolated to the start of the temperature jump between isothermal plateaus (Fig. 1). This gives $-dm/dt$ at two temperatures (T_1 and T_2) from which ΔH_{sub} may be obtained:

$$\Delta H_{sub} = R\left[\ln\left(\frac{\{dm/dt(T_1)\sqrt{T_1}\}}{\{dm/dt(T_2)\sqrt{T_2}\}}\right)\right]\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{6}$$

Note that it is no longer necessary to know the mass of the vaporising species provided that it does not change significantly during the change in temperature. A plot of the enthalpy of sublimation of 2,4-DHB as a function of temperature is shown in Fig. 2. The gap in the data around 220°C spans the melting region – the points above this temperature refers to the enthalpy of vaporisation and are less reliable due to probable decomposition of the sample.
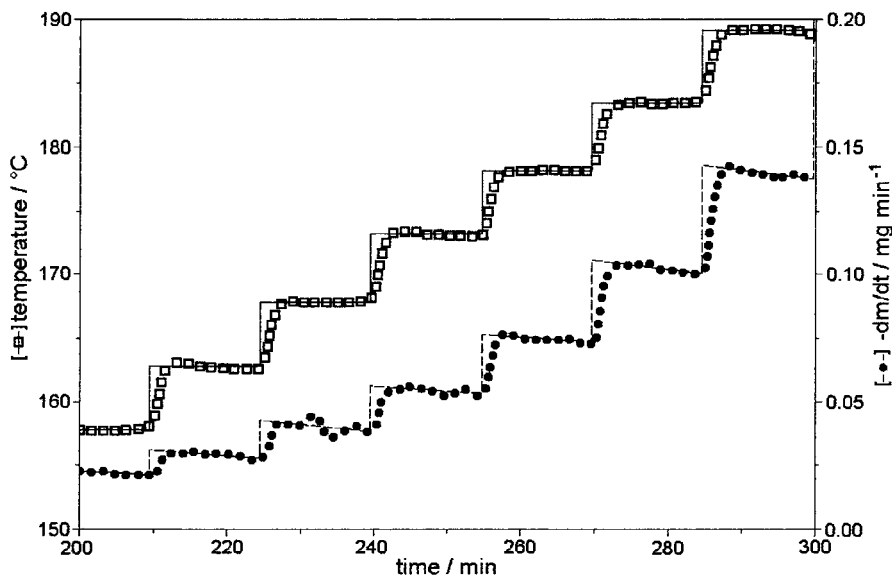

Fig. 1. Temperature (□) and rate of mass loss, $-dm/dt$ (●) versus time for 2,4-dihydroxybenzoic acid. Solid and broken lines indicate constructions used to determine average temperatures and rates of mass loss respectively over each temperature jump.
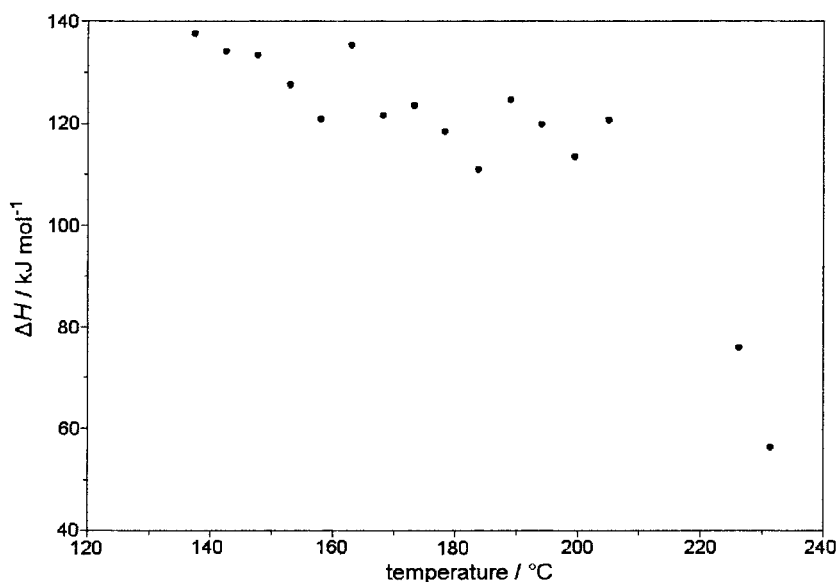
Fig. 2. Enthalpy of sublimation versus temperature for 2,4-dihydroxybenzoic acid; gap in data at melting region; results above this temperature refer to the enthalpy of vaporisation (see text).

Benzoic acid has been extensively used as a standard for combustion and adiabatic calorimetry and has been recommended by IUPAC as a calibration and test material for enthalpy of sublimation measurements [21]. De Kruif [22] has reviewed the literature data for this material and provides a table of vapour pressures over a wide temperature range. These results were used to determine the enthalpy of sublimation of benzoic acid and compared with that obtained from this work (Table 1). As discussed above, enthalpies of sublimation are a weak function of temperature, therefore average values were determined from each experiment. The standard error in these determinations amounts to about $\pm 7\%$.

Sublimation can occur at laser powers below threshold, but at threshold enough molecules have sublimed to be detected. Even when there is zero laser power, monolayer desorption can be thought to take place, at low pressures (3×10^{-6} mbar), which are commonly attained in mass spectrometers. Sublimation of the matrix purely due to the vacuum is not important and its contribution to the MALD processes is thought to be negligible. The sudden input of energy into the sample may also lead to sample vaporisation (i.e., solid–liquid–gas) rather than pure sublimation (solid–gas). This processes depends on the density of the gas cloud. It is known that the gas plume expands supersonically into the vacuum causing the sample to cool. Molecules that have similar absorption characteristics at a specific wavelength, would have similar sublimation properties.

Inspection of Table 1 shows that, although the best performing 2,5-DHB has the lowest enthalpy of sublimation, the worst performing compound, 2,4-DHB does not have the highest enthalpy of sublimation. This position is occupied by 3,4-DHB which, although not the best performing compound of this series, has also been successfully used as a matrix. Therefore, it

Table 1

Normal melting temperatures and enthalpies of sublimation of *x,y*-dihydroxybenzoic acid (DHB) isomers. (T_m values are at $p=1$ bar)

Substance	T_m (°C)	$-\Delta H_{sub}$ (kJ mol^{-1})
Benzoic acid	122.3	89 ± 6 [a]
2,3-DHB	206.3	116 ± 4
2,4-DHB	228.5	126 ± 6
2,5-DHB	205.7	109 ± 3
2,6-DHB	170.5	111 ± 7
3,4-DHB	201.7	153 ± 9
3,5-DHB	235.7	135 ± 6

[a] Denotes mean of two experiments, data of De Kruif [22] gives $\Delta H_{sub}=-(90 \pm 2)$ kJ mol^{-1}.

can be concluded that some other factor may be important in determining yield in the MALD process. (The matrix rating is based on analyte desorption at the lowest laser power, where signal-to-noise ratio was 3). The analyte molecules are not thought to adversely affect the sublimation of the matrix, since they are in trace amounts and the laser desorption of the matrix is similar to its desorption threshold in the MALD process [10].

4. Conclusions

Our results indicate that sublimation enthalpy is not the principle factor in the MALD process. The hypothesis that the sublimation distinguishes between 2,5-dihydroxybenzoic acid being a good matrix and 2,3-dihydroxybenzoic acid being a bad matrix is not the case. It must be noted that the matrix would also sublime at laser powers above the threshold. The model proposed by Vertes et al. [14] leads to bulk desorption of the matrix. In this desorption energy transfer to the analyte occurs. Also the mechanical process of desorption can aid the analyte molecules via reaction momentum into the gas phase [23] ('popcorn' model). In the 'bottle-neck' model competitive kinetics between sublimation and fragmentation was considered, with a 'good' matrix possessing low sublimation (phase-transition) temperature [15]. The performance of these materials as MALDI matrices may depend on some other physio-chemical property such as absorption coefficient or proton affinity in conjunction with sublimation.

Acknowledgements

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