Characterization of technical waxes
Comparison of chromatographic techniques and matrix-assisted laser-desorption/ionization mass spectrometry

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Abstract

Technical waxes and paraffins were investigated using supercritical fluid chromatography (SFC), matrix-assisted laser-desorption/ionization mass spectrometry (MALDI-MS) and size-exclusion chromatography (SEC). SEC enables the simultaneous determination of molecular masses up to high values and molecular mass distributions. The resolution of homologous species, however, is poor. In this respect the use of SFC is advantageous. Proper resolution of homologues may be provided up to $M_r \approx 1200$ without great difficulty. To compare these chromatographic results MALDI-MS was applied for the determination of molecular masses and mass distributions in technical waxes for the first time. Using reflectron mode excellent MALDI mass spectra could be achieved in the molecular mass range up to 1000 Da. The mean values of the molecular masses calculated by MALDI-MS were in sufficient agreement with those of SFC. In the linear mode of MALDI-MS molecular masses could be determined to nearly 3000 Da. Beyond this mass range SEC still remains the method of choice. Advantages and difficulties of the used methods are discussed in this paper.

Keywords: Molecular mass determinations; Molecular mass distributions; Waxes; Paraffins

1. Introduction

Technical used hydrocarbons can roughly be divided into paraffins, Fischer–Tropsch waxes and polyolefin waxes differing with respect to chemical structure and molecular mass [1].

The properties important for practical purposes, such as melt viscosity, hardness and melting point, should be already targeted at the development and production of batches of waxes. Many of these properties depend on both the molecular mass range

and the content of low-molecular-mass components. Knowing the exact composition, the performance properties can be predicted, and production processes can be controlled [2].

New developments in capillary gas chromatography (cGC), especially the introduction of thermostable stationary phases and non-discriminative injection devices, have made the use of high-temperature capillary gas chromatography for investigation of waxes applicable [3,4]. However, the decomposition of paraffins at temperatures above 350°C represents one of the important restrictions for the investigation of paraffins by cGC [5,6]. First applica-

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tions of supercritical fluid chromatography (SFC) have been reported, which demonstrate the possibility of a fingerprint characterization of high-molecular-mass paraffins and waxes until a chain length of C_{52} (>700 Da) [3,7]. However, the main problem in SFC is to determine waxes and paraffins consisting of longer aliphatic chains.

A variety of mass spectrometric techniques have been applied previously to analyze higher molecular masses of paraffins and low-molecular-mass poly(ethylene)s, for example field-desorption/ionization mass spectrometry (FD-MS) [8–10] and laser-desorption Fourier transform mass spectrometry (LD-FT-MS) [11].

In the last years matrix-assisted laser-desorption/ionization mass spectrometry (MALDI-MS) was established as a powerful technique for the determination of the mass of large biomolecules and synthetic polymers [12–16]. This method is based on the principle of mixing a dissolved specimen with a matrix and their crystallization. The specimen is desorbed and ionized by a short laser pulse. The molecular mass is determined by the time of flight (TOF).

The ionization of compounds using MALDI-MS is normally facilitated by the attachment of hydrogen or alkali metal ions of the aromatic matrix to the heteroatoms of the investigated compounds. Because of the lack of these sites, non-polar hydrocarbons give no results under these conditions of ionization.

Our aim was to investigate whether MALDI-MS could be used for determining molecular masses and mass distributions in these non-polar substances by adding silver salts to the matrix.

Kahr and Wilkins [11] have shown that mixtures of suitable metal salts with analyte samples can lead to an effectively chemical ionization of alkanes and alkenes using LD-FT-MS. Gold was an attractive first choice for a chemical ionization reagent; however, when gold chloride was used, some polymers fragmented significantly or lost end-groups. This behavior could result from the relatively high ionization energy of gold (9.2 eV). The lower ionization energy of silver (7.6 eV) therefore seems to be more useful for the investigation of alkanes and overcomes this problem of ionization of non-polar hydrocarbons [11,17].

2. Experimental

2.1. MALDI-MS

The MALDI-MS investigations were performed using a Kratos Kompact MALDI 3 mass spectrometer in linear and reflectron mode. A solution of 2,5-dihydroxybenzoic acid (1% solution in ethanol-water 1:1, v:v) for the investigation of paraffins and 2-nitrophenyl octyl ether (1% solution in xylene) for the waxes was dropped onto the slide. After drying, silver nitrate (5% solution in water) was added, and the matrix was dried again. Then the samples (1–5 mg/ml in toluene or xylene) were dropped onto the matrix and the silver nitrate. A co-crystallization of matrix and sample molecules can be assumed, because aromatic solvents were used for the samples which also redissolve the matrix. The ionization of molecules was performed by laser desorption at 337 nm and 3 ns pulse width. The ions were accelerated with 20 kV in the positive ion mode. Within the molecular mass range up to 1000 Da they were detected in reflectron mode, whereas the detection of molecules with higher molecular masses could be achieved in the linear mode.

2.2. SFC

The SFC chromatograms were obtained with a Dionex 602-D SFC (Dionex, Lee Scientific Division, USA). The supercritical separations were carried out with linear temperature programming from 100 to 200°C and simultaneous pressure programming from 10 to 40 MPa. Carbon dioxide was used as the eluent. The separation column consisted of a fused silica column (SB-Biphenyl-30, 10 m × 50 μm I.D., Dionex, USA). Flame ionization detection (FID) was used at 380°C. The temperature of the injector was 100°C.

2.3. SEC

SEC investigations were performed using a Hewlett-Packard chromatograph containing three columns (30 × 0.75 cm I.D.) filled with PL-gel (100, 1000, 10 000 Å pore size, Polymer Labs., UK). The chromatography was carried out at a temperature of

135°C using o-dichlorobenzene as the solvent and 0.1% ionol as an antioxidant. The peaks were detected by an IR detector (Du Pont Instruments) at a wavelength of 3.4 μm. The chromatographic system was calibrated using polyethylene standards (Polymer Labs. Ltd.).

All solvents and matrix substances were obtained from Aldrich (analytical grade) and the samples were from Hoechst.

3. Results and discussion

Fig. 1 shows the application of SEC of six different waxes. SEC can provide simultaneous information about molecular masses and molecular mass distributions. Separation of single homologous species, however, cannot be achieved in the normal case.

In the low-molecular-mass range the use of SFC is advantageous in this respect. Proper resolution of homologues may be provided up to $M_r \approx 1200$ without great difficulty, as is shown in Fig. 2. One major problem in SFC represents the decrease of solubility by increasing the chain length of the n-alkanes. SFC separations of higher-molecular-mass materials are not recommended because of enlarged retention using SB-Biphenyl-30 columns. Optimization is possible with the use of non-polar SB-Methyl-100 columns or the application of higher temperatures and densities of carbon dioxide. Thus, separation of homologous species with chain lengths $> C_{100}$ had
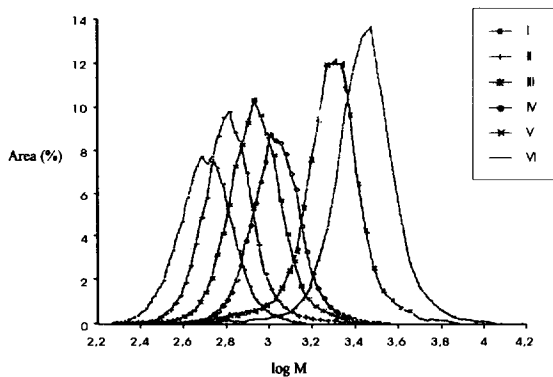

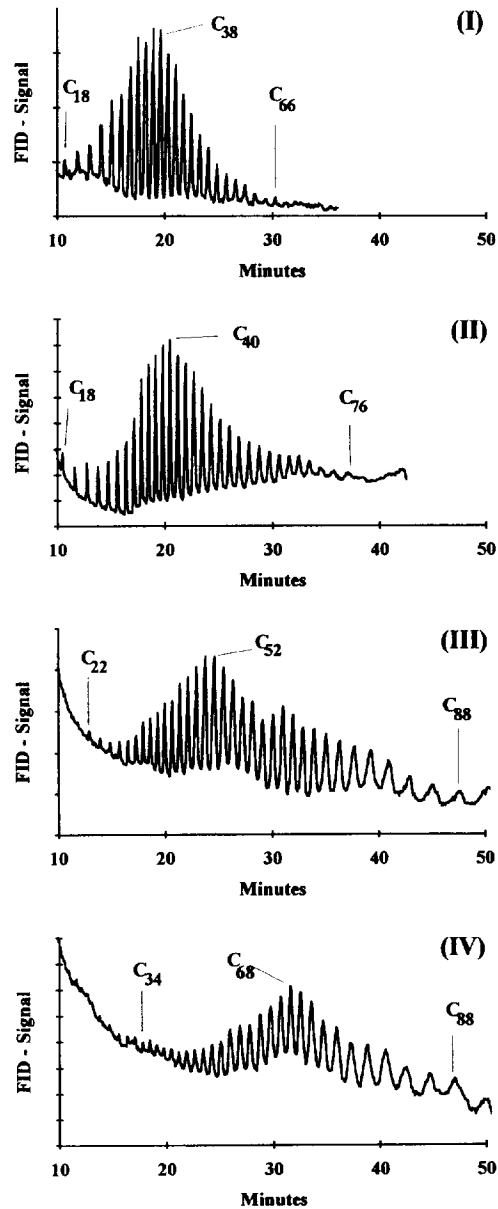Fig. 1. SEC of waxes I, II, III, IV, V and VI.

Fig. 2. SFC chromatograms of waxes I, II, III and IV.

been realized [18]. The identification of unknown substances requires the use of standards or the combination of SFC with spectroscopic methods. Therefore, the chromatographic system was calibrated with different n-alkanes.

It has to be remembered that the FID signal

intensity not only depends on the amount of the eluted component, but also on its composition and chemical structure [20]. Therefore, the molecular mass distributions were calculated considering the relative mass response of the homologues, i.e. an increment of 100 for each methylene group of the molecule [21]. An excellent agreement between SEC and SFC was obtained by comparing the molecular masses and mass distributions of wax 500 and wax 655 (Table 1). The lower mass values calculated from the SFC chromatograms for wax 850 and wax 1000 can be explained by the exclusion of homologues $> C_{88}$ due to the poor resolution in this range.

Using MALDI-MS the range of detection and resolution of single species are higher than in SFC. In Fig. 3 the MALDI mass spectra of the investigated waxes are depicted. As with the use of SFC, single homologues could clearly be separated. The best results below molecular mass 1000 Da were obtained in the reflectron mode, whereas waxes consisting of alkanes with molecular masses above 1000 Da were only detected in the linear mode of MALDI-MS. Masses above 3000 Da are not yet detectable by MALDI-MS.

For investigating a possible discrimination of ion masses below 500 Da some low-molecular-mass natural and technical paraffins were chosen. The MALDI spectra of these paraffins are shown in Fig. 4, and the corresponding SFC chromatograms in Fig. 5. The homologues in the mass spectra shown in Fig. 4 show double peaks which are caused by the ionization of wax molecules by the two isotopes of silver ([107]Ag, [109]Ag). The lower-molecular-mass homologues obtainable with SFC ($<C_{22}$) were not found in the MALDI spectra. The slope of the mass

spectra (Fig. 4) clearly indicates a discrimination of ions below molecular mass 500 Da (respectively masses of wax molecules < 390 Da). Below 350 Da we found a lot of masses resulting from the matrix and silver ions. Molecules with lower masses are sucked off.

The molecular mass distributions of the waxes calculated by SEC, SFC and MALDI-MS proved to be very low, as shown in Table 1. The number-average molecular masses M_n calculated by MALDI-MS are about 6–14% higher than the SEC data. The weight-average molecular masses M_w up to approximately 900 Da show about 10% higher values in MALDI-MS compared with SEC results, whereas the differences in the range from 900 to 2000 Da are very low. The polydispersity P ($=M_w/M_n$) calculated by MALDI-MS, appears to be narrower then with SEC and SFC. The reason for this behavior must be found in the broadening of the peaks in SEC as well as in the discrimination of lower molecular masses in MALDI-MS.

Similar observations had been made investigating poly(methylmethacrylate)s by SEC and MALDI-MS [19].

4. Conclusions

The range of applicability of the methods used for the determination of molecular masses and mass distributions of waxes and paraffins is estimated as shown in Fig. 6. The limits of the investigated methods overlap over a broad range.

Using SFC molecular masses of homologues of waxes and paraffins up to 1200 Da can be separated with proper differentiation.

Table 1
Molecular mass distributions of technical waxes calculated by different chromatographic techniques and MALDI-MS (M_w: weight-average molecular mass; M_n: number-average molecular mass; P: polydispersity M_w/M_n

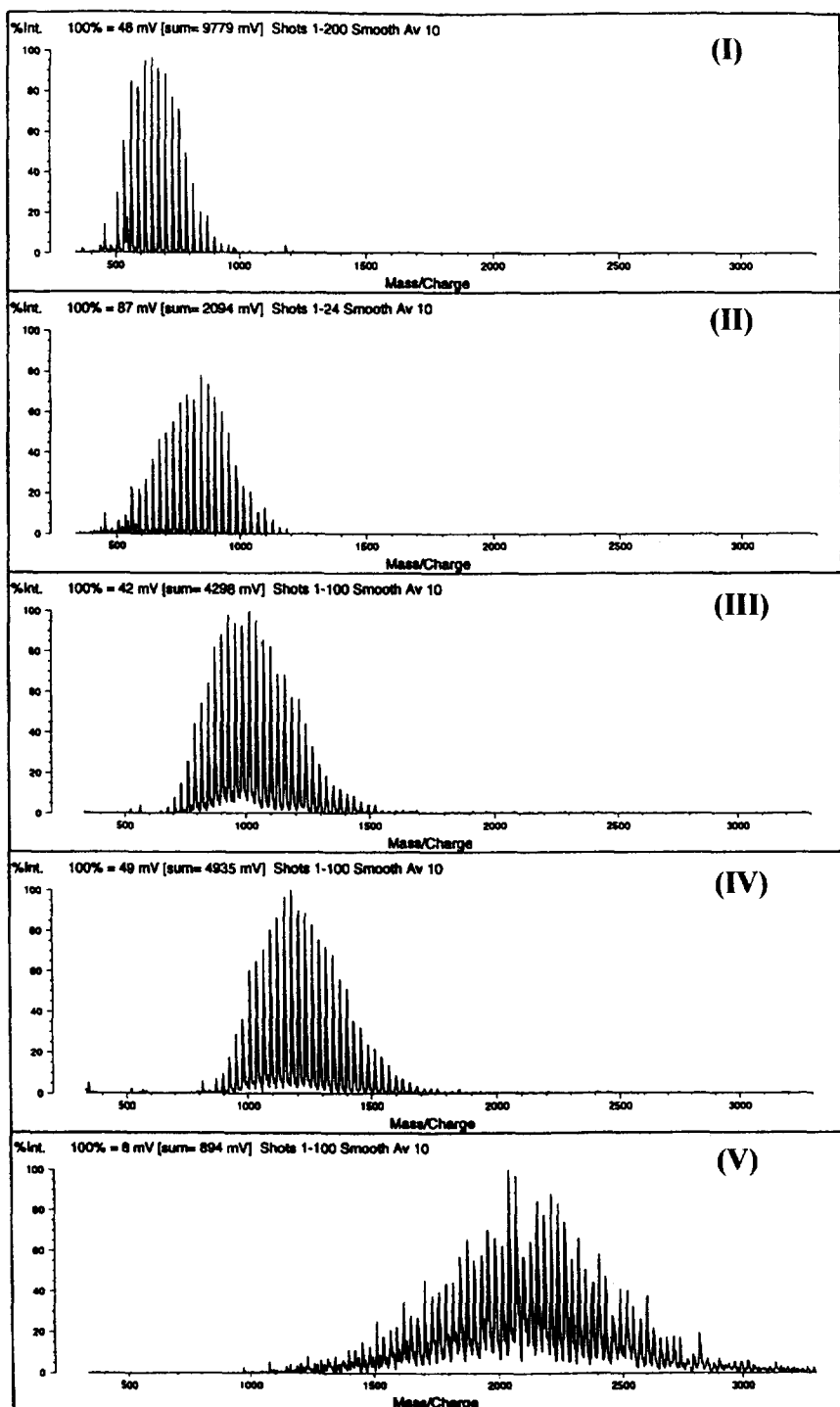| Wax	SEC			SFC			MALDI-MS		
	M_w	M_n	P	M_w	M_n	P	M_w	M_n	P
I	527	483	1.09	522	492	1.06	572	558	1.03
II	680	624	1.09	661	607	1.09	742	725	1.02
II	905	821	1.10	868	811	1.07	938	914	1.03
IV	1121	1040	1.08	987	948	1.04	1129	1109	1.02
V	2066	1802	1.15	–	–	–	2061	2014	1.02
VI	2964	2583	1.15	–	–	–	–	–	–

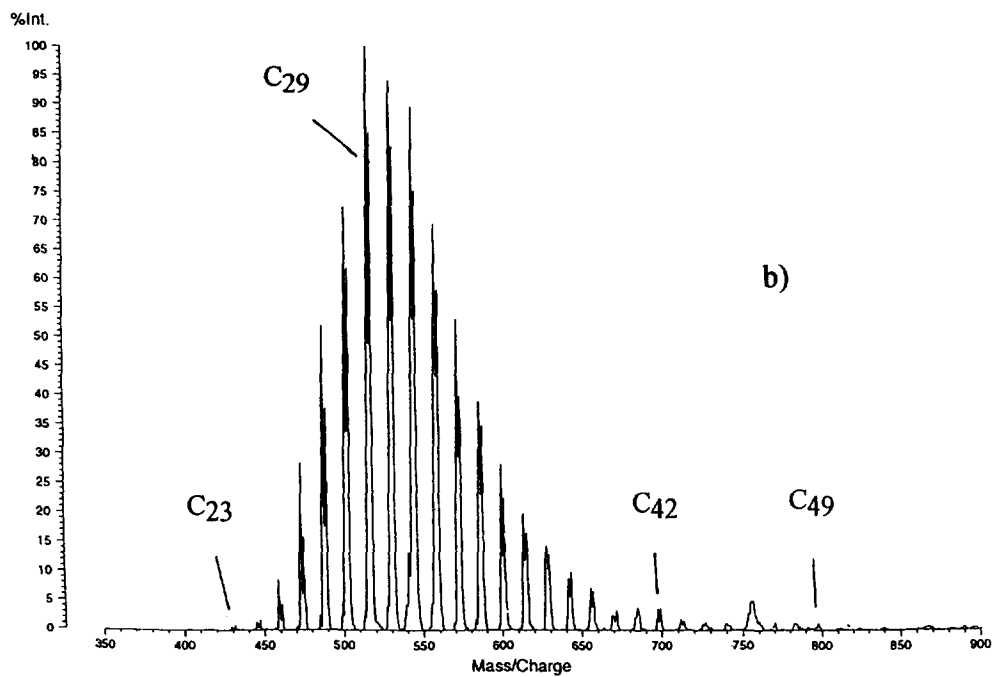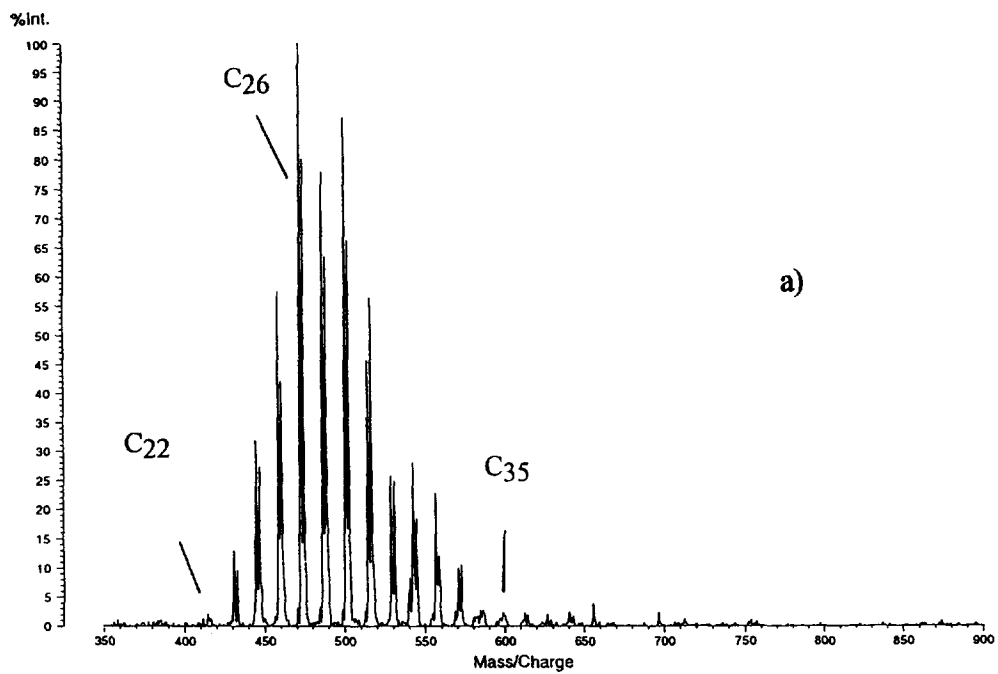Fig. 3. MALDI mass spectra of waxes I, II, III, IV and V.

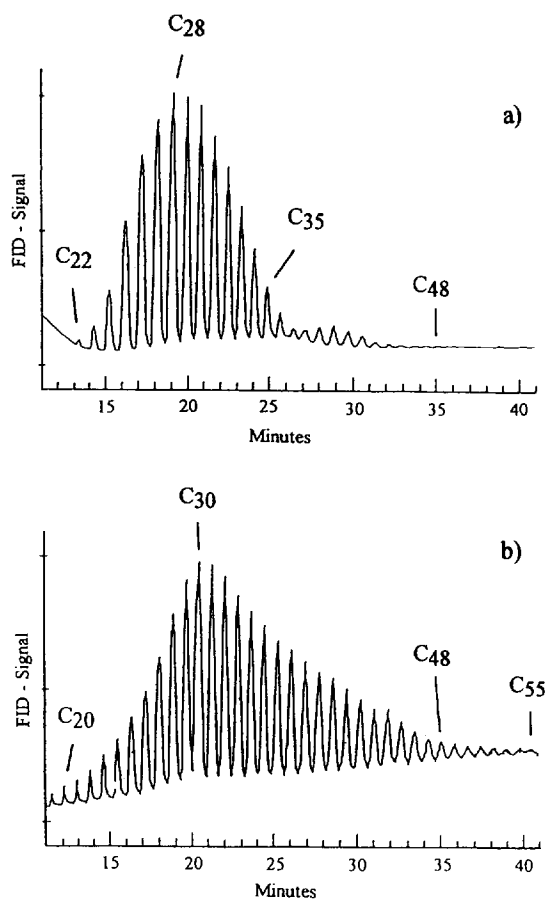Fig. 4. MALDI mass spectra of candle wax (a) and paraffin wax (b).

Fig. 5. SFC chromatograms of candle wax (a) and paraffin wax (b).

MALDI-MS realized by addition of silver salts to the matrix can be a useful method to complement the application of SFC and SEC. It seems to be possible
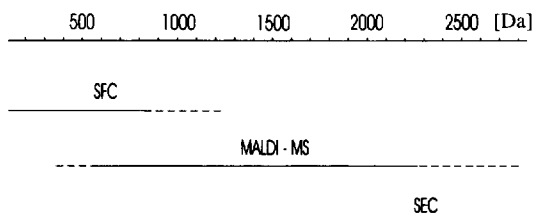

Fig. 6. Range of applicability of SEC, SFC and MALDI-MS for the determination of molecular masses (scale on top) and mass distributions of waxes.

to characterize fractions of SEC eluates by MALDI-MS and to use these fractions for the calibration of SEC columns and systems in the low-molecular-mass range of approximately C_{30} up to C_{200}.

Beyond this mass range SEC remains yet the method of choice for the determination of molecular masses and mass distributions of waxes and paraffins.

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