

# Direct Chemical Ionization Mass Spectrometry of Short-chain Polysaccharides

J. O. Metzger\* and E. Bruns-Weller

Department of Chemistry, University of Oldenburg, W-2900 Oldenburg, Germany

Underivatized short-chain polysaccharides such as two inulins from different plants containing up to 35–40 monosaccharide units with molecular weights of up to 6500 Da and dextran T 1.5 containing up to 16–20 monosaccharide units with molecular weights of up to 3200 Da have been investigated by direct chemical ionization. Under soft ionization conditions such as ammonia chemical ionization and reduced ion-source temperature, it was possible to obtain spectra of the native polysaccharides showing dominant ion series corresponding to ammonia adduct ions of oligosaccharides, and also ion series corresponding to ammonia adduct ions of anhydro-oligosaccharides.

Oligo- and polysaccharides with molecular weights of up to some  $10^6$  Da represent a group of biologically very important compounds, either as free carbohydrates or as constituents of glycoconjugates. Mass spectrometric techniques such as fast-atom bombardment (FAB) have been applied both for molecular weight determination and identification of these compounds up to a molecular weight of 4000.<sup>1</sup> Recently, <sup>252</sup>Cf plasma-desorption mass spectrometry<sup>2</sup> and matrix-assisted laser-desorption mass spectrometry<sup>3</sup> were used for mass determination of neutral nonderivatized oligo- and polysaccharides up to molecular weights of approximately 4000 and 10 000 Da,<sup>4</sup> respectively.

Direct chemical ionization mass spectrometry (DCI-MS) is a valuable tool for the investigation of small oligosaccharides with up to 6 sugar units.<sup>5</sup> DCI-MS has also been applied for the investigation of the pyrolysis products of polysaccharides.<sup>5,6</sup> In almost all cases, the formation of a main ion series corresponding to ammonia adducts of anhydro-oligosaccharides has been observed, which results from a thermal degradation during heating of the DCI-wire. Recently Boon *et al.*, successfully applied DCI-MS to obtain a pyrolysis mass spectrum from cellulose with ammonia adduct ions of anhydro-oligomers of up to 12 sugar units (1962 u).<sup>7</sup> The formation of oligomers in polysaccharide pyrolysates has been proven by high performance liquid chromatography/mass spectrometry (HPLC/MS)<sup>8</sup>, gas chromatography/mass spectrometry (GC/MS)<sup>7</sup> and by gel-permeation chromatography (GPC)/NMR and GPC/FAB/MS.<sup>9</sup> This paper presents the results of DCI-MS of underivatized short-chain polysaccharide polymers such as two different inulins and dextran T 1.5. Inulins are plant reserve poly-saccharides of the Compositae. These are linear D-fructose polymers with 1,2-glycosidic linkages (fructane) and with a degree of polymerization of up to 35–40 which varies according to the plant species and the life cycle. Dextran T 1.5 is a branched 1,6-D-glucose polymer with an average molecular weight of 1500, produced by enzymatic synthesis.

## EXPERIMENTAL

### Materials

Inulins from different plants (Jerusalem artichokes and dahlia tubers) were purchased from Sigma Chemical Co (St. Louis, MO, USA). Dextran T 1.5 was pur-

\* Author to whom correspondence should be addressed.

chased from Fluka Biochemika (Buchs, Switzerland). The samples were dissolved in hot water ca 70 °C at a concentration of 10 µg/µL. 1–2 µL solvent was deposited onto the DCI-wire. Prior to insertion of the probe into the CI source the solvent was evaporated by a stream of air.

### Mass spectrometry

Experiments were carried out with a MAT212 magnetic sector mass spectrometer (Finnigan MAT, Bremen, Germany) equipped with a SS200 data system (mass range of up to 2000 u) and with a MAT95 magnetic sector mass spectrometer, equipped with a Decstation 21100 data system (mass range to 17 500 u). Both systems were fitted with a DCI-probe. The operational conditions for the MAT212 were as follows: accelerating voltage: 2 kV, electron energy: 230 eV, emission current: 6 mA and pressure of reactant gas (ammonia): 20 Pa. The ammonia pressure was optimized so that the ammonia clusters would give the highest measured abundance. The source temperature was kept at 100 °C. The DCI-wire was heated up to 1500 °C at 10 °C/s using linear current programming. Positive-ion ammonia chemical ionization spectra were scanned from 90–2000 u with a cycle time of 4.3 s and an instrumental resolution of approximately 1000. The operational conditions for the MAT95 were: accelerating voltage: 2.5 kV, electron energy: 140 eV, emission current: 0.2 mA, pressure of reactant gas approximately 20 Pa. The reactant gas pressure was optimized as described above. The source temperature was kept at 80 °C. The spectra were scanned from 2000–7000 u with a cycle time of 0.74 s for inulins and from 500–4000 u with a cycle time of 1.1 s for dextran T 1.5, respectively. The instrumental resolution was 1000. The spectra were acquired during heating of the DCI-wire to 1500 °C at 16 °C/s using linear current programming.

## RESULTS AND DISCUSSION

As has often been reported in the Literature, we established that under standard ion-source conditions, that is *inter alia* using approximately 200 °C ion-source temperature, all the spectra over the desorption profile of the polysaccharide show only pyrolysis product ions: more or less dehydrated oligosaccharide units of up to 6 sugar units are produced from the polymeric chain. To

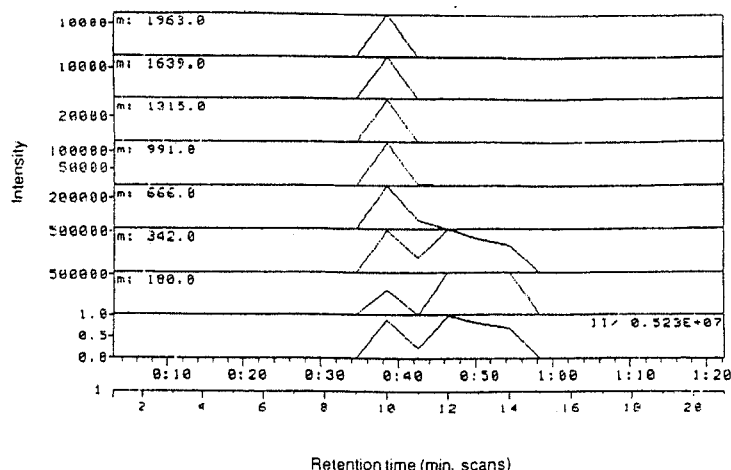


Figure 1. Total-ion current traces and time-resolved mass traces of various ammonia adduct anhydro-oligomer ions of an inulin (MAT212).

enhance the desorption of intact molecules and to avoid the competitive process of thermal decomposition and mass spectrometric fragmentation we found the important parameter to be the CI source temperature: low source temperatures support the production of intense molecular ion species.

Figure 1 shows the total-ion current and time-resolved mass traces of higher molecular weight ions of an inulin over the evaporation profile under ammonia DCI conditions and an ion-source temperature of 100 °C. This form of representation shows the abrupt appearance as well as disappearance of higher molecular weight compounds in a specific stage of vaporization at the beginning of the desorption profile under low source-temperature conditions. Following the explo-

sive desorption of relatively large polymers, only lower molecular weight ions are detected at higher DCI-wire temperatures. From this it can be concluded that after a very short time of desorption of intact molecules the pyrolytic process occurs.

Figure 2 shows the spectrum of an inulin taken at the desorption maximum of higher molecular weight compounds. As a result of low resolution the mass assignment sometimes differed slightly from the correct masses. The spectrum exhibits an ion series corresponding to ammonia adducts of saccharide ions of oligomers of up to 12 fructose units. The series starts at  $m/z$  198 with mass increments of 162 u (corresponding to the mass of a single fructose unit):  $m/z$  360, 522, 684... up to  $m/z$  1980. Furthermore, a main additional

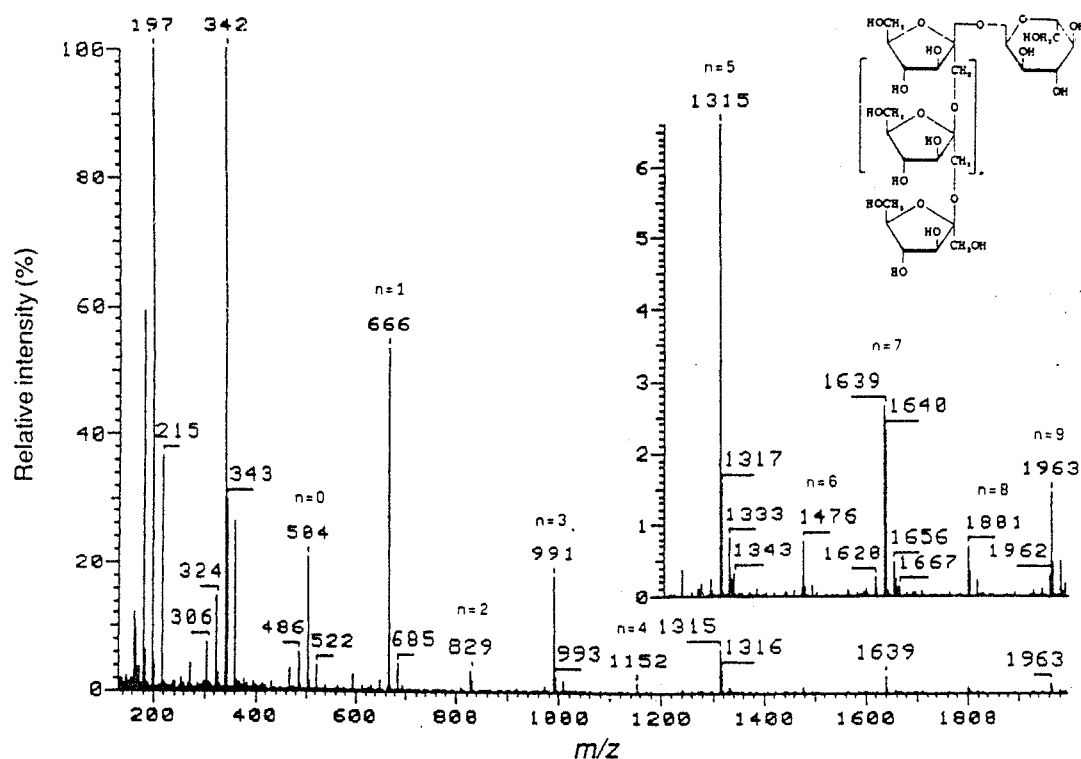


Figure 2. Positive ammonia DCI mass spectrum of an inulin scanned from  $m/z$  150-2000 (MAT212). The various ion series species differ in mass by 162 u. The numbers of the sugar units are available from the formula scheme and the numbers of the  $n$ -fructose units, given by the ions of the anhydro-oligomers

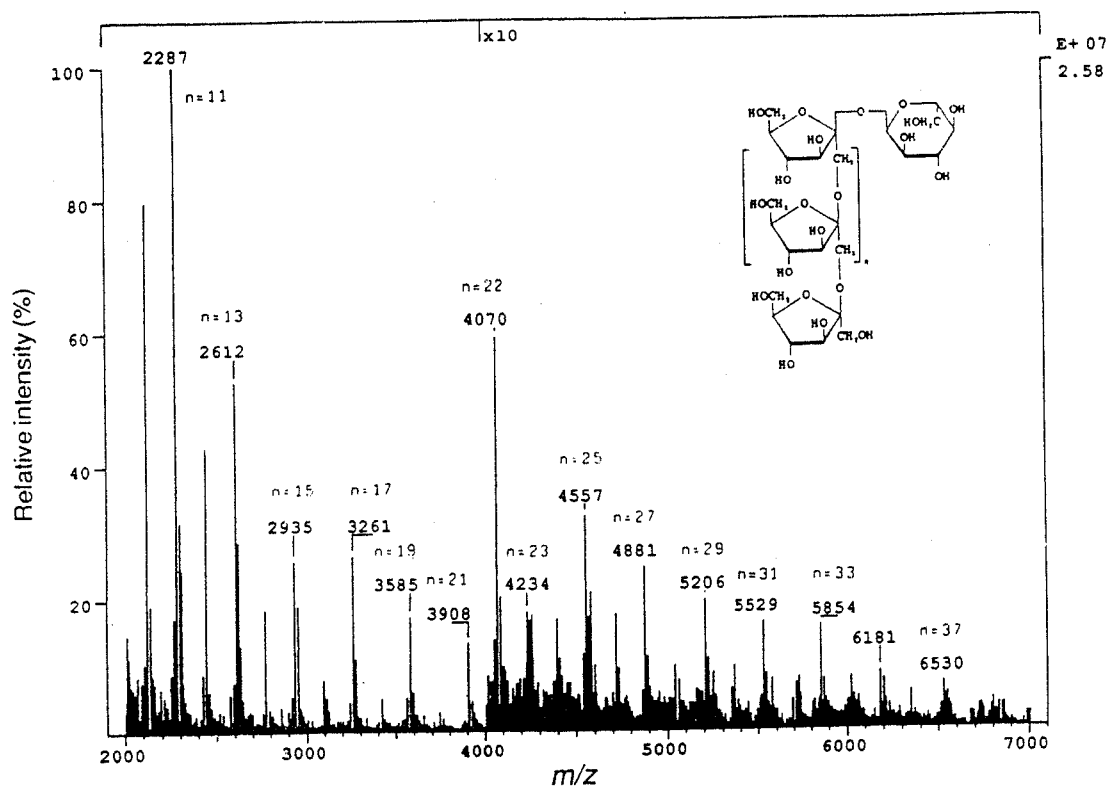


Figure 3. Positive ammonia DCI mass spectrum of dahlia inulin, scanned from  $m/z$  2000–7000 (MAT95).

ion series corresponding to ammonia adducts of anhydro-oligomers is observed (for example  $m/z$  504, 666... up to  $m/z$  1962). In the lower mass range an ion series interpreted as representing ammonia adducts of dianhydro-oligomers appears (for example, peaks at  $m/z$  324, 486).

Until now we could not determine whether anhydro-oligomer ion series are formed by pyrolytic processes during desorption, by CI fragmentation in the ion source or if both events occurred simultaneously. But there is some reason to believe that, at first, intact oligomers are desorbed from the DCI-wire and that then the quasimolecular ions are subjected to mass fragmentation. For example, in the mass spectrum of inulin the intensities of masses corresponding to oligomers and anhydro-oligomers with even numbers of sugar units in the molecule are higher than the intensities of masses corresponding to odd numbers of sugar units. Oligomers and corresponding anhydro-oligomers with even numbers of sugar units seem to form more stable ions. Pyrolytic processes cannot preferentially give rise to a special ion series, it can only be caused by a preferred mass fragmentation. A reason for preferred mass spectrometric formation of ion series with even numbers of sugar units in the molecule cannot be given at the moment.

The results led us to expect, under low ion-source temperature conditions, the possibility of detecting polymer ion series in a much higher molecular weight range. This hypothesis was confirmed by measurements with the MAT95. The inulins showed the same desorption reaction as described above: high molecular weight compounds are produced during a very short time interval. Figure 3 shows the spectrum of dahlia inulin during this time interval under ammonia CI conditions

and an ion-source temperature of 80 °C. The desorption process proceeded so fast that even at the maximum scan rate of the mass spectrometer it was not possible to scan over the whole mass range necessary. Because of this, the scan range was limited to  $m/z$  2000–7000. As a result of the very fast scanning and low resolution, mass assignment was difficult and not always absolutely correct, especially in the high mass range.

The influence of low source temperature under DCI conditions on the production of molecular-ion species is apparent from the resulting dahlia inulin spectrum: ion series are detected up to 40 sugar units. As described above, three ion series are observed: ammonia adducts of oligomers, anhydro-oligomers and dianhydro-oligomers. The preferred formation of ion series corresponding to even numbers of sugar units in the molecule can be clearly seen, but ion series with odd numbers of sugar units are also visible up to high masses. Because the spectrum of artichoke inulin is very similar to the spectrum of dahlia inulin, this spectrum is not shown.

To prove the applicability of DCI-MS to another group of carbohydrates we employed dextran T 1.5, a branched glucan with mainly 1,6-glycosidic linkages and with an average molecular weight of 1500 Da. Figure 4 shows the spectrum of dextran T 1.5 acquired under the same conditions as described for inulins; only the mass range differed. Dextran T 1.5 shows similar DCI-MS behaviour to that described for inulins: at the beginning, the desorption profile shows a maximum range of ion masses. The spectrum during this time interval is given in Fig. 4. Ion series are the ammonia adducts of oligomers where up to 18 sugar units were detected, ammonia adducts of anhydro-oligomers and

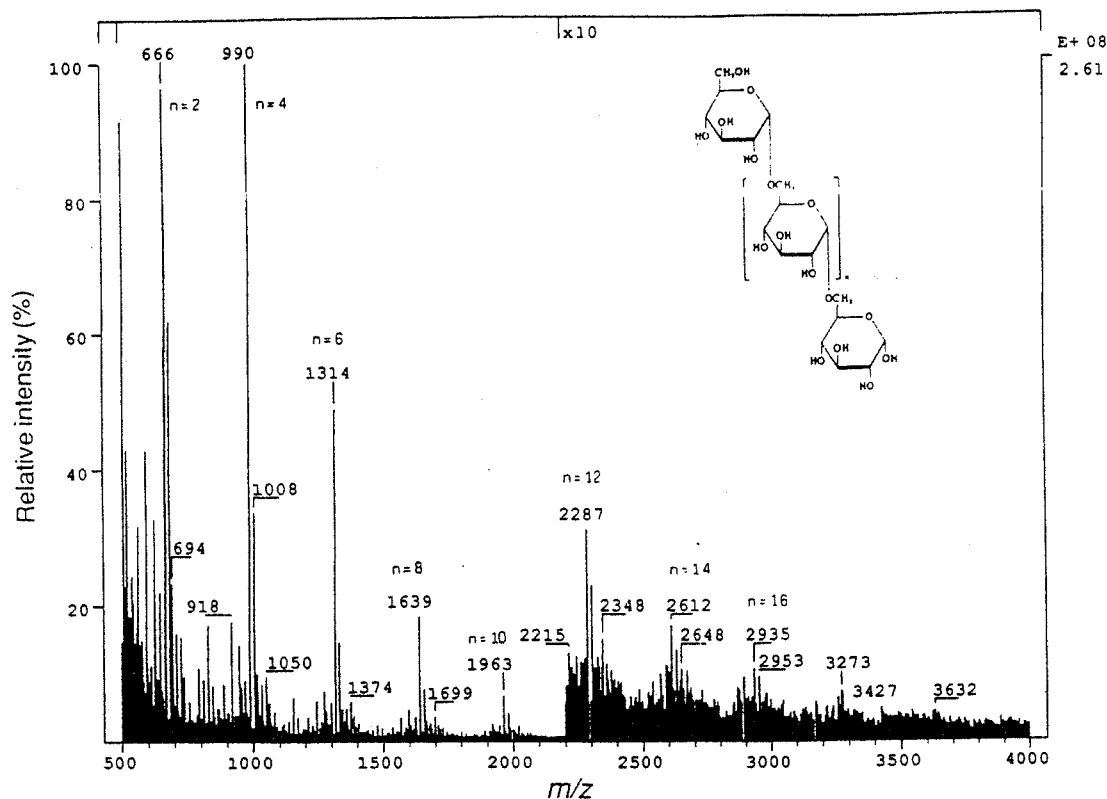


Figure 4. Positive ammonia DCI mass spectrum of dextran T 1.5, scanned from  $m/z$  500–4000 (MAT95).

(with low intensity) ammonia adducts of dianhydro-oligomers. Furthermore an ion series is observed up to high mass range which is probably formed by the reverse aldolization cleavage of a sugar unit as proposed<sup>6,10</sup> for other glucans. The ion series differs from that for ammonia adducts of anhydro-oligomers by 60 u, i.e. peaks are observed at  $m/z$  1050, 1374, 1699. Masses corresponding to ion series with even numbers of sugar units are obviously preferred. Masses of ion series with odd numbers are only to be seen in the low-mass range with low intensity.

## CONCLUSION

Under controlled experimental conditions and especially using low ion-source temperatures, thermal decomposition and fragmentation of molecular ions can be avoided for all short-chain polysaccharides studied. Investigations carried out so far have shown that the accessible mass range for carbohydrates lies approximately in the 7000 u range. DCI-MS seems to be a promising technique for the determination of carbohydrate polymers.

## Acknowledgement

The authors would like to thank H. Münster, Finnigan MAT, Bremen, for DCI-MS measurements using MAT95.

## REFERENCES

1. A. Dell, N. H. Carman, P. R. Tiller, J. E. Thomas-Oates, *Biomed. Environ. Mass Spectrom.* **16**, 19 (1988).
2. J. O. Metzger, C. Bicke, R. Woisch, F. Hillmann, W. Tuszynski, *12th International Mass Spectrometry Conference*, Amsterdam, Abstract, S. 393 (1991).
3. B. Stahl, M. Steup, M. Karas, F. Hillenkamp, *Anal. Chem.* **62**, 1219 (1990).
4. B. Stahl, U. Bahr, M. Karas, M. Steup, F. Hillenkamp, *12th International Mass Spectrometry Conference*, Amsterdam, Abstract, S. 197 (1991).
5. A. K. Ganguly, N. F. Cappuccino, H. Fujiwara, A. K. Bose, *J. Chem. Soc. Chem. Commun.* 148 (1979).
6. A. C. Tas, A. Kerkenaar, G. F. LaVos, J. Van Der Greef, *J. Anal. Appl. Pyrolysis* **15**, 55 (1989).
7. J. A. Lomax, J. M. Commandeur, P. W. Arisz, J. J. Boon, *J. Anal. Appl. Pyrolysis* **19**, 65 (1991).
8. P. W. Arisz, J. A. Lomax, J. J. Boon, *Anal. Chem.* **62**, 1219 (1990).
9. P. Köll, G. Borchers, J. O. Metzger, *J. Anal. Appl. Pyrolysis* **17**, 319 (1990).
10. A. Pouwels, G. B. Eijkel, P. W. Arisz, J. J. Boon, *J. Anal. Appl. Pyrolysis* **15**, 71 (1989).