

Summary of PhD thesis

Mono and dimer adduct ions for structural investigation of polymers and chiral molecules by mass spectrometry

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I Introduction and aims of work

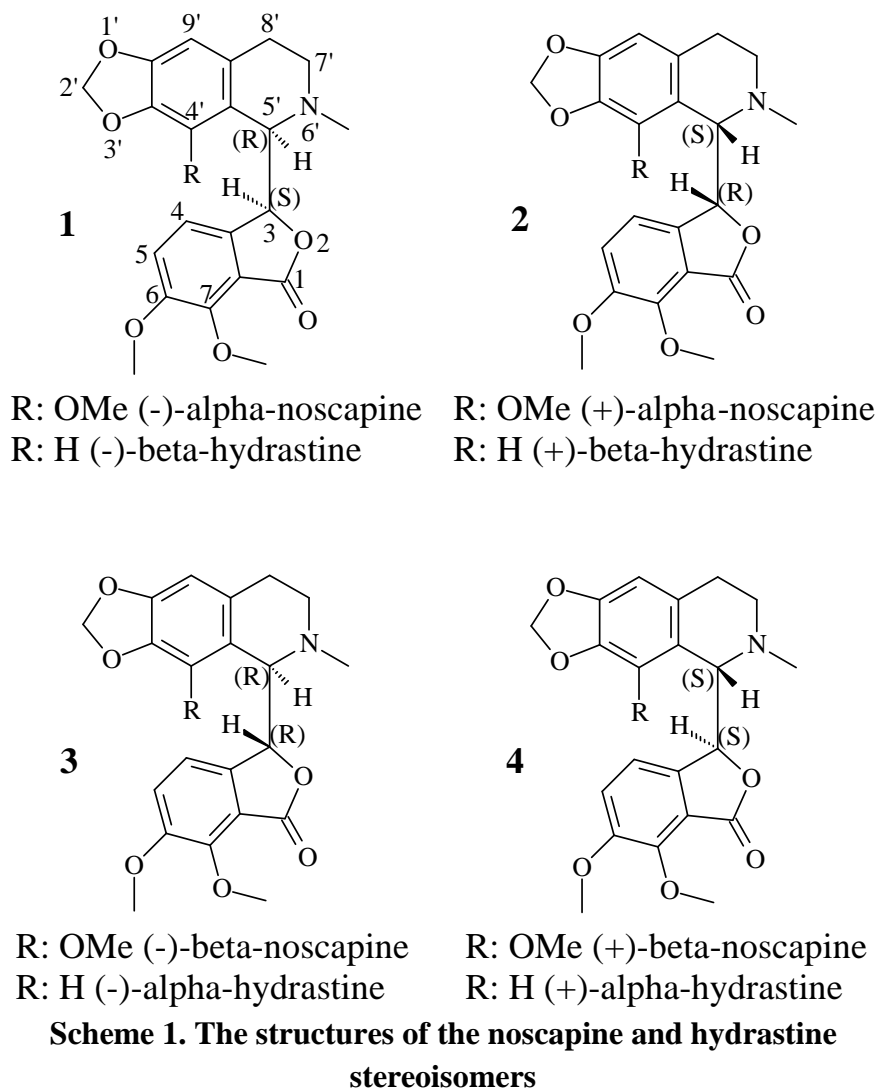
The differentiation of stereoisomers with mass spectrometry requires special methods. Fragmentation studies of the structural isomers often provide acceptable results, however the stereoisomers cannot be distinguished. Under electrospray (ESI) conditions non-covalent dimers are often generated. The masses of these dimer adduct ions are the same but the energy dependent dissociation or isotopic labelling allow us to study and distinguish these stereo isomers.

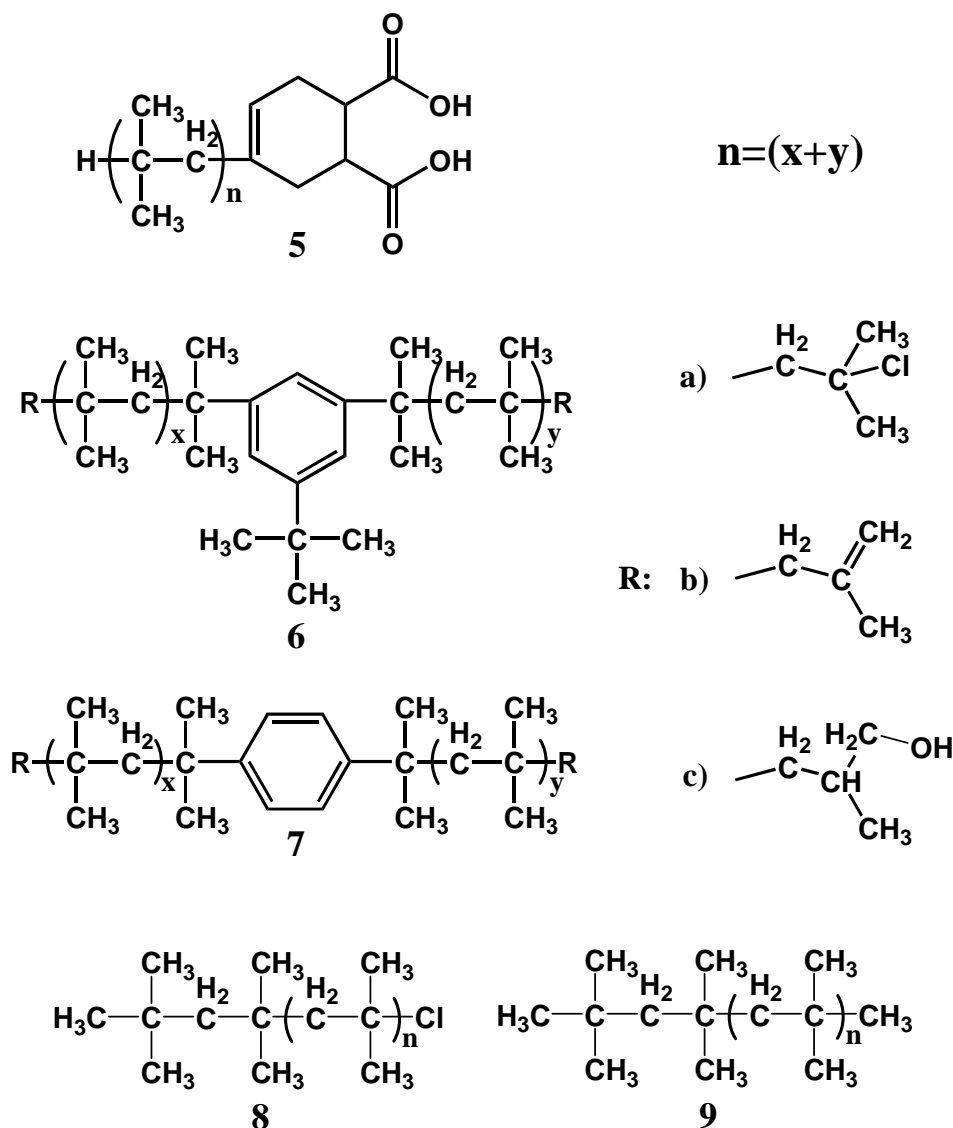
Soft ionization techniques allow large molecules even polymers to be ionized and open new ways of structural investigations of these molecules. Furthermore, mass spectrometry offers a unique opportunity to characterize polymers because each macromolecule appears separately in the mass spectrum. The ionization of a polymer sample depends on its polarity, molecular weight and molecular weight distribution. The two most frequently used ion sources for polymer characterization are the MALDI (matrix-assisted-laser-desorption/ionization) and the ESI.

One of the aims of my work was to study the differentiation of the noscapine and hydrastine stereoisomers utilizing tandem mass spectrometric methods. Noscapine and hydrastine have similar structures and they both contain two chirality centres, therefore four stereoisomers exist. The fragmentation pathways of the stereoisomers are the same, thus they cannot be differentiated in that way. However, the analysis of the dimer adduct ions may lead to the distinction of the stereoisomers. Therefore, the energy dependent collision induced dissociation (CID) of the homodimers ($[2M+Cat]^+$) and heterodimers ($[M+Tyr+Cat]^+$, $[M+Lys+Cat]^+$) of noscapine and hydrastine stereoisomers were studied, where Cat stands for Li^+ , Na^+ , K^+ and Cs^+ ions. The survival yield method and the characteristic collision energies are capable of quantifying the relative stability of the homo- and heterodimers.

The generation of the adduct ions of nonpolar polymers is a hard task due to the lack of any effective site for the attachment of ions. Special ion sources, such as APPI (atmospheric pressure photoionization) are capable of ionizing nonpolar polymers, however the process requires hazardous chemicals and anhydrous conditions.

Another goal of my work was to investigate the applicability of the ESI and DART (direct analysis in real time) ion sources for the characterization of nonpolar polymers. The adduct ions generated by the ESI and DART ion sources can offer stability and structural information. The structures of the investigated compounds are presented on **Scheme 1** and **2**.





Scheme 2. The structures of the polyisobutylene derivatives

II Methods

A MicroTOF-Q type Qq-TOF MS instrument (Bruker Daltonik, Bremen, Germany) was used for the MS and MS/MS measurements. For the MS/MS experiments, nitrogen was used as the collision gas. The pressure in the collision cell was determined to be 1.2×10^{-2} mbar. The precursor ions for MS/MS were selected with an isolation width of 4 m/z units. The mass spectra were recorded by means of a digitizer at a sampling rate of 2 GHz. The DART, APPI and ESI ion sources were utilized by the MicroTOF-Q instrument.

4 kV spray voltage was utilized in the ESI source and N_2 was applied as drying gas. The drying temperature was 200 °C and the flow rate was 4.0 L/min. The mass spectra were calibrated externally using the exact masses of clusters $[(NaTFA)_n + TFA]^+$ generated from the electrosprayed solution of sodium trifluoroacetate (NaTFA).

The DART SVP ion source was purchased from IonSense (IonSense, Inc., Saugus, MA, USA). The polymer samples were manually introduced into the DART gas stream and were inserted into the middle of the gap. The DART system was operated in both negative and positive modes with helium 5.0 (purity >99.999%). The temperature of the helium reagent gas was varied from 200 °C to 450 °C.

APPI quadrupole time-of-flight mass spectrometric (APPI-Qq-TOF MS) measurements were performed in negative ion mode with a MicroTOF-Q type Qq-TOF MS instrument (Bruker Daltonik GmbH, Bremen, Germany) equipped with an APPI source (PhotoMate Kr discharge lamp, VUV photons of 10.0 and 10.6 eV in intensity ratio of 4:1, respectively) from Syagen Ltd. (Syagen Technology, Inc., Tustin, CA). The PIB solutions were introduced directly into the APPI source with a syringe pump (Cole-Parmer Ins. Co., Vernon Hills, IL) at a flow rate of 25 L/min together with a carrier flow of carbon tetrachloride at a flow rate of 200 μ L/min by means of a T-piece. The APPI source heater was kept at 450 °C.

III New scientific results

III.1 Differentiation of the noscapine and hydrastine stereoisomers applying tandem mass spectrometry

III.1.1 I determined the characteristic collision energy (CE_{50}) of the homodimers based on their energy dependent collision induced dissociation and I proved that the diastereomers can be distinguished.

I studied the distinguishability of the noscapine and hydrastine isomers by tandem mass spectrometry. I used four cations, namely Li^+ , Na^+ , K^+ and Cs^+ . I established that the only fragmentation process is the dissociation of the dimers to ionized and neutral noscapine and hydrastine. In the case of the lithiated dimer adduct ions, additional backbone fragmentation was observed at higher collision energies.

I determined CE_{50} values for all types of homodimers and I concluded that the CE_{50} values of the diastereomers were different, therefore they could be differentiated. On the other hand, between the CE_{50} values of the enantiomer pairs no difference could be found. Based on the SY curves I found that the sodiated adduct ions were more suitable for the differentiation of the stereoisomers than the lithiated ones. The homodimers of hydrastine provided similar results so the diastereomer pairs of hydrastine can also be differentiated. I determined the order of the stability of the homodimers which was the following: $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Cs}^+$. Furthermore, I determined the activation energies of the fragmentation which were in good agreement with the results of quantum chemical calculations.

III.1.2 I found that based on the CE_{50} values of the heterodimers the enantiomer pairs can be distinguished and the difference between the diastereomers increased.

I pointed out that the application of the dimer adduct ions with a chiral selector had benefits for the differentiations. Additionally, the heterodimers were suitable for distinguishing even the enantiomer pairs. I utilized L-, D-tyrosine and L-, D-lysine as chiral auxiliary reagents along

with Na^+ and Li^+ ions. The survival yields of the noscapine-L-tyrosine heterodimers with Na^+ and Li^+ ions can be found in **Fig 1**.

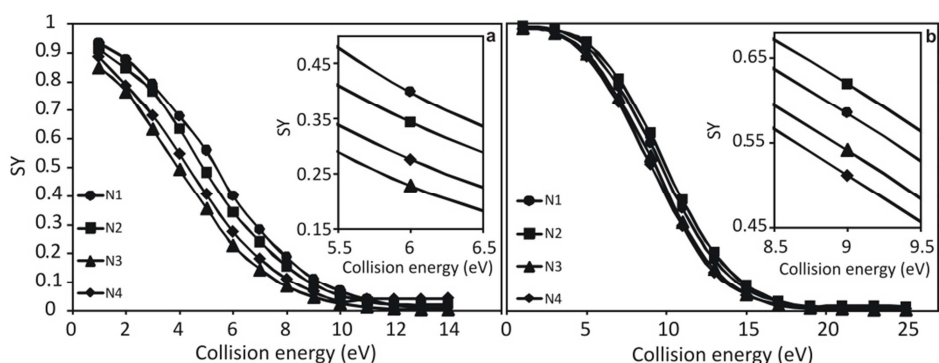


Figure 1. The survival yield (SY) versus collision energy curves of the sodiated and lithiated noscapine-L-tyrosine heterodimers. The figure insets show the zoomed survival yield curves in the range of 5.5 to 6.5 eV and 8.5 to 9.5 eV.

III.1.3 I proved that in the case of biner samples the stereoisomeric purity can be determined using the corresponding CE_{50} values

I found linear relationship between the stereoisomeric purity and the CE_{50} values of two component samples, thus it can be utilized for the determination of stereoisomeric purity. In order to test the validity of the method, samples with known stereoisomeric composition were analyzed by calibration. I found good agreement between the measured and calculated values.

III.2 Application of the DART ion source for the ionization of nonpolar polyisobutylenes

III.2.1 I proved that the intact polymers can be characterized through the formation of $[M+Cl]^-$ and $[M-H]^-$ ions in negative ion mode

I showed that the DART ion source was capable of the fast characterization of nonpolar polymers. The chlorine and olefin telechelic polymer adduct ions were generated by Cl^- ions, while in the case of the polyisobutylene with succinic acid end group, only the deprotonated ions appeared. **Figure 2.** shows the mass spectrum of the chlorine telechelic polyisobutylene.

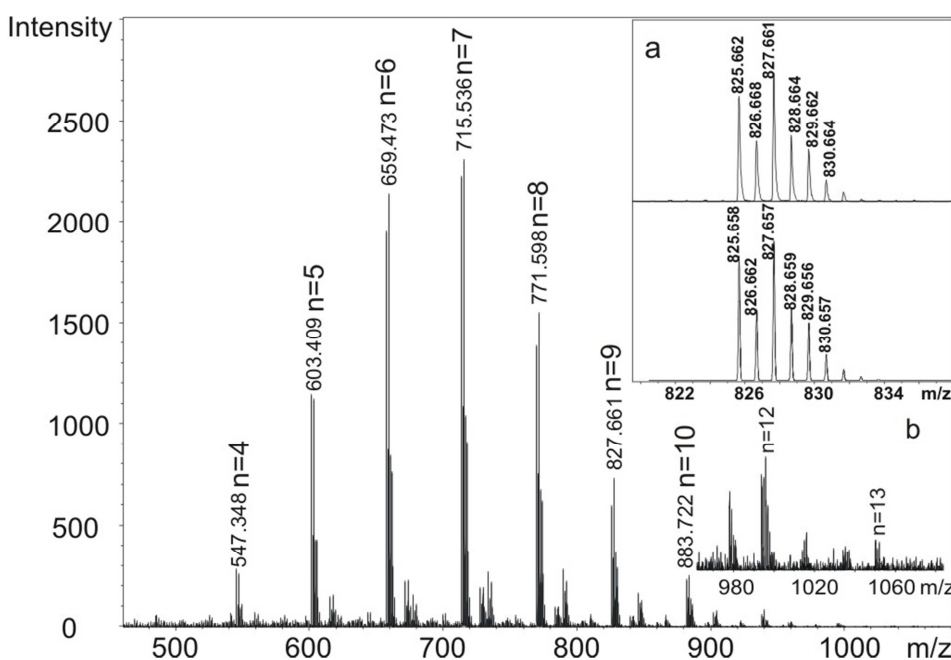


Figure 2. The DART-(-)MS mass spectrum of the chlorine telechelic polyisobutylene

Based on the peaks in the mass spectrum and the accurate masses the polymer could be identified and members of the series with mass over 1000 Da also occurred. To the best of my knowledge these were the highest masses which were detected in case of nonpolar polymers applying DART-MS.

III.2.2 I demonstrated that the generated adduct ions were not suitable for tandem mass spectrometric investigations

I achieved the tandem mass spectrometric measurement of the $[M+Cl]^-$ and $[M-H]^-$ ions. I determined that the only fragmentation step was the dissociation of the polymer and the chloride ion. However, the polyisobutylene with succinic acid end group yielded a fragment ion with the loss of a CO_2 molecule, but further fragmentation processes did not occur.

III.2.3 I showed that the polyisobutylene derivatives can generate adduct ions with ammonium ions in positive ion mode

I detected a series of polyisobutylene-ammonium ion adducts in the case of the olefin and chlorine telechelic polymers. In the positive ion mode further series and fragment ions appeared in the MS spectra indicating the possibility of tandem mass spectrometric investigations. **Figure 3.** shows the DART-(+)MS spectrum of the chlorine telechelic polyisobutylene.

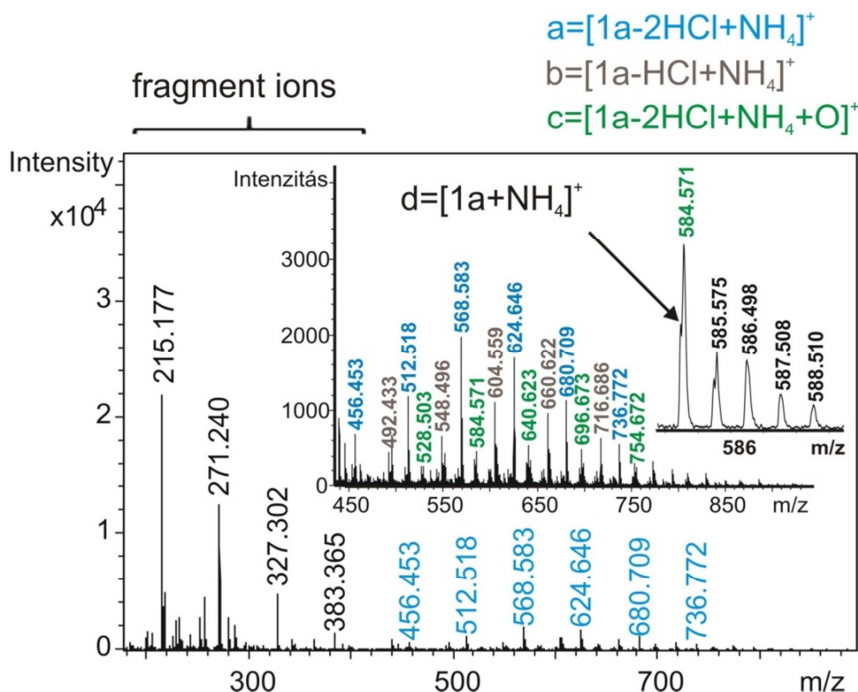


Figure 3. The DART-(+)MS spectrum of the chlorine telechelic polyisobutylene

I identified four different series in the case of the chlorine telechelic polyisobutylene. These series were the $[6a+NH_4]^+$, $[6a-HCl+NH_4]^+$, $[6a-$

$2\text{HCl}+\text{NH}_4]^+$ and $[\mathbf{6a}-2\text{HCl}+\text{O}+\text{NH}_4]^+$. An additional series was detected in the mass spectrum of olefin telechelic polyisobutylene which corresponded to the $[\mathbf{6a}-2\text{HCl}+2\text{O}+\text{NH}_4]^+$ series.

I determined that the main fragmentation step of the adduct ions was the loss of the polyisobutylene chains generating resonance stabilized benzyl-type cations. Additionally, I showed that the oxidation of the polymers took place at the olefin end groups.

III.3 Ionization of polyisobutylene derivatives by electrospray ion source

III.3.1 I demonstrated that the ESI ion source is capable of ionizing nonpolar polyisobutylenes with chloride and nitrate ions

I studied the ionization of polyisobutylenes with different end-groups including chlorine, olefin, hydroxyl telechelic and methyl, initiated from aliphatic and aromatic initiators by electrospray ionization mass spectrometry (ESI-MS). I used NH_4Cl and NH_4NO_3 for the generation of $[\text{M}+\text{Cl}]^-$ and $[\text{M}+\text{NO}_3]^-$ adduct ions. The ESI ion source needed polar solvent for the sufficient ionization while the polyisobutylenes could be solved in nonpolar solvents. Therefore, I used solvent mixtures (acetone:dichloromethane), but I also investigated other types of solvents. **Figure 4.** shows the mass spectrum of the hydroxyl telechelic polyisobutylene measured in methanol.

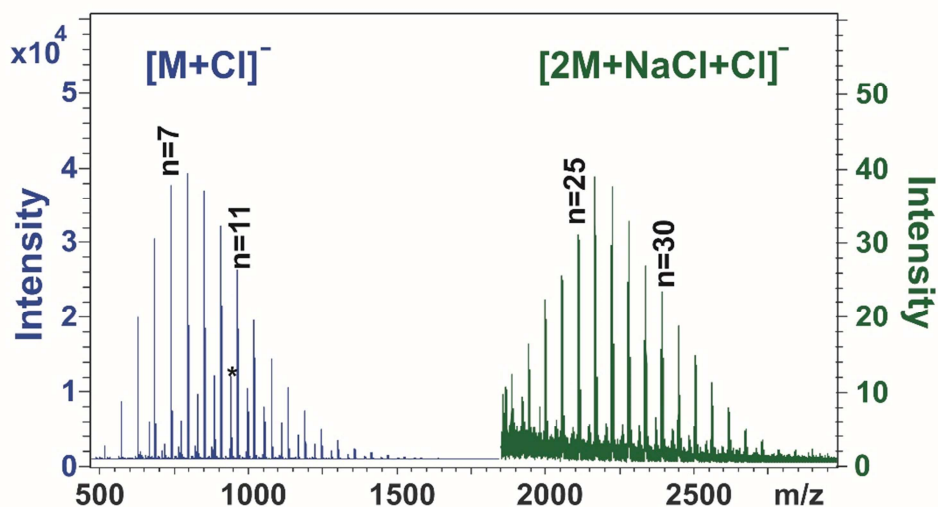


Figure 4. ESI-MS spectrum of hydroxyl telechelic polyisobutylene ionized by Cl^- ions. The numbers at the top of the peaks stand for the total number of isobutylene units (n). Solvent: ethanol

In **Figure 4**, two series appeared at low m/z values. These series belong to the chlorinated and nitrated polymers. Furthermore, I identified other series between m/z 1800-2700. That series belonged to the $[2M+NaCl+Cl]^-$ adduct ions. The formation of dimer ions is typical for the ESI ion source, but the dimerization of such a nonpolar and high molecular weight polymer was not published before.

III.3.2 I determined that for the formation of the adduct ions the presence of a polar solvent is required

I investigated the effect of the composition of the solvent mixtures on the ionization in the range of 0-90% acetone content. I showed that at least 20% acetone was necessary for the detection of the polymer series. Increasing the amount of acetone the intensities were also increasing. In the case of the chlorine telechelic polymer a maximum was reached, while the intensity of the hydroxyl telechelic polymer was the highest at 90% acetone content.

III.3.3 I determined the relative stability of the chlorinated and nitrated adduct ions.

I studied the collision induced dissociation of the adduct ions in MS and MS/MS modes. I experienced that with the increase of the collision energy the measured number average molecular weight was shifted to higher m/z values. I determined also that the $[M+Cl]^-$ adduct ions had higher dissociation energies than the corresponding $[M+NO_3]^-$ ions. These results were supported by quantum chemical calculations.

IV Possible application of the results

The method developed for the differentiation of the noscapine and hydrastine stereoisomers can be applied to distinguish other classes of chiral components. The method can also be suitable for fast identification of stereoisomers and stereoisomeric compositions.

I proved that nonpolar polymers can be ionized by DART and ESI ion sources generating different adduct ions. These investigations provide new ways in the characterization of nonpolar polymers by mass spectrometry. Besides the characterization of the polymers the additional appeared series can be applied for unique structural investigations in the case of other nonpolar polymers. From the mass spectrometric point of view, these results are important, because I proved that these ion sources are capable of ionizing nonpolar polymers extending the number of investigable components.

Acknowledgement

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Doctoral School: Doctoral School of Chemistry

List of publications related to the dissertation

Foreign language scientific article(s) in international journal(s) (3)

1. Nagy, L., **Nagy, T.**, Deák, G., Kuki, Á., Purgel, M., Narmandakh, M., Iván, B., Zsuga, M., Kéki, S.: Can Nonpolar Polyisobutylenes be Measured by Electrospray Ionization Mass Spectrometry? Anion-Attachment Proved to be an Appropriate Method. *J. Am. Soc. Mass Spectrom.* 27 (3), 432-442, 2016. ISSN: 1044-0305.
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IF:2.379 (2014)



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List of other publications

Foreign language scientific article(s) in international journal(s) (10)

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9. Banerjee, S., Tripathy, R., Cozzens, D., **Nagy, T.**, Kéki, S., Zsuga, M., Faust, R.: Photoinduced Smart, Self-Healing Polymer Sealant for Photovoltaics.
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IF: 2.642

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