

**Short thesis for the degree of Doctor of Philosophy  
(PhD)**

**Application of Mass-Remainder Analysis  
(MARA) in the characterization of polymers  
and epoxidized oils**

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Doctoral School of Chemistry  
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## 1. Introduction and Objectives

To handle the large number of peaks generated from mass spectrometric measurements requires special data mining methods. We developed new methods, namely Multi-step Mass-remainder Analysis (M-MARA) to analyse the composition of vegetable oils, characterize polymer blends, and tandem mass-remainder analysis (TAMARA) method to investigate and differentiate various types of polyether polyols. Our methods eliminate the periodicities present in a complex chemical system, and therefore, provides highly simplified plots. With  $MR_3$  versus  $m/z$  and/or  $MR_2$  versus  $MR_1$  plots, where  $MR_i$  is the Mass-remainder of the  $i^{\text{th}}$  step, we can easily determine the sample is whether a single copolymer or polymer blend. Soft ionization methods such as MALDI-TOF and ESI-QTOF allowed our samples to be ionized and provided high accuracy masses. Therefore, because of the overlapping peaks as well as isobar and isomer compounds, we applied tandem mass spectrometric measurements using ESI-QqTOF mass spectrometry to obtain structural information on the polyether polyols

with different initiators. MS/MS was also used to study fragmentation pathways of the epoxidized triglyceride adducts, which enables to estimate the epoxidized fatty acid compositions of these types of oils.

Plotting third-step MARA depicts the straight lines along the x-axis, where they describe the DBE values. The various polyether polyols can easily be differentiated based on the number of lines, where characteristic DBE values are 0-1 for the linear diol polyethers, 0-2 for the three-arm, and 0-4 for the six-arm polyether polyols.

## 2. Experimental Methods

The MALDI-TOF MS measurements were carried out with a Bruker Autoflex Speed mass spectrometer equipped with a time-of-flight mass analyser. In all cases, 19 kV (ion source voltage 1) and 16.65 kV (ion source voltage 2) were used. For reflectron mode, 21 kV and 9.55 kV were applied as reflector voltage 1 and reflector voltage 2, respectively.

A Maxis II type Qq-TOF MS instrument equipped with an Apollo II electrospray ion source was used. The spray voltage was 4.5 kV. The resolution of the instrument was 40,000 at  $m/z$  400 (FWHM), and the mass accuracy was  $<2$  ppm (external calibration).  $N_2$  was utilized as the drying gas (200 °C, 4.0 L/min), nebulizer gas (0.5 bar) and collision gas. The mass spectra were recorded by means of a digitizer at a sampling rate of 2 GHz. The spectra were calibrated externally by ESI tune mix, from Bruker. The spectra were evaluated with the Compass Data Analysis 4.4 software. The sample solutions were introduced directly into the ESI source with a syringe pump) at a flow rate of 3  $\mu$ L/min.

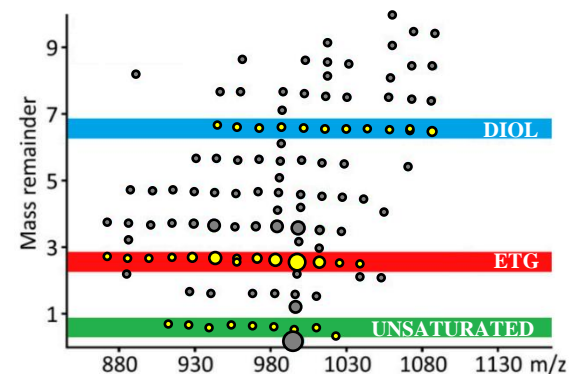
### 3. New Scientific Results

#### 3.1 We first applied the mass-remainder analysis (MARA) for the analysis of epoxidized vegetable oils

**3.1.1.** We determined the number of carbon atoms and epoxide groups of the epoxidized soybean and linseed oils.

**3.1.2.** We applied the mass-remainder analysis (MARA) method to explore the peaks with lower intensity such as double bonds (or epoxide groups) by showing them graphically (**Fig. 1**).

**3.1.3.** We determined the polymer quantities of the epoxidized soybean and linseed oils such as number average molecular weight  $M_n$ , the average number ( $n_n^{NEG}$ ) and average number of epoxide groups weighted by the number of epoxide groups ( $n_w^{NEG}$ ), the polydispersity of the number of epoxide groups ( $n_n^{NEG}/n_w^{NEG}$ ), average number of carbon atoms ( $n_n^C$ ), and the degree of epoxidation (DOE).

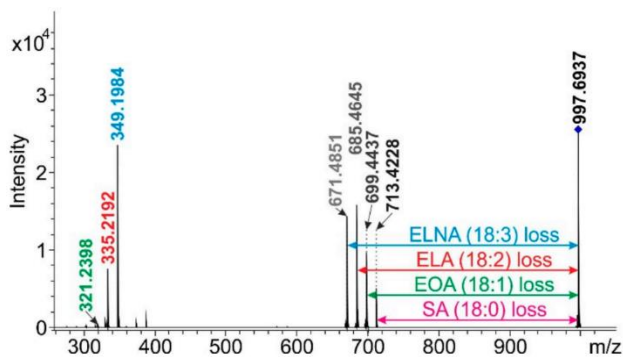


**Figure 1** MR versus  $m/z$  plot of the MALDI-TOF spectrum of the epoxidized soybean oil.

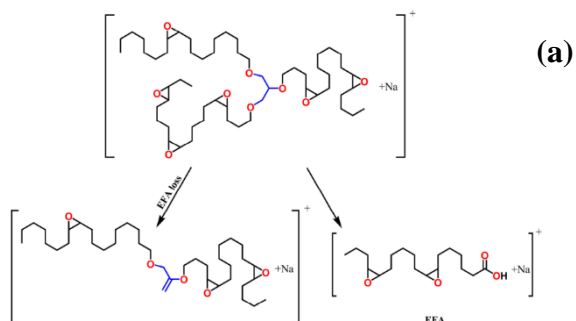
**3.1.4.** Epoxidized soybean and linseed oils were measured with different ionization agents by MS/MS. We found that fragmentation of the triglycerides with sodium cation is the simplest (**Fig. 2**).

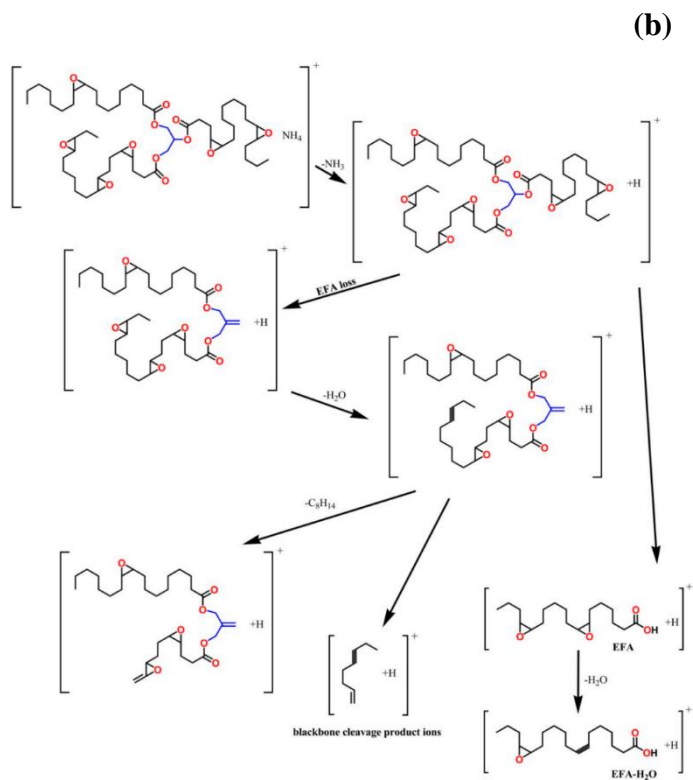
**3.1.5.** We determined the possible structures of the product ions formed from the epoxidized soybean oil using tandem mass spectrometry. We identified epoxidized diglycerides and single epoxidized fatty acids with different epoxide groups.

**3.1.6.** Finally, we proposed the fragmentation pathways for the sodiated and ammoniated ETG adducts (**Fig. 3**).



**Figure 2** MS/MS spectrum of the sodiated ETG adduct at m/z 997 of the soybean oil (70 eV).





**Figure 3** Proposed fragmentation pathways of (a) sodiated and (b) ammoniated EFA

**3.2. We developed a new data-mining method called multi-step mass analysis (M-MARA) for the characterization of polymers/copolymers and their blends.**

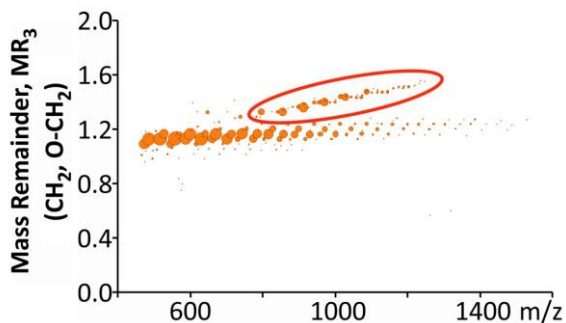
**3.2.1.** We developed a multi-step mass analysis (M-MARA) method to differentiate a polymer sample whether it is a blend or a single copolymer.

**3.2.2.** Applying Eq (1) we plotted  $MR_3$  *versus*  $m/z$  graph, where two copolymers with the different repeat units in the blend system can easily be observed (**Fig. 4**).

$$MR_3 = [(m/z \text{ MOD } R_1) \text{ MOD } R_2] \text{ MOD } R_3 \quad \text{Eq(1)}$$

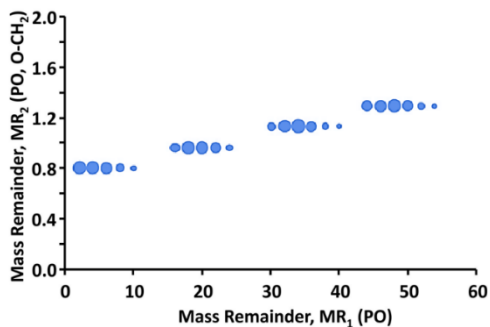
where  $R_1$ ,  $R_2$  and  $R_3$  are 58.04, 14.01 and 1.97, respectively.

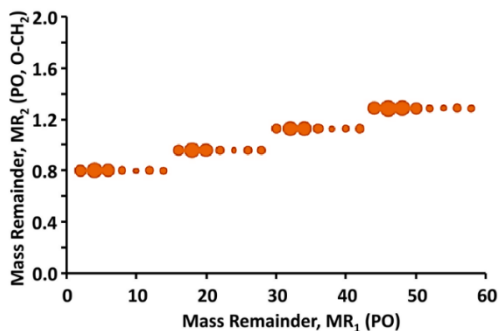
**3.2.3.** We found that for the differentiation of two copolymers with the same repeat units in blends,  $MR_2$  *versus*  $MR_1$  plot is suitable, when the intensity distribution of the peaks is considered.



**Figure 4** MR<sub>3</sub> versus m/z plot of the PCL/PLA and PE3100 blend system (PE3100 copolymer is circled)

**3.2.4.** We were also able to compare the copolymer and blends where both copolymers have almost the same molecular weight, using MR<sub>2</sub> *versus* MR<sub>1</sub> plot. Therefore, only a single copolymer shows unimodal distribution, while polymer blend reveals bimodal intensity distribution (**Fig. 5**).





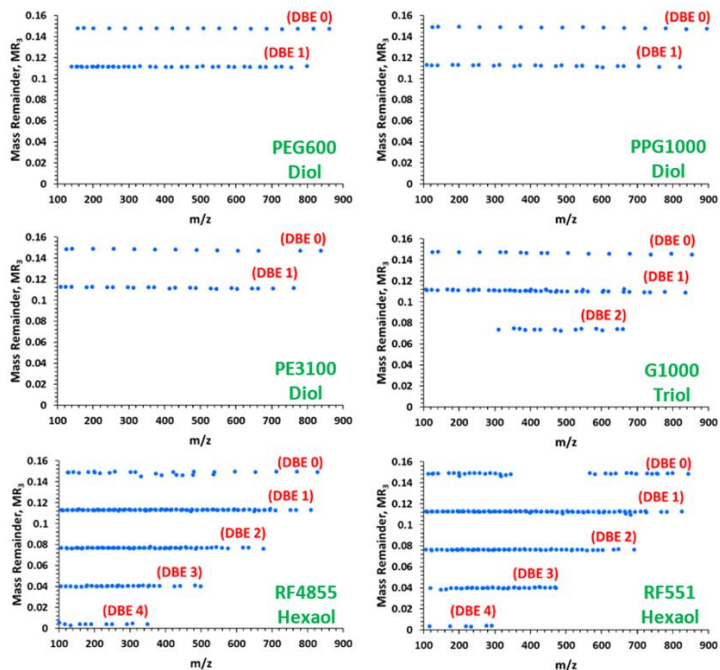
**Figure 5** MR<sub>2</sub> versus MR<sub>1</sub> plot (blue) PE8100 copolymer (orange) the blend of PE3100 and RPE1740 copolymers

**3.3. We developed a third-step Mass-Remainder tandem mass spectrometry method (TAMARA) for the analysis of polyether polyols.**

**3.3.1.** We developed a third-step tandem mass analysis (TAMARA) method using Eq (1) where R<sub>n</sub> numbers are 58.04, 1.97 and 0.16, to depict ultra-simplified plot to identify various types of polyether polyols.

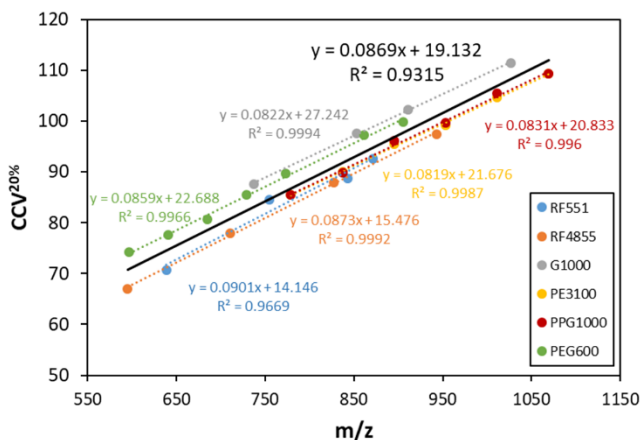
**3.3.2. Figure 6** suggests that maximum DBE value of 1, 2 and 4 indicates diol, glycerol- and sorbitol-based polyether polyols, respectively.

**3.3.3.** Based on the fragmentation of the diol type polyether polyols by MS/MS, we suggested product ion series with different chain terminations: hydroxyl, vinyl and formyl.



**Figure 6**  $MR_3$  versus  $m/z$  plots of the MS/MS spectra of different polyether polyols.

**3.3.4.** We found that the characteristic collision voltage (where survival yield (SY) is 0.2), which can be used to tune the spectrometer to get structural information, especially to compare the polyether polyols based on the DBE values in M-MARA plots (**Fig. 7**).



**Figure 7** Characteristic collision voltage (CCV<sup>20%</sup>) versus m/z plots of the polyether polyols.

#### **4. Possible Applications of this work**

Since epoxidized vegetable oils are environmental-friendly and raw materials for biopolymers, characterization of other compounds of natural origin using our mass-remainder analysis method are easy and effective. In addition, fragmentation patterns, structures and compositions of polymer-like samples can be investigated.

Multi-step Mass-remainder analysis can especially be effective for quality control in polymer/copolymer production to save time and to increase accuracy and precision of the processes.

Furthermore, various industrially important copolymers as well as another type of distinctions such as the type and the number of heteroatoms, rings or aromatic moieties can be analysed and differentiated using tandem mass-remainder analysis method.

## 5. List of Publications



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Registry number: DEENK/68/2022.PL  
Subject: PhD Publication List

Candidate: Mahir Hashimov  
Doctoral School: Doctoral School of Chemistry  
MTMT ID: 10067176

### List of publications related to the dissertation

#### Foreign language scientific articles in international journals (3)

1. Nagy, T., Kuki, Á., Hashimov, M., Zsuga, M., Kéki, S.: Multistep Mass-Remainder Analysis and its Application in Copolymer Blends.  
*Macromolecules*. 53 (4), 1199-1204, 2020. ISSN: 0024-9297.  
DOI: <http://dx.doi.org/10.1021/acs.macromol.9b02409>  
IF: 5.985
2. Hashimov, M., Kuki, Á., Nagy, T., Zsuga, M., Kéki, S.: Tandem Mass-Remainder Analysis of Industrially Important Polyether Polyols.  
*Polymers*. 12 (12), 1-10, 2020. EISSN: 2073-4360.  
DOI: <http://dx.doi.org/10.3390/polym12122768>  
IF: 4.329
3. Kuki, Á., Nagy, T., Hashimov, M., File, S., Nagy, M., Zsuga, M., Kéki, S.: Mass Spectrometric Characterization of Epoxidized Vegetable Oils.  
*Polymers*. 11 (3), 1-9, 2019. EISSN: 2073-4360.  
DOI: <http://dx.doi.org/10.3390/polym11030394>  
IF: 3.426





**List of other publications**

Foreign language scientific articles in international journals (1)

4. Róth, G., Nagy, T., Kuki, Á., Hashimov, M., Vonza, Z., Timári, I., Zsuga, M., Kéki, S.:

Polydispersity Ratio and Its Application for the Characterization of Poloxamers.

*Macromolecules*, 54 (21), 9984-9991, 2021. ISSN: 0024-9297.

DOI: <http://dx.doi.org/10.1021/acs.macromol.1c01552>

IF: 5.985 (2020)

**Total IF of journals (all publications): 19,725**

**Total IF of journals (publications related to the dissertation): 13,74**

The Candidate's publication data submitted to the IDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

02 February, 2022



## 6. Conferences

### *Presentations related to the dissertation:*

1. **Hashimov, M;** Nagy, T. ; Kuki, Á.; Zsuga, M.; Kéki, S. Tandem Mass Spectrometric Characterization of Sorbitol-Initiated Polyethers. XXV. International Conference on Chemistry 2019, Cluj-Napoca, Romania.
2. File, S.; Kuki, Á.; Nagy, T.; **Hashimov, M.;** Nagy, M.; Zsuga, M.; Kéki, S. Characterization of epoxidized soybean and linseed oil by mass spectrometry. XXV. International Conference on Chemistry 2019, Cluj-Napoca, Romania.
3. Kuki, Á.; Nagy, T.; **Hashimov, M.;** File, S.; Nagy, M.; Zsuga, M.; Kéki, S. Determination of the fatty acid compositions of epoxidized vegetable oils by tandem mass spectrometry. XXV. International Conference on Chemistry 2019, Cluj-Napoca, Romania.
4. Nagy, T. ; Kuki, Á.; **Hashimov, M;** Zsuga, M.; Kéki, S. Mass-remainder Analysis (MARA): a multifunctional tool for characterization of copolymers and copolymer blends. ESOPS 2020, Linz, Austria.
5. Nagy, T.; Kuki, Á.; **Hashimov, M.;** Zsuga, M.; Kéki, S. Characterization of copolymer blends by Mass-Remainder Analysis. XXV. International Conference on Chemistry 2020, Cluj-Napoca, Romania.
6. **Hashimov, M.;** Kuki, Á.; Nagy, T.; Zsuga, M.; Kéki, S. Application of Multistep Mass-Remainder Analysis for the evaluation copolymer tandem mass spectra.

XXV. International Conference on Chemistry 2020, Cluj-Napoca, Romania.

7. **Hashimov, M.**; Kuki, Á.; Nagy, T.; Zsuga, M.; Kéki, S. Importance of Mass-Remainder Analysis (MARA) in Polymer Characterization. Young Researchers' International Conference on Chemistry and Chemical Engineering (YRICCCE III) 2021, Cluj-Napoca, Romania.

*Other presentations:*

8. **Hashimov, M.**; Nagy, T.; Kuki, Á.; Zsuga, M.; Kéki, S. Rapid determination of PEG 400 excreted in the urine. XXVII. International Conference on Chemistry 2021, Cluj-Napoca, Romania.
9. Nagy, T.; Roth, G.; Kuki, Á.; **Hashimov, M.**; Timari, I.; Vonza, Zs.; Zsuga, M.; Kéki, S. Application of polydispersity ratio on the analysis of copolymers. XXVII. International Conference on Chemistry 2021, Cluj-Napoca, Romania.
10. **Hashimov, M.**; Nagy, T.; Kuki, Á.; Zsuga, M.; Kéki, S. Determination of polyethylene glycol 400 excreted in the urine by MALDI coupled to time-of-flight mass spectrometry. 10th Interdisciplinary Doctoral Conference (IDK2021) 2021, Pécs, Hungary

***Posters related to the dissertation:***

1. **Hashimov, M.**; Nagy, T. ; Kuki, Á.; Zsuga, M.; Kéki, S. Characterization of various polyether polyols by Mass Spectrometry. ESOPS 2020, Linz, Austria.
2. Kuki, Á.; Nagy, T.; **Hashimov, M.**; File, S.; Nagy, M.; Zsuga, M.; Kéki, S. Mass spectrometric characterization of epoxidized vegetable oils for biopolymers. ESOPS 2020, Linz, Austria.
3. **Hashimov, M.**; Kuki, Á.; Nagy, T.; Zsuga, M.; Kéki, S. Mass spectrometric characterization of epoxidized vegetable oils. 1st UFAZ-ASOIU-UNISTRA scientific conference 2021, Baku, Azerbaijan.

***Other posters:***

4. **Hashimov, M.**; Kuki, Á.; Nagy, T.; Zsuga, M.; Kéki, S. Rapid detection of fire-retardant compounds by direct analysis in real-time mass spectrometry, European Meeting on Fire Retardant Polymeric Materials (FRPM21) 2021, Budapest, Hungary

## 7. AWARDS

### 1. Young Researchers Fellowship 2019

**Research topic:** Study of reactivity and fragmentation of epoxidized triglycerides by mass spectrometry

**Issue date:** April 2019

**Issuer:** European Union and the European Social Fund, Debrecen Venture Catapult Program - Széchenyi 2020

### 2. Young Researchers Fellowship 2021

**Research topic:** Fractionation and characterization of epoxidized vegetable oils and a study of the evolution of physical properties of polymers formed by photopolymerizations of different epoxidized triglycerides

**Issue date:** January 2021

**Issuer:** European Union and the European Social Fund, Debrecen Venture Catapult Program - Széchenyi 2021