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Final Report

FK18 128440

In the European Union, Commission regulation 10/2011 lays down specific measures for plastic food contact materials (FCMs). It aims at restraining the transfer of potentially harmful constituents of FCMs into food. Experimental conditions for migration studies are described in detail. Four years ago, we started a work that was originally aimed at meeting certain challenges in the analytical methods used for the resulting samples and collecting information on the organic and inorganic compounds migrating from commercially available plastic FCMs. Due to the scarcity of information in the inorganic field we started the work with a wide range of polymers. Whereas, in the case of organic compounds we focused on polypropylene (PP) and polylactic acid (PLA) based plastics. (PP is a commonly used but rarely investigated polymer in FCM production, while PLA has narrower but raising market, overgrowing all other bioplastics.) In the course of our work, we met several unexpected but exciting questions both in the field of determining certain types of migrating compounds and - more surprisingly - in the field of designing migration experiments as well. Table 1. summarizes the topics we originally aimed at, along with the ones we decided to pursue, sometimes at the cost of dismissing some less compelling ones. Changes in personnel and difficulties due Covid measures also incited some adjustments in the focus of the work.

Table 1. Topics of Project 128440

Topic	Type	Comments
Untargeted GC-MS screening for the identification of migrating organic compounds	Initial	PP and PLA based, commercially available FCMs have been tested. [1,2] Results of the PP samples have been published in the Microchemical Journal. [3]
Soundness of GC-MS based identification	Newly adopted	We have demonstrated how reliability and productivity of identification based on GC-MS measurements can be improved. [3] Parameters influencing LRI have also been studied. [4]
Building strategy for using screening data for quantification	Initial	Results demonstrated problems with generally used approaches and verified suspected difficulties with others. [5] No satisfying solution has been found.
Screening of migrating metals and metalloids	Initial	Neither realistic, nor exaggerated circumstanced resulted in the migration of extreme and/or interesting metals or metalloids. [6] With the leave of Katalin Perényi the topic has been closed
Method development for the sample preparation of GC-MS screening	Initial	Dispersive liquid-liquid microextraction (DLLME) proved to be a promising and environmentally friendly technique for this task. [7,8]
Screening for migrants with LC-MS and setting up multicomponent methods for the analysis of potential migrants	Initial	The topic has been dismissed. Both the instrument and its experts had been allocated to study primer aromatic amines, and how their migration is to be tested.
Setting up a database of migrants	Initial	https://reka.wirec.eu/
Kinetics of migration from PP and PLA into fatty food simulants	Newly adopted	Correlation of migration and swelling has been studied in depth. [9,10,11,12]
Methods of studying migration of primer aromatic amines (PAAs)	Newly adopted	Methods have been developed for the determination of 24 PAAs. [13] Problems with their stability during migration experiments have also been demonstrated. [14]

1. Screening of migrating metals and metalloids

Our work with the migration of metals and metalloids started with observing blank values using thoroughly cleaned glass vials, brand-new polypropylene (PP) test tubes and previously acid-cleaned PP test tubes (brand-new and earlier used ones). Only the latter two provided satisfactorily low and reproducible value.

We targeted more than 20 elements, most of which were authorized in 10/2011/EC. Others, like Cr, St, Ni and Pb were chosen based on their toxicity and frequent occurrence in our preliminary experiments. Two different set of migration circumstances have been applied. The first was 6 days at 60 °C in 6V/V% acetic acid. This was followed by -the still overestimating- 10 days at 60°C in food simulant B (=3m/V% acetic acid) which is supposed to model one-year storage at room temperature. [6]

The highest migrated concentrations (0.1-1 mg/L) in 6V/V% acetic acid were observed for Al, K, iron, Ni, Zn, Sr and Ba. Most of the metals were released from coloured disposable polystyrene plates and foamed polystyrene plastic trays. Anyway, concentrations and even the total concentration of migrated metals did not exceed the limits set in 10/2011/EC.

With the circumstances described in the regulation 114 plastic FCMs (PP, PET, PLA, PS, PE and other) have been tested. Migration of common, harmless ions, like Na, K, Ca, Mg, Al, Fe, Mn was generally an order of magnitude higher from PLA probes than from PP or PET. Probably because the contact temperature was close to the crystallization temperature of the PLA, and some of the probes got damaged during the treatment. Migration of Al exceeded the special migration limit (SML) set in 10/2011/EC in four cases, migration of manganese once, all of them in PLA samples. Another noteworthy result was that in some cases the migrated concentration of K and Mg was alone higher than 60 mg/kg. As these elements are not toxic these results are not worrying at all. However, it suggests that the overall migration from certain PLA-s could exceed the overall migration limit.

This topic was not pursued further due to the limited interestingness of these results and the untimely leave of Katalin Perényi, our expert on the analysis of elements.

2. Screening of unknown migrants with GC-MS

GC-MS is an outstanding technique for the identification of volatile and semi-volatile organic compounds. Single quadrupole GC-MS instruments are widely available, they are cost effective and relatively easy to operate. Furthermore, this technique supports the use of mass spectral libraries for the easy interpretation of the results. However, the common procedure of accepting the results of these tentative identifications involves serious risks of miss-identification. We have demonstrated that using confirmatory methods (GC-TOF, analytical standards, retention indeces) and having expertise in the studied field can remarkably increase both reliability and productivity of the identification. [3] For this we used the results of migration experiments of 53 PP based, commercially available FCMs. As fatty food simulant isooctane was applied at 60 °C for 10 days. Beside several n-alkanes, 45 other compounds have been identified. Of these, only 15 are included in the Union List (10/2011/EC).

Using *van den Dool* and *Kratz* type linear retention index (LRI) for confirmation purposes have been found very cost effective. However, a mayor problem with LRI values is that for any given compound they may vary greatly depending on the source of the data. Fortunately, these data are published along with the experimental settings of their determination. Reproducing these experiments for every compound would be expensive, if not impossible. Instead, choosing the data obtained with the most similar settings is rational. To increase the reliability of this choice the effect of chromatographic parameters on LRI was investigated. Heating rate, film thickness and composition of the stationary phase all have notable influence. Whereas, carrier gas type (He or H_2), flow rate, internal diameter and

aging of the column had negligible effect only. The manuscript describing these results is under construction.

Right after the identification of unknowns the typical question is their concentration. For precise quantitation analytical standards are crucial, though often costly. As a result, laboratories often settle for using the calibration of other compounds. But this approach can lead to extreme over- or underestimations due to the differences in the relative sensitivities of the MS for various compounds. We aimed at examining if a certain strategy in choosing the compound used for calibration can improve the reliability of the concentration estimation. We found that appropriateness of a compound to become a calibration standard to another compound is not correlated with their similarity in volatility, molecule size or retention. By extending the set of our test compounds with compound groups, such as fatty acid methyl esters, polycyclic aromatic hydrocarbons, alkylbenzenes, phthalates, alkyl-alkanes, phenols and halogenated alkanes we could prove that closeness in structure is a good indicator of closeness in detector response.

Sample preparation is also a crucial point in GC-MS screening. In the case of water samples most commonly liquid-liquid extraction is used. This provides a GC compatible matrix for the sample and hopefully, gets most of the compounds of interest into the instrument. We have tested isooctane and hexane as alternatives to dichloromethane as extraction solvents. We have also started the development of a dispersive liquid liquid microextraction (DLLME) method, which could be an environmentally friendly but still effective alternative to traditional LLE in this field. [7,8]

3. LC-MS measurements

Though in the planning phase of this project, compiling a list of compounds for which targeted screening methods are to be developed seemed promising, we have turned this topic down in an early stage. During literature search it became obvious that aiming at comprehensiveness cannot give us even remotely satisfying results. Meanwhile, primer aromatic amines (PAAs) caught our attention. These allergenic, genotoxic and/or carcinogenic compounds are not supposed to migrate from FCMs. The limit for the sum of PAAs is set to 10 µg/kg in regulation 10/2011/EC. However, a lower individual limit, 2 µg/kg has been recently introduced for the carcinogenic PAAs in 2020/1245/EC. As the majority of the previously published methods are no longer compliant with the current regulation, a UHPLC-MS/MS method was developed to enable food packaging compliance testing for PAAs not only from 3% (w/v) acetic acid, but also from 10% (v/v) ethanol food simulant. Since the latest amendment of 10/2011/EC refers to the list of the 22 restricted PAAs of EU Regulation No. 1907/2006, these PAAs were selected as target compounds along with aniline and p-toluidine. The method was successfully validated and applied for the analysis of plastic kitchenware samples. Eight out of the ten were compliant with both the current overall migration limit and the individual limit of 2 µg/kg. However, 2 samples released ten times more 2,4-diaminotoluene than the new individual migration limit. Additionally, neither aniline nor 4,4'-diaminodiphenylmethane could be quantified in these samples as both of their amounts exceeded the upper limit of the working range. These results show that despite the strict legislation and continuous compliance testing, problematic products can still be found in the retail market within the EU. [13]

During this method development we experienced stability problems on several occasions. To provide data for better regulations and guidelines, the stability of 24 primary aromatic amines (PAAs) was investigated under several storage conditions in all aqueous food simulants of 10/2011/EC. Eleven carcinogenic PAAs appeared to be less stable under at least one of the investigated conditions. PAAs were the least stable in 3% (m/V) acetic acid. This is highly problematic because this food simulant represents the worst-case scenario regarding PAA migration testing. PAAs were more stable in

3 mmol/L HCl solution, as well as in ethanol containing food simulants. Decreased temperature also improved PAA stability, whereas shortened storage time improved PAA recovery. [14]

Or studies on the migration of PAAs continued with a comparison of aqueous food simulants to see if 3% (m/V) acetic acid is really the best choice of them for these compounds. The raw data of these experiments are still under evaluation.

4. Kinetics of migration

Migration is a complex process, the result of diffusion, dissolution, and equilibrium. Therefore, a deeper understanding of this phenomenon is based on kinetic tests. Even though the effect of swelling on migration is a generally expected, the common approach for the evaluation of the kinectic tests is to assume that Fick's second law of diffusion applies and calculate diffusion coefficients. Kinetic studies on the migration from PP and PLA usually aim at demonstrating the applicability of a tested compounds to become the active agent of active packaging. Thus, they focus on the release of antioxidants or antimicrobial compounds from thin plastic films. However, FCMs are often thicker than thin foils. So, we carried out our tests with 30 × 10 × 2 mm test specimens, which contained antioxidants (BHT, Ionox 220, Irgafos 168), UV absorber (Uvinul 3039) and plasticizers (TBAC and TOTM). As fatty food simulants, both ethanol 95 v/v% and isooctane were used. The relatively thick test specimens ensured that non-Fickian behaviour could be unambiguously observed on multiple occasions. Accordingly, instead of estimated diffusion coefficients, the focus in the evaluation of kinetic curves of both migration concentrations and swelling was placed on variography, to determine objectively the starting points of long-lasting plateaus as well as short halts in the increase. A strong correlation between migration and swelling was observed: the kinetic curves showed that migration always followed swelling. Also, more intensive swelling results increased migration of the additivies. The importance of these results is that they can be used to lower the costs of migration experiments, by reducing the number of experiments to be conducted [9]. E.g. PLA is swelled by ethanol 95 v/v% and not by the other alternative fatty food simulant, isooctane. Whereas PP is the other way around. So, when 10/2011/EC establishes that these solvents can replace the D2 food simulant together, and the higher result is to be accepted, than based on our results only the solvent which swells the plastic is must be tested. [9]

The migration kinetics was further investigated with four stabilizer-type additives migrating from polypropylene (PP) and polylactic acid (PLA) with different tributyl acetyl citrate plasticizer content. The results confirm that the presence of plasticizer in the plastics enhances swelling and thus the migration of additives, as well as elevated temperature. The plasticizer content is generally in strong correlation with migration rate of additives. Migration kinetic experiments conducted at different contact temperatures showed that the role of temperature apparently exceeds that of plasticization. Nevertheless, hierarchical cluster analysis revealed similarity in the migration mechanisms of stabilizers from plastics with low plasticizer content at high temperature, to that at low temperature from plastics with high plasticizer content. [10]

5. Database of migrating compounds

Data on identified migrants have been compiled and organized into a searchable database, which is available at reka.wirec.eu. For login it requires an email address to which it sends a link to enable free access. The database currently includes over 600 hundred compounds with over 2000 identification hits. (Each compound in each experimental setting where it was reported to be identified is considered a hit.)

Three types of searches allow access to the entries. One starts from the common identifier (Name, CAS No.) of the compounds. Another is focusing on the sources and extraction media which resulted

the identification. And last, but not least a basic mass spectrum search is also included to help the identification of the user's unknown compound.

6. Changes in personnel and effects of Covid

Zoltán Nyiri, Csaba Kirchkeszner and Katalin Perényi all left their university positions during the project. Due to their special expertise and experience in the field their early leave caused mayor difficulties in all cases. Changes in the FTE of the other researches and the joining of Aina Horváth (10/2021-09/2022) only partially could make up for these losses. Meanwhile, the studies involving LC-MS greatly benefited from the increased participation of Bálint Szabó.

Covid restrictions severely affected the second and third period of the project. Possibilities of sharing results at conferences became very limited. The closing of the university meant that students could not participate in lab work for months. Furthermore, changing the way of teaching at the university and the home schooling of children put a lot of extra burden on our researchers.

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