PhD Thesis

Investigation of plastic additives migrating from food contact materials

Zsolt Bodai

Eötvös Loránd University
Doctoral School of Chemistry
Director: Prof. György Inzelt
Analytical, Colloid and Environmental Chemistry
Program Director: Prof. Gyula Záray

Supervisor: Zsuzsanna Eke, PhD, assistant professor

ELTE, Institute of Chemistry
Joint Research and Training Laboratory on Separation Techniques (EKOL)

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1. INTRODUCTION

Modern food supply chains necessitate the processing and packaging of foodstuffs, and an enormous variety of materials have been derived for the protection of food quality. It is critical for consumer safety that the interaction between these materials and food be assessed, such that migration is identified and quantified. Of particular interest are material additives, such as antioxidants and ultraviolet stabilisers, which are applied to maintain polymer integrity through the processing, packaging and storage sequence.

In 2011 the European Commission issued a regulation (10/2011/EC) regarding plastic materials and articles intended to come into contact with food. According to this regulation, migration studies have to be performed with plastic materials and articles intended to come into contact with food. The regulation defines food simulants as suitable test surrogates for different food-types, based on typical physical chemistry properties. There are five liquid food simulants and one solid food simulant on this regulation. Liquids simulants are ethanol 10% (V/V) (Food simulant A), acetic acid 3% (m/V) (B), ethanol 20% (V/V) (C), ethanol 50% (V/V) (D1), vegetable oil (D2). E food simulants for solid foods: poly(2,6-diphenyl-p-phenylene oxide), particle size:60–80 mesh, pore size: 200 nm

The application of these food simulants simplifies the analytical tasks of migration studies remarkably. However, it is not absolutely clear how representative these six food simulants are of all foods, and thus whether safety assessments are complete. Surprisingly few articles have been published which compare the migration into food and related food simulant. A. Sanches Silva and co-workers [2] showed that migration of diphenyl-butadiene (DPBD) into orange juice is lower than migration into related food simulants (3% (m/V) acetic acid in water). This difference probably occurred due to fibres in orange juice. Reinas and co-workers [3] studied the migration of Irgafos 168 and Irganox 1076 into rice and authorized food simulant. Migration was slower into rice than into the food simulants. These limited data question the suitability of the authorized simulants and suggest that further investigation of their adequacy in the assessment of additive migration is needed. This can be especially true for such complex colloid systems as the milk which has to be modelled with 50% (V/V) ethanol – water solution.

The EC regulation defines test conditions for each migration experiment, such as contact time and temperature. Typically, extreme variables are used to maximise test stringency.

(“worst foreseeable use”). However, for undefined reasons, once the “worst foreseeable use” is 3 to 30 days, a test time of 10 days is taken, presumably on the assumption that additive migration will reach equilibrium well before this time. Again, this will have specific relevance for extended shelf-life (ESL) milk, which expire more than 10 days. Premature testing passes by over exposition by contrast unnecessary efforts could be omitted if shorter contact time also gives relevant migration results.

Migrated concentrations of the targeted antioxidants and ultraviolet stabilisers (See 2. Aims for target compounds) are usually reported to be lower than the limits of detection when migrations experiments are performed into polar food simulant [4-7]. Migration only occur into non-polar food simulant [5,7,8] or at elevated temperature [9]. Based on these articles the solubility of the target compounds in 10/2011/EC polar food simulant can be limited.

2. AIMS

My overall objective was to investigate additive migration discrepancy between real and simulant food substrate and thus expand our knowledge in ‘real-world’ food safety. During my PhD I measured five antioxidants (Irgafos 168, Irganox 1010, Irganox 3114, Irganox 3790 and Irganox 565) and six UV stabilizers (Cyasorb UV-1164, Tinuvin P, Tinuvin 234, Tinuvin 326, Tinuvin 327 and Tinuvin 1577) listed in 10/2011/EC as candidate compounds for this purpose.

My first aim was to develop a new analytical method that was suitable for quantitative determination of the target compounds in milk. Although previous studies had described quantitative analytics in other media (e.g. in standard solutions, plastic, or simulants [4-13]) none had measured these compounds simultaneously, and none in milk. A liquid chromatography mass spectrometry approach was selected owing to its superior sensitivity and specificity of detection. Moreover, a new sample preparation method was also developed. During the migration studies huge number of samples were expected so a simplicity, speed and robustness of the sample preparation were main requirements. Final methods for

chromatography, mass spectrometry and sample preparation were validated to ensure reliability.

After the validation, my next aim was to compare migration of Irganox 3114 and Tinuvin P from high-density polyethylene (HDPE) into milk and the relevant food simulant and to determine the minimal necessary contact time to reach equilibrium. During the contact time experiments I expected increasing concentration at the beginning of the migration after which the concentration should reach the equilibrium. Separation of these two domains can be hard because of the deviation of the measurements. So I decided to test the applicability of semivariograms (a function well known in geostatistics) in the establishment of the minimum contact time.

Finally, solubility of the target compounds in polar food or food simulant can be limited. I intended to compare the specific migration limits (SMLs) of my target compounds with the solubility values in liquid food simulants and two beverages (fruit juice and coke). Before the determination of solubility values, sample preparation method development was necessary for D2 food simulant and beverages. Determination of solubility based on MSZ 21485-3 Hungarian standard method is a time-consuming process unsuited to high throughput sample preparation. Therefore I also aimed at building a streamlined preparation method that offered comparable recovery.

3. METHODS

3.1. ANALYTICAL METHODS

Eleven compounds were measured in foods (1.5% and 3.5% fat content milk, fruit juice and coke) and food simulants (acetic acid, ethanol water solutions and oil). All of the samples were measured with an Agilent 1100 HPLC coupled to API 2000 triple quadrupole mass spectrometry system for the quantitative determinations. Chromatographic separation was performed on a Kinetex PFP column using 0.1% (V/V) formic acid in methanol and 1 mmol/l ammonium-formate in water (pH 2.8 adjusted with formic acid). Positive electrospray ionization (ESI) was used with the Multiple Reaction Monitoring (MRM) mode.

For acetic acid and ethanol water solutions the HPLC-MS/MS system provided the selectivity so further sample preparation wasn’t needed. In the cases of foods and oil (D2 food simulant) proper sample preparation method had to be developed and analytical performance characteristics had to be determined. Milk samples were prepared with liquid-liquid extraction combined with low temperature purification. In the case of beverages samples a valve system
was used to prevent the contamination of the mass spectrometer. Oils were diluted to mitigate ion suppression in the ESI source.

3.2. MIGRATION EXPERIMENTS

After the method development custom-made HDPE was formed with different amount of Tinuvin P and Irganox 3114. These HDPE sheets were soaked into milk with different fat contents and authorized food simulant (D1 food simulant, 50% (V/V) ethanol water solution) during the migration experiments.

Minimal contact time was determined with D1 food simulant with both additives. Semivariogram was applied to establish equilibrium metrics.

3.3. SOLUBILITY EXPERIMENTS

Prior to solubility experiments, common pitfalls of sample preparation (e.g. low recovery from syringe filter or matrix effect in the ion-source) were examined and optimised step by step in order to obtain accurate results.

Solubility experiments were based on MSZ 21485-3 Hungarian standard method: 10 mg aliquots of Tinuvin 327 were measured in nine vials and filled with 50% (V/V) ethanol water solution (D1 food simulant). Each was magnetically stirred at 30°C in a water bath, at 24, 48 and 72 hours, three vials were removed to a 25°C water bath and allowed to equilibrate with stirring for 24 hours. The vials were centrifuged and the supernatant filtered into an HPLC vial, and immediately injected into the HPLC.

This cumbersome method was not suited to the solubility estimation of eleven compounds in seven test substrates. Therefore a streamlined approach was taken which took advantage of sonication rather than stirring to improve solubility. Thus, daily sampling was replaced with sampling at 25, 50 and 75 minutes sonication, and 2 hours equilibration was applied instead of 24 hours. Nine aliquots of 10 mg Tinuvin 327 were measured into vials and prepared with this new method. The results of the two methods were compared with t-test. There were no significant different with 95% probability between the two methods so further solubility experiments were performed with the new method in order to compare the solubility values with the SMLs listed in 10/2011/EC regulation.
4. **RESULTS**

4.1. **CHARACTERISTICS OF THE ANALYTICAL METHOD**

HPLC-MS method was successfully developed for the determination of all target compounds in a single chromatographic run however different sample preparation methods had to be used for the beverages, milks and food simulants. Limits of detection are between 0.5 and 10 ng/ml in the case of food simulants and beverages and between 0.25 and 10 µg/kg in the for milk samples. Also for milk samples, accuracy values are between 93 and 109% with all of the tested concentrations (25, 100, 500 µg/kg, 1 and 2.5 mg/kg). RSD values are lower than 13% in three prepared samples at each concentration levels.

Due to the low solubility of the target compounds accuracies could not be tested by addition of the standard into food simulants and beverages. Therefore accuracies were determined step by step through testing the pitfalls of the analytical method. Recoveries from syringe filter are between 95 and 107% and the matrix effect, which occurred in the ESI ion source, between 68 and 120%.

4.2. **RESULTS OF THE MIGRATION EXPERIMENTS**

After an initial increase the measured concentrations became constant during the determination of minimal contact time. It was difficult to separate the increasing part from the equilibrium part to the naked eye. For quantitate determination of the proper contact time semivariograms were fitted. Minimum required contact time is 57 min for Tinuvin P and it is 23 min for Irganox 3114.

D1 food simulant mimicked milk samples well during the migration experiments with HDPE sheets containing different concentrations of Tinuvin P or Irganox 3114. All migration curves are similar; additive saturation took place above 1% (m/m) additive in the HDPE. Average migrated concentrations from 1, 3 and 5% (m/m) Tinuvin P containing HDPE into 1.5%, 3.5% fat milk and D1 food simulant are 41, 58 and 63 mg/kg respectively. In the case of Irganox 3114 0.0047 mg/kg migrated into 1.5% fat milk, 0.0045 mg/kg into 3.5% fat milk and 0.039 mg/kg into food simulant. SMLs are 30 mg/kg for Tinuvin P and 5 mg/kg for Irganox 3114.
4.3. **RESULTS OF THE SOLUBILITY STUDY**

Using the standard method for determination the solubility of Tinuvin 327 in D1 food simulant, solubility was found to be 1.72 µg/ml with 6% RSD. With the improved sonication-based method it was found to be 1.73 µg/ml with 4% RSD. Shapiro-Wilk test proved the normal distribution of the data. The p-value is 0.22 during the F-test which meant there is no significant difference on the variance of the data with 95% probability. According to the F-test results equal variance two-sample t-test was performed. The p value is 0.84 which means there is no significant difference at 95% probability level between the two tested methods. Further experiments were performed with the improved sonication-based method. Acquired solubility values with this method are shown in Table 1.

Table 1. Solubility values determined with the sonication based method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Food simulant</th>
<th>Beverage</th>
<th></th>
<th></th>
<th></th>
<th>coke</th>
<th>juice</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D1</td>
<td>D2</td>
<td>coke</td>
<td>juice</td>
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<tr>
<td>Tinuvin 1577</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&gt;1000</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>Cyasorb UV-1164</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&gt;1000</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<tr>
<td>Irgafos 168</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&gt;1000</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<tr>
<td>Irganox 1010</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<td>&lt; LOD</td>
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<td>Irganox 3790</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>8.8</td>
<td>&gt;1000</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<tr>
<td>Irganox 565</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<tr>
<td>Irganox 3114</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>0.23</td>
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<tr>
<td>Tinuvin 326</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>2.21</td>
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<td>&lt; LOD</td>
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<tr>
<td>Tinuvin 327</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>1.73</td>
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<tr>
<td>Tinuvin 234</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<td>&lt; LOD</td>
<td>&lt; LOD</td>
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<tr>
<td>Tinuvin P</td>
<td>0.15</td>
<td>0.18</td>
<td>1.96</td>
<td>104</td>
<td>&gt;1000</td>
<td>0.34</td>
<td>0.14</td>
</tr>
</tbody>
</table>

LOD: Limit of detection
5. **New Scientific Conclusions in the Thesis**

- The developed HPLC-MS/MS and sample preparation methods are suitable for the determination of 11 compounds listed in 10/2011/EC regulation in milk, beverages and authorized food simulants.

- A geostatistical method, semivariogram was successfully applied to determination of minimal contact time with Tinuvin P and Irganox 3114. This statistical method has been never used in the field of chemistry. Based on the results of the minimal contact time experiments, analytical measurements can be performed before the 10th day.

- The 50% (V/V) ethanol water solution (D1 food simulant) was an appropriate milk simulant for the studied additives, offering similar migration of Tinuvin P and Irganox 3114 from HDPE sheets. Migrated concentrations were slightly higher in D1 than in milk but these results are reassuring regarding food safety.

- In the case of Irganox 3114 the migrated concentrations at 5°C were two order of magnitude lower than the SML even when ten times higher concentration of additive was applied in HDPE sheet than the recommended concentration by BASF. Whereas Tinuvin P migrated in the same order of magnitude as the SML when it was applied in the recommended concentration in the HDPE.

- Solubility experiments were based on MSZ 21485-3 Hungarian standard. Since this method had been time and tool consuming faster method was developed. Reliability of the improved sonication-based method was proved by determination of Tinuvin 327 solubility with both method.

- Solubility of the target compounds at 25°C in beverages and A, B and C food simulants were one to three order of magnitude lower than their specific migration limits. Solubility in D1 food simulant could exceed the SML only in the case of Tinuvin P and Irganox 3790. Solubility in D2 food simulant exceeded the corresponding SML for all the compounds. Based on these solubility results we can easily decide which migration experiments are needed.
6. Publication in the topic of the thesis

6.1. Articles

Analysis of potential migrants from plastic materials in milk by liquid chromatography mass spectrometry with liquid-liquid extraction and low temperature purification
Zsolt Bodai, Bálint Sámuel Szabó, Márton Novák, Susanne Hámori, Zoltán Nyiri, Tamás Rikker, Zsuzsanna Eke

Migration of Tinuvin P and Irganox 3114 into milk and the corresponding authorised food simulant
Zsolt Bodai, Csaba Kirkheszner, Márton Novák, Zoltán Nyiri, József Kovács, Norbert Magyar, Béla Iván, Tamás Rikker, Zsuzsanna Eke
Food Additives and Contaminants, 2015, 32 (8), pp 1358-1366

Solubility study of antioxidants and UV stabilizers into beverages and food simulants
Zsolt Bodai, Péter Pál Jakab, Márton Novák, Zoltán Nyiri, Bálint Sámuel Szabó, Tamás Rikker, Zsuzsanna Eke
Submitted to Food Additives and Contaminants

6.2. Posters

LC-MS/MS method development for the determination of triazines and triazoles migrating from food packing materials
Zs. Bodai, M. Novák, Zs. Eke
HPLC 2013, 2013, Amsterdam, Netherlands

Analysis of UV Stabilizers and Antioxidants in Milk by High Performance Liquid Chromatography and Mass Spectrometry
Zsolt Bodai, Susanne Hámori, Márton Novák, Zsuzsanna Eke
HPLC 2014, 2014, New Orleans, USA

Élelmiszerekkel érintkezésbe kerülő műanyagok adalékanyagainak modellelderítése alapján
Élő oldalakon és üdítőkben való oldódásának vizsgálata LC-MS/MS technikával
Péter Pál Jakab, Zsolt Bodai, Bálint Sámuel Szabó, Tamás Rikker, Zsuzsanna Eke

Investigation of minimum contact time for migration studies of Tinuvin P and Irganox 3114
Zsolt Bodai, Bálint Sámuel Szabó, Csaba Kirchkeszner, Márton Novák, Zoltán Nyiri, József Kovács, Norbert Magyar, Zsuzsanna Eke
21st International Symposium on Separation Science 2015, 2015, Ljubljana, Slovenia

Comparison of the solubility of plastic additives in food simulants and beverages
Jakab Péter Pál, Bodai Zsolt, Szabó Bálint Sámuel; Eke Zsuzsanna
10th Balaton Symposium on High-Performance Separation Methods, 2015, Siófok, Hungary