Certified reference materials for food packaging specific migration tests: development, validation and modelling

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Proefschrift

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#### Abstract

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This thesis compiles several research topics during a feasibility study for the certification of 6 reference materials for specific migration testing of food packaging materials. The overall results of the certification exercise, covering results for 3 certification parameters (initial concentration of migrants, specific migration value and diffusion coefficient) from 4 participating laboratories were evaluated. The development and validation of analytical methods for the nylon 12 monomer laurolactam was described. The new methods were applied during two studies. Alternative fatty food simulants for nylon 12 were evaluated, and nylon 12 films were subjected to two food simulants at either side simultaneously, in order to simulate their use as sausage casings. Mathematical models simulating both one- and two-sided migration were described and a way to estimate diffusion and partitioning coefficients of the migrants - including their confidence intervals - was introduced. Effects of gamma-irradiation on some certified reference material candidates were also investigated. Amounts of common polymer additives (Irganox 1076 and Irgafos 168) from polyolefins decreased with higher irradiation doses due to the degradation of these additives in the polymer, however, the overall migration did not significantly change. With rising irradiation doses, the sensory quality with respect to odour increased for polystyrene and decreased for the other polymers investigated.

*Keywords:* certified reference materials; diffusion; food contact materials; food packaging; laurolactam; migration modelling; nylon; specific migration

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#### Symbols and abbreviations

-	Atomic Emission Detector
-	Polymer Specific "Conductance" Coefficient
-	Comité Européen de Normalisation
-	Initial Concentration of a Migrant in a Polymer
-	Certified Reference Material
-	Central Science Laboratory
-	Dichloromethane
-	Diethylhexyladipate
-	Diffusion Coefficient (cm/s <sup>2</sup> )
-	Diphenylbutadiene
-	European Disposables And Nonwovens Association
-	Food Analysis Performance Assessment Scheme
-	Flame Ionisation Detector
-	Gas Chromatography
-	Gel Permeation Chromatography
-	General Purpose Polystyrene
-	High Density Polvethylene
-	High Impact Polystyrene
-	High Performance Liquid Chromatography
-	Half-Width of Confidence Interval
-	Fraunhofer IVV
-	Partitioning Coefficient between Polymer and Food
-	I ow Density Polyethylene
-	Limit of Detection
-	Limit of Quantification
-	Modified Polyphenylene Oxide
-	Mass Spectrometry
-	Mean Squares Between Laboratories
-	Mean Squares Within Laboratory
_	Polvamide
-	Polvethylene Terephtalate
-	Polypropylene
-	Polystyrene
-	Polyvinyl chloride
-	Relative Variation
-	Size Exclusion Chromatography
-	Standard Deviation
-	Specific Migration
-	Tetrahvdrofuran
_	Trimethylolpropane
-	Ultraviolet

General introduction

#### 1.1 Introduction

Food packaging is intended to protect food from environmental influences such as microbial or chemical degradation, air (oxygen), light or water. In the last decades, the use of plastic materials for packaging of foods has increased tremendously and nowadays plastics have become the most frequently applied packaging material (Figure 1.1). In Germany,  $3.5 \times 10^9$  kg of plastic packaging materials were produced for the German market in 2003; roughly 40 kg per person (Industrieverband Kunststoffverpackungen 2004, Emminger 2004). This increase in the use of plastics as packaging materials is due to its many advantages. Compared to glass and metal, plastics are more flexible, significantly lighter in weight and less energy is needed for their production and processing. However, plastics do have some disadvantages. For instance, they are less heat resistant, more permeable with respect to gases and water, and transport of low molecular compounds can occur, such as of monomers or additives from plastics into foodstuffs as the result of diffusion. The increased use of plastic packaging materials and the possible interaction phenomena have created the need for more research in the field of food and packaging applications.



Figure 1.1 Development of packaging material use in Germany (Industrieverband Kunststoffverpackungen 2004).

#### 1.2 Migration testing

Figure 1.2 shows mass transport processes that can and will happen when food is packaged using plastic materials. This is the reason why food contact plastics have to undergo tests in order to evaluate whether transfer of compounds from the plastic into foods (migration) takes place (EEC 1989). Among migration tests, one should differentiate between overall and specific migration testing. The first part of both tests is the same; a test specimen is exposed to a food simulant at certain temperature and time conditions that are realistic for the practical application of the plastic (e.g. 2 hours at 100°C for a material that is cooked along with the food). With overall migration testing, the sum of constituents that migrated into a foodstuff is measured and should not exceed 10 mg/dm<sup>2</sup> or 60 mg/kg of foodstuff (it is assumed that 1 kg of food is wrapped in 6 dm<sup>2</sup> of packaging, EC 2004). For specific migration tests, the amount of a certain monomer or additive listed in directive 2002/72/EC (EC 2002) is measured in a food simulant after the exposure. These specific migration limits (SML) are likewise given in mg/dm<sup>2</sup> and mg/kg.



#### Figure 1.2 Illustration of mass transport phenomena between food and food packaging

Directive 85/572/EEC (EEC 1985) lays down which food simulant should be used for what type of food. Plastics that are supposed to be in contact with aqueous foods, have to be tested with distilled water. Vinegar and some fruit juices are simulated by an aqueous solution of 3% acetic acid, liquors and wine by a solution of 10% ethanol or actual alcoholic strength if concentration exceeds 10%. Olive oil is used as simulating agent for meat products and fatty foods (Table 1.1).

Table 1.1	Food	simulant	to	be	used	according	to	Directive
	85/572	2/EEC (EEC	1985	)				

Examples of type of food	Food simulant	
Water, honey	Distilled Water (or water of equivalent quality)	(Simulant A)
Vinegar, fruit drinks	3% Acetic acid (w/v) in aqueous solution	(Simulant B)
Wine, liquors	10% Ethanol (v/v) in aqueous solution	(Simulant C)
Meat, cheese	Olive Oil	(Simulant D)

Because of the complex analytical problems involved, specific migration testing – especially when using simulant D, olive oil – is very error-prone, time consuming and as a result, expensive. Therefore, alternative and substitute tests have been developed. According to Directive 97/48/EC (EC 1997) 95% ethanol, isooctane and modified polyphenylene oxide (Tenax<sup>®</sup>) can be used as such alternative matrices, for instance when the application of olive oil is technically not feasible. This way, the analytical part of migration testing becomes significantly less difficult and migration testing less expensive and more accurate.

Mathematical models are another means to facilitate plastics compliance testing. Directive 2002/72/EC (EC 2002) allows the use of computer based migration modelling, when "generally recognised diffusion models" are applied. In most cases, mass transfer from a plastic into food follows Fick's law of diffusion and is therefore generally predictable by an accepted equation (Crank 1975). Certain pieces of information are fundamental for such migration modelling: the initial concentration of the migrant in the polymer  $(C_{P,O})$ , the diffusion coefficient  $D_P$ , and the partitioning coefficient  $K_{P,F}$ , describing the migrants' partitioning between plastic and food phase. For  $K_{P,F}$ , a worst case estimation can be obtained by setting  $K_{P,F} = 1$ , thus assuming a high solubility of the migrant in the food. The estimation of  $D_{P}$  however, is more complex. Piringer (1993, 1994). has established an empirical relationship of the diffusion coefficient with the relative molecular weight of the migrant, the temperature and the polymer type. A material specific coefficient  $A_{P}$ , based on experimental data collected in literature and expressing the "diffusion conductance" of a polymer, was introduced. For some polymers, however, not many experimental data are available and consequently, their  $A_P$  values are not very reliable.

#### 1.3 Certified reference materials

Many governmental, but also commercial control laboratories have introduced quality management systems. These generally comprise the exact documentation of all routine, development and validation work, the use of statistical control charts and the participation in interlaboratory comparison studies or proficiency testing schemes. Certified reference materials (CRMs) are used as tools for such quality control. CRMs are samples with properties that have been certified, mostly by a large group of laboratories that have proved to be able to accurately measure the analyte of interest. CRMs are available from several organisations. The most complete list of CRMs is available from the Joint Research Centre of the EU and can be found at: http://www.irmm.jrc.be/rm/cat.html.

#### 1.4 Motivation for and outline of this thesis

The situation described above was the motivation to initiate a project for the development of the know-how to produce certified reference materials (CRMs) for specific migration testing. These were to have three certification parameters:

- 1. The initial concentration of the migrant in the plastic  $(C_{P,O})$ .
- 2. The specific migration (SM) of a migrant into a food simulant under defined time and temperature conditions.
- 3. The diffusion coefficients of the migrants in the plastic,  $D_{\rm P}$ .

The scientific community would benefit from these in the following ways:

- a) The availability of CRMs (with certified  $C_{P,O}$  and SM) as an established tool for laboratories to check their performance would improve the overall quality and reliability of results (Quevauviller, 1999).
- b) Alternative fatty food simulants (95% ethanol, isooctane, Tenax<sup>®</sup>) have only been established and validated for overall migration testing. If CRMs were available, validation studies comparing results applying olive oil and alternatives would be possible.
- c) The availability of a certified  $D_P$  for migrants in plastics frequently used for food packaging purposes would enhance the reliability of  $A_P$  values and consequently, of migration modelling.

**Chapter 2** gives a detailed description of the current state of the art in the introduction followed by a description of the main results with respect to two of the three certification parameters ( $C_{P,0}$  and SM). The outcome of the project with respect to the third certification parameter, the diffusion coefficients of the polymers tested, is given in **Chapter 3**. Additionally, with experimental data and all necessary input for migration modelling available, a comparison is given of experimental and predicted values, which allows to validate the model that Migratest (commercial software to predict migration) is based upon .

During the early phase of the project, whilst screening literature for analytical methods, it was observed that no information was available about analytical methods, specific migration or diffusion data regarding nylon 12 (and its monomer laurolactam). Therefore, analytical methods for the migrant laurolactam were developed and validated. Methods using gas chromatography applying flame ionisation detection (GC-FID), high performance liquid chromatography applying ultraviolet detection (HPLC-UV) and HPLC applying mass spectrometric detection (HPLC-MS) were developed for the detection of laurolactam in nylon 12 or in food simulants. The results of this work are described and discussed in **Chapter 4**.

**Chapter 5** describes the application of these methods to the estimation of diffusion coefficients – one of the certification parameters – for laurolactam in nylon 12 films. Furthermore, a comparison of alternative fatty food simulants as well as a new approach for the determination of diffusion coefficients including confidence intervals is presented.

In **Chapter 6**, the approach taken in the previous chapter is extended. The simultaneous migration of monomers from nylon films into two directions is investigated experimentally and by migration modelling. This is due to the practical applications of these films – often used as artificial sausage casings – where they are simultaneously subjected to fatty food on one side and boiling water on the other.

Some of the candidate materials that were developed have also been irradiated using gamma-irradiation in order to evaluate changes in migrant concentrations, their migration behaviour as well as their effect on sensory properties. The results of this study are described in **Chapter 7**.

All the results of the previous chapters are evaluated in a general discussion and conclusion in **Chapter 8.** 

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# 2

Feasibility study for the development of certified reference materials for specific migration testing. Part 1: Initial migrant concentration and specific migration.

#### Abstract

This chapter describes a project with the main objective to develop the know-how to produce certified reference materials (CRMs) for specific migration testing. Certification parameters discussed in this chapter are the initial concentration of the migrant in the polymer (CP,0) and the specific migration into a food simulant under certain temperature / time conditions. 16 preliminary candidate CRMs were defined and produced. The most important polymers (low and high density polyethylene (LDPE and HDPE), polypropylene (PP), polystyrene (PS), polyethylene terephtalate (PET), plasticized polyvinyl chloride (PVC), rigid PVC, polyamides (PA)) and additives as well as monomers representing different physicochemical properties as target substances for migration were chosen. Stability and homogeneity of the migrants in the materials were tested and methods for the determination of the certification parameters were developed and validated. Of the 16 materials produced the 6 most suitable (LDPE // Irganox 1076 / Irgafos 168, LDPE // 1,4-diphenyl-1,3-butadiene (DPBD), HDPE // Chimassorb 81 / Uvitex OB, PP homo // Irganox 1076 / Irgafos 168, HIPS, 1 % mineral oil // styrene, PA 6 // caprolactam) were selected. The feasibility of CRM production for the 6 candidate materials was demonstrated and a trial certification exercise was performed with participation of all 4 partner laboratories. All 6 materials showed suitable properties for future production as certified reference materials.

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#### 2.1 Introduction

To assure consumers' protection, EU-Framework Directive 89/109/EEC (EEC 1989) states that food contact materials must not transfer their constituents to foodstuffs in quantities which could endanger human health or bring about an unacceptable change in the composition of the foodstuffs. In the specific Directive 2002/72/EC (EC 2002) for plastics, the overall migration and the specific migration of certain monomers and additives are limited, and their migration has to be tested to show materials' compliance.

Migration tests can be divided into two distinct phases. The first is the migration exposure itself, i.e. the contact of the plastic material to the food simulant. The second is the quantification of the migrants by chemical analysis for specific migration and by gravimetric analysis for overall migration. It is the first phase that sets migration methodology apart and establishes it as a distinct discipline. The major source of error lies in the initial choice and execution of the migration exposure rather than in the subsequent analysis (Castle 2001).

Reference materials for specific migration are not available anywhere. Even proficiency test schemes such as FAPAS<sup>®</sup> (Food Analysis Performance Assessment Scheme, UK) offer only spiked simulant samples for testing specific methods. There are certified reference materials DANREF (Lund *et al.* 2000) and CRMs 537, 538 and 539 (Lord *et al.* 1998) for overall migration into olive oil but they are not suitable for specific migration testing since the migrants have not been qualified in these materials.

Migration testing in olive oil (the simulant for fatty foods) is not only rather imprecise but also very time consuming and therefore expensive. Specific migrants are often difficult to measure in oil with the sensitivity required in the legislation. Some migrants are also not stable in the oil simulant. For these reasons, alternative and substitute tests have been and will be further developed. According to EC Directive 97/48/EC (EC 1997) 95% ethanol, isooctane and modified polyphenylene oxide (Tenax<sup>®</sup>) can be used as such alternative matrices, for instance when the application of olive oil is technically not feasible. But these alternatives have been validated predominantly for overall migration rather than for specific migration. Alternative methods such as rapid extraction tests (EN 1186-15. 2004) using organic solvents also need to be validated for specific migration purposes.

The prediction of migration using mathematical models is a further tool to streamline testing for compliance. Article 8 paragraph 4 of EU Plastics Directive 2002/72/EC and previously the 6<sup>th</sup> amendment of Directive 90/128/EEC (EC 2002, 2001) allows verification of compliance with

specific migration limits (SML) by an analysis for the corresponding maximum concentration of the substance in the finished product (QM). The correspondence between the SML for any substance and its concentration QM in the plastic must be established by experimentation or by using "generally recognised diffusion models". In most cases, mass transfer from a plastic into food obeys Fick's law of diffusion and is therefore generally predictable by an accepted equation (Crank 1975) when the partition coefficient between plastic and food (simulant) and the diffusion coefficient of the migrant in the plastic material are known or workable default parameters are used. For the partition coefficient  $K_{PF}$ a worst case estimation can be obtained by setting  $K_{P,F} = 1$  if the migrants are highly soluble in the food or simulant. The diffusion coefficient  $D_{P}$ depends on polymer type, molecular mass and shape of the migrant, and the temperature. Because the determination of the diffusion coefficient is time consuming and error-prone, only a limited number of reliable  $D_{P}$ values exists and a need exists for more and better estimation procedures need. An estimation procedure developed by Piringer (1993 and 1994) uses an empirical, so-called  $A_{P}$ -value as a characteristic property of the polymer to describe its basic diffusivity. This approach of estimating the DP-values is statistically based and if more experimental results are used then the parameter AP can be made more reliable. The collection of available migration data was one objective of an EU-Project SMT4-CT98-7513 (Hinrichs and Piringer 2001) but it was concluded that further migration studies aimed specifically at leading to AP and DP values were necessary. Quite often, not all the critical parameters, e.g. exact migration parameters or proper characterisation of the polymer, can be picked out of the literature. Therefore, reliable specific migration / diffusion data obtained from well-defined polymer-migrant systems are necessary. An important aspect for both the migration modelling and the worst case

An important aspect for both the migration modelling and the worst case QM approach is the initial concentration of the migrants in the polymer (CP,0). Recent reports from proficiency trials (Bart et al. 2001, Ritter et al. 2003) have shown that there was a large variability of interlaboratory results and that the general quality of the analysis of polymers for their initial concentration of additives needs to be improved.

#### 2.2 Structure of the research project

The situation described above was the motivation to initiate a research project with the acronym "Specific Migration" and EU contract no. G6RD-CT2000-00411 within the 5th Framework Programme of the European Commission. The project started in January 2001 with duration

of 3 years. The objective of the project was to develop the know-how needed to produce and certify reference materials for specific migration testing. The certification parameters were the initial concentration of the migrants in the polymer ( $C_{P,0}$ ), the specific migration (SM) into a food simulant under defined time/temperature conditions and the diffusion kinetic characteristics of the material. This chapter will focus on the  $C_{P,0}$  and SM results only. The diffusion kinetics studies are published in a separate chapter (Stoffers et al. 2004b). 16 candidate materials were defined, produced, and the stability and homogeneity were tested in the first phase of the project. For those 16 materials, methods for the three certification parameters were developed. Of the candidate CRMs the 6 most suitable materials were selected. Larger batches of these materials were produced and the feasibility of CRM production was investigated.

#### 2.3 First selection of material candidates

16 materials containing one or two migrants each were selected (See Table 2.1). Both the polymers and the migrants were selected to cover a wide range of different structures and physical-chemical properties. The migrants should be relevant for industrial and enforcement migration testing. Therefore, additives or stable monomers used to manufacture food contact plastics - where possible with specific migration limits (SML) - were selected from the Plastics Directive 2002/72/EC (EC 2002). The substances cover a range of polarity and chemical types as well as a range of molecular weights and volatility. The materials were to be designed such that migration levels could be achieved which were measurable with high accuracy and precision. On the other hand, total extraction at the given migration contact conditions should not take place. This was because if total migration did occur, then future use of the candidate CRMs would not reveal any mistakes made in the time / temperature conditions applied. Therefore, for some polymer-migrant combinations, commercial plastics were not available and pilot materials were produced especially for the purpose of this project.

#### 2.4 Packaging and storage

All materials were cut into pieces of roughly 10 x 10 cm. In order to prevent mass transfer from or to packaging materials, sets of 3 films were first wrapped into aluminium foil and subsequently packed into polyethylene bags. Only polystyrene materials with styrene as the migrant

were packed in packs of 5 due to the high volatility of styrene. The outer two plaques were used as additional protection and were to be removed and discarded prior to using the material. This way, the loss of styrene at the surface of the plaques was minimised. During the first phase of the project, approximately 60 packs of each of the 16 materials were produced and packed, for the trial certification exercise in the second phase, 500 packs of each of the selected 6 materials were made.

#### 2.5 Homogeneity / Stability

Homogeneity was tested with regard to the parameters: thickness, concentration of the migrants and density. 10 sample sheets or plagues taken from throughout the production order were examined. Thickness was tested at 10 different locations of each sheet, the concentration of the migrants and density were tested in duplicate for each sheet. All data were evaluated using the SOFT-CRM software (Bonas et al. 1998). The software performs the analysis of variance (ANOVA) and a Snedecor F-test at two levels of significance (a=0.05 and 0.01) to assess the significance of the observed differences between sheets and also within sheets. The output of this test needed however to be used with care since its validity depended on the precision of the analytical method. If the between sheets standard deviation was 5% or less, the material was judged "fit for purpose" even if software output for the respective parameter was "not homogeneous". Detailed homogeneity results can be found in Table 2.1 for the 16 materials produced in the first phase and Table 2.3 for the 6 materials produced in the trial certification exercise.

Stability was monitored over a period of 12 months for the first 16 materials and the CRM candidates applying storage temperatures of 5°C (reference temperature), 20°C and 40°C (reference temperature for styrene -20°C). The parameters tested were the concentration of the migrants and density. The samples were stored at the respective temperatures and periodically analysed every two months. The day-to-day variability of the analytical methods for migrant concentration was relatively high, which made the detection of significant trends over time difficult. Hence, for the certification exercise, isochronous testing was applied (Lamberty *et al.* 1998). This is based on a storage design that allows all analytical measurements to be done on the same day at the end of the study. The advantage of isochronous stability testing is that it requires only repeatability conditions, whereas classical designs (i.e. a single fixed starting point culminating in sample analysis being spread out over the lifetime of the stability tests) require both repeatability and long

term reproducibility conditions. Consequently, this design allowed the quality of the results to be improved compared to the preliminary investigations. Stability data were also evaluated using the SOFT-CRM software (Bonas *et al.* 1998). As a rule of thumb, rates of degradation processes in organic materials generally double with an increase in temperature of 10°C. Hence, if stability at 40°C could be proved over a period of one year, the material should be expected to be stable over 4 years at 20°C or 11 years at 5°C.

No	Polymer	Migrant	PM/REF	SML (mg/kg)	MW (Daltop)	Thickness	Density
				(mg/kg)	(Darton)	(µm)	(g/cm²)
1	LDPE	Irganox 1076	68320	6	531	$993 \pm 11$	0.917 ± 0.002
		Irgafos 168	74240		647		
2	LDPE	Diphenyl- butadiene			206	497 ± 2	0.920 ± 0.005
3	HDPE	lrganox 1076	68320	6	531	1043 ± 9	$0.948 \pm 0.002$
		Irgafos 168	74240		647		
4	HDPE	Chimassorb 81	61600	6	326	355 ± 4	0.933 ± 0.006
		Uvitex OB	38560	0.6	431		
5	PP	lrganox 1076	68320	6	531	378 ± 2	0.899 ± 0.003
		Irgafos 168	74240		647		
6	PP	Chimassorb 81	61600	6	326	383 ± 3	0.892 ± 0.015
		Uvitex OB	38560	0.6	431		
7	PP	Erucamide	52720		337	437 ± 3	$0.881 \pm 0.001$
		Trimethylol- propane	94960	6	134		
8	HIPS 6-7						
	% mineral	lrganox 1076	68320	6	370	2009 ± 3	1.025 ± 0.012
	oil						
9	HIPS 1 % mineral oil	Styrene	24610		104	2000 <sup>2</sup>	1.012 ± 0.014
10	GPPS	Styrene	24610		104	2000 <sup>2</sup>	1.034 ± 0.006
11	PET	Cyclic Trimer	15760	30	106	105 ± 3	1.427 ± 0.015
12	PET	Tinuvin 1577	51700	0.05	425	22 ± 0.4	1.360 ± 0.043
13	PVC rigid	Organotin stabilizer	94400			251 ± 2	1.360 ± 0.016
14	plast.PVC	DEHA	31920	18	370	650 ± 22	$1.2\overline{48 \pm 0.002}$
15	PA nylon	Caprolactam	14200	15	113	119 ± 1	1.122 ± 0.003
16	PA 12	Laurolactam	19490	5	197	197 ± 5	$1.002 \pm 0.002$

Table 2.1	Polymer-migrant	combinations	and	results	of	homo-
	geneity testing					

<sup>1</sup> and standard deviation (SD)

<sup>2</sup> no SD is given because mean squares within units (MSW) < between units (MSB).

## 2.6 Selection of specific migration conditions

Several aspects were taken into account for the selection of the conditions for specific migration testing. The conditions were to represent the real use of that type of plastic. Additionally, the migration was to be in the range of approximately 30 to 70% of total mass transfer. Furthermore, a certain variety of food simulants (fatty and aqueous) as well as temperatures was to be covered. The specific migration conditions can be seen in Table 2.2 for phase 1 and Table 2.7 for the trial certification exercise.

No	Polymer	Migrant	SM conditions	SM	
	-			(mg/dm²) <sup>1</sup>	
1	LDPE	lrganox 1076	Olive oil 100°C	2.17 ± 0.15	
		Irgafos 168	Sunflower oil 100°C	1.35 ± 0.065	
2	LDPE	Diphenylbutadiene	Olive oil 20°C	0.0504 ± 0.0054	
3	HDPE	Irganox 1076	Sunflower oil 40°C	0.437 ± 0.033	
		Irgafos 168	Sunflower oil 40°C	0.159 ± 0.018	
4	HDPE	Chimassorb 81	Olive oil 70°C	0.575 ± 0.014	
		Uvitex OB	Olive oil 70°C	0.122 ± 0.003	
5	PP	lrganox 1076	Olive oil 100°C	0.831 ± 0.061	
		Irgafos 168	Sunflower oil 100°C	$0.825 \pm 0.008$	
6	PP	Chimassorb 81	Olive oil 70°C	$0.650 \pm 0.022$	
		Uvitex OB	Olive oil 70°C	0.138 ± 0.003	
7	PP	Erucamide	Olive oil 70°C	0.718 ± 0.043	
		Trimethylolpropane	Water 70°C	$0.0303 \pm 0.0017$	
8	HIPS 6-7 %	lrganox 1076	Sunflower oil 70°C	< l.o.d.	
	mineral oil				
9	HIPS 1 %	Styrene	Olive oil 20°C	$0.014 \pm 0.0007$	
	mineral oil				
10	GPPS	Styrene	Olive oil 40°C	0.00499 ±	
				0.0003	
11	PET	Cyclic Trimer	Olive oil 175°C	11.1 ± 0.3	
12	PET	Tinuvin 1577	Olive oil 121°C	0.287 ± 0.047	
13	PVC rigid	Organotin stabilizer	Olive oil 100°C	0.429 ± 0.025	
14	plast.PVC	DEHA	Olive oil 40°C	10.2 ± 0.3	
15	PA nylon	Caprolactam	Water 100°C	1.55 ± 0.10	
16	PA 12	Laurolactam	Water 70°C	0.766 ± 0.079	

#### Table 2.2Results of SM testing in phase 1

<sup>1</sup> and standard deviation (SD)

Table 2.3:	Results	of	home	ogene	eity i	nvestiga	tions	in	phase	2:
	thickness	, de	ensity	and	initia	concen	tratior	n of	migrar	nts.

No	Polymer	Migrant	Thickness <sup>1</sup> (um)	Density <sup>1</sup> (g/cm <sup>3</sup> )	Migrant
			(P)	(9, )	(mg/kg)
01-2	LDPE	Irganox 1076	1011 ± 18	0.916 ± 0.001	601 ± 6
		Irgafos 168			536 ± 12
02-2	LDPE	DPBD	444 ± 7	$0.912 \pm 0.003^{-2}$	154 ± 3
04-2	HDPE	Chimassorb 81	310 ± 4	$0.942 \pm 0.004$	901 ± 3
		Uvitex OB			459 ± 5 <sup>2</sup>
05-2	PP	Irganox 1076	411 ± 1	$0.909 \pm 0.001$	1392 ± 61 <sup>2</sup>
		Irgafos 168			1538 ± 47
09-2	PS	Styrene	1946 ± 5	$1.027 \pm 0.005^{-2}$	365 ± 10 <sup>2</sup>
15-2	PA6	Caprolactam	107 ± 2	1.13 ± 0.01	2067 ± 19

<sup>1</sup> with between units standard deviation (SD)

<sup>2</sup> within units SD is given because mean squares within labs (MSW) < between labs (MSB).

#### 2.7 Methods used in the project

As mentioned earlier, the three certification parameters were the initial concentration of the migrants in the polymer ( $C_{P,0}$ ), the specific migration into a given food simulant under defined exposure conditions and the diffusion characteristics of the polymer – migrant combination. Methods for these three parameters were developed or adapted (from existing methods) and validated. For every polymer – migrant combination, a method for each certification parameter was available. A list of the methods used in phase one can be found on the project homepage (www.ivv.fraunhofer.de/growth/), those applied in the certification exercise are summarised in Tables 2.2 and 2.3. The diffusion measurement testing methods are published in a separate chapter (Stoffers *et al.* 2004b).

	1			
No	Polymer	Migrant	C <sub>P,0</sub> method	t l
01-2	LDPE	lrganox 1076	Precipitation /	HPLC/UV
		Irgafos 168	dissolution	
02-2	LDPE	DPBD	Extraction w/ hexane	Normal phase
				HPLC/FLD
04-2	HDPE	Chimassorb 81	Extraction w/ THF	HPLC/UV
		Uvitex OB		
05-2	PP	lrganox 1076	Precipitation /	HPLC/UV
		Irgafos 168	dissolution	
09-2	PS	Styrene	Dissolution in DMA	HS-GC/MS
15-2	PA6	Caprolactam	Extraction w/ 95%	GC/FID
			ethanol	

#### Table 2.4Methods applied in certification exercise for $C_{P,o}$ determination.

#### Table 2.5Methods applied in certification exercise for specific<br/>migration measurement.

No	Polymer	Migrant	SM method				
01-2	LDPE	lrganox 1076	Dilution with acetone	HPLC /UV or FLD			
		Irgafos 168					
02-2	LDPE	DPBD	Dilution with hexane	Normal phase			
				HPLC/FLD			
04-2	HDPE	Chimassorb 81	Dilution with acetone	HPLC/UV			
		Uvitex OB					
05-2	PP	lrganox 1076	Dilution with acetone	HPLC /UV or FLD			
		Irgafos 168					
09-2	PS	Styrene	Direct	HS-GC/MS			
15-2	PA6	Caprolactam	Direct	HPLC-UV			

## 2.8 Selection and preparation of materials for the certification exercise

Of the 16 candidate materials first selected, 5 materials were found to be unsuitable due to inhomogeneity (materials 6, 13), instability (material 7) or difficulties during migration measurement (materials 8, 11). Of the remaining materials, Material 12 was excluded from the list because the migrant (Tinuvin 1577 in PET) was not relevant for most of the test laboratories. Both polyamides were found to be suitable, but polyamide 6 (material 15) was chosen because it is used more frequently than PA12. Material 14 (PVC // diethylhexyl adipate (DEHA)) was selected to be in the trial certification exercise, however, no industrial partner with the ability to produce the amounts of thick plasticized PVC film needed could be found. As a substitute, material 2 was chosen to be in the trial certification exercise. The 6 new materials were produced in new larger batches (materials 01-2, 02-2, 04-2, 05-2, 09-2, 15-2); one using inhouse facilities of one of the partner labs, one was a commercial product, and 4 were tailor-made by external industry facilities. Larger quantities of the materials than in the first phase of the project were needed, therefore, 500 packs with 3 sheets or plaques (5 plaques each of material 09-2) were packed and labelled.

#### 2.9 Technical evaluation

A short summary of the methods applied in the certification exercise can be found in Tables 2.4 and 2.5. As can be observed, the same methods were applied for materials 01-2 and 05-2 (LDPE and PP with Irganox 1076 and Irgafos 168) as they both contained the same migrants. During the method development for these materials, several methods for determination of  $C_{P,O}$  were evaluated (dissolution, static extraction, soxhlet extraction). The best results were achieved using a dissolving / precipitation method (dissolution in hot toluene, precipitation with methanol). To compensate for any recovery losses of analytes encapsulated during the precipitation process, an internal standard (Tinuvin 234) was used. A difficulty that was encountered during all methods evaluated was the partial oxidation of the Irgafos 168 phosphite to the phosphate. The Irgafos 168 phosphate and Irganox 1076 peaks coeluted when using GC-FID with a common column such as DB-1, therefore, this method could not be used. When using reverse phase HPLC, all peaks could be well separated from each other. In order to solve the problem of partial oxidation, two sets of calibration standards were prepared – one for the Irgafos 168 phosphite and one for the phosphate form. The latter was fully oxidised with tetrahydrofuran or the peroxides in this solvent. Hence, it was possible to quantify the amounts of both forms of Irgafos 168 in the extract. The total concentration in the polymer was then calculated by adding up both amounts. For the specific migration determination, considerable effort had to be directed to the development of a method for Irganox 1076 and Irgafos 168 in oil. No effective way to separate the analytes from the oil could be found. Therefore, the oil was diluted with acetone and directly injected into the liquid chromatograph. For Irganox 1076 fluorescence detection (FLD) was used and for Irgafos 168 ultraviolet detection (UV) was used resulting in a slightly lower sensitivity for the latter migrant. Since olive oil caused more interference peaks than sunflower oil, the latter was used for the specific migration experiments.

For material 02-2, due to the lipophilic character of both the polymer and the migrant, extraction of DPBD from the LDPE film could easily be completed by static extraction with hexane. For the SM method the olive oil was diluted with hexane. The next step of analysis in both methods consisted of normal phase HPLC using an FLD or UV detector. Normal phase HPLC caused problems when changing a system from reverse phase to normal phase HPLC. Remains of water in the system deactivated the sorption of the normal phase column. A description of how to implement this change was inserted into the respective method descriptions. Both DPBD methods were adapted from Castle *et al.*(2001).

All other  $C_{P,0}$  and SM methods were straightforward. No difficulties were observed when methods were transferred from one laboratory to the other partners'.

## 2.10 Results for the certification exercise and confidence intervals

We will now present the raw data of the trial certification exercise. Within our small-scale interlaboratory studies with only 4 participating labs, we have judged the relative 95% half-width confidence intervals (relative HWCI) < 5% excellent; <10% very good; <20% good and <30% acceptable. The 95 % half-width confidence intervals were obtained from the Soft CRM program (Bonas *et al.* 1998). It includes both between and within laboratory variation. We want to emphasize that during a certification of reference materials in the future, a significantly larger group of laboratories will be necessary to obtain smaller confidence intervals of the certified values.

Table 2.6	Results of	$C_{P,0}$	investigations:	mean,	standard	deviation
	(SD) and ha	alfw	idth 95% confic	dence ir	nterval (HV	VCI).

No	Polymer	Migrant	Mean C <sub>P,0</sub> (mg/kg)	C <sub>P,0</sub> SD	С <sub>Р,0</sub> - 95% HWCI
01-2	LDPE	Irganox 1076	618	39	60
		Irgafos 168	585	58	114
02-2	LDPE	Diphenylbutadiene	121	2	4
04-2	HDPE	Chimassorb 81	891	36	42
		Uvitex OB	443	19	24
05-2	PP	Irganox 1076	1384	177	189
		Irgafos 168	1726	390	424
09-2	PS	Styrene	354	33	52
15-2	PA6	Caprolactam	2116	31	60

Table 2.7Results of specific migration tests; mean, standard<br/>deviation (SD) and half width 95% confidence interval<br/>(HWCI)

No	Food simulant	SM	Mean SM	SM	SM - 95%
	for SM	conditions	(mg/dm²)	SD	HWCI
01-2	Sunflower oil	2h / 100°C	1.79	0.09	0.21
	Sunflower oil	2h / 100°C	1.23	0.17	0.42
02-2	Olive oil	4h / 20°C	0.17	0.04	0.04
04-2	Olive oil	2h / 70°C	0.91	0.27	0.29
	Olive oil	2h / 70°C	0.20	0.04	0.04
05-2	Sunflower oil	4h / 100°C	1.55	0.10	0.16
	Sunflower oil	4h / 100°C	0.99	0.86	1.37
09-2	Olive oil	10d / 40°C	0.013	0.001	0.001
15-2	Water	2h / 40°C	0.99	0.12	0.19

## 2.10.1 Material no. 01-2: Low density polyethylene containing Irganox 1076 and Irgafos 168 as migrants

Material 01-2 is a LDPE film having a thickness of approximately 1000 microns and containing commonly applied amounts of Irganox 1076 (antioxidant) and Irgafos 168 (processing stabilizer). The material was tailor-made for the purpose of this project by Basell Polyolefins GmbH, Wesseling, Germany. The material was found to be homogeneous in

terms of thickness, density and concentration of the migrants (also see Table 2.3). Certification exercise results are shown in Tables 2.4 and 2.5. As can be seen in those tables, good agreement was obtained between results of the individual laboratories involved in the trial certification exercise. For Irganox 1076 content in the material ( $C_{P,0}$ ), the mean of lab means  $\pm$  relative half-width of 95% confidence interval (HWCI) was 618  $\pm$  9.7%, for Irgafos 168 results were a bit worse: 585  $\pm$  19.5%. The  $C_{P,0}$  results for material 1-2 are therefore evaluated as very good for Irganox 1076 and good for Irgafos 168.

The conditions for specific migration testing were 2 hours at 100°C, tested with sunflower oil as food simulant. As can be observed in Figure 2.2, results from one partner laboratory were statistically identified as an outlier (by both the Dixon and Nalimov outlier tests). This one laboratory did have large analytical problems with both analytes in oil matrices. The data were therefore eliminated. The mean of the remaining three lab means and the *relative variation* (RV) for specific migration of Irganox 1076 was 1.79 mg/dm<sup>2</sup> ± 12 %. The result of Irgafos 168 specific migration was 1.23 mg/dm<sup>2</sup> ± 34 %. Laboratories reported problems with the analysis owing to the low concentration and interferences from the sunflower oil. The detection limit of Irganox 1076 and Irgafos 168 in sunflower oil was in the range of 10 µg/g at the participating laboratories. During the migration test 35 % (Irganox 1076) and 28 % (Irgafos 168) of the initial amount of the additives was transferred into the simulant.

Regarding the data of Irganox 1076, this material appears to be a very good or good choice for a certified reference material. With respect to  $C_{P,0}$  (19% RV) of Irgafos 168, the material is suitable as reference material for determination of the content in the material. The data for specific migration of Irgafos 168 were not acceptable for a reference material mainly owing to analytical problems (partial oxidation of analyte / UV detection in oil). Nonetheless, we would suggest to include this migrant in a future CRM after having improved the analytical methodology. For the specific migration data, it needs to be explored whether the analytical method still can be improved or if the migration contact time should be extended in order to increase the concentration in the oil, which should facilitate analysis.



Figure 2.1  $C_{P,0}$  certification exercise results including within lab and between labs 95% confidence intervals: 01-2 Irganox 1076 and Irgafos 168 in LDPE.



Figure 2.2 Specific migration certification exercise results including within lab and between labs 95% confidence intervals: 01-2 Irganox 1076 and Irgafos 168 from LDPE into sunflower oil 2 hours / 100°C.

# 2.10.2 Material no. 02-2: Low density polyethylene containing 1,4-diphenyl-1,3-butadiene (DPBD) as a migrant

Material 02-2 is an LDPE film with a thickness of approximately 450  $\mu$ m, spiked with 200 mg/kg of the fluorescent dye 1,4-diphenyl-1,3-butadiene (DPBD). The film was produced in the facilities of the project partner Fraunhofer IVV, Freising, Germany and was found to be homogeneous in terms of thickness, density and concentration of the migrants (Table 2.3). As can be seen in Table 2.6 and Figure 2.3, the laboratories' results for  $C_{P,0}$  determination matched quite well. The mean of means value for  $C_{P,0}$  ± relative HWCI obtained from the trial certification exercise was 121.4 mg/kg ± 3,1 % which can be considered excellent. The Cochran test on variances identified one outlier at 95 % confidence interval. The referring laboratory did not use a fluorescence detector for the analysis, which was the detection method, recommended for the analyte and obtained a higher variation of results (see Figure 2.3, Lab 2). The result was not eliminated.

Specific migration into olive oil was tested at 20°C for 4 hours (total immersion), 35 % of the initial DPBD migrated into the oil. The mean of means specific migration value  $\pm$  relative HWCI obtained was 0.17 mg/dm<sup>2</sup> $\pm$  23.5 %. The results for the SM certification exercise (24% RV) are acceptable; especially due to the low concentrations to be analysed here.

Overall, the material-migrant combination LDPE containing diphenylbutadiene is considered as a very good choice for a certified reference material.



Figure 2.3 Certification exercise results including within lab and between labs 95% confidence intervals :  $02-2 C_{P,0}$  DPBD in LDPE; specific migration of DPBD from LDPE into olive oil 4 hours /  $20^{\circ}$ C

# 2.10.3 Material no. 04-2: High density polyethylene containing Chimassorb 81 and Uvitex OB as migrants

Material 04-2 is a HDPE film with a thickness of approximately 300 microns containing the two commonly applied polymer additives Chimassorb 81 (UV absorber) and Uvitex OB (fluorescent whitening agent). The film was tailor-made for the purpose of this project by Rapra Technology Ltd, Shropshire, England and was found to be homogeneous in terms of thickness, density and concentration of the migrants in the polymer (Table 2.2). The  $C_{P,0}$  results of the certification exercise can be seen in Table 2.6 and Figure 2.4. Very good results with regard to the  $C_{P,0}$  determination of both analytes have been obtained. For the concentration of Chimassorb 81 in the polymer, the mean of lab means and relative HWCI were 891.3 mg/kg ± 4.7 %. The respective data for Uvitex OB were 443.2 mg/kg ± 5.4 %.

Specific migration into olive oil was tested at 70°C for 2 hours (singlesided contact). The results are summarised in Table 2.7. After contact 35 % Chimassorb 81 and 16 % Uvitex OB were transferred from the film into the oil. The mean of lab means and relative HWCI for Chimassorb in HDPE was 0.91 mg/dm<sup>2</sup>± 32 %, the respective result for Uvitex OB was 0.20 mg/dm<sup>2</sup> ± 20.0 %. SM results Chimassorb 81 (32% RV) are just outside the acceptable range applied here, those for Uvitex OB (20% RV) are considered acceptable. In order to include the specific migration of Chimassorb 81 into oil in a certification study, the analytical method for Chimassorb 81 in olive oil would have to be improved.


Figure 2.4 C<sub>P,0</sub> certification exercise results including within lab and between labs 95% confidence intervals: 04-2 Chimassorb 81 and Uvitex OB in HDPE.



Figure 2.5 Specific migration certification exercise results including within lab and between labs 95% confidence intervals: 04-2 Chimassorb 81 and Uvitex OB from HDPE into olive oil 2 hours / 70°C.

#### 2.10.4 Material no. 05-2: Polypropylene containing Irganox 1076 and Irgafos 168 as migrants

Material 05-2 is a PP film with a thickness of approximately 1000 microns and contains commonly applied amounts of Irganox 1076 (antioxidant) and Irgafos 168 (processing stabilizer). Like material 01-2, the material was made for the purpose of this project by Basell Polyolefins GmbH, Wesseling, Germany. It was found to be homogeneous in terms of thickness, density and initial concentration of the migrants (Table 2.3). The results of the certification exercise for the concentrations of both additives in the material are shown in Table 2.6 and Figure 2.6. For Irganox 1076 the mean of lab means  $\pm$  relative HWCI was 1384 mg/kg  $\pm$ 13.7 %. There was more variation in the results for Irgafos 168: 1726 mg/kg  $\pm$  24.7 %. Results for Irganox 1076 are considered good for  $C_{P,0}$ and acceptable for Irgafos 168.

The conditions for specific migration testing were 4 hours at 100°C (single-sided contact), tested with sunflower oil as food simulant. The results are summarised in Table 2.7 and Figure 2.7. The mean of means with relative HWCI of the specific migration test for Irganox 1076 was 1.55 mg/dm<sup>2</sup>± 10 % and therefore considered good. The result of specific migration of Irgafos 168 was 0.99 mg/dm<sup>2</sup> ± 138 %. Laboratories reported large problems with the analysis mainly owing to interferences from the sunflower oil and the migration value just above the detection limit (10  $\mu$ g/g). The same problems that arose with material 01-2 occurred here but much more obvious due to the lower migration value. The results of specific migration of Irgafos 168 are not acceptable for a reference material. At migration contact 29 % Irganox 1076 and 16 % Irgafos 168 were transferred into the simulant. Increasing the temperature or extending the contact time in order to increase the amount of migrating Irgafos 168 would not be recommendable because changes in the material or oxidation effects in the oil would be expected. To include a specific migration value in the certified parameters of such a material, the simulant might have to be changed to an alternative fat simulant such as 95 % ethanol.



Figure 2.6  $C_{P,0}$  certification exercise results including within lab and between labs 95% confidence intervals: 05-2 Irganox 1076 and Irgafos 168 in PP.



Figure 2.7 Specific migration certification exercise results including within lab and between labs 95% confidence intervals: 05-2 Irganox 1076 and Irgafos 168 from PP into sunflower oil 4 hours / 100°C.

## 2.10.5 Material no. 09-2: Polystyrene containing styrene as a migrant

Material 09-2 consists of PS plaques with a thickness of approximately 2 mm, containing the monomer styrene retained in the polymer after production. The plates were produced by BASF GmbH, Ludwigshafen, Germany for the purpose of this project and were found to be homogeneous in terms of thickness, density and concentration of styrene in the polymer (Table 2.3). The laboratories' results for  $C_{P,0}$  determination matched quite well (Table 2.6 and Figure 2.8). The mean of means value for  $C_{P,0}$  and relative HWCI obtained from the trial certification exercise was 354 mg/kg ± 14.7 %. The  $C_{P,0}$  results for styrene are considered good. Specific migration into olive oil was tested at 40°C for 10 days (single-sided contact). The mean of means specific migration value and relative HWCI obtained was 0.013 mg/dm<sup>2</sup> ± 7.7 %. The specific migration result is quite low and only 1.8 % of initial styrene migrated during the oil contact, but very good in terms of precision.

## 2.10.6 Material no. 15-2: Polyamide 6 containing caprolactam as a migrant

Material 15-2 is a PA6 film with a thickness of approximately 100 microns containing the monomer caprolactam at a common concentration level. It is a commercially used film provided by MF-Folien GmbH, Kempten, Germany and was found to be homogeneous in terms of thickness, density and concentration of styrene in the polymer (Table 2.3). Among the laboratories'  $C_{P,0}$  results, one outlier was identified according to the Dixon and Nalimov outlier tests. The respective laboratory did have problems with the analysis of this particular analyte, therefore, the outlier was eliminated. The mean of lab means value and HWCI for  $C_{P,0}$  after elimination of the outlier were 2116 mg/kg ± 2.8 % (including the outlier 2190 mg/kg ± 10.8 %), which is considered excellent.

Specific migration into water was tested at 40°C for 2 hours (single-sided contact) and the mean of means obtained was 0.99 mg/dm<sup>2</sup> with a relative 95% H.W C.I of 19 % meaning that 39% of the caprolactam present in the polymer migrated into the water. Results can be seen in Figure 2.9 as well as Table 2.7. Results for specific migration testing are considered good (19% RV). All in all this material seems to be a very good choice for a certified reference material especially for specific migration in contact with aqueous foods.



Figure 2.8 Certification exercise results including within lab and between labs 95% confidence intervals : 09-2  $C_{P,0}$  of styrene in PS; specific migration of styrene from PS into olive oil 10 days / 40°C.



Figure 2.9 Certification exercise results including within lab and between labs 95% confidence intervals : 15-2  $C_{P,0}$  of caprolactam in PA6; specific migration of caprolactam from PA6 into water, 2 hours / 40°C.

## 2.11 Discussion

The results of this feasibility study are very encouraging for a possible certification study in the future. The 6 candidate materials passed the certification exercise. Only Chimassorb 81 and Irgafos 168 caused analytical problems in the matrix oil, which were not solved in the project. This could be overcome by using 95 % ethanol as alternative fat simulant for these migrants.

The 6 candidate CRMs contain three polyolefins and three non-polyolefins that are among the most used food packaging materials and therefore very relevant for testing laboratories. The selection covers a broad range of polymers as well as migrant polarities. Also, a range of molecular sizes from the rather volatile styrene (104 daltons) and the small caprolactam (113 daltons) to the large Irgafos 168 (647 daltons) is represented. Furthermore, several contact times and temperatures have been evaluated. Material 02-2 (LDPE // DPBD) was tested at 20°C, material 01-2 (LDPE // Irganox 1076 / Irgafos 168) was tested at 100°C. Polystyrene (material 09-2) was tested for 10 days, materials 01-2, 04-2 and 15-2 have been tested for 2 hours. It was attempted to have migration values where 30% to 70% of the total amount of migrant in the polymer were transferred to the food simulant. This was roughly managed with three exceptions. The amount of Uvitex OB migrated from HDPE into olive oil and the amount of Irgafos 168 migrated from PP into sunflower oil were both 16% of the respective initial concentrations in the polymer. And, due to the rather inert character of polystyrene, only 1.8% of the styrene migrated into olive oil after 10 days.

Of the 7 migrants present in the 6 materials, 4 have specific migration limits and are therefore very relevant for conformity testing laboratories (Irganox 1076 and Chimassorb 81: 6 mg/kg, Uvitex OB: 0.6 mg/kg and Caprolactam: 15 mg/kg). Of the remaining three migrants the processing stabiliser Irgafos 168 is frequently used, styrene is a monomer that is always present in polystyrenes and DPBD is a recommended test substance to experimentally establish the diffusion behaviour of polymers according to the guide "Estimation of migration by generally recognised Diffusion models in support of Directive 2002/72/EC" Annex B (published in EU Commission Practical Guide, Section 3 Annex I. EC 2003a).

The additives are also very important for determination of the concentrations in the materials, which is - beside conformity issues - important especially for industry laboratories. Recent proficiency tests have shown the difficulties during polymer additive analysis and the necessity of reference materials for it (Bart *et al.* 2001, Ritter *et al.* 2003). As a rather volatile monomer it is a good substance to check a

laboratories' proficiency to deal with volatile food contact substances. On the other hand, styrene is discussed as a substance to be regulated by an SML in future (Synoptic Document, EC 2003b). DPBD is the test substance for the fatty contact test (EN 14481 2003). With this test, the fatty character of food in contact with packaging can be investigated (Castle *et al.* 2001). The LDPE reference film of that test (certified reference material BCR-593) has a thickness of only 150 µm. This way, total transfer of the migrant into the fat can happen within a short time. With the thicker material 02-2, time dependent transfer into foods can be studied excellently which is why this material is already used in various research projects (e.g. EU project, www.foodmigrosure.com).

There is high interest in the methods developed within the project, therefore it is planned to publish the compilation of methods either as EU-Report, or in scientific journals. The results of the project as well as materials themselves have a great potential for further use in migration method validation (including mathematical modelling and proficiency testing, see chapters 3 and 8 for details) and migration research. We have previously published the results of our investigations where materials from and methods developed during this project have been applied (Stoffers et al. 2003a, 2003b, 2004a). Furthermore, a new European research project based on these results and materials has already started ("Foodmigrosure" QLRT-2001-02390) with the aim of modelling migration from food contact materials in foods itself as cost efficient tool for exposure estimates. Mathematical modelling gets more and more important for conformity testing of food contact materials. With the future reference materials, test laboratories can check their proficiency in determining the migration kinetics as a basis for modelling the behaviour of polymers with unknown diffusion properties and check their proficiency in modelling the data.

This feasibility study gives the basis for future production of very useful CRMs for specific migration testing as well as determination of additive content in the material. The data and materials produced during the project have already shown their benefits for migration research with the aim to simplify compliance testing and increase consumer safety.

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# 3

Feasibility study for the development of certified reference materials for specific migration testing. Part 2: Estimation of diffusion parameters and comparison of experimental and predicted data

### Abstract

This chapter describes the second part of a project with the main objective to develop the know-how to produce certified reference materials (CRMs) for specific migration testing. Certification parameters discussed in this chapter are the diffusion coefficient  $D_{P}$ , the respective polymer-specific coefficient  $A_{P}$  of the migrant polymer combinations and the partitioning coefficient  $K_{P,F}$  describing the partitioning of the migrant between the polymer and a food simulant. The parameters were determined for 16 preliminary candidate CRMs, each parameter was determined by one laboratory. The 6 materials most suitable as reference materials were selected and the parameters were then determined by 4 laboratories. The coefficients resulting from this small scale interlaboratory comparison study can be regarded as the most reliable values available to date. These coefficients were applied for a comparison of experimental and predicted migration data. The experimental migration data arose from the same project and were determined by one laboratory for the first 16 materials and subsequently by 4 laboratories for the 6 materials selected in the second phase. Overall, experimental and predicted migration data fit together guite well. Roughly half of the predicted data were within  $\pm 10\%$ , almost all predicted data were within  $\pm 40\%$  compared to the experimental data.

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## 3.1 Introduction

In Chapter 1 the motivation for as well as results of the EU funded project "Specific Migration" – a feasibility study for the development of certified reference materials for specific migration testing has been described (Stoffers *et al.* 2004b). Within this project, 3 possible certification parameters – the initial concentration of the migrant in the polymer ( $C_{P,0}$ ), specific migration of the migrant into a food simulant under certain time / temperature conditions (SM) and diffusion characteristics of the polymer - were determined. Chapter 1 (Stoffers *et al.* 2004b) focused on the results of  $C_{P,0}$  and SM. In this chapter the outcome of the project with regard to the diffusion kinetics investigations and a comparison of experimental and predicted migration data will be presented.

### 3.1.1 Theory

The transport of components within a polymer can be generally described by Fick's second law of diffusion.

$$\frac{\partial C_P}{\partial t} = D_P \frac{\partial^2 C_P}{\partial x^2}$$
 (equation 3.1)

where  $C_P$  is the concentration of a migrant in a food contact polymer at time t at a distance x from the origin of the x-axis (for single-sided contact) and  $D_P$  is the constant diffusion coefficient in the polymer. When Crank (1975) established an analytical solution for Fick's second law, one of his assumptions was that the migrant transport from polymer to food takes place at the contact side and the mass balance is described as:

$$K_{P,F}\left(\frac{V_F}{A}\right)\frac{\partial C_F}{\partial t} = -D_P \frac{\partial C_P}{\partial x}$$
 at  $x = L_P$   $t > 0$  (equation 3.2)

where  $K_{P,F}$  is the partitioning coefficient describing the partitioning of the migrant between the polymer and the food,  $V_F$  is the volume of food and A is the contact area. All further details on the solution of this equation for migration have been described in detail earlier (Piringer 1994, Piringer and Baner 2000).

Piringer and co-workers (Piringer 1993 and 1994, Brandsch et al. 2000) have developed an empirical relationship of the diffusion coefficient with

the relative molecular weight of the migrant, the temperature and the polymer type. A material specific coefficient  $A_P$ , expressing the "diffusion conductance" of a polymer was introduced. The Arrhenius type relationship between  $A_P$  and the parameters above is given as:

$$D_{p} = 10^{4} \cdot e^{A_{p} - 0.1351 \cdot M_{r}^{2/3} + 0.003 \cdot M_{r} - 10454/T}$$
 (equation 3.3)

where  $A_P$  is the polymer specific coefficient,  $D_P$  is the diffusion coefficient (cm<sup>2</sup> s<sup>-1</sup>),  $M_r$  is the relative molecular weight of the migrant in Daltons and T is the temperature in K.

For regulatory purposes,  $D_P$  is replaced by an upper limit diffusion coefficient  $D_P^*$ . This value exceeds diffusion coefficients available within the database gathered in the EU funded project SMT4-CT98-7513 at the 95% confidence level. (Hinrichs and Piringer 2001). Applying such a  $D_P^*$ will give a worst case / overestimated migration (Practical Guide, EC 2003). A new approach to determine the worst case  $D_P^*$  values for migration modelling has recently been developed and will be introduced shortly (Begley *et al.* 2004).

## 3.2 Materials and methods

The polymers used have been described in detail in the previous chapter no. 2 (Stoffers *et al.* 2004b). The experimental methods are described in the following section.

### 3.2.1 Migration experiments

Migration experiments were carried out at three different temperatures using food simulants that did not penetrate the polymers. The testing conditions applied during the diffusion experiments can be found in Table 3.2. Experiments were carried out in migration cells for one-sided contact with a circular contact area of 0.48 dm<sup>2</sup> (MigraCell, FABES GmbH, Munich, Germany). The sample film was fixed between the lid and the beaker tightened against both with two PTFE-coated o-rings. The migration cell was held together by a clamp. The upper side of the film was the food contact side; in case of very thin films (<100µm), a metal plate was fixed on the non food contact area. For the migration tests the cells were pre-heated at the desired temperature (see Table 3.2), the

desired amount of food simulant was then filled into the cell through the opening of the lid and the cell was then incubated at a defined temperature. After each defined time interval, 250µl of the food simulant was removed from the migration cell. Upon cooling down, 100µl of this solution was placed into an autosampler vial, and 50µl of an internal standard solution was added. This solution was then analysed by HPLC or GC. The simulant removed from the migration cells during sampling was not replaced. Gandek (1986) investigated the error caused by replacing the simulant during diffusion measurements and found out that it was smaller when the simulant was not replaced.

For diffusion measurement of styrene from polystyrene into Tenax<sup>®</sup>, the following approach was taken: For each sampling time, one migration test cell was set up. The cells were filled with approximately 2 g of Tenax<sup>®</sup>. After the respective time intervals, the Tenax<sup>®</sup> was removed from the cell and extracted with two 10 ml aliquots of diethyl ether. 50 µg of internal standard (m-methylstyrene) was then added to the combined extracts and the solvent was evaporated to a volume of 5 ml under a gentle air stream.

#### 3.2.2 Analytical methods

Some of the analytical methods applied have been previously reported in the literature. See Table 3.1 for details. All other methods are described in the following section.

## 3.2.2.1 Irganox 1076 and Irgafos 168, Chimassorb 81 and Uvitex OB

Simulant samples were analysed, after addition of internal standard (Tinuvin 234 for Irganox 1076 and Irgafos 168, Tinuvin 327 for Chimassorb 81 and Uvitex OB), using an a Shimazu Class 10 HPLC system with ultraviolet (UV) detection according to the following HPLC conditions: The column was a Sphereclone ODS2 (250 x 4.6 mm) and the mobile phase was 1 ml/minute of 95% aqueous ethanol (isocratic). The UV wavelength monitored was 230 nm for Irganox 1076 and Irgafos 168, Chimassorb detection was performed at 290 nm and Uvitex OB at 374 nm. The injection volume was 20  $\mu$ l.

Material Nos.	Migrant	Food simulant	Analytical method	Reference
1, 3, 5, 8, 01-2, 05-2	lrganox 1076	95% ethanol	HPLC / UV	
1, 3, 5, 01-2, 05-2	Irgafos 168	95% ethanol	HPLC / UV	
2, 02-2	DPBD	95% ethanol	HPLC / FLD	Castle <i>et al.</i> 2001
4, 6, 04-2	Chimassorb 81	95% ethanol	HPLC / UV	
4, 6, 04-2	Uvitex OB	95% ethanol	HPLC / UV	
7	TMP	water	LC/MS	
7	Erucamide	95% ethanol	GC/FID	Cooper and Tice 1995
9, 10, 09-2	Styrene	Tenax®	HS-GC/MS	
12	Tinuvin 1577	PET	HPLC-UV	
13	Organo-Tin	PVC	GC/AED	EDANA 2001
14	DEHA	soft PVC	GC/FID	O'Brien <i>et al.</i> 1997
15, 15-2	Caprolactam	Isooctane	GC/FID	Franz and Rijk 1997
16	Laurolactam	lsooctane	GC/FID	Stoffers et al. 2003a

Table 3.1Analytical methods applied for diffusion kinetics testing

#### 3.2.2.2 Styrene

Ether extracts of Tenax<sup>®</sup> were analysed, after addition of internal standard (m-methylstyrene), using a GC/MS system applying the following conditions: The column was a DB17 (30m x 0.25mm, 0.15µm film layer), the mass selective detector was operated in selected-ion-monitoring-mode (m/z 78 and 104 for styrene and 91 and 117 for m-methylstyrene).

#### 3.2.2.3 Tinuvin 1577

Simulant samples (isooctane) were evaporated to dryness, and redissolved in acetonitrile. After addition of internal standard (Chimassorb 81) they were analysed using a Shimazu Class 10 HPLC system with ultraviolet (UV) detection according to the following HPLC conditions: The column was a Phenomenex Luna C18 (250 x 4.6 mm) and the mobile phase was 1.5 ml/minute of 95% acetonitrile and 5% tetrahydrofuran (isocratic). The UV wavelength applied was 274 nm. The injection volume was 20 µl.

#### 3.2.2.4 1,1,1-Trimethylolpropane (TMP)

Water samples were directly analysed using HPLC/MS: Thermo Finnigan LCQ Deca (ion trap) applying atmospheric pressure chemical ionisation according to the following HPLC conditions: The column was a Nucleosil 10CN (250 x 4.6 mm) with the mobile phase being 30% methanol in water at a flow of 1.0 ml/min. The detector was operated in MS/MS (SRM) mode, parent molecule m/z 134.9, fragment m/z 80.3. The injection volume was 20  $\mu$ l.

### 3.2.3 $D_P$ , $K_{P,F}$ and $A_P$ determination

During the first phase of the project diffusion kinetics at three different temperatures of each of the 16 materials were investigated by one laboratory only. The 6 most suitable newly produced materials were subjected to diffusion testing by all four laboratories participating in the project. After the kinetics studies described above had been completed, the diffusion coefficient  $D_P$ , and the partitioning coefficient  $K_{P,F}$  were determined either by manual curve-fitting using Migratest Lite 2001 (changing coefficients until the predicted curve fitted the experimental data) or by automated curve fitting using the software Athena Visual Workbench (www.athenavisual.com) as described in a different chapter (Stoffers *et al.* 2003b). In both cases,  $A_P$  was then calculated from the determined  $D_P$  value using equation 3.3.

## 3.2.4 Comparison of experimental and predicted migration data

Within the project, the migration tests as well as the kinetic measurements have been performed using the 16 preliminary materials produced in the first phase and then the 6 candidate CRMs produced in the second phase. The 16 materials produced in phase 1 were tested for specific migration by one laboratory and the diffusion kinetics were derived by one of two partner laboratories. In the second phase of the project, all 6 candidate CRMs were investigated in terms of specific migration and diffusion kinetics by all 4 project partners. Hence, experimental migration data and all parameters required for migration modelling were available. For verification of the  $A_{P}$ ,  $D_{P}$  and  $K_{P,F}$  values derived from the diffusion kinetics measurements, the results of the specific migration experiments into oils or water at specific time and

temperature conditions were used (see Stoffers *et al* 2004b for details). The migration results obtained in the specific migration experiments were compared with predicted migration values obtained from the migration model (Practical Guide EC 2003), using the derived  $A_P$ ,  $D_P$  and  $K_{P,F}$  values. Where no  $A_P$ -values for the exact testing temperatures were available, they were linearly interpolated (e.g. for experimental migration at 70°C the  $A_P$ -value was estimated from the available data for 60 and 80°C). The experimental and predicted values were then compared and evaluated.

## 3.3 Results / Discussion

Diffusion kinetics of the first 16 materials were determined in one of the partner laboratories. Experimental data were used for parameter estimation in order to determine  $D_P$  and  $K_{P,F}$  values. The respective  $A_P$ values were then calculated applying equation 3.3. The results are listed in Tables 3.2, 3.3 and 3.4. No data could be generated for material 8 (migration below detection limit), or for materials 11 and 13 (no curvefitting possible due to scattered results). For the 6 materials selected for the certification exercise, all four partner laboratories submitted their experimental diffusion data and again, the best fitting  $D_P$ ,  $A_P$  and  $K_{PF}$ values were determined. An example of experimental results of the four laboratories along with the fitted graph can be found in Figure 3.1 (diffusion of Chimassorb 81 from HDPE into 95% ethanol). The mean results of the individual 6 materials investigated in the trial certification exercise are also presented in Tables 3.2, 3.3 and 3.4.  $D_P$  results are discussed below (all  $D_P$  values in cm<sup>2</sup>/s) for the 6 candidate CRMs. In order to assess the results in this chapter, we have calculated a guotient by considering the range in which the laboratories' results were. By dividing the maximum value by the minimum value observed by either of the laboratories, we obtained a quotient. We considered the results good when the results of all partner labs were inside one order of magnitude (quotient less than 10), very good when the quotient was less than 5), acceptable when the quotient was between 10 and 15, and not acceptable when it was above 15.



Figure 3.1 Experimental diffusion data along with data predicted by migration model after curve-fitting; Chimassorb 81 from HDPE into a solution of 95% ethanol at a) 40°C, b) 60°C and c) 80°C.

### 3.3.1 $D_P$ determination

3.3.1.1 Material no. 01-2: LDPE containing Irganox 1076 and Irgafos 168

Material 01-2 is a LDPE film having a thickness of 1011 µm and containing 618 mg/kg of Irganox 1076 (antioxidant) and 584 mg/kg Irgafos 168 (processing stabilizer). For migration kinetics into 95% ethanol, very good agreement was obtained with all four laboratories at 40°C for both analytes with diffusion coefficients ( $D_p$ ) in the range of 1.5 E-09 to 3.0 E-09 for Irganox 1076 and 2.7 E-10 to 4.5 E-10 for Irgafos 168. At 60°C, two laboratories obtained higher results than possible from total mass transfer calculations (migration >  $C_{P,0}$  due to the presence of interfering substances in the exposed simulant) for Irganox 1076 and were eliminated. Very good agreement was obtained for the other two laboratories with  $D_P$  values ranging from 9.9 E-09 to 1.2 E-08. For Irgafos 168, the agreement between all four labs was also very good with  $D_{P}$ values of 2.7 E-09 and 7 E-09. At 80°C, two laboratories obtained higher results than possible from total mass transfer calculations of Irganox 1076 (as previously) and were eliminated. Very good agreement was obtained for the other two laboratories with  $D_{P}$  values of 3.5 E-08 and 6.0 E-08. For Irgafos 168, and after removal of one laboratory owing to migration >  $C_{P,0}$  the agreement between labs was very good with  $D_P$  values ranging from 1.6 E-08 to 2.1 E-08.

3.3.1.2 Material no. 02-2: LDPE containing 1,4-diphenyl-1,3butadiene (DPBD)

Material 02-2 is an LDPE film with a thickness of 444  $\mu$ m, containing 121 mg/kg of the fluorescent dye DPBD. For migration kinetics into 95% ethanol, good agreement was obtained with all four laboratories at 40°C with  $D_P$  values in the range of 1.1 E-08 to 7.1 E-08. At 60°C, very good agreement was obtained for all four laboratories with  $D_P$  values ranging from 0.5 E-07 to 1.3 E-07. At 80°C, very good agreement was also obtained for all four laboratories with  $D_P$  values ranging from 1.8 E-07 to 4.2 E-07.

## 3.3.1.3 Material no. 04-2: HDPE containing Chimassorb 81 and Uvitex OB

Material 04-2 is a HDPE film with a thickness of 310 µm containing 891 mg/kg of the UV absorber Chimassorb 8 and 1 443 mg/kg of the fluorescent whitening agent Uvitex OB. For migration kinetics into 95% ethanol, very good agreement was obtained with all four laboratories at 40°C for Chimassorb 81 with  $D_p$  values in the range of 4.0 E-10 to 9.8E-10. For Uvitex OB at 40°C, acceptable agreement was obtained with  $D_p$  values ranging from 1.3 E-11 to 1.4 E-10. At 60°C, very good agreement was obtained with  $D_p$  values ranging from 5.1 E-09 to 9.3 E-09 for Chimassorb 81 and 7.7 E-10 to 1.0 E-09 for Uvitex OB. At 80°C agreement between the four labs was very good with  $D_p$  values ranging from 2.5 E-08 to 3.7 E-08 for Chimassorb 81 and 6.8 E-09 to 1.0 E-08 for Uvitex OB.

## 3.3.1.4 Material no. 05-2: Polypropylene containing Irganox 1076 and Irgafos 168

Material 05-2 is a PP film with a thickness of 410 µm and containing 1384 mg/kg of Irganox 1076 and 1726 mg/kg of Irgafos 168. For migration kinetics into 95% ethanol, very good agreement was obtained with all four laboratories at 40°C for Irganox 1076 with  $D_P$  values in the range of 6.0 E-12 to 7.0 E-12. For Irgafos 168 at 40°C, very low migration values were obtained and two laboratories reported the value below their limit of detection. The other two laboratories had very good agreement with  $D_P$  values of 2.7 E-13 and 6.0 E-13. At 60°C, one laboratory obtained higher results than possible from total mass transfer calculations (migration >  $C_{P,0}$ ) for Irganox 1076 (as previously) and was eliminated. Very good agreement was obtained for the other three laboratories with  $D_P$  values ranging from 1.3 E-10 to 2.2 E-10. For Irgafos 168 at 60°C, the agreement between all four labs was very good with  $D_P$ values ranging from 9.0 E-12 to 2.0 E-11. At 80°C, two laboratories obtained higher results than possible from total mass transfer calculations of Irganox 1076 (as previously) and were eliminated. Very good agreement was obtained for the other two laboratories with  $D_{P}$  values of 2.1 E-09 and 2.4 E-09. For Irgafos 168, the agreement between labs was also very good with  $D_{P}$  values ranging from 2.3 E-10 to 7.0 E-10.

#### 3.3.1.5 Material no. 09-2: Polystyrene containing styrene

Material 09-2 consists of PS plaques with a thickness of 1946  $\mu$ m, containing 354 mg/kg of residual styrene monomer in the polymer. Regarding diffusion kinetics onto Tenax<sup>®</sup>, good agreement was obtained with all four laboratories at 40°C with  $D_P$  values in the range 1.0 E-13 to 5.0 E-13 for styrene. At 60°C, very good agreement was obtained for three of the four laboratories with  $D_P$  values ranging from 0.9 E-12 to 1.1 E-12 (after removing the data from one outlying laboratories with  $D_P$  values ranging from 3.5 E-12 to 7.0 E-12 (after removing the data from one outlying laboratories with  $D_P$  values ranging the data from one outlying laboratories with  $D_P$  values ranging the data from one outlying laboratories with  $D_P$  values ranging from 3.5 E-12 to 7.0 E-12 (after removing the data from one outlying laboratories with  $D_P$  values ranging from 3.5 E-12 to 7.0 E-12 (after removing the data from one outlying laboratories with  $D_P$  values ranging from 3.5 E-12 to 7.0 E-12 (after removing the data from one outlying laboratories with  $D_P$  values ranging from 3.5 E-12 to 7.0 E-12 (after removing the data from one outlying laboratories with  $D_P$  values ranging from 3.5 E-12 to 7.0 E-12 (after removing the data from one outlying laboratory).

#### 3.3.1.6 Material no. 15-2: Polyamide 6 containing caprolactam

Material 15-2 is a PA6 film with a thickness of 107  $\mu$ m containing 2116 mg/kg of the monomer caprolactam. For migration kinetics into isooctane, very good agreement was obtained with three of the four laboratories at 40°C with  $D_P$  values in the range of 0.7 E-12 to 1.0 E-12 for caprolactam. One laboratory could not report any results because the concentration of the migrant in the solvent was below their limit of detection. At 60°C, good agreement was obtained for the four laboratories with  $D_P$  values ranging from 1.3 E-12 to 7.7 E-12. At 80°C, good agreement was also obtained for the four laboratories with  $D_P$  values ranging from 1.0 E-11 to 8.3 E-11.

#### 3.3.2 $K_{P,F}$ determination

Partitioning coefficients were not one of the certification parameters of the project covered by this chapter. Their determination strongly depends on the duration of the migration experiments. In order to determine  $K_{P,F}$  values with small confidence intervals, it is necessary to have enough data point close to the partitioning equilibrium. With the relatively thick materials applied, it was not always possible to reach equilibrium within a reasonable time frame. Furthermore, the partitioning coefficient in oil and applied simulants is likely to differ due to the difference in solubility of the migrants. This is likely to be one of the reasons for the deviations when comparing experimental and predicted migration values. A general trend that could be observed is that the migrants affinity for the food simulant phase generally increased with temperature.

No.	Polymer	Migrant	No. of labs	Food simulant		D	iffusion c	oefficient	:s (cm <sup>2</sup> s <sup>-1</sup> )		
			considered		5°C	20°C	40°C	60°C	70°C	80°C	100°C
01	LDPE	Irganox 1076	1/1/1	95% Ethanol			2.2E-09	1.2E-08		4.8E-08	
01	LDPE	Irgafos 168	1/1/1	95% Ethanol			3.3E-10	3.3E-09		1.4E-08	
02	LDPE	DPBD	1/1/1	95% Ethanol			1.2E-08	8.7E-08		3.3E-07	
03	HDPE	Irganox 1076	1/1/1	95% Ethanol			3.9E-11	4.3E-10		3.3E-09	
03	HDPE	Irgafos 168	1/1/1	95% Ethanol			2.1E-12	7.2E-11		4.5E-10	
04	HDPE	Chimassorb	1/1/1	95% Ethanol			5.0E-10	2.7E-09		1.9E-08	
04	HDPE	Uvitex OB	1/1/1	95% Ethanol			4.5E-11	4.5E-10		4.7E-09	
05	PP (homo)	Irganox 1076	1/1/1	95% Ethanol			8.7E-12	2.1E-10		3.0E-09	
05	PP(homo)	Irgafos 168	1/1/1	95% Ethanol			2.4E-13	8.8E-12		2.0E-10	
90	PP(random)	Chimassorb	1/1/1	95% Ethanol			5.7E-10	4.2E-09		2.5E-08	
06	PP(random)	Uvitex OB	1/1/1	95% Ethanol			3.5E-11	7.0E-10		6.2E-09	
07	ЬР	TMP	1/1/1	Water			7.0E-13	3.0E-11		1.1E-9	
07	ЬР	Erucamide	1/1/1	95% Ethanol			2.0E-11	8.3E-10		2.0E-08	
60	HIPS(oil)	Styrene	1/1/1	Tenax®			8.1E-13	6.0E-12	3.5E-11		
10	GPPS	Styrene	1/1/1	Tenax®			3.0E-13	2.2E-12	7.9E-12		
12	PET	Tinuvin 1577	1/1/1	Isooctane						1.6E-14	7.1E-13
14	plast. PVC	DEHA	1/1/1	50% Ethanol	2.7E-12	2.3E-11	3.3E-10				
15	PA6	Caprolactam	1/1/1	Isooctane			5.7E-12	4.2E-11		2.3E-10	
16	PA12	Laurolactam	1/1/1	Isooctane			3.0E-12	6.7E-12		1.3E-10	
01-2	LDPE	Irganox 1076	4/2/2	95% Ethanol			2.1E-09	1.1E-08		4.8E-08	
01-2	LDPE	Irgafos 168	4/4/4	95% Ethanol			3.7E-10	4.8E-09		1.9E-08	
02-2	LDPE	DPBD	4/4/4	95% Ethanol			3.1E-08	9.7E-08		2.8E-07	
04-2	HDPE	Chimassorb	4/4/4	95% Ethanol			7.1E-10	6.5E-09		3.3E-08	
04-2	HDPE	Uvitex OB	4/4/4	95% Ethanol			2.6E-11	8.6E-10		8.8E-09	
05-2	ЬР	Irganox 1076	4/3/2	95% Ethanol			6.3E-12	1.8E-10		2.3E-09	
05-2	ЬР	Irgafos 168	2/4/4	95% Ethanol			4.4E-13	1.3E-11		4.1E-10	
-60	HIPS	Styrene	4/3/3	Tenax®			2.3E-	1.0E-		5.7E-12	
15-	PA6	Caprolacta	4/4/4	lsooctane			8.0E-	4.5E-		4.6E-11	

Table 3.2Diffusion coefficients  $D_p$  determined by one partner<br/>laboratory for materials 1-16 and mean values of 4<br/>laboratories for materials 01-2 – 15-2

Table 3.3Polymer specific coefficient  $A_p$  values determined by one<br/>partner laboratory for materials 1-16 and mean values of<br/>4 laboratories for materials 01-2 – 15-2

No.	Polymer	Migrant	No. of labs			Pol	ymer sp	ecific A <sub>P</sub>	value		
			considered	5°C	20 °C	40 °C	C° 00	70 °C	80 °C	100 °C	121 °C
01	LDPE	lrganox 1076	1/1/1			12	11		11		
01	LDPE	Irgafos 168	1/1/1			11	11		11		
02	LDPE	DPBD	1/1/1			10	10		10		
03	HDPE	lrganox 1076	1/1/1			7.5	7.9		8.1		
03	HDPE	Irgafos 168	1/1/1			5.5	7		7		
04	HDPE	Chimassorb 81	1/1/1			8.2	7.9		7.9		
04	HDPE	Uvitex OB	1/1/1			6.8	7.1		7.6		
05	PP (homo)	lrganox 1076	1/1/1			9	7.2		8		
05	PP(homo)	Irgafos 168	1/1/1			3.3	4.9		6.2		
90	PP(random)	Chimassorb 81	1/1/1			8.3	8.3		8.3		
90	PP(random)	Uvitex OB	1/1/1			6.5	7.5		7.9		
07	ЬР	TMP	1/1/1			-0.5	1.1		3.1		
07	РР	Erucamide	1/1/1			5.1	6.8		8.2		
60	HIPS(oil)	Styrene	1/1/1			-1	, ,	-1			
10	GPPS	Styrene	1/1/1			-2	-2	-2.5			
12	PET	Tinuvin 1577	1/1/1						-6.5	-4.3	-1.3
14	plast. PVC	DEHA	1/1/1	7.6	7.8	8.2					
15	PA6	Caprolactam	1/1/1			1.3	1.1		0.8		
16	PA12	Laurolactam	1/1/1			1.4	0.7		1.7		
01-2	LDPE	lrganox 1076	4/2/2			11	11		11		
01-2	LDPE	Irgafos 168	4/4/4			11	11		11		
02-2	LDPE	DPBD	4/4/4			11	10		9.3		
04-2	HDPE	Chimassorb 81	4/4/4			8.4	8.7		8.5		
04-2	HDPE	Uvitex OB	4/4/4			6.9	7.7		8.3		
05-2	РР	lrganox 1076	4/3/2			5.6	6.9		7.7		
05-2	РР	Irgafos 168	2/4/4			3.8	5.2		6.9		
09-2	HIPS	Styrene	4/3/3			-2.5	-2.8		-2.9		
15-2	PA6	Caprolactam	4/4/4			-0.6	-1.4		-0.9		

## Table 3.4Partitioning coefficients $K_{P,F}$ determined by one partner<br/>laboratory for materials 1-16 and mean values of 4<br/>laboratories for materials 01-2 – 15-2

Migrant	No. of labs			Pa	rtitioning	coefficie	nt		
	Considered	5°C	20 °C	40 °C	C° 0∂	70 °C	80 °C	100 °C	121 °C
lrganox 1076	1/1/1			0.1		0.4	1		
Irgafos 168	1/1/1			0.1		2	2		
DPBD	1/1/1			4		4	5		
lrganox 1076	1/1/1			0.1		3	15		
Irgafos 168	1/1/1			ε		50	70		
Chimassorb 81	1/1/1			0.1		0.1	0.1		
Uvitex OB	1/1/1			0.1		1	1		
lrganox 1076	1/1/1			0.1		2.5	20		
Irgafos 168	1/1/1			L		20	300		
Chimassorb 81	1/1/1			0.2		0.2	1		
Uvitex OB	1/1/1			0.1		2	2		
TMP	1/1/1			L		1	1		
Erucamide	1/1/1			0		1	1		
Styrene	1/1/1				1	1	1		
Styrene	1/1/1				1	1	1		
Tinuvin 1577	1/1/1	0.1	0.1	0.1					
DEHA	1/1/1						0.1	0.1	0.1
Caprolactam	1/1/1			20		0.1	0.1		
Laurolactam	1/1/1			0.1		0.1	600		
Irganox 1076	4/2/2			1		2	1		
Irgafos 168	4/4/4			1		1	5		
DPBD	4/4/4			L		1	L		
Chimassorb 81	4/4/4			l		2	4		
Uvitex OB	4/4/4			1		1	11		
lrganox 1076	4/3/2			1		6	15		
Irgafos 168	2/4/4			0.1		67	1238		
Styrene	4/3/3			-		-	-		
Caprolactam	4/4/4			149		325	844		

## 3.3.3 Validation of estimated parameters of diffusion kinetics model

#### 3.3.3.1 Preliminary study - Materials 01 to 16

As can be seen in Table 3.4, the experimental and predicted data of materials 1 to 16 fit together quite well. Of the 19 migration values predicted, 8 (or 42%) were in the range of  $\pm 10\%$  compared to the experimental migration values and a total of 16 (84%) predicted values were in the range of  $\pm 40\%$  compared to the experimental data. Some exceptions in detail:

- The predicted migration value of Irgafos 168 from HDPE at 40°C into sunflower oil was 41% lower than the migration value determined experimentally. This observation can be attributed to the higher partitioning coefficient of the additive between HDPE and 95% ethanol compared to sunflower oil. Due to the polarity and the molecular weight of 647 AU, the solubility of Irgafos 168 in sunflower oil is higher than in 95% ethanol. The determination of a  $K_{P,F}$  value for Irgafos 168 in sunflower oil is recommended for future studies. When applying a  $K_{P,F}$  value of 10, the deviation is only 11%, with a  $K_{P,F}$  value of 1, the deviation is only 7%.
- The predicted migration value of Tinuvin 1577 from PET into olive oil is 3 times higher than the migration value determined experimentally. It is not clear why there was such a big difference. The  $A_p$  value determined experimentally and applied in the comparison was -1.3 while the practical guide suggests a value of 2 ( $A'_p = 6$ ,  $\tau = 1577$ , T = 121°C (Practical Guide, EC 2003). Errors are possible during the contact phase (high temperatures applied here are difficult to handle), or it is possible that the migrant has been blooming out. No plausible explanation could be found.
- Using water as food simulant for polyamides causes swelling of the polymer that will cause an accelerated diffusion process (Helmroth *et al.* 2002). The  $A_p$ -value for polyamides has been determined with isooctane where no or much less swelling occurs and therefore, the data cannot be compared. This effect can be observed very clearly with polyamide 6, which is very hydrophilic and therefore absorbs a lot of water, less with polyamide 12. Detailed results in this matter can be found in two different chapters describing materials from this project (Stoffers *et al.* 2003b, 2004a)

#### 3.3.3.2 Certification exercise - Materials 01-2 to 15-2

The experimental and predicted migration data with respect to the 6 materials selected for the trial certification exercise fit together even better (see Tables 3.3, 3.4, 3.5 and Figure 3.4). This is not unexpected as a lot more experimental data points per migrant-polymer combination were available. Of the 9 migration values compared, 8 values (or 89%) were in a range of  $\pm 40\%$  of the experimental data. The only exception here is again the PA6 as mentioned in the section above (diffusion kinetics in isooctane, specific migration in water). Excluding this value which can be easily justified - 100% of the predicted data are in  $a \pm 40\%$ range. The only value in this range, where the calculation delivers a significant underestimation of the experimental data is that of material no. 02-2 (LDPE containing DPBD). When looking for a possible explanation for this outlier the following fact becomes evident: The diffusion characteristics for this material were determined for 40°, 60° and 80°C (mean  $A_P$  values of 10.7, 10.0 and 9.3 respectively). When calculating a migration value for the conditions applied (4 hours at 20°C), an extrapolated  $A_{P}$  value had to be used, which was estimated to be 11.4. It is therefore recommended to verify this result and determine an  $A_{P}$ value at 20°C for this material in future studies.

Table 3.5	Comparison of experimental and predicted s	pecific
	migration for materials 1-16 and mean values	of 4
	laboratories for materials 01-2 – 15-2	

No.	Polymer	Migrant	$A_{P}$	$D_P$	$K_{P,F}$	Time	Temp.	Food	Migratio	Migratio	Deviation
								Simulant	experime	predicted	[%]
				[cm <sup>2</sup> /s]			[0°]		[zmb/gm]	[mg/dm²]	
1	LDPE	Irganox	10.8	2.3E-	0.1	2 h	100	olive oil	2.2	2.4	8.4
-	LDPE	Irgafos	10.5	7.0E-	0.1	2 h	100	sunflower	1.35	1.26	-6.7
2	LDPE	DPBD	10	1.2E-	5	6 h	20	olive oil	0.050	0.049	-2
С	HDPE	Irganox	7.5	4.0E-	15	10 d	40	sunflower	0.44	0.40	-7.8
С	HDPE	Irgafos	5.5	2.2E-	80	10 d	40	sunflower	0.16	0.09	-41
4	HDPE	Chimassor	7.9	7.0E-	0.1	1 h	70	olive oil	0.58	0.49	-12
4	HDPE	Uvitex OB	7.4	1.6E-	0.1	1 h	70	olive oil	0.12	0.12	-1.7
5	PP(homo)	Irganox	8.3	1.9E-	0.1	1 h	100	olive oil	8.0	1.13	37
5	PP(homo)	Irgafos	6.5	1.3E-	0.1	4 h	100	sunflower	8.0	0.71	-14
9	PP(rando	Chimassor	8.3	1.1E-	0.1	1 h	70	olive oil	0.65	0.63	-2.8
9	PP(rando	Uvitex OB	7.9	2.6E-	0.1	1 h	70	olive oil	0.14	0.15	10
7	dd	Trimethylo	2.1	2.1E-	1	4 h	70	water	0.03	0.04	20
7	dd	Erucamide	7.5	4.2E-	0.1	2 h	70	olive oil	0.72	0.75	3.5
6	HIPS(oil)	Styrene	, ,	8.3E-	1	10 d	20	olive oil	0.014	0.016	14
10	GPPS	Styrene	-2	3.0E-	1	1 d	40	olive oil	0.005	0.007	40
12	PET	Tinuvin	-1.3	1.4E-	0.1	2 h	121	olive oil	0.29	0.85	193
14	soft PVC	DEHA	7.5	1.7E-	0.1	1 d	20	olive oil	10	13	31
15	PA6	Caprolata	1.3	7.0E-	0.1	2 h	40	water	1.55	0.07	-95
16	PA12	Laurolacta	1.7	5.1E-	0.1	1 d	70	water	22.0	0.55	-29
38018	LDPE	Irganox	10.7	1.7E-	0.9	2h	100	sunflower	1.8	2.1	15
38018	LDPE	Irgafos	10.8	9.5E-	1.0	2h	100	sunflower	1.2	1.5	22
38019	LDPE	DPBD	11.5	5.4E-	0.5	4h	20	olive oil	0.17	0.11	-36
38021	HDPE	Chimassor	8.5	1.3E-	1.5	2h	70	olive oil	0.91	0.9	<u>,</u>
38021	HDPE	Uvitex B	8	2.9E-	0.5	2h	70	olive oil	0.2	0.22	7
38022	PP(homo)	Irganox	7.7	1.4E-	0.1	4h	100	sunflower	1.6	1.8	20
38022	PP(homo)	Irgafos	7.5	3.5E-	0.1	4h	100	sunflower	L	1.3	25
38026	HIPS(oil)	Styrene	-2.5	1.8E-	1.0	10d	40	olive oil	0.013	0.017	31
38032	PA6	Caprolact	-0.6	1.0E-	844	2h	40	water	66.0	0.02	-98



Figure 3.2 Experimental and predicted migration (experimental results with within laboratory standard deviation n=4) of migrants from polyolefins into sunflower oil / olive oil



Figure 3.3 Experimental and predicted migration (experimental results with between laboratory 95% confidence intervals) of migrants from polyolefins into sunflower oil / olive oil



Figure 3.4 Experimental and predicted migration (experimental results with within laboratory standard deviation / between laboratory 95% confidence intervals) of migrants from non-polyolefins into food simulants

## 3.4 Conclusions

One of the main objectives of the EU-project described here was to generate materials that are well-characterised with known properties, e.g. their diffusion parameters necessary for migration modelling. Since the determination of diffusion parameters is time consuming and error prone, only a limited number of reliable  $D_P$ -values exists. Many data found in literature with respect to diffusion data lack critical info, such as a proper characterisation of the polymer. The empirical formula for estimation of  $D_{P}$  by  $A_{P}$ -values rests upon a data base of diffusion coefficient found in literature. Therefore, one of the main conclusions within EU-Project SMT4-CT98-7513 (Hinrichs and Piringer 2001, Beglev et al. 2004) was that further studies aimed specifically at  $A_P$  and  $D_P$  values were necessary. One important output of the project discussed here is the collection of reliable diffusion data. For the first time, diffusion coefficients were determined in a small scale interlaboratory comparison study, meaning that the data established here can be considered the most reliable values relevant for this purpose available to date. Another important outcome of this project was the possibility to compare reliable experimental migration data on one side and predicted migration data on the other. This itself has been done before (O'Brien *et al.* 1999, O'Brien and Cooper 2001). However, in our case, the diffusion coefficients applied had been determined for the exact same materials. Hence, a validation of the migration modelling approach itself was possible. Overall, the experimental and predicted results agreed very well. Deviations observed are likely to originate from analytical variations or in different solubilities of the migrants in oil (applied during specific migration testing) and food simulants (applied during diffusion testing). It is foreseeable that the materials and knowledge developed in this project will be of great help in assisting future progress in migration modelling of food contact materials.

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# 4

Development and validation of analytical methods for monomeric and oligomeric migrants from nylon 12 packaging materials

## Abstract

Analytical methods for the determination of laurolactam - the monomer of nylon 12 - as well as the cyclic dimer and trimer were established. High performance liquid chromatography using ultraviolet (HPLC-UV) and mass spectrometric detection (HPLC-MS) were both found suitable to identify and quantify monomer, cyclic dimer and trimer well below the specific migration limit (SML) of laurolactam being 5 mg/kg of food (simulant). Gas chromatography with flame ionisation detection (GC-FID) showed to be an appropriate method for the detection of only laurolactam in aqueous and fatty food simulants. Food simulants could be analysed directly by all three methods, or after a change of solvents. For olive oil, a method for sample clean-up by size exclusion chromatography (SEC) was established.

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## 4.1 Introduction

In food packaging applications, beside nylon 6, nylon 12 is the most important polyamide. Nylon 12 is produced by polycondensation of dodecanolactam. The monomer, laurolactam (PM/Ref 19490, CAS 00947-04-6), is listed in section A of Directive 90/128/EEC with a specific migration limit (SML) of 5 mg/kg (EEC 1990, EC 2001). Looking at the "EU-cube", one kg of food being packaged in 6 dm<sup>2</sup> of packaging material, the maximum permitted migration of laurolactam per dm<sup>2</sup> of packaging material is 0.833 mg. During production, laurolactam as well as lower oligomers will remain in the polyamide. Due to their small molecule size and therefore relatively high diffusivity the monomer as well as the lower oligomers are able to migrate into foods. The chemical structures of these compounds are presented in Figure 4.1.

Among the applications of polyamide 12 as food packaging material, sausage and cooked meat wraps are the most common ones. Due to their application, where the casings are subjected to hot water or hot steam on the outside, and high temperature fatty food on the inside, an evaluation of suitability for food contact applications will typically include both migration into water and olive oil, the latter being the official fatty food simulant.

With one exception (ISO 2002), no references in literature can be found concerning analytical methods for laurolactam or its oligomers, neither in polymers nor in foods. The similarity in structure between caprolactam and laurolactam - their main difference being the molecular weight (caprolactam 113, laurolactam 197 daltons) - was reason for us to assess analytical methods for the determination of caprolactam, in order to have a good starting point to develop an analytical procedure for the determination of laurolactam and oligomers.

Several authors have reported methods for chemical analysis of both caprolactam and its oligomers in the polymer itself, in food simulants and in foods. Analysis by gas chromatography equipped with a flame ionisation detector (GC-FID) was described by Franz and Rijk (1997), Pogorzelska and Mielniczuk (2001) and in an ISO method (ISO 2002). Apart from their GC-FID method, Franz and Rijk (1997) described a GC method with mass selective detection for confirmation purposes. Analysis by high performance liquid chromatography using ultra-violet detection (HPLC-UV) was reported frequently (Soto-Valdez *et al.* 1997, Begley *et al.* 1995, Bonifaci *et al.* 1991, Barkby and Lawson 1993, Gramshaw and Soto-Valdez 1998). Among the HPLC-UV methods, all authors used reverse phase columns, Barkby and Lawson (1993), however, developed both normal and reverse phase HPLC methods.
Since caprolactam has a rather polar character, its determination in olive oil and fatty food is not too complex. Caprolactam can be extracted from oil with water as described by Franz and Rijk (1997) and Pogorzelska and Mielniczuk (2001), with aqueous solutions of methanol (Soto-Valdez *et al.* 1997), or with acetonitrile (Begley *et al.* 1995). Laurolactam, however, has a less polar character and it can therefore be expected that it cannot be separated from oils in the same way. Another way to separate caprolactam from a food was described by Gramshaw and Soto-Valdez (1998). They developed a method to measure caprolactam and its oligomers in chicken. In their method, the migrants were first extracted from the meat, the extract was subsequently cleaned-up by column chromatography using silica gel as stationary phase and a mixture of hexane/ethyl acetate/methanol as mobile phase. Similar to the waterextraction of caprolactam from oil, the polarity of caprolactam is used in this approach as well.

In the present chapter, we summarize the results of our attempts to develop and validate methods for the determination of laurolactam and nylon 12 oligomers in polymers as well as in food simulants including olive oil.



#### Figure 4.1 Structure of nylon 12 monomer (laurolactam), dimer and trimer

#### 4.2 Materials and reagents

All reagents and solvents were of analytical quality unless specified otherwise. Water, methanol (both HPLC grade) and laurolactam were purchased from Fluka, capryllactam (2-azacyclononanone) from Aldrich. Isooctane and ethanol were purchased from Merck. The olive oil used came from Minerva. The azeotropic mixture of ethyl acetate and cyclohexane was distilled at IVV using solvents from Merck. Nylon 12 dimer and trimer standards were produced in-house. For this purpose 50 g or 50 dm<sup>2</sup> of a polyamide 12 monofilm (Grilamid L25, EMS-Chemie, Germany) was extracted with a 500 ml of a 95% ethanol/water mixture for 3 days at 60°C. The extract was evaporated to dryness and the residue was purified using preparative HPLC. Conditions are described below in the apparatus section. 5 mg of the dimer and 1 mg of the trimer fraction were collected in total. The purity of the standard fractions collected ranged between 98 and 99% (determined by area percentage in full scan by HPLC-MS).

#### 4.2.1 Sample preparation

Several food simulants have been investigated in this study. Standard solutions of laurolactam were prepared in methanol, isooctane, 95% ethanol and in olive oil. Migration tests were set up using 1 dm<sup>2</sup> of film fully immersed in 100 ml of food simulant (isooctane, 10% ethanol, 50% ethanol and 95% ethanol in water and in olive oil) for 10 days at 40°C. For repeatability testing, solutions of 10% ethanol, 50% ethanol, 95% ethanol, isooctane and olive oil were spiked at the SML of 5 mg/l (5 mg/kg for olive oil). Six aliquots were taken and analysed. Samples and standard solutions were directly injected into the respective analytical devices unless stated otherwise in the apparatus sections below.

#### 4.2.2 Apparatus

Gas chromatography was carried out using a Hewlett Packard 5890 equipped with a flame ionisation detector (FID). The column was a 30 m x 0.32 mm DB624 1.8  $\mu$ m film (J&W Scientific). The carrier gas was hydrogen with a column head pressure of 65 kPa and a split rate of 1:20. Prior to injection, 0.1 ml of a 210 $\mu$ g/ml solution of capryllactam was added to 0.5 ml of any standard or sample. Injection volume was 1  $\mu$ l. The injector port and FID were heated to 220°C and 240°C respectively.

The oven temperature was programmed from 180°C held for 2 minutes, to 240°C at 10°C/min with a final hold for 5 minutes. Retention times for capryllactam (internal standard) and laurolactam were 6.6 and 9.5 minutes respectively.

High performance liquid chromatography was performed with a Shimazu Class 10A equipped with a diode array detector (Shimazu SPD-M10A) and a 100 µl loop. Injection volume was 50 µl. The column used was a Hypersil ODS5 with 125 x 4 mm inner diameter and 5 µm particle size. The column was thermostated at 40°C. Total flow was 1 ml/min. The eluents were water (A) and methanol (B). The gradient went from 30% to 90% solvent B in 8 minutes where it was held for 10 minutes. The concentration of methanol was reduced back to 30% in 0.1 minutes and held for 6.9 minutes to equilibrate. The retention times of the analytes were: laurolactam 9.4, dimer 10.8, trimer 12.2, and tetramer 12.9 minutes. The detection wavelength used was set at 207 nm. During preparative HPLC for purification of dimer and trimer standards, a Shimazu fraction collector (FRC-10A) was used. 50 µl of a solution containing approximately 1% (w/v) of total residue as described in the reagents section were injected and fractions belonging to the peaks were collected. Yield for the tetramer was not sufficient to obtain standards that could be used for calibration purposes.

High performance liquid chromatography with mass spectrometric detection (HPLC-MS) was carried out using a Thermo Finnigan LCQ Deca (ion trap) applying atmospheric pressure chemical ionisation (APCI, Vaporizer Temperature: 450 °C, Corona current: 5.0 mA, heated capillary: 225°C). Detection was performed in full scan mode (m/z = 100- 1200), using the following mass traces for guantification: laurolactam m/z = 198.2, dimer m/z = 395.4, trimer: m/z = 592.6. Quantification was achieved by external calibration using standard solutions of the analytes. Isooctane samples and standards (0.5 ml) were evaporated using a stream of nitrogen and redissolved in 0.5 ml of methanol prior to injection. Other samples and standards were directly injected. The column used was the same as reported in the HPLC-UV section, the gradient conditions were similar to those described above for HPLC-UV. Total flow was 0.8 ml/min, injection volume was 15 µl. The methanol concentration was held at 30% for 3 minutes, then increased to 90% in 6 minutes where it was held for 6 minutes before returning to 30% for 10 minutes. The respective retention times: laurolactam: 8.1, dimer: 8.9, trimer: 9.9 and tetramer: 10.8 minutes.

For size exclusion chromatography (SEC), the following instruments were used: the mobile phase pump was a Gynkotek N480 pumping an azeotropic mixture of ethyl acetate and cyclohexane (56:44, w:w) at a

flow of 3 ml/min. The column used was a Pharmacia XK26/40, 26 mm i.d., bed height 370 mm, filled with Bio-Beads SX-3 swollen in the eluent described above. The detector was a refraction index detector (Gynkotek RI SE-51), the autosampler was a Gilson Abimed 231XL equipped with a syringe pump 402. The fraction collector used was a Gilson Abimed 201. A 1 g oil aliquot was dissolved in eluent and filled up to a total weight of 10 g. 4 ml were injected into the SEC. The fraction containing the oil was determined by injection of a blank (10% w/w olive oil in eluent), the fraction containing laurolactam was determined by injection of a 10 000  $\mu$ g/g laurolactam standard in eluent. In Figure 4.2, it can be seen that the olive oil peak eluted roughly between 23 and 34 minutes. The following fraction, containing all analytes, was completely collected and analysed as follows: The collected fraction (approximately 75 ml) was evaporated in a rotary evaporator at 60°C to about 1 ml, quantitatively transferred into a 10 ml volumetric flask, and dried down using a stream of nitrogen. The residue was redissolved in 10 ml methanol and a 15 µl aliguot was injected into the HPLC/MS system. For calibration, seven oil standards spiked with 0, 1, 5, 10, 25, 50 and 100 µg/g laurolactam respectively were treated the same way as the test samples.



## Figure 4.2 Chromatogram from size exclusion chromatography showing traces of a 10000 µg/g laurolactam standard (bold line) and olive oil blank (normal line) injection

#### 4.2.3 Statistical evaluation

All analytical data were evaluated using "DINTEST", a programme for the statistical evaluation of analytical data according to the guidelines of the German norm DIN 32645 (Schmitt and Herbold 2002). By means of this norm, the limits of detection (LOD) and limits of quantification (LOQ) are determined from the Y – intercept and its confidence interval after linear regression. A confidence level of 95% was applied.

#### 4.3 Results and discussion

#### 4.3.1 GC-FID

A chromatogram from GC-FID analysis can be found in Figure 4.3 (standard solution 20 µg/ml laurolactam and 35µg/ml in 95% ethanol). Linearity was observed over a large range of concentrations (from 0.01 µg/ml to 200 µg/ml). The limits of detection and guantification in organic solvents (95% ethanol, isooctane) determined according to DIN 32645 were found to be 0.04 µg/ml and 0.15 µg/ml respectively. Chromatograms from migration solutions or extracts that had been in contact with nylon 12 films did not show any kind of interferences. This is not unexpected since the samples used were monofilms and did not to contain any kind of additives or other substances that might cause interferences. With a typical area-to-volume ratio of 10 dm<sup>2</sup>/liter during migration tests, the limit of detection corresponds with 4 µg/dm<sup>2</sup>, the limit of guantification corresponds with 15 µg/dm<sup>2</sup>. For comparison, the SML for laurolactam is 833 µg per dm<sup>2</sup> for a given test condition with 6 dm<sup>2</sup> in contact with 1 liter of food simulant, so the method is well suitable for migration testing into organic solvents.



Figure 4.3 Typical GC-FID chromatogram of a migration solution (95% ethanol) with capryllactam as internal standard and laurolactam as migrant

#### 4.3.2 HPLC-UV

A chromatogram from reverse phase HPLC can be found in Figure 4.4 (95% ethanol migration solution after 10 days at 40°C). Laurolactam, cyclic dimer, trimer and tetramer were separated adequately. The rising baseline originated from the change in composition of the eluent during the run-time, methanol having a higher absorbance at 207 nm than water. Linearity was observed up to a concentration of 30 µg/ml. Figure 4.5 shows the calibration curves of laurolactam, di- and trimer. The limits of detection for laurolactam, di- and trimer in 95% ethanol were 1.5, 1.2 and 0.5 µg/ml, limits of quantification were 5.7, 4.4 and 2.0 µg/ml, respectively. The tetramer peak could be detected and its identity was confirmed by HPLC-MS (see below), however, since no standards were available, guantitative data or response factors cannot be reported here. Response factors at 207 nm, calculated as ratios of the slopes of the respective calibration graphs, are  $0.68 \pm 0.01$  for the cyclic dimer (slope dimer / slope laurolactam) and  $0.85 \pm 0.02$  for the cyclic trimer (slope trimer / slope laurolactam). The ranges given were derived from the

standard errors of the analytical procedures.

The relative dimer response factor, expressed as the ratio of the calibration graphs' slopes: dimer (quantified at m/z = 395.4) / laurolactam (at m/z = 198.2) ranged from 1.2 to 1.4 due to a relatively high day to day variability of the analytical device. The corresponding relative trimer response factor (trimer quantified at m/z = 592.6) ranged from 2.7 to 3.2. The standard error of the procedures observed was 2%. For conservative reasons, the lowest response factors observed should be used in case they are used for quantitative determination of dimer or trimer.



Figure 4.4 Typical chromatogram from HPLC-UV obtained for the separation of laurolactam (1) and its oligomers (2, 3 and 4 for di-, tri- and tetramer respectively)



Figure 4.5 Calibration data for laurolactam, cyclic dimer and cyclic trimer for HPLC-UV in 95% ethanol.

#### 4.3.3 HPLC-MS

A chromatogram from reverse phase HPLC-MS can be seen in Figure 4.6 (95% ethanol migration solution after 10 days at 40°C). Laurolactam and its cyclic oligomers could be fully separated and detected up to the tetramer.

External calibration resulted in the following limits of detection and quantification: laurolactam: 0.05  $\mu$ g/ml and 0.20  $\mu$ g/ml; dimer: 0.04  $\mu$ g/ml and 0.17  $\mu$ g/ml and trimer: 0.03  $\mu$ g/ml and 0.14  $\mu$ g/ml. No standards for the tetramer were available. The LODs, but also the LOQs are well below the specific migration limit of laurolactam being 5 mg/kg. Likewise, the LOD and LOQs expressed in  $\mu$ g/dm<sup>2</sup> are significantly lower than the corresponding SML.



Figure 4.6 Chromatogram and mass spectra for peaks of laurolactam, cyclic dimer and cyclic trimer in the chromatogram from HPLC-MS in full scan (laurolactam m/z = 198.2, dimer m/z = 395.4, trimer: m/z = 592.6)

## 4.3.4 Determination of laurolactam and oligomers in olive oil.

The limits of detection and quantification of laurolactam in olive oil were 0.57  $\mu$ g/g and 2.1  $\mu$ g/g respectively. With a typical area-volume ratio of 10 dm<sup>2</sup>/liter during migration tests, the limit of detection corresponds

with 57  $\mu$ g/dm<sup>2</sup>, the limit of quantification corresponds with 210  $\mu$ g/dm<sup>2</sup>. Also at these somewhat higher detection characteristics, the method is still very suitable for migration testing into olive oil. In case lower LOD and LOQ are needed, the residue after the drying down step can easily be redissolved in 1 ml instead of 10 ml. By doing so, the LOD and LOQ can be decreased by a tenfold.

#### 4.3.5 Discussion of method validation parameters

Several validation parameters will now be discussed in detail:

**Sensitivity**: The limits of detection and quantification are described in the respective instruments' sections and in Table 4.1. The methods proved to be sufficiently sensitive for the purpose of migration testing into food simulants including olive oil.

**Repeatability**: Solutions of 10% Ethanol, 50% Ethanol, 95% Ethanol, Isooctane as well as an olive oil sample were spiked at the SML level of 5 ppm (m/v for solvents, m/m for olive oil). 6 aliquots were taken and analysed as described in the methods section. The coefficients of variation (c.v. for variation between aliquots) are summarized in Table 4.2.

**Linearity**: Regression coefficients of calibration graphs were typically >0.995 within the calibration area. Therefore, it can be stated that linearity was observed for all methods described.

**Specificity**: For solvents and migration solutions, no interfering peaks could be detected. Baseline noise was highest for the HPLC-UV method, which explains the relatively high LOD and LOQ. HPLC-MS was most specific when looking at the respective mass traces only, as one would expect. For olive oil samples, SEC gave an adequate purification, and no interfering peaks could be detected in HPLC-MS or GC-FID chromatograms.

**Trueness**: Concentrations of laurolactam in spiked food simulants as described in the repeatability section as well as in migration solutions were determined using external standards. Concentrations found in the spiked food simulants corresponded with 95 to 105% of the spiked level. No bias between the detection methods could be demonstrated.

**Recovery:** For the solutions from migration experiments, no loss is expected or likely as aliquots were directly injected into the respective detection systems. Any loss of analytes that might have occurred during clean-up of olive oil samples or evaporation of isooctane samples was compensated by the simultaneous clean-up / evaporation of standards as described in the experimental section.

Table 4.1 Limits of detection (LOD) and quantification (LOQ) of laurolactam, cyclic dimer and cyclic trimer in  $\mu$ g/ml and in quantities that can be detected or quantified per dm<sup>2</sup> of food contact area

		Laurolactam		Dimer		Trimer	
Method		µg/ml	µg/dm²*	µg/ml	µg/dm² *	µg/ml	µg/dm² *
GC-FID	LOD	0.04	4				
	LOQ	0.15	15				
HPLC-UV	LOD	1.5	146	1.2	115	0.5	50
	LOQ	5.7	566	4.4	440	2.0	200
HPLC-MS	LOD	0.05	5	0.04	4	0.03	3
	LOQ	0.20	20	0.17	17	0.14	14

\* assuming a area-to-volume ratio of 10 dm<sup>2</sup>/liter during migration testing

Table 4.2Observed repeatabilities (coefficients of variation - c.v.)for different methods and food simulants.

	Olive oil	lsooctane	95% ethanol	50% ethanol	10% ethanol
GC-FID		1.0%	1.4%		
HPLC-UV			0.46%	1.2%	0.64%
HPLC-MS	2.9%	2.3%	2.3%	2.3%	2.0%

#### 4.4 Conclusions

The developed methodology based on GC-FID is highly suitable for the quantitative determination of laurolactam in the EU-official food simulants as well as in aqueous mixtures of ethanol and isooctane. The level of quantification achievable with this method is well below the SML of laurolactam.

Furthermore, laurolactam and its oligomers up to the tetramer could be well separated and quantified by an HPLC procedure using UV-detection at 207 nm or, alternatively and more sensitively, mass spectrometric detection. Both detection methods were capable of detecting laurolactam and the cyclic di- and trimer, significantly below the SML. Since all migration and extracts containing solutions were directly injected into the HPLC systems, even substantially lower quantification limits appear to be easily achievable by application of a concentration step. Signal to noise ratios were very high, so no significant increase of interferences is expected when a tenfold concentration is applied.

Also for olive oil, LOD and LOQ were extensively lower than the SML. Due to the difference in molecular size between oil on the one hand and laurolactam and polyamide oligomers on the other hand, separation of migrants from a fatty matrix is effectively accomplished using size exclusion chromatography. It is justified to expect that extracts from real foodstuffs, such as ham, meat, or other can be cleaned-up by this or a similar approach, which makes the presented analytical method also applicable for real food matrices. Finally, due to the general non-availability of nylon 12 oligomers (n=2, n=3) the relative response factors as established above can be used for their quantitative determination via the commercially accessible monomer laurolactam.

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# 5

A study on alternative fatty food simulants and diffusion kinetics of nylon 12 food packaging<sup>.</sup>

#### Abstract

The migration of laurolactam and cyclic di- and trimer of nylon 12 was evaluated using three different films and five food simulants (olive oil, isooctane, 95% ethanol and 50% ethanol and water). Substitute test conditions for migration into olive oil according to EU directive EC/97/48 were applied using 95% ethanol and isooctane. Results showed that 95% ethanol overestimated while isooctane underestimated respective migration into olive oil. Water proved to be the best olive oil substitute as migration of laurolactam into water and olive oil using the same temperature gave similar results. Additionally, diffusion kinetics of laurolactam were investigated by migration kinetic studies using isooctane and olive oil. Diffusion coefficients determined with isooctane were significantly higher than those found using olive oil. It was proved that isooctane had an interaction and olive oil was inert to the polymer. The diffusion conductance coefficient  $A_P$  for nylon 12 determined using olive oil ranged from 0.3 to 0.6.

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#### 5.1 Introduction

Among food packaging materials, nylon 6 (polyamide 6) and nylon 12 (polyamide 12) are the most frequently used polyamides. A considerable amount of research has been carried out concerning the release of nylon 6 mono- and oligomers into water (Barkby and Lawson 1993), fatty food simulants (Franz and Rijk 1997, Pogorzelska and Mielniczuk 2001, Soto-Valdez *et al.* 1997) and foods (Barkby and Lawson 1993, Begley *et al* 1993, Gramshaw and Soto-Valdez 1998). Hardly anything, however, is known about the migration behaviour of the nylon 12 monomer (laurolactam, PM/Ref 19490, CAS 00947-04-6) and oligomers into foods and food simulants (see Figure 5.1 for structures of mono- and lower oligomers).

Until recently, laurolactam was listed in section B of directive 90/128/EEC (EEC 1990). This list contains monomers and additives that are permitted only in at least one member state and are not yet fully harmonised at EU level. This meant that a nylon 12 film for food contact use had to comply with the overall migration limit of 60 mg/kg or 10 mg/dm<sup>2</sup>. In the latest amendment to directive 90/128/EEC (EC 2001) however, laurolactam has been transferred from section B into section A with a specific migration limit (SML) of 5 mg/kg corresponding with 0.833 mg/dm<sup>2</sup>.

In some of the studies on nylon 6 packaging materials mentioned above, it was shown that mono- *and* oligomers were migrating into several food simulants (Barkby and Lawson 1993, Begley *et al* 1995). Migration of oligomers with molecular weights as high as 1017 Dalton (nylon 6 nanomer) was observed. Consequently, due to the similarities in structure of the two polymers, a similar behaviour of the nylon 12 oligomers may be anticipated. As diffusion and migration strongly depends on the molecular weight of migrants, it can be expected that migration of nylon 12 oligomers up to the pentamer (986 Dalton) might occur.

The other expectation is that mono- and oligomers are covered by overall migration tests. According to the "Practical guide for users of European directives" (EC 2003) intermediate products such as oligomers may be present in packaging materials even though they are not listed. It is stated that these substances were taken into account when a technical dossier submitted for the authorisation of a substance to be put in the positive list was evaluated.



#### Figure 5.1 Structure of nylon 12 monomer (laurolactam), dimer and trimer

Typical applications of nylon 12 films are sausage and cooked meat casings. During the process of cooking meat or sausages, the nylon film will be exposed to hot water or steam on the outside and fatty food on the inside. To show compliance with EU legislation, specific migration into water and olive oil, the official fatty food simulant, has to be evaluated. Typical migration testing conditions are 2 hours at 100°C. Table 4 in EU directive 97/48/EC (EC 1982 and 1997) gives detailed conditions for olive oil and fat substitute testing. Suggested substitute testing conditions are: isooctane 1.5 hours at 60°C; 95% ethanol 3.5 hours at 60°C; modified polyphenylene oxide (MPPO, Tenax<sup>®</sup>) 2 hours at 100°C. The time and temperature conditions given for isooctane and 95% ethanol are based on a large pool of data obtained mainly from overall migration test results (Alnafouri and Franz 1999, Van Battum 1996). Both solvents have the necessary ability to penetrate certain polymers (isooctane for apolar polymers such as polyolefins, 95% ethanol for polar polymers such as polyamides and PET) to extract all potential migrateable substances at comparable rates within shorter times. Numerous studies have focused on finding the best specific migration substitute testing conditions for polyolefins (Linssen et al. 1992, Baner et al. 1992 and 1995, Cooper et al. 1998, Alnafouri and Franz 1999). However, no data are available for specific migration substitute tests for polyamides.

#### 5.2 Objectives

The objective of this study was to investigate into the migration characteristics of nylon 12 and particularly to study the migration kinetics of the monomer laurolactam in contact with several food simulating liquids as well as the migration behaviour of the lower oligomers. From these studies conclusions were expected on the selection of an appropriate alternative fat simulant as well as on the missing  $A_P$ -value for modelling migration from nylon 12 food packaging materials.

#### 5.3 Theory of diffusion

The transport of components from a polymer to foods is generally described by Fick's second law of diffusion.

 $\frac{\partial C_P}{\partial t} = D_P \frac{\partial^2 C_P}{\partial x^2}$  (equation 5.1)

where  $C_P$  is the concentration of a migrant in a food contact polymer at time *t* at a distance *x* from the origin of the x-axis and  $D_P$  is the constant diffusion coefficient in the polymer. Crank (1975) established an analytical solution for Fick's second law. His findings were not specially formulated for migration problems, but for diffusion phenomena in general. The initial and boundary conditions formulated by Crank and needed to solve the partial differential equation can be "translated" into a number of assumptions for migration processes (Gandek 1989, Chung 2001, 2002). It is assumed that

• the migrant initially is homogeneously distributed in the polymer

 $C_P = C_{P,0}$  at  $0 < x < L_P$  t = 0 (equation 5.2)

where  $L_P$  is the thickness of the polymer and  $C_{P,0}$  is the initial concentration of the migrant in the polymer.

• the food initially is migrant-free

 $C_F = 0$  at t = 0 (equation 5.3)

where  $C_F$  is the concentration of the migrant in the food

 there is no migration and no concentration gradient at the left (noncontact) side.

$$\frac{\partial C_P}{\partial x} = 0$$
 at  $x = 0$   $t > 0$  (equation 5.4)

 the migrant transport takes place at the right (contact) side of the polymer and the mass balance is described as

$$K_{FP}\left(\frac{V_{F}}{A}\right)\frac{\partial C_{P}}{\partial t} = -D_{P}\frac{\partial C_{P}}{\partial x}$$
 at  $x = L_{P}$   $t > 0$  (equation 5.5)

where  $K_{P,F}$  is the partitioning coefficient describing the partitioning of the migrant between the polymer and the food,  $V_F$  is the volume of food and A is the contact area. Further assumptions are described in detail elsewhere (Gandek 1989, Chung 2001, 2002). Figure 5.2 gives an illustration of the Fickian diffusion from a polymer into a liquid food and the conditions described above. The concentration profile in the polymer at  $t_1$  is marked  $C_P(x, t_1)$ . It can be seen that the concentration at the interface  $(x = L_P)$  is derived from the concentration in the food:  $C_F(t_1) = K_{P,F} \cdot C_P(x, t_1)$ . At a later time  $t_2$  the overall amount of migrant in the polymer has decreased, but the concentration at  $x = L_P$  has increased. Therefore the concentration in the food  $C_F(t_2) = K_{P,F} \cdot C_P(x, t_2)$  has increased as well.



Figure 5.2 Schematic diagram of diffusion from a polymer into a liquid food (Chung 2001). Concentration profiles of migrant in polymer and liquid food.  $(t_1 < t_2)$ .

It must be stated that the above model is generally accepted to be used for two-sided migration as well. In this case, half the thickness and both sides of the film are considered as contact area (CEN 2002).

Essential for migration estimation by modelling is knowledge about the diffusion coefficient  $D_P$ . Piringer and co-workers (Piringer 1993 and 1994) have developed an empirical relationship of the diffusion coefficient with the molecular weight of the migrant, the temperature and the polymer type. An empirical material constant  $A_P$ , expressing the diffusion conductance of a material was introduced. The relationship between  $A_P$  and the parameters above is given as:

$$D_{\rho} = 10^{4} \cdot e^{A_{\rho} - 0.1351 \cdot MW^{2/3} + 0.003 \cdot MW - 10454/T}$$
 (equation 5.6)

where  $A_P$  is the diffusion conductance,  $D_P$  is the diffusion coefficient (cm<sup>2</sup> s<sup>-1</sup>), MW is the molecular weight of the migrant in Dalton and T is the temperature in K.

The model developed by Piringer and co-workers has proved to work satisfactorily for polyolefins (O'Brien *et al.* 1999, O'Brien and Cooper 2001) and some non-polyolefins (O'Brien and Cooper 2002), however only little data are available for polyamides.

#### 5.4 Experimental

#### 5.4.1 Sample materials

Three different nylon 12 films were used. Two of them were commercial sausage casings, the third material used in this study originated from an EU project for the development of a reference material for specific migration testing (Störmer 2004).

- Film A: a nylon 12 monofilm, sausage casing, 39 µm thickness
- Film B: a bilayer sausage casing, 29µm nylon 12, 19 µm nylon 6
- Film C: a PA12 monofilm, certified reference material candidate, 196 µm thickness

#### 5.4.2 Reagents

All reagents and solvents were of analytical quality unless specified otherwise: water and methanol were HPLC grade from Fluka. Olive oil

was from Minerva, Italy. The azeotropic mixture of ethyl acetate and cyclohexane was glass distilled in our laboratories. Nylon 12 dimer and trimer standards were produced in-house. Details were described earlier (Stoffers, 2003).

#### 5.4.3 HPLC/MS

A Thermo Finnigan LCQ Deca (ion trap) applying atmospheric pressure chemical ionisation (APCI, Vaporizer Temperature: 450 °C, Corona current: 5.0 mA, heated capillary: 225°C) was used. Detection in full scan mode (m/z = 100 – 1000), the following mass traces were used for quantification: laurolactam m/z = 198.2, dimer m/z = 395.4, trimer: m/z = 592,6. A Hypersil ODS5 with 125 x 4 mm inner diameter and 5  $\mu$ m particle size was used; 0.8 ml/min total flow of the mobile phase, injection volume 15  $\mu$ l. Quantification by external standards. Isooctane samples (0.25 ml) and standards were evaporated using a stream of nitrogen and redissolved in 0.25 ml methanol prior to injection. Other samples and standards were directly injected. Gradient: Going from 30% A (methanol) 70% B (water) for 3 minutes, to 90% A in 6 minutes, held for 6 minutes, returning to in 1 minute to 30% A for 10 minutes. Retention times were: laurolactam: 8.1, dimer: 8.9, trimer: 9.9.

#### 5.4.4 Size exclusion chromatography (SEC)

Pump: Gynkotek N480, mobile phase: azeotropic mixture of ethyl acetate and cyclohexane (56:44, w:w), flow: 3 ml/min, column: Pharmacia XK26/40, 26 mm i.d., bed height 370 mm, filled with Bio-Beads SX-3 swollen in the eluent described above, refraction index detector: Gynkotek RI SE-51, autosampler: Gilson Abimed 231XL equipped with a syringe pump 402, fraction collector: Gilson Abimed 201.

#### 5.4.5 Olive oil sample clean-up

A 0.5 g oil aliquot was filled up to a total weight of 5 g using the GPC mobile phase mentioned above. 4 ml of this solution were injected into the SEC. The fraction containing the oil was determined by injection of a blank (10% w/w olive oil in eluent), the fraction containing laurolactam was determined by injection of a 10 000  $\mu$ g/ml laurolactam standard in eluent. The olive oil peak eluted roughly between 23 and 34 minutes. The

following fraction was completely collected and analysed as follows: The collected fraction (approximately 75 ml) was evaporated at 60°C and 350 mbar in a rotary evaporator to about 1 ml, quantitatively transferred into 10 a ml volumetric flask, and dried down using a stream of nitrogen. The residue was redissolved in 10 ml methanol and a 15  $\mu$ l aliquot was injected into the HPLC/MS system. For calibration, oil standards spiked with 0, 1, 5, 10, 25 and 50  $\mu$ g/g laurolactam respectively were cleaned-up along with the test samples. Oligomer concentrations were determined using response factors determined by injections of laurolactam, dimer and trimer standards.

## 5.4.6 Determination of the initial concentration $(C_{P,0})$ of laurolactam and oligomers in the polymer

1 g film samples cut into 0.5 cm squares were weighed accurately into 50 ml Erlenmeyer flasks (three replicates per film). 25 ml of 95% ethanol was added. The flasks were closed and stored at 60°C for 24 hours. 1 ml aliquots were transferred to autosampler vials for analysis. The remaining solvent was discarded, the film pieces were briefly rinsed with 95% ethanol, the solvent was discarded, and the film pieces were dried. Then, another 25 ml of solvent were filled into the flasks. 1 ml aliquots from the second extraction were transferred to autosampler vials for analysis. The samples were analysed by HPLC along with external standards. The first extraction was considered complete if less then 5% was found in the second extraction cycle.

#### 5.5 Migration experiments

### 5.5.1 Migration into olive oil, isooctane, 95% ethanol, 50% ethanol and water.

Migration tests were carried out according to European Standard EN1186 (CEN 1998). The following test conditions were used: olive oil 2h/100°C; isooctane 1.5h/60°C; 95% ethanol 3.5h/60°C; 50% ethanol 2h/reflux 90°C and water 2h/reflux 100°C.

Overall and specific migration were determined by full immersion testing. 1 dm<sup>2</sup> of film was folded fan-like so that it could be easily placed into a cylinder and full contact was ensured. The contact area was 2 dm<sup>2</sup> (1 dm<sup>2</sup> on both sides), and the volume used was 100 ml. Prior to the incubation period, cylinders and solvents were preheated to the respective testing temperatures. Film B consisted of a PA6 side and a PA12 side, therefore, only 1 dm<sup>2</sup> was considered as contact area. Upon exposure, 1 ml aliquots were filled into autosampler vials to determine specific migration (isooctane samples after a change of solvents as described above). The remaining volume was used to determine overall migration by evaporation of the solvent in a stainless steel beaker and subsequent gravimetric determination of the residue (CEN 1998).

Cylinders as described above were also used for diffusion kinetics experiments. 250µl samples of isooctane or 0.5g samples of olive oil were taken using a pipette after 1, 2, 4, 8, 24 and 48 hours of incubation. Solvent samples were filled into autosampler vials and stored at 4°C until analysis. Olive oil samples were diluted 10 times with an azeotropic mixture of ethyl acetate and cyclohexane and stored at 4°C until analysis.

#### 5.6 Kinetic migration modelling

Experimental data were used to estimate the diffusion coefficient  $D_P$ , the partitioning coefficient  $K_{P,F}$  and the diffusion conductance  $A_P$ . Numerical integration of Fick's second law was applied using the software Athena Visual Workbench (www.athenavisual.com). The initial and boundary conditions were used as stated in the theory section. The parameters of the model were estimated using non-linear regression. Parameter output is given with 95% confidence intervals.

#### 5.7 Results and discussion

## 5.7.1 Initial concentrations of mono- and oligomers.

All three films were tested for initial concentrations of laurolactam as well as the cyclic di- and trimer. Since a second extraction cycle always contained less than 5% of the first cycle, the first extraction was considered complete.

Table 5.1 Mean concentrations of laurolactam, cyclic dimer and cyclic trimer found in films A, B and C in concentrations (mg/kg) and in migration potentials (mg/dm<sup>2</sup>) for full two-sided migration testing. (n=3; relative standard deviations  $\leq 5\%$ )

	Film A		Film B		Film C	
	mg/kg	mg/dm <sup>2</sup>	mg/kg	mg/dm <sup>2</sup>	mg/kg	mg/dm <sup>2</sup>
Laurolactam	2645	0.52	2925	0.84	2364	2.36
Dimer	9460	1.84	11083	3.19	8983	8.98
Trimer	3755	0.73	4418	1.27	3767	3.77

As can be observed in Table 5.1, the respective concentrations of mono-, di- and trimer were in the same orders of magnitude and at similar ratios for the three materials. The dimer concentration was found to be the highest, being approximately 1% of the total weight of the films. Since film C is thicker than the two others, its migration potential, given in area related mg/dm<sup>2</sup> is significantly higher. As mentioned in the materials section, migration data from this film must not be used to draw conclusions about the safety of sausage casings in general. This film was solely used as a reference material to be able to investigate the migration and diffusion behaviour better.

## 5.7.2 Overall and specific migration into food simulants

All three films were also evaluated for migration into olive oil, isooctane, 95% and 50% ethanol and water. Test conditions and results are summarized in Table 5.2. Looking at the amounts of laurolactam migrated into olive oil, it is apparent that all three films did meet the EU SML of 0.833 mg/dm<sup>2</sup> under the conditions applied here.

Before discussing further results, it must be emphasized that migration is controlled by three key factors investigated here: the diffusivity of the migrant in the polymer, its partitioning between polymer and food, and the initial concentration of the migrant in the polymer. It can be observed that significant amounts of the nylon 12 dimer and smaller amounts of the trimer migrated into olive oil. Due to the higher molecular weight (laurolactam 197, dimer 394, trimer 591 Dalton), the diffusivities of the dimer and trimer are lower than that of laurolactam. The high migration of the dimer is therefore likely to be caused by the high initial concentration of the dimer in the polymer. The amount of trimer migrating into olive oil is lower than that of the mono- and dimer. This can easily be explained by a lower diffusion of the molecule due to its higher molecular weight.

For all three films investigated here, compliance with the SML of laurolactam was proved, but the limit would be exceeded if the amounts of migrated mono- and oligomers had to be summed up.

It can be seen from Table 5.2 that the substitute test conditions for isooctane (1.5 hours at 60°C) underestimate the migration of the monomer as well as that of the lower oligomers into olive oil. This is not unexpected as isooctane with a temperature of 60°C does not possess the aggressiveness towards nylon 12 to extract the same amount as migrated into olive oil at 100°C in 2 hours. For polar polymers such as polyamides, 95% ethanol ought to be used as an olive oil substitute. Using this solvent, a stronger solvent-polymer interaction can be expected. As can be observed from Tables 5.1 and 5.2 this is the case as 95% ethanol caused total extraction of laurolactam. Also, large amounts of the oligomers investigated were extracted. It is evident that conditions suggested for 95% ethanol significantly overestimate the migration into olive oil. It is apparent from the 50% ethanol results that a relatively strong interaction with the polymer must have taken place here as well. Laurolactam migration into this solvent mixture from films A and B is comparable or somewhat higher than into olive oil, results from film C show overestimation with respect to olive oil results.

Applying water, the laurolactam migration observed is in the same order of magnitude as into oil. There are two possible causes for this. If water does cause swelling of the material that enables an enhanced diffusion or extraction of laurolactam, but then the solubility of laurolactam in water must have been limited. This case does not appear to be very likely since the amounts migrated here corresponded with concentrations of 10 µg/ml in water at a temperature of 100°C. (Experience in our lab showed that the solubility of laurolactam in water at room temperature is in the range of several hundred µg/ml.) The other possible explanation is that water will not cause any swelling in the polymer, but the migration was limited by diffusion. As olive oil and water migration took place at the same temperature, it is likely that both migration processes were limited by diffusion and results therefore match so well. As a consequence water would be an appropriate substitute for laurolactam migration into olive oil, where the same exposure time could be applied when the temperature equals or is below 100°C. It must be noted, however, that oligomer migration into water could not be observed. This is most likely due to their limited solubility in water.

Another interesting finding is that the specific migration of laurolactam into water observed from films A and C is higher than the respective overall migration. An explanation for this could be that the laurolactam evaporated along with the water during the evaporation step. In order to verify this, and to find out whether this is also the case for solvents having lower boiling points, the following test was performed.

Migration solutions (50% and 95% ethanol, 3 replicates each), having been in contact with film C for 24 hours at 40°C, were tested for contents of laurolactam and evaporated according to the procedure for overall migration testing. Subsequently, the residue was redissolved in 95% ethanol. It was found that migration of 2.3 mg/dm<sup>2</sup> had taken place into both solvents. The amount of laurolactam found in the residues, however, corresponded with 0.1 mg/dm<sup>2</sup>. This points out that approximately 95% of the laurolactam disappeared during the evaporation step, and seems to confirm that laurolactam will evaporate along with solvents at temperatures of 80°C and above. A likely explanation why this effect was not observed regarding film B is that the film released a significant amount of nylon 6 mono- and oligomers. A part of these migrants evidently did not evaporate along with the water and was subsequently found during gravimetric determination of the overall migration. LC-MS results confirmed this assumption as polyamide 6 mono- and oligomers up to the heptamer could be identified in the relevant solutions (see Figure 5.3 for chromatogram). The effect observed here can cause problems in another regard: The EU allows laboratories to evaluate the overall migration of a certain polymer to show compliance with a certain SML. If the overall migration is found to be below the specific migration limit, the polymer complies with EU legislation. If this approach had been applied for a film that releases laurolactam e.g. at a level of 1 mg/dm<sup>2</sup>, it can be assumed that overall migration of this material had been below the SML of  $0.833 \text{ mg/dm}^2$ .

Table 5.2	Comparison of laurolactam and nylon 12 oligomers overall
	and specific migration (mg/dm <sup>2</sup> ) from sample films into
	olive oil, isooctane, 95% ethanol, 50% ethanol and water

Food simulant	Test conditions	Test sample	Overall migration	Monomer migration	Dimer migration	Trimer migration
				0.6	იფ/din ეგ	0 1
		А		0.5	0.5	0.1
				0.4	0.4	0.1
Olive oil	2h/100°C	В		0.4	0.4	0.1
				0.5	0.4	0.1
		C		0.5	0.3	0.1
			0.4	0.2	0.1	0.0
		А	0.4	0.2	0.1	0.0
			0.6	0.2	0.1	0.0
Isooctane	1.5h/60°C	В	0.6	0.2	0.1	0.0
		6	0.7	0.2	0.1	0.0
		C	0.9	0.2	0.1	0.0
	3.5h/60°C	А	1.8	0.5	1.5	0.9
			3.3	0.5	1.5	0.8
95%		В	2.5	0.8	2.0	1.2
ethanol			2.5	0.9	2.1	1.4
		С	11.5	2.3	3.8	2.6
			8.6	2.3	4.0	2.7
	2h/reflux	А	1.8	0.5	1.0	0.5
			1.8	0.5	1.0	0.5
50%		D	2.4	0.6	0.8	0.6
ethanol		В	3.0	0.8	0.8	0.4
		C	7.4	1.9	*	0.3
		Ĺ	7.5	1.9	*	0.3
		А	0.1	0.5	0.0	0.0
	2h/reflux		0.1	0.5	0.0	0.0
		В	1.9	0.5	0.0	0.0
vvaler			1.4	0.5	0.0	0.0
		С	0.0	0.6	0.0	0.0
			0.1	0.6	0.0	0.0

analysis not possible owing to technical problems



- Figure 5.3 HPLC/MS chromatogram showing traces of a migration solution (film B, water, 2 hours at 100°C) with peaks of nylon 6 (up to n = 7) and nylon 12 oligomers (m=1 and 2).
- Table 5.3Estimates of diffusion and partitioning coefficients as well<br/>as  $A_P$  values and their 95% confidence intervals found by<br/>kinetic modelling of migration into isooctane and olive<br/>oil.

Simulant	Temperature	Diffusion Coefficient D <sub>P</sub>	Partitioning Coefficient K <sub>PF</sub>	Diffusion Conductance $A_P$	
		(cm²/s)			-
	40°C	$3.6 \pm 1.8 \cdot 10^{-12}$	1246 ± 320	1.8	(1.1 – 2.2)
lsooctane	60°C	$3.1 \pm 1.0 \cdot 10^{-11}$	140 ± 56	2.0	(1.6 – 2.2)
	80°C	$3.0 \pm 0.5 \cdot 10^{-10}$	10 ± 7	2.4	(2.3 – 2.6)
	40°C	$1.1 \pm 0.3 \cdot 10^{-12}$	2963 ± 374	0.6	(0.3 – 0.9)
Olive oil	60°C	$7.8 \pm 2.6 \cdot 10^{-12}$	495 ± 153	0.6	(0.2 – 0.9)
	80°C	$3.4 \pm 0.5 \cdot 10^{-11}$	34 ± 20	0.3	(0.1 – 0.4)



Figure 5.4 Migration of laurolactam from film C into olive oil and isooctane at 40, 60 and 80°C. Experimental data are shown as points, calculated data as lines.

#### 5.7.3 Diffusion kinetics / graphs

Of the solvents used in the first part of this study, isooctane was thought to be the least aggressive towards nylon 12. Therefore, isooctane, along with olive oil, was chosen for a kinetics study. The purpose of this study was to gain information about diffusion of laurolactam, the interaction of olive oil and nylon 12, the partitioning of laurolactam between the polymer and liquids, and finally, about the diffusion conductance coefficient  $A_P$  of nylon 12.

As can be seen in Figure 5.4 and Table 5.3, the parameters  $D_P$ ,  $K_{P,F}$  and  $A_P$ could be estimated within acceptable confidence intervals. In this regard, a short remark with respect to the confidence intervals of the partitioning coefficients seems appropriate as a  $K_{P,F}$  value with a confidence interval as e.g. 10  $\pm$  7 being  $\pm$  70% might look imprecise. However, a  $K_{P,F}$  value varying between 3 and 17 here, will result in migration results varying from 1.7 mg/dm<sup>2</sup> for  $K_{PF}$  =3 and 1.4 for  $K_{PF}$  =17 being in the range of ± 8% (laurolactam migration after 48 hours. As considerable differences can be observed between diffusion coefficients determined with isooctane and olive oil, it must be concluded that an interaction between isooctane and polyamide must have taken place. This was not expected for polyamide has a polar structure and isooctane is apolar. Therefore, a short experiment was performed to verify this assumption. During 48 hours contact time with isooctane at 80°C, it was shown that the mass of film C increased by 1.8%, while that of a film that had been in contact with olive oil decreased by 0.4%. These data suggest that isooctane penetrates polyamide 12 and therefore changes the polymer structure.

These diffusion coefficients translate into mean  $A_P$  values of 1.8 to 2.4 for diffusion determined with isooctane, and 0.3 to 0.6 for diffusion into olive oil (see Table 5.3 for respective 95% confidence intervals). In Table 5.3, it can be observed that the partitioning coefficients decrease with increasing temperature. This means that the solubility of laurolactam in the liquids increases more than that in the polymer, which is a common phenomenon. But it can also be seen that laurolactam seems to have a slightly higher affinity for isooctane than for olive oil in general. The partitioning coefficients of laurolactam (with respect to the polymer on the one side and olive oil or isooctane on the other) are in the same order of magnitude meaning that solubility of laurolactam in the liquids tested did not play an important role for the large difference in migration. What can be concluded from this, is that the  $A_P$  values determined with olive oil seem to be the most appropriate to be used for further migration modelling purposes.

#### 5.8 Conclusions

- Migration of laurolactam into water proved to be the best alternative estimation for its migration into olive oil; ethanol-based solvents gave overestimation and isooctane gave underestimation.
- Diffusion coefficients determined with isooctane were significantly higher than those found using olive oil.
- It was proved that isooctane penetrated nylon 12, while olive oil was inert to the polymer.
- The diffusion conductance coefficient *A<sub>P</sub>* for polyamide 12 determined using olive oil ranged from 0.3 to 0.6.

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# 6

Modelling of simultaneous two-sided migration into water and olive oil from nylon food packaging<sup>.</sup>

#### Abstract

Nylon 6 and nylon 12 food packaging materials used as sausage casings are typically exposed to fatty food on one side and boiling water on the other during the cooking process. To simulate the migration behaviour under these conditions, a special migration cell was constructed and filled with olive oil on one side of the polymer and water on the other side to find out what amounts of the migrants will transfer to either side and phase at 100 °C. Results show that when a nylon 6 film is exposed to conditions described above, total mass transfer of the monomer – caprolactam – into the water phase occurs after 2 hours at 100°C. Nylon 12 sausage casings release similar amounts of their monomer – laurolactam – into both, the aqueous and oil phase. An existing computer migration model was adapted to simulate the situation of simultaneous two-sided migration applying previously determined diffusion and partitioning coefficients. The suitability of the model was confirmed by experimental data.

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#### 6.1 Introduction

Nylon 6 (polyamide 6, polycaprolactam) and nylon 12 (polyamide 12, polylaurolactam) are the most frequently used polyamides for food packaging applications. Their monomers caprolactam (Ref No 14200, CAS No 0105-60-2) and laurolactam (Ref No 19490, CAS No 00947-04-6) are listed in section A of directive 2002/72/EC (EC 2002) with specific migration limits (SML) of 15 mg kg-1 and 5 mg kg-1, respectively (2.5 mg/dm<sup>2</sup> and 0.833 mg/dm<sup>2</sup> considering that 1 kg of food is packaged in 6 dm<sup>2</sup> of film according to the EU cube model). Figure 6.1 shows the chemical structures of caprolactam and laurolactam.

Typical applications of nylon 6 and nylon 12 films are sausage and cooked meat casings. Such meat products usually contain 20-40% fat. During the process of cooking meat or sausages, the nylon film will be exposed to hot water or steam on the outside and fatty food on the inside. In order to show compliance with EU legislation, specific migration into water and olive oil, the official fatty food simulant, has to be evaluated. Typical migration testing conditions are 2 hours at 100°C. While previous research has always focussed on the migration of monomers into either water or fatty food simulants (Begley et al 1993, Barkby and Lawson 1993, Gramshaw and Soto-Valdez 1998, Soto-Valdez et al. 1997, Stoffers et al. 2003b), during practical applications, the film will be exposed to hot water and fatty food simultaneously. The idea behind our investigations was therefore to find a way to simultaneously test specific migration into two totally different food simulants at either side of a food packaging material. We have furthermore tried to simulate this situation by computer modelling and draw conclusions about the partitioning of nylon monomers in the multi-phase system: fatty phase – polymer – water.



Figure 6.1 Structures of nylon 6 and nylon 12 monomers (caprolactam left and laurolactam right)
### 6.2 Theory of two-sided diffusion

The basic principles of modelling migration from a polymer into foods have been described in detail in literature (e.g. Gandek *et al.* 1989, Piringer 1993). In short,  $C_P$  the concentration of a migrant in a food contact polymer at time t at a distance x from the origin of the x-axis is described by Fick's second law of diffusion (Crank 1975).  $D_P$  is the diffusion coefficient and  $K_{P,F}$  is the partitioning coefficient (between polymer and food) in this system. A few assumptions have to be made in order to use this model:

- a) There is no chemical interaction between food and polymer
- b) The migrant initially is homogeneously distributed in the polymer
- c) Both phases (water phase on one side and oil phase on the other side of the polymer) are initially migrant-free
- d) No mass transfer limitation in the foods, so the migrant is always homogeneously distributed in the respective phases.

In the previous chapter no. 5 (Stoffers 2003b) these assumptions have been translated into formulas. In the case of two-sided migration, the transport phenomena from the polymer into the oil phase can be described as:

$$K_{P,O}\left(\frac{V_O}{A}\right)\frac{\partial C_O}{\partial t} = -D_P \frac{\partial C_P}{\partial x}$$
 at  $x = 0$   $t > 0$  (equation 6.1)

where  $K_{P,O}$  is the coefficient describing the partitioning of the migrant between the polymer and the oil,  $C_O$  is the concentration of the migrant in the oil,  $V_O$  is the volume of oil, A is the contact area. Diffusion from the polymer into the water phase can be described similarly as:

$$K_{P,W}\left(\frac{V_W}{A}\right)\frac{\partial C_W}{\partial t} = D_P \frac{\partial C_P}{\partial x}$$
 at  $x = L_P$   $t > 0$  (equation 6.2)

where  $K_{P,W}$  is the coefficient describing the partitioning of the migrant between the polymer and the water,  $C_W$  is the concentration of the migrant in the water,  $V_W$  is the volume of water and  $L_P$  is the thickness of the polymer. Figure 6.2a illustrates the situation described above.

In addition, a new coefficient is introduced describing the partitioning between the oil and water phases, both phases being separated by a polymer film layer.

$$K_{W,O} = \frac{K_{P,O}}{K_{P,W}}$$
 (equation 6.3)

This partitioning coefficient gives information about the affinity of a certain migrant for the water or oil phase. Values of  $K_{W,O} > 1$  indicate a higher affinity of the migrant for water or an aqueous phase, values  $K_{W,O} < 1$  indicate a higher affinity for oil or fatty foods. Information given by this partitioning coefficient is especially interesting for the development of a new migration modelling tool that is currently taking place in the EU funded project "Foodmigrosure" (www.foodmigrosure.com).

Since one multilayer film (next to three monolayers) was used in this study, the model described above had to be extended such that this kind of film could be described properly.





Figure 6.2 Schematic diagram of two-sided diffusion into oil and water from a) monofilm b) multilayer film

First of all, it had to be considered that different diffusion coefficients ( $D_{P1}$  and  $D_{P2}$ ) apply in the two layers. Furthermore, it had to be assumed that partitioning processes take place between the two layers: a partitioning coefficient  $K_{P1,P2}$  describes the partitioning of a migrant between the two polymers. This, and the coefficients describing the partitioning between the polymers and food simulants are illustrated in Figure 6.2b.

### 6.3 Sample materials

Four different nylon films were used. Two of them were commercial sausage casing, the other two materials originated from an EU project for the development of a reference material for specific migration testing (Störmer and Stoffers 2004). A short description of the materials used can be found in Table 6.1.

### 6.4 Reagents & Apparatus

All reagents and solvents were of analytical quality unless stated otherwise: water was HPLC grade from Fluka, Bucks, Switzerland. Virgin olive oil was from Minerva, Voghera, Italy. The glass migration cells were purchased from Gassner Glastechnik GmbH, Munich, Germany. All details concerning the analysis of laurolactam in water and olive oil have been described earlier (Stoffers et al. 2003a and 2003b). In short: aqueous samples containing laurolactam were directly analysed by LC-MS. Olive oil samples containing laurolactam were first cleaned-up by size exclusion chromatography (SEC) and then analysed by LC/MS. For water samples containing caprolactam, the same method as for laurolactam was applied. For the analysis of caprolactam in olive oil, the method of Franz and Rijk (1997) was used: olive oil containing known amounts of caprolactam and olive oil samples used during migration experiments were first diluted with heptane, then extracted with a mixture of ethanol and water. Instead of analysing by GC/FID the aqueous phase was injected into the LC/MS system.



Figure 6.3	Migration cell used in this study
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# 6.5 Two-sided migration into olive oil and water.

Migration tests were carried out according to the principles of European Standard EN1186-1 (CEN 1998). But instead of exposing the films to a food simulant on one side only, both sides of the cells were filled with food simulants. For this, two upper parts of glass migration cells were combined to one as shown in Figure 6.3.

# Table 6.1Thickness and migration potential of films A, B, C and D<br/>considering one-sided migration testing (Stoffers *et al.*<br/>2003b and Störmer and Stoffers 2004)

Film	Film Thio	ckness (µm)	Migration Pot	ential (mg/dm²)
	Nylon 6	Nylon 12	Caprolactam	Laurolactam
A*		39		1.0
B*	19	29	0.4	0.8
С		196		4.7
D	107		2.2	

<sup>\*</sup>commercial sausage casings

Prior to the incubation period, migration cells, water and olive oil were preheated to the testing temperature (100°C). The lower part of the migration cell was completely filled with water (approximately 85 ml, the exact weight was recorded). The film with a diameter of 7 cm having an area of 0.385 dm<sup>2</sup> was then placed in the cell, the upper part was assembled and the cell was closed. Next, a thin layer (approximately 8.5 ml, the exact weight was recorded) of olive oil was filled into the upper part of the cell. The cell was then incubated at 100°C for two hours. All experiments described were carried out in triplicate. After the incubation period, two 1 ml water aliquots were filled into autosampler vials, two olive oil aliquots per replicate were prepared to determine levels of caprolactam and / or laurolactam as described in the section above.

### 6.6 Migration modelling

Numerical integration of Fick's second law was performed using the software Athena Visual Workbench (www.athenavisual.com). The initial and boundary conditions applied here have been described in detail earlier (Stoffers *et al.* 2003b). Values for diffusion as well as partitioning coefficients ( $D_P$  and  $K_{P,F}$ ) originated from the EU project "Specific Migration" (Störmer and Stoffers 2004, Stoffers *et al.* 2003b). For the multilayer film system as described in the theory section, a numerical model written in Microsoft Excel was used. In this model the polymer-layers were discreticised as a grid model which was integrated in time using Fick's second law and the equations 6.1 and 6.2 by Euler's method.

### 6.7 Results

The method for two-sided migration testing as described in the experimental section worked quite well and was very reproducible. Coefficients of variations between three replicates were typically around 5%. All results reported below are means of three replicate migration tests.



Figure 6.4 Observed migration of monomers from films A, B, C and D (all 2 hours at 100°C)

Table 6.2	Diffusion and partitioning coefficients applied for
	migration modelling (Stoffers et al. 2003b and Störmer
	and Stoffers 2004)

Film	Со	efficients app	lied
	$D_P$ (cm <sup>2</sup> /s)	<b>К</b> <sub>Р,О</sub>	$K_{P,W}$
А	5 E-10	1	1
B (caprolactam)	5 E-10	1000	1
B (laurolactam)	5 E-10	1	1
С	5 E-10	1	1
D	3 E-08	100	1
D (one-sided)	5 E-10	100	

### 6.7.1 Laurolactam migration from films A and C into water and olive oil

The results from the two-sided migration experiments using mono-layered films A and C are presented in Figures 6.4a and 6.4c and show that similar amounts of laurolactam have migrated into the two phases. In both cases, a mean of 0.3 mg migrated into the water phase, and 0.2 mg migrated into the oil phase. From film A (39µm), almost total mass transfer took place. Film C (196µm) contained 1.8 mg meaning that 26% of the laurolactam migrated. The diffusion coefficient  $D_{P}$  and the partitioning coefficient  $K_{PF}$  values determined in previous studies (Stoffers et al. 2003b, Störmer and Stoffers 2004) were used to model the twosided migration from nylon 12 (Table 6.2). The slightly higher migration on the water side observed could not be simulated by our model, as only one constant  $D_{P}$  for the complete film was applied. Figure 6.5a and 6.5b show concentration profiles in the polymer at different times between t0 = 0 and t6 = 2h. It becomes visible that the application of the two-sided model with two liquids having the same partitioning coefficients gives, as is to be expected, the same profiles as full immersion testing in one food or solvent. Furthermore, the graphs obtained using the coefficients from the previous study do correspond to the experimental data guite well. Almost total mass transfer can be observed in the concentration profile for film A looking at t6 (2 hours) whereas only a minor fraction of the laurolactam migrated out of the thicker film C at t6. When regarding the concentration in the liquids during the testing period, the effect of the different volumes applied becomes obvious. The same amounts of laurolactam migrated into either direction, but different volumes of food simulants were present, thus the concentration in the olive oil was calculated to be higher. This also matches the experimental observations. A practical consequence of these findings is that if a high solubility of a migrant in a food simulant is given, lower volumes of food simulant can be or even should be applied to increase the sensitivity of the migration test without affecting the masses migrating.

Describing the results in a partitioning coefficient between oil and water phase as suggested in the theory section, for nylon 12 the coefficient is  $K_{W,Q} = 1$ , based upon the assumed other K-values.

### 6.7.2 Caprolactam migration from film D into water and olive oil

The results from the two-sided migration experiment using film D show that 99% of the caprolactam migrated into the water phase (see Figure 6.4d). The total migration of 1 mg from film D into the water means that nearly quantitative mass transfer of caprolactam from the film took place under the testing conditions applied here. Since nylon 6 has a much more hydrophilic character than nylon 12, in contact with water – especially boiling water – the water rapidly penetrates the polymer and swelling occurs. Therefore the diffusion process takes place at a much higher rate due to the increased  $D_P$  in the swollen matrix. The  $D_P$  for this matrix was determined in a separate experiment (details not shown). It was found to be two orders of magnitude higher than in the dry polymer. We used the partitioning coefficient between the polymer and water  $K_{P,W} = 1$  as given by Baner (2000) and a value of  $K_{P,O} = 100$  was assumed for the partition between polymer and oil. When looking at the outcome of the model using these coefficients, (Figure 6.5c) the assumption seems to be correct. Describing the results in a partitioning coefficient between oil and water phase as suggested in the theory section, for nylon 12 the coefficient is  $K_{W,O}$  = 100. Interestingly, the model shows that the caprolactam concentration in the oil initially rises up to a value of about 13 mg kg<sup>-1</sup> before decreasing to a value of 1 mg kg<sup>-1</sup> after 2 hours. This means that the caprolactam initially having migrated into the oil permeated back through the swollen film into the water phase. In order to compare this situation of contact on both sides with one-sided contact, we have modelled the situation that the film has a one-sided contact with oil and no swelling occurs ( $D_P$  taken from Störmer and Stoffers (2004), see Table 6.2 for details.). As visible in Figure 6.4, the migration itself is slower than during two-sided migration due to the usage of a lower  $D_{P}$ . The concentration of caprolactam in oil would reach the 9 mg kg<sup>-1</sup> after

two hours, meaning that the migration into olive oil measured by onesided testing would be considerably higher than the respective migration determined by two-sided testing.

### 6.7.3 Caprolactam and laurolactam migration from film B into water and olive oil

Figure 6.4b shows the results from the two-sided migration experiment using multi-layered film B. Roughly two thirds of the laurolactam migrated into the water phase, one third migrated through the nylon 6 layer into the oil phase, confirming once again that laurolactam has similar affinities for water and olive oil. Also, it can be seen that due to its hydrophilic character 99% of caprolactam migrated through the nylon 12 layer into the water phase, while only 1 % could be found in the oil phase. As for the modelling of this multilayer-system, no swelling was assumed since the hydrophilic nylon 6 layer was not in direct contact with water. It was also assumed that both monomers caprolactam and laurolactam have similar affinities for both polymers, translating into partitioning coefficients  $K_{P1,P2} = 1$  (Table 6.2). Figure 6.6 shows the outcome. Figure 6.6a describes the diffusion of caprolactam from the nylon 6 layer into the nylon 12 layer and into the two food simulants. Figure 6.6b shows the respective migration of laurolactam from the nylon 12 layer (t6 = 1h for illustration purposes). The phenomenon of caprolactam first migrating into the oil and then permeating back through the polymer into the water can be observed here as it could with film D. Also, it can be observed that the coefficients applied here seem to be in the right order of magnitude since the results of the model match the experimental observations guite well.



Figure 6.5 Modelled migration from films A, C and D (diffusion and partitioning coefficients given in Table 6.2; t0 = 0 hours, t6 = 2 hours).



Figure 6.6 Modelled migration from film B (diffusion and partitioning coefficients given in Table 6.2; figure a) t0 = 0 hours, t6 = 2 hours, figure b) t6 = 1 hour). a) caprolactam, b) laurolactam.

### 6.8 Discussion

It was shown that the approach of simultaneous two-sided migration testing is technically feasible and can generally provide information about the fate of migrants from a polymer film in between two different foods, food simulants or other liquids. The partitioning coefficient  $K_{W,O}$  introduced here is a convenient tool that can instantly give information about the fate of migrants (in equilibrium) being subjected to such a two-sided system.

The results on two-sided migration from nylon 12 films here confirm the outcome from a previous study where specific migration of the same films had been investigated by full immersion testing (Stoffers *et al.* 2003b). The migration of laurolactam into water and oil – tested individually – had been similar, and in a range of  $\pm$  20 % compared to results found here. This confirms our recent suggestion (Stoffers *et al.* 2003b) to consider water as an appropriate olive oil substitute for nylon 12 specific migration

testing.

Even though the respective results are similar, there is an evident difference in laurolactam migration into water (mean 0.7 mg dm<sup>-2</sup>) and oil (mean 0.5 mg dm<sup>-2</sup>) phases. It is very unlikely that the lower migration into the oil phase is the result of limited solubility in oil.  $K_{P,O}$  would have to be in the range of several hundred to see this kind of effect, but it was determined in olive oil at 80°C as  $K_{P,O} = 34 \pm 20$  (Stoffers *et al.* 2003b). Hence, a slightly higher diffusion coefficient on the water side caused by penetration of water into the polymer can be assumed. A short experiment was performed to test this assumption. During 48 hours contact time with water at 100°C, it was shown that the mass of film C increased by 0.5%, while that of a film that had been in contact with olive oil decreased by 0.3%. These data suggest that water penetrates nylon 12 and likely causes higher diffusion.

Migration of caprolactam from film D into water was 1 mg, which means that almost total mass transfer of the monomer from the film took place after 2 hours at 100°C. This is even though the film is significantly thicker than sausage casings usually are (the model film used here is more than 100µm thick as opposed to the normal 30-50 µm for commercial products). This is in itself nothing new (Barkby and Lawson 1993). It has, however, been shown here that if a nylon 6 film is simultaneously exposed to water and olive oil on either side, still all of the caprolactam will migrate to the water side. As for the modelling applied for nylon 6 films, we want to emphasize that applying a constant  $D_P$  does not describe the process taking place in the film correctly. The diffusion coefficient increases two orders of magnitude during the migration process along with change from dry to the swollen wet polymer. However, this swelling process takes place very quickly. Therefore, using a high  $D_{P}$  from the beginning will approximate reality using this model as much as possible. The outcome of our model suggests that the migration testing time is of very high importance. When comparing the concentrations of caprolactam in olive oil in Figures 6.5c and 6.5d, it becomes obvious that one-sided migration testing gives an overestimation compared to two-sided migration. A kinetic study in a two-sided system would be necessary to validate the outcome given in Figure 6.5c.

It has furthermore been shown that migration modelling in a multi-layer system is possible when using assumptions as described in the theory section. It would be interesting to extend the investigations in this chapter to a system where a multilayer film consists of at least two totally different polymers. In that case the effect of partitioning between the two polymer layers could become more obvious. A comment about the situation in real life seems appropriate: as sausages as well as bacon and

meat have a water content of 20-50%, mass transport and partitioning processes between water and oil phase are taking place also in the food itself. Furthermore, sausage casings usually have a maximum thickness of 50 microns, and we have measured monomer concentrations in the range of a maximum of 3000 mg kg<sup>-1</sup>. This means that in a worst case situation, total mass transfer from the film expressed in amounts per area would be roughly 1.5 mg dm<sup>-2</sup>. This itself would exceed the SML of laurolactam being 0.83 mg dm<sup>-2</sup>, but not the one of caprolactam at 2.5 mg dm<sup>-2</sup>. But considering directive 85/572/EEC (EEC 1985) giving products in category 06.04 – processed meat products (ham, salami, bacon and others) – a reduction factor of X/4 is applicable, which means that the result of the migration tests X can be divided by 4. Assuming the above, this means that the corrected maximum migration after full mass transfer of the films would be 0.4 mg dm<sup>-2</sup>, well below the SML values for caprolactam and laurolactam. This means that if the thickness of a nylon 6 or nylon 12 sausage casing is less than 50 µm, and the concentration of the monomer is less than 3000 mg kg<sup>-1</sup>, then no migration testing is necessary, because the SML of caprolactam or laurolactam cannot be reached.

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Migration and sensory evaluation of irradiated specific migration certified reference material candidates

### Abstract

The effects on ionising irradiation on polymer additives, monomers and polymers themselves have been investigated. Changes of initial concentrations of certain additives and monomers, a change in their specific migration as well as sensory changes of the polymers were examined. Polymer stabilizers such as Irganox 1076 and Irgafos 168 used in polyethylene were found to be degraded by ionising radiation. Decreased concentrations of stabilisers in polyolefins led to lower specific migration, however, not to lower overall migration into food simulants. Irganox 1076 levels in polystyrene did not change up to irradiation doses of 54 kGy. Sensory properties of LDPE, HDPE, PA6 and PA12 worsened, while sensory properties (i.e., odours) of PS improved with increasing irradiation doses.

This chapter is based on the published article:

Stoffers, N.H., Linssen, J.P.H., Franz, R. and Welle, F. 2004. Migration and sensory evaluation of irradiated polymers. *Radiation Physics and Chemistry.* **71**, 203-206

### 7.1 Introduction

Packaging materials are being sterilised by ionising radiation as an alternative to other sterilisation methods e.g. chemical or heat treatment. Sterilisation takes place in commercial irradiation plants with ionising radiation from <sup>60</sup>Co-sources and electron accelerators, respectively. Authorities' specific regulations for irradiated goods, low consumer acceptance and effects of ionising radiation on polymers and polymer additives however, led to a low application level of irradiation for sterilisation of packaging materials in the past.

In this research, the effects of gamma-irradiation on polymer additives, monomers and polymers themselves have been investigated. It was looked into changes of the initial concentrations of certain additives and monomers, a change in their specific migration as well as sensory changes of the polymers (i.e., changes in odours).

### 7.2 Materials and methods

#### 7.2.1 Materials

The polymers used in this study came from a project with the aim to develop certified reference materials for specific migration testing (Störmer and Stoffers 2004). All films were monolayer packaging films. The materials and migrants investigated in this research are listed in Table 7.1.

Tahla 71	Dolymor_mia	rant combinatio	nc invectionted i	n this study
			ns mvesugateu n	II LINS SLUUY
	, , ,			

No.	Polymer	Thickness	Migrant(s)
1	Low density polyethylene	1 mm	lrganox 1076
	(LDPE)	1 111111	and Irgafos 168
З	High density polyethylene	1 mm	lrganox 1076
	(HDPE)	1 111111	and Irgafos 168
10	Polystyrene (PS)	2 mm	lrganox 1076
15	Polyamida 6 (PAG)	0.1 mm	caprolactam
	Folyannide o (FAO)	0.1 11111	and lower oligomers
16	Polyamida 12 (PA12)	0.2 mm	laurolactam
	POlyannice TZ (PATZ)	0.2 11111	and lower oligomers

In the packaging industry, a typical dose for sterilization of the packaging material is 10 - 25 kGy. All polymers were irradiated in commercial irradiation plant with <sup>60</sup>Co with two different high radiation doses in order to generate a sufficient worst case scenario and to induce measurable effects. The polymers were irradiated with two doses: 29 kGy and 54 kGy.

#### 7.2.2 Analytical methods

Headspace gas chromatography (HS-GC) with either flame ionisation detector (FID) or mass spectrometry detection (MS) was used for the screening of volatile substances and for guantification of radiolysis products in the polymers. Each sample was analysed in the following way: The polymer sample (1.0 g for PS, LDPE and PP) was cut in small pieces and placed in a 22 ml headspace vial. After equilibration for 1 h at appropriate temperatures (see below) the samples were analysed by headspace gas chromatography HS GC (FID). Quantification was achieved by external calibration. Gas chromatograph: Perkin Elmer AutoSystem XL, column: J&W Scientific DB 1 - 30 m - 0.25 mm i.d. - 0.25 um film thickness, temperature program: 50 °C (4 min), rate 20 °C min<sup>-1</sup>, 320 °C (15 min), pressure: 50 kPa helium, split: 10 ml min<sup>-1</sup>. Headspace autosampler: Perkin Elmer HS 40 XL, oven temperature: 120 °C (PP, LDPE), 150 °C (PS), 200 °C (PET), needle temperature: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), transfer line: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), equilibration time: 1 h, pressurization time: 3 min, inject time: 0.02 min, withdrawal time: 1 min.

Identification of volatile radiolysis products was achieved by HS GC (MS). Gas chromatograph: Hewlett Packard 6890, column: Macherey-Nagel Optima 1 MS - 30 m - 0.25 mm i.d. - 0.25 µm film thickness, temperature program: 40 °C (5 min), rate 10 °C min<sup>-1</sup>, 320 °C (1 min), pressure: 1.3 bar helium, split: 1:20. Mass detector: Hewlett Packard 5973 Mass Selective Detector (MSD), MS-conditions: electronic ionisation, full scan, scan range 34-700 daltons. Headspace autosampler: Perkin Elmer HS 40 XL, oven temperature: 120 °C (PP, LDPE), 150 °C (PS), 200 °C (PET), needle temperature: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), transfer line: 140 °C (PP, LDPE), 170 °C (PS), 210 °C (PET), time: 1 h, pressurization time: 3 min, inject time: 0.06 min, withdrawal time: 1 min. The mass spectra were compared for identification with the commercial NIST database.

Semi-volatile radiolysis products were determined by gas chromatography with FID detector after extraction of the polymers. Extraction of LDPE /

HDPE: 1.0 g PET of each sample was transferred into glass vials. Then 10 ml of dichloromethane (DCM) were added. The vials were sealed and placed for 24 hours at 40 °C. The swollen polymers were cooled to room temperature. After 24 h at 40 °C the extracts were analysed by GC/FID. Gas chromatograph: Hewlett-Packard HP 5890II, column: Supelco SE 10 - 30 m - 0.32 mm i.d. - 0.32 µm film thickness, temperature program: 40 °C (5 min), rate 15 °C min<sup>-1</sup>, 240 °C (15 min), pressure: 50 kPa hydrogen, split: 10 ml min<sup>-1</sup>. For identification and quantification, external standards of the additives Irganox 1076 and Irgafos 168 as well as radiolysis products 1,3-di-*tert*-butylbenzene (1,3-DBB) and 2,4-di-*tert*-butyl-phenol (2,4-DBP) were applied.

All details with regard to the other analytical as well as specific migration methods that have been applied in this study can be found in the previous chapters and in Störmer and Stoffers (2004).

### 7.2.3 Sensory Examinations

The sensory examination was conducted by a trained panel of six persons. The applied method was in accordance to DIN 10955. Odour evaluation: The irradiated polymer samples were placed in a preserving jar and stored for 1 d at room temperature (23 °C). Subsequently sensory differences were determined by the sensory panel. The applied evaluation scale ranges from I = 0 (no noticeable odour) to I = 4 (strong odour).

### 7.3 Results / discussion

### 7.3.1 Concentrations of migrants and radiolysis products

As can be observed in Table 7.2, a significant decrease of phosphite additives (Irganox 1076 and Irgafos 168) in HDPE and LDPE could be observed after irradiation doses of 29 and 54 kGy. Irgafos 168 was completely gone after a dose of 29 kGy. For Irganox 1076, a higher irradiation dose generally led to a higher decrease. Figure 7.1 shows chromatograms of extracts of LDPE subjected to different irradiation dosages. Transformation products that could be identified were 1,3-di*tert*-butylbenzene (1,3-DBB) and 2,4-di-*tert*-butyl-phenol (2,4-DBP). Levels of 1,3-DBB increased with increasing irradiation doses, while 2,4-DBP seemed to undergo further degradation during the irradiation process.

Both products have been identified earlier (Buchalla *et al.* 1999, 2000, Welle *et al.* 2000, 2002) and seem to originate from degraded Irgafos 168.

The antioxidant Irganox 1076 in PS was found to be relatively radiation resistant. No degradation of Irganox 1076 could be observed up to a dose of 54 kGy, which corresponds with observations of Kawamura (2004). As can be seen in Table 7.2, the sum of volatiles (mainly styrene monomer) was found to decrease with increasing irradiation dose. An obvious matrix effect with respect to the stability of antioxidants could be observed.

Levels of PA monomers and lower oligomers were not affected by gamma-irradiation. During headspace GC analysis of PA 12, aliphatic hydrocarbons (C9, C10) were found in irradiated samples which have been formed during the irradiation process. As can be observed in Figure 7.2, several other volatile substances were formed during the irradiation process in both PA films, non of which could be identified unfortunately.

Polymer	Irradiation dose	Irgaphos 168	lrganox 1076	1,3-DBB	2,4-DBP	unidentified (RT 30 min)	total volatiles
	0 kGy	599	693	0	0	0	0
LDPE	29 kGy	0	172	111	86	85	13
	54 kGy	0	69	153	56	53	14
	0 kGy	492	650	0	0	0	0
HDPE	29 kGy	0	226	94	38	37	15
	54 kGy	0	140	135	29	38	21
	0 kGy		540				237
PS	29 kGy		540				168
	54 kGy		550				17

Table 7.2	Concentrations (mg/kg) of phenolic antioxidants and	k
	degradation products in unirradiated and irradiated	ł
	LDPE, HDPE and PS	



Figure 7.1 GC-FID chromatograms of DCM extracts of LDPE films irradiated with 0, 29 and 54 kGy



Figure 7.2 Headspace GC chromatograms of polyamide films irradiated with 0 and 54 kGy

#### 7.3.2 Overall Migration

It was shown that overall migration results of all films investigated were not influenced by irradiation even up to high irradiation doses of 54 kGy which corresponds with observations of Welle *et al.* (2000). In all cases, the overall migration was below or far below the migration limit of 10 mg/dm<sup>2</sup> given by EU regulations. A likely reason for unchanged overall migration is based on the overall migration testing procedure where volatile substances are lost during the evaporation step in sample preparation. It can therefore be concluded that the overall migration test that is based on gravimetric procedures is not suitable for detection of changes in overall migration.

### 7.3.3 Specific Migration

Specific migration of Irgafos 168 and Irganox 1076 from LDPE and HDPE into 95% ethanol was evaluated (4 hours at 70°C). Specific migration decreased with increasing radiation dose. As these additives were degraded by irradiation, this is not unexpected. Margue et al (1998) stated that the reduced specific migration of additives irradiation is beneficial with regard to food safety, but this conclusion is only one part of the migration behaviour of irradiated polymers. The concentration of additive-related degradation products e.g. 1,3-DBB or 2,4-DBP increases during irradiation. To make a statement about the food safety of irradiated polyolefins in general, these degradation products would have to be included into the specific migration testing as well. As specific migration of these irradiation products was not investigated in our project, we would prefer not to make a statement about this issue in general. We did however find several unidentified peaks in the chromatograms of migration solutions of the LDPE material increasing with increasing radiation dosages which confirms our consideration above.

PS proved to be the most inert polymer as no migrants could be found in any of the migration solutions that had been in contact with 95% ethanol for 4 hours at 70°C. No significant changes could be observed with respect to the specific migration of polyamide monomers caprolactam and laurolactam. It is, however recommended to do further research into volatiles degradation products as mentioned in section 7.3.1.



Figure 7.3 GC-FID chromatograms of 95% ethanol specific migration solutions having been in contact with LDPE for 4 hours at 70°C



Figure 7.4 Specific migration results of polyamide 6 and polyamide 12.

### 7.4 Sensory Examinations

The influence of irradiation on the sensory behaviour was investigated with all polymer samples which are all intended for packaging of food products. The results of sensory examinations of LDPE, PS, PA 6 and PA 12 are summarised in Table 7.3. HDPE results are not shown as no significant changes of sensory properties could be observed.

Volatile substances formed during irradiation affected the sensory properties of the investigated LDPE. A typical off-odour occurred after irradiation with increasing irradiation dose. A slight decrease in odour intensity and "improvement" of quality could be observed with PS. This can easily explained by an decrease of the styrene level as described in Section 7.3.1 above. The perceived sensory aspects with respect to odour of the polyamides worsened with increasing irradiation dose. A correlation to the sensory properties can therefore be concluded.

Polymer	Irradiation dose	Description of odour	Intensity
	unirradiated	weak PE odour, dull, dusty	2.0
LDPE	29 kGy	strong PE odour, wax-like, dull, burnt, spicy, slightly chemical	3.5
	54 kGy	distinct PE odour, burnt, spicy	3.0
	unirradiated	burnt, strong plastic odour, slightly wax-like, slightly chemical	3.0
PS	29 kGy	burnt, strong plastic odour, slightly wax-like, slightly chemical	3.0
	54 kGy	noticeable plastic odour, burnt, slightly stinging	2.5
	unirradiated	weak plastic odour, slightly smoky	1.5
PA 6	29 kGy	distinctly cheesy – sweaty	3.0
	50 KGy	strong plastic odour, distinctly cheesy – sweaty	3.5
	unirradiated	slightly sweet plastic odour	2.0
PA 12	29 kGy	strong plastic odour, cheesy – sweaty, slightly burnt	3.0
	54 kGy	very strong plastic odour, cheesy – sweaty, burnt	3.5

Table 7.3	Results of the sensory examinations (odour) of irradiated
	packaging materials in comparison to the unirradiated
	reference sample

### 7.5 Conclusions

- It can be shown from the results of this study that irradiation of packaging materials can lead to formation of volatile compounds during irradiation.
- Commonly used polymer stabilizers such as Irganox 1076 and Irgafos 168 used in polyethylene were degraded by ionising radiation; commonly found degradation products were identified.
- Polymers will not be sufficiently stabilised after irradiation. The use of stabilizers relatively resistant to irradiation is recommended (Kawamura 2004)
- Decreased concentrations of stabilisers in polyolefins leads to lower specific migration, however, not to significantly lower overall migration into food simulants
- Irganox 1076 in polystyrene was found to be unaffected by radiation as its concentration did not change up to irradiation doses of 54 kGy.
- Levels of PA monomers and oligomers were not affected by ionising irradiation, but the amounts of volatiles was increased.
- The odours originating from LDPE, PA6 and PA12 worsened, while those of PS improved with increasing irradiation doses.

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# 8

### General discussion

### 8.1 Introduction

This thesis is a compilation of a number of research topics during a feasibility study for the certification of 6 reference materials for specific migration testing of food packaging materials. Within the feasibility study, various new analytical methods were developed and validated. The methods for migrants from nylon 12 materials were applied investigating the migration behaviour of nylon 12 sausage casings. Additionally, a way to estimate diffusion and partitioning coefficients and their confidence intervals based on experimental data, was introduced. In this final chapter, the results are reviewed, compared with results from literature, and recommendations for future work are made.

### 8.2 Certified reference materials

The feasibility study in which results from four laboratories were collected and evaluated has been successfully completed. The CRM candidates include three polyolefins and three non-polyolefins that are among the most frequently used food packaging materials. The selection covers a broad range of polymers and migrant polarities and molecular sizes. Furthermore, several contact times and temperatures have been evaluated. Of the 7 migrants present in the 6 materials, 4 have specific migration limits (SMLs) and are therefore relevant for conformity testing laboratories. Of the remaining three migrants, Irgafos 168 is a commonly used processing stabiliser, and styrene is a monomer that is usually present in polystyrene type packaging materials, and currently being discussed as a substance to be regulated by an SML in future (EC 2003a). Finally, DPBD is an established fatty food contact model migrant. It has previously been used to investigate packaging materials in contact with fatty foods (Castle et al. 2001).

Most of the methods developed and validated within the project have proved to be rugged and easy to transfer from one laboratory to the other. Difficulties that have been encountered were during the determination of two migrants (separation of Irgafos 168 and Chimassorb 81 peaks from interfering oil peaks) in sunflower and olive oil, and the  $C_{P,0}$  determination by dissolution of polyolefins followed by precipitation. A further focus on these methods in order to obtain lower limits of detection is desirable in order to obtain more accurate migration tests.

Some of the materials have been requested by the FAPAS<sup>®</sup> (Food Analysis Performance Assessment Scheme) organisation for one of their next proficiency testing trials in the future. Furthermore, several materials are currently in use during the project "Foodmigrosure", an EU funded project with the goal to establish a physical-chemical migration model that can mathematically describe the migration processes from plastics into actual foodstuffs (http://www.foodmigrosure.org).

# 8.3 Concentration of migrant in the polymer - $C_{P,0}$

The CRM candidates developed are of particular interest for testing laboratories and for the scientific community because of their certified  $C_{P,O}$  value. In a recent interlaboratory comparison study, a large variability in results between laboratories was observed. It was concluded that the general quality of polymer additive analysis needed to be improved (Ritter *et al.* 2003, Bart *et al.* 2001). As mentioned in chapter 1, the use of CRMs, proficiency testing or interlaboratory comparison testing would be appropriate ways to do this. Materials 01-2, 04-2 and 05-2 developed in this project would be excellent choices in this respect.

In addition to the work described in the previous chapters, a short comparison of different sample preparation and extraction methods for additives in polyolefins was started using some of the CRM candidates. Accelerated solvent extraction (ASE) was compared with dissolution / precipitation and static extraction applying different solvents. For the latter, two preparation methods – grinding to powder versus cutting into 1 mm stripes – were evaluated. Unfortunately, not all methods have undergone a full validation process yet (recovery not available for all experiments). Also, not enough data were gathered to make a complete assessment of different methods. The available data are shown in Table 8.1.

Table 8.1	Comparison	of	different	sample	preparation	and
	extraction tee	chnic	jues for	the d	letermination	of
	additives in p	olyo	letins.			

	Stati	c extract	ion - Eth	anol	Dissolu Precipi	ution - tation	Sta extrac DCI	tic tion – M ³	Accele Solv Extra (A:	rrated ent ction SE)
No. Polymer / Analyte	Pow	/der	Pow	/der	Pow	/der	Pow	der	Pow	der
	Conc.1	Rec. <sup>2</sup>	Conc.	Rec.	Conc.	Rec.	Conc.	Rec.	Conc.	Rec.
01 LDPE / Irg.1076	601	1,03	627	1,09	580	~	805		452	
02 LDPE / DPBD	1			1			135		108	
04 HDPE /Chim.81	931	<del>、</del>	930	0,98	783	1,09	879	66'0		
04 HDPE /Uvitex OB	471	<del>、</del>	456	1,03	442	1,06	609	1,21		
05 PP / Irg.1076	1296	1,25	941	1,28	1409	0,83	1303	1	1032	
<sup>1</sup> Conc. :	= Concer	itration ir	n mg/kg,	<sup>2</sup> Rec. =	: Recover	y, <sup>3</sup> DC	M = dichl	orometh	ane	

It is interesting to see that static extraction of additives from polyolefins with ethanol produced results that are very close to the values from the project obtained by static extraction with dichloromethane (DCM). No differences as a result of a grinding step before the extraction could be observed. Extraction with DCM is a commonly applied method for this purpose, but ethanol has the advantage that it is the mobile phase applied during HPLC determination and thus no solvent exchange step is necessary after extraction. Furthermore, it is relatively harmless and easy to handle. Hence, this method could be considered for future activities in this regard. This will be an improvement since the method of choice during the second part of the certification exercise (dissolution in boiling toluene with subsequent precipitation with methane) proved to be very time consuming and somewhat difficult to apply.

#### 8.3.1 Diffusion data and $A_{P}$ values

Due to the complex analytical procedures and high costs involved, it is desirable to use migration modelling instead of experimental testing where available and allowed by legislation (Chapters 1, 2, EC 2003b). For migration modelling, two fundamental pieces of information are needed: the diffusion coefficient  $D_P$  for a certain migrant in a certain polymer at a certain temperature, and the coefficient  $K_{P,F}$  describing a migrant's partitioning between polymer and food. For the latter, a worst-case approach can easily be implemented by setting  $K_{P,F} = 1$ , thus assuming a high solubility of the migrant in the food. The diffusion coefficient  $D_P$  however, depends on several factors, being

- Properties of the migrant (e.g.molecular weight *M<sub>r</sub>*, structure)
- Properties of the polymer (e.g. free volume, structure)
- External factors (e.g. temperature, pressure, absorption of food components)

The experimental determination of  $D_P$  values is more time-intensive and costly than the measurement of the actual migration, and as a result, only a limited number of diffusion coefficients is available.

With this in mind, a polymer specific diffusion "conductance" coefficient was introduced by Piringer (1993, 1994). An Arrhenius-type relationship of the diffusion coefficient with the relative molecular weight of the migrant, the temperature and the polymer type was established and a polymer coefficient  $A_p$  describing the diffusion "conductance" of a polymer was introduced. Provided a reliable  $A_p$  has been established, a

worst-case diffusion coefficient  $D_{P}^{*}$  can be estimated for any migrant (molecular weight less than 1000 Daltons, EC 2001) that is toxicologically relevant for a given temperature.

$$D_{P}^{*} = 10^{4} \cdot e^{A_{P} - 0.1351 \cdot M_{r}^{2/3} + 0.003 \cdot M_{r} - 10454/T}$$
 (equation 8.1)

where  $D_{P}^{*}$  is the upper bound diffusion coefficient (cm<sup>2</sup> s<sup>-1</sup>),  $A_{P}$  is the polymer specific coefficient,  $M_{r}$  is the molecular weight of the migrant in Daltons, *T* is the temperature in K (Piringer 1993).

For some polymers,  $A_P$  needs a temperature correction according to the following equation:

$$A_{P} = A'_{P} - \frac{\tau}{T}$$
 (equation 8.2)

where  $A'_{P}$  is the temperature independent  $A_{P}$  and is the polymer specific temperature coefficient in K. For the empirical relationship given, = 0 (EC 2003b) represents one group of plastics, e.g. LDPE, and = 1577 (EC 2003b) represents another important group of plastics, e.g. HDPE and PP. Table 8.2 below shows a list of  $A'_{P}$  and values as currently applied in the Practical Guide for food contact materials (EC 2003b).

Table 8.2Polymer specific conductance coefficient  $A'_{\rho}$  and polymer<br/>specific temperature coefficient  $\tau$  along with their<br/>application temperature for selected Polymers as<br/>currently applied in the Practical Guide for food contact<br/>materials (EC 2003b)

Polymer	A' <sub>P</sub>	τ (Κ)	T (°C)
LDPE/LLDPE	11.5	0	< 90
HDPE	14.5	1577	< 100
PP	13.1	1577	< 120
HIPS	1.0	0	< 70
PA	2.0	0	< 100

Table 8.3 Polymer specific conductance coefficient  $A'_{p}$ , upper limit  $A'_{p}^{*}$  and polymer specific temperature coefficient  $\tau$  as suggested by Begley *et al.* (2004), compared with updated values from this thesis (CRM).

Polymer		Mean A' <sub>P</sub>	S.D.	n	t- value	A' <sub>P</sub> *	τ
LDPE	Begley	10.0	1.0	27	1.70	11.7	0
	CRM	10.6	0.7	40	1.68	11.7	0
	Begley & CRM	10.3	0.9	67	1.67	11.8	0
HDPE	Begley	10.0	1.9	49	1.68	13.2	1577
	CRM	12.6	0.8	36	1.69	13.9	1577
	Begley & CRM	11.4	2.0	85	1.67	14.8	1577
PP	Begley	9.4	1.8	53	1.67	12.4	1577
	CRM	10.2	2.5	36	1.69	14.4	1577
	Begley & CRM	9.6	2.2	89	1.66	13.3	1577
HIPS	Begley	-2.7	1.7	33	1.69	0.1	0
	CRM	-2.5	0.6	14	1.76	-1.4	0
	Begley & CRM	-2.6	1.4	47	1.68	-0.2	0
PA _	Begley	-1.5	2.0	31	1.70	1.9	0
	CRM	-0.7	1.0	14	1.76	1.1	0
	Begley & CRM	-1.3	1.8	45	1.68	1.7	0

Initially, so-called upper limit  $A'_{P}^{*}$  values were defined as to lead to conservative  $D_{P}^{*}$  values by making sure that they would result in an overestimation of 95% of the diffusion coefficients available within the database (Hinrichs and Piringer 2001). Table 8.2 shows the values as they are currently listed in the Practical Guide for food contact materials (EC 2003b). Just recently, the data have undergone a new statistical evaluation (Begley *et al.* 2004). In this approach, the mean  $A'_{P}$  values and standard deviations for each polymer were calculated. In order to

calculate an upper limit  $A'_{P}^{*}$ , the standard deviation, multiplied with the student t-factor corresponding to the number of  $A'_{P}$  values available and the right-side 95% confidence level was added to the mean  $A'_{P}$  value. The new data from this thesis were evaluated according to the same approach. The upper limit  $A'_{P}^{*}$  was calculated based on the "old" data, the "new" data from this thesis, and, as average, all data available and can be found in Table 8.3.

It should be mentioned that migration from a food package has a square root dependence on the diffusion coefficient, therefore small differences in  $A_P'$  do not translate into large differences in migration. Overall, it can be stated that the new data from this thesis confirm previous observations. For PP, HIPS and PA, the values calculated from data in this thesis significantly differ from those from Begley *et al.* (2004). However, when comparing the outcome based on *all* data with those currently applied (Table 8.2) it can be concluded that there is no need to make changes to the Practical Guide for food contact materials (EC 2003b).

### 8.4 Diffusion coefficient determination

One of the certification parameters in the feasibility study was the diffusion coefficient. Kinetic studies regarding the diffusion of migrants from a polymer into a food simulant were completed. The shape of the kinetic curve depends on two coefficients, being the diffusion coefficient  $D_P$ , and the partitioning coefficient  $K_{P,F}$  (Chapter 5, Stoffers *et al.* 2003). In chapter 5, an approach to estimate diffusion and partitioning coefficients applying the software Athena Visual Workbench (www.athenavisual.com) was reported. After having entered the mathematical fundamentals (Fick's law and appropriate boundary conditions) into the software, the following further input data were needed:

- initial concentration of the migrant in the polymer  $-C_{P,O}$
- polymer thickness and density
- food (simulant) volume and density
- contact area.

Once these have been inserted, experimental observations (migration data), concentrations of a migrant in the food (simulant) at a certain time are necessary. The software then produces an estimation of the diffusion and partitioning coefficient  $D_P$  and  $K_{P,F}$  including their 95% confidence intervals.

Looking at the raw data that the Piringer model and consequently the current legislation is based upon (Begley *et al.* 2004, EC 2003b), it can be noticed that many of the  $A'_{P}$  values reported were based on one experimental observation only meaning that a specific migration test was performed, not a kinetic study. In order to facilitate the determination of  $D_{P}$  from this experimental observation, the partitioning coefficient was set to a default value of 1 (high solubility in food simulant) or 1000 (low solubility), and the curve was adjusted to fit one data point. Obviously, the  $D_{P}$  values obtained from only one experimental observation are not very reliable. If one applied the software mentioned here for an experiment with only one observation, a very wide 95% confidence interval would be the consequence.

With this confidence interval, the  $D_P$  and  $A'_P$  could be given something of a "quality stamp", e.g. a weighting factor. When calculating a mean  $A'_P$ , a result with a narrow confidence interval and a higher weighting factor would count more than an unreliable result. This way, an unreliable outlying result would hardly have effect on the calculation of a mean value.

Alternatively, all independently collected raw data could be gathered, and the mean of those could be determined. This way, the differences in the size of individual data sets would be taken into account in a proper statistical way.

### 8.5 Follow-up project

A real certification would be the next logical step after completion of this feasibility study. However, several more laboratories would be needed to participate. These would first have to prove their suitability in a small interlaboratory comparison study. Only the laboratories that conform to specified criteria would then be invited to participate in the actual certification exercise. With a larger number of laboratories and more input data, the mean results would be based on a larger number of raw data and the confidence intervals of the certification parameters would likely become narrower.

The participating laboratories themselves would already profit from partaking in such a certification study. They would see their own results in a different, wider perspective and could – if necessary – improve the quality of their work.

### 8.6 Nylon research / two-sided migration

Since there has been a special focus on nylon materials in this thesis, some discussion regarding the practical consequences of the results seems appropriate.

As described in the chapters 5 and 6 (Stoffers *et al.* 2003 and 2004), nylon materials are often used as sausage casings, and as such they are exposed to fatty food on one side and steam or hot water on the other. Therefore, migration tests with both water and olive oil have to be completed to assess the compliance of a nylon sausage casing. When alternative fatty food simulants for nylon 12 materials were evaluated, solutions containing different concentrations of ethanol overestimated, and isooctane underestimated the migration into olive oil. However, the migration results into water matched the olive oil results best. This result was confirmed when nylon 12 materials were tested by two-sided migration testing as described in chapter 6 (Stoffers *et al.* 2004). As a consequence, for specific migration compliance purposes, one would only have to test a nylon 12 film with water. When regarding the complex analytical method needed to detect the migrant laurolactam in olive oil, this approach can save time and money.

As described, it was also tried to simulate the practical application of sausage casings with a special migration cell. The simultaneous migration into water and olive oil was monitored. Along with the nylon 12 test films, a nylon 6 sausage casing was evaluated using this approach. The relevant migrant – the monomer caprolactam – was only found in the water phase. Obviously, this is due to the hydrophilic character of the nylon 6 monomer caprolactam. But what is the practical consequence? The water phase applied during the boiling process of sausages is not used for consumption. So if one wanted to compare the use of nylon 6 and nylon 12 for these applications, with nylon 6, all of the migrants tend to migrate into the phase that is normally not used for consumption, whereas about half of the nylon 12 migrants do migrate into the actual food. Therefore, assuming the toxicity of both monomers is comparible, it can be suggested that the use nylon 6 for purposes as mentioned above is safer than that of nylon 12.

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#### Summary

The core of this thesis is a project with the main objective to develop the know-how to produce certified reference materials (CRMs) for specific migration testing necessary for food contact plastics. Parameters for certification were:

- The initial concentration of migrants in the polymer  $(C_{P,0})$ .
- The specific migration into a food simulant under certain temperature / time conditions.
- The diffusion coefficient  $D_P$  of the migrant in the polymer

16 preliminary CRMs were defined and produced. The most important polymers (LDPE, HDPE, PP, PS, PET, PVC, PA) and additives were chosen as well as monomers representing different physicochemical properties as target substances for migration. Stability and homogeneity of the migrants in the materials were tested and methods for the determination of the certification parameters were developed and validated. Of the 16 materials produced the 6 most suitable CRM candidates were selected (LDPE // Irganox 1076 / Irgafos 168, LDPE // 1,4-diphenyl-1,3-butadiene (DPBD), HDPE // Chimassorb 81 / Uvitex OB, PP homo // Irganox 1076 / Irgafos 168, HIPS, 1 % mineral oil // styrene, PA 6 // caprolactam). The feasibility of CRM production for the 6 candidate materials was demonstrated during a certification exercise with participation of 4 partner laboratories. All 6 materials showed suitable properties for production as CRMs. The diffusion and partitioning coefficients determined were applied for a comparison of experimental and predicted migration data, both based on results from the same materials. Roughly half of the predicted data were within  $\pm 10\%$ , almost all predicted data were within  $\pm 40\%$  compared to the experimental data.

Within the project, several analytical methods needed for the determination of the certification parameters were developed and validated. As an example, methods for the determination of the nylon 12 monomer, cyclic dimer and trimer were described. High performance liquid chromatography using ultraviolet (HPLC-UV) and mass spectrometric detection (HPLC-MS) were both found suitable to identify and quantify monomer, cyclic dimer and trimer and trimer well below the specific migration limit (SML) of laurolactam being 5 mg/kg. Gas chromatography with flame ionisation detection (GC-FID) showed to be an appropriate

method for the detection of only laurolactam in aqueous and fatty food simulants. Food simulants could be analysed directly, or after a change of solvents by all three methods. For olive oil, a method for sample clean-up by size exclusion chromatography (SEC) was established.

The new methods were applied in a study, where the migration of laurolactam and cyclic di- and trimer of nylon 12 was evaluated using three different films and five food simulants (olive oil, isooctane, 95% ethanol and 50% ethanol and water). Substitute test conditions for migration into olive oil according to EU directive EC/97/48 were applied using 95% ethanol and isooctane. Results showed that 95% ethanol overestimated while isooctane underestimated respective migration into olive oil. Water proved to be the best olive oil substitute as migration of laurolactam into water and olive oil using the same temperature gave similar results. Additionally, diffusion kinetics of laurolactam were investigated by migration kinetic studies using isooctane and olive oil. Diffusion coefficients determined with isooctane were significantly higher than those found using olive oil. It was proved that isooctane interacted with the polymer whereas olive oil was inert. The diffusion conductance coefficient  $A_P$  for nylon 12 determined using olive oil ranged from 0.3 to 0.6.

Since nylon food packaging materials used as sausage casings are typically exposed to fatty food on one side and boiling water on the other during the cooking process, a special migration cell was constructed and filled with olive oil on one side of the polymer and water on the other side to simulate the migration behaviour under these conditions. When a nylon 6 film was exposed to conditions described above, total mass transfer of the monomer into the water phase occurred after 2 hours at 100°C. Nylon 12 sausage casings released similar amounts of their monomer into the aqueous and oil phases. An existing computer migration model was adapted to simulate the situation of simultaneous two-sided migration applying previously determined diffusion and partitioning coefficients. The suitability of the model was confirmed by experimental data.

In addition, the effects on ionising irradiation on polymer additives, monomers and polymers themselves have been investigated. Changes of initial concentrations of certain additives and monomers, a change in their specific migration as well as changes in sensory properties (odour) of the polymers were examined. Polymer stabilizers such as Irganox 1076 and Irgafos 168 used in polyethylene were found to be degraded by ionising radiation. Decreased concentrations of stabilisers in polyolefins led to lower specific migration, however, not to lower overall migration into food simulants. Irganox 1076 levels in polystyrene did not change up to irradiation doses of 54 kGy. Sensory properties of LDPE, HDPE, PA6 and

PA12 worsened, while sensory properties of PS improved with increasing irradiation doses.

Finally, the results of the thesis were given a critical review and the implementation of the results were discussed. An incorporation of the diffusion data obtained into the migration modelling approach currently applied by the EU was simulated and suggested.

# Zusammenfassung

Diese Arbeit beschreibt ein Projekt mit der Zielsetzung, das Know-how zu entwickeln, um zertifizierte Referenzmaterialien (Englisch CRMs) für die spezifische Migrationsprüfung zu produzieren. Zertifizierungsparameter sind:

- Die Ausgangskonzentration der Migranten im Polymer ( $C_{P,O}$ ).
- Die spezifische Migration in ein Lebensmittelsimulanz bei bestimmter Temperatur und Zeit.
- Der Diffusionskoeffizient  $D_P$  des Migranten im Polymer

In der ersten Phase des Projekts wurden 16 vorläufige CRMs definiert und produziert. Die wichtigsten Polymere (LDPE, HDPE, PP, PS, PET, PVC und PA) und Migranten (Additive und Monomere) mit den unterschiedlichsten Eigenschaften wurden ausgewählt. Stabilität und Homogenität der Migranten in den Materialien wurden geprüft und Methoden für die Bestimmung der Zertifizierungsparameter entwickelt und validiert. Von den 16 Materialien wurden die 6 besten ausgewählt und neu und im größeren Maßstab produziert (LDPE // Irganox 1076 / Irgafos 168, LDPE // 1,4-diphenyl-1,3-butadiene (DPBD), HDPE // Chimassorb 81 / Uvitex OB, PP homo // Irganox 1076 / Irgafos 168, HIPS mit 1 % Mineralöl // Styrol, PA 6 // Caprolactam). Eine Zertifizierungsübung im kleinen Rahmen, unter Mitarbeit von 4 Laboren wurde für die 6 Materialien erfolgreich durchgeführt. Alle 6 Materialien zeigten brauchbare Eigenschaften für die zukünftige Produktion von Referenzmaterialien. Diese im Projekt bestimmten  $D_P$  und  $A_P$  Koeffizienten wurden benutzt, um experimentelle und berechnete Migrationsdaten miteinander zu vergleichen. Dabei lagen ungefähr die Hälfte der berechneten Daten innerhalb von ±10%, fast alle berechneten Daten innerhalb von  $\pm 40\%$  der experimentellen Daten.

Innerhalb des Projektes wurde eine Reihe analytischer Methoden, die für die Zertifizierungsparameter benötigt wurden, entwickelt und validiert. Als Beispiel werden in dieser Arbeit analytische Methoden für die Bestimmung des Nylon 12 Monomers Laurolactam beschrieben. HPLC mit UV und MS Detektion wurden verwendet, um das Monomer, sowie Di- und Trimer weit unter dem gültigen SML von 5 mg/kg in Lebensmittelsimulanzien nachzuweisen. Außerdem wurde eine GC-FID Methode für die Bestimmung von Laurolactam in organischen Lösungsmitteln (z.B. Ethanol, Isooktan) entwickelt und validiert. Für

eine für Probenaufreinigung Olivenöl wurde Methode durch Gelchromatographie (SEC) entwickelt. Die neuen Methoden wurden in einer Studie angewendet, in der die Migration von Laurolactam und Nylon 12 Oligomeren mit drei unterschiedlichen Folien und fünf Lebensmittelsimulanzien (Olivenöl, Isooktan, 95% Ethanol, 50% Ethanol und Wasser) untersucht wurde. Migrationstests mit Simulanzien wurden entsprechend EU Richtlinie EC/97/48 angewendet. Tests mit 95% Ethanol überschätzten, die mit Isooktan unterschätzten die Migration in Olivenöl signifikant. Die besten Ergebnisse zur Abschätzung der Migration in Olivenöl bei gleichen Bedingungen lieferte Wasser. Zusätzlich wurde die Diffusion von Laurolactam in Isooktan und Olivenöl verglichen. Die beim Übergang in Isooktan bestimmte Diffusion war signifikant größer als die vergleichbare in Olivenöl. Ein kurzer Test bestätigte, daß im Gegensatz zum Öl, Isooktan in das Polymer eindringt und somit Laurolactam extrahiert. Der mit Olivenöl ermittelte  $A_{P}$  Wert für Nylon 12 lag zwischen 0.3 und 0.6.

Nylon wird bei Kunstdärmen eingesetzt und ist dabei auf der einen Seite in Kontakt mit fettigen Lebensmitteln, und auf der anderen mit heißem Wasser oder Wasserdampf. Um diese Situation zu simulieren, wurde eine spezielle Migrationszelle konstruiert, die auf der Seite mit Wasser und auf der anderen mit Olivenöl befüllt werden kann. Nylon 6 Folien, die auf diese Weise getestet wurden, gaben 100% des Monomer auf die Wasserseite ab. Die getesteten Nylon 12 Folien gaben in etwa gleiche Mengen ihres Monomers in beide Richtungen ab. Ein vorhandenes Migrationsmodell wurde angepaßt, um die zweiseitige Migration simulieren zu können. Die Eignung des Modells wurde durch experimentelle Daten bestätigt.

Zusätzlich sind einige der CRM Materialien verwendet worden, um die Effekte von Gamma Bestrahlung auf Additive, Monomere und die sensorischen Eigenschaften der Polymere zu untersuchen. Die Konzentration von Additiven wie Irganox 1076 und Irgafos 168 in LDPE nahm durch die ionisierende Strahlung rasch ab. Die geringere Konzentration der Migranten führte erwartungsgemäß auch zu einer geringeren spezifischen Migration, nicht jedoch zu einer verminderten Gesamtmigration. Die sensorische Eigenschaften von LDPE, HDPE, PA6 und PA12 verschlechterten sich, während die von PS bei höheren Bestrahlungsdosen sich verbesserten.

Abschließend wurden die Ergebnisse der Arbeit kritisch durchleuchtet und ihre praktische Anwendung diskutiert. Die in der Arbeit bestimmten Diffusionsdaten wurden mit den in der EU Gesetzgebung derzeit verwendeten Daten verglichen und in sie eingegliedert.

### Samenvatting

In dit proefschrift wordt een onderzoek beschreven met als belangrijkste doelstelling het ontwikkelen van de know-how om gecertificeerde referentie materialen (Certified Reference Materials, CRMs) voor specifieke migratietesten te kunnen produceren. De certificatie parameters zijn:

- de initiële concentratie van migranten in het polymeer ( $C_{P,0}$ ),
- de specifieke migratie in een voedselsimulant bij bepaalde temperatuur en tijd.
- de diffusie coëfficiënt *D<sub>P</sub>* van het polymeer.

16 voorlopige CRMs werden gedefinieerd en geproduceerd. De belangrijkste polymeren (LDPE, HDPE, PP, PS, PET, PVC, PA) met additieven en monomeren met verschillende eigenschappen werden gekozen. De stabiliteit en de homogeniteit van de migranten in de CRMs werden bepaald en methodes voor de bepaling van de certificatie parameters werden ontwikkeld en gevalideerd. Uit de 16 voorlopige materialen werden de 6 meest geschikte kandidaten geselecteerd (LDPE / Irganox 1076 / Irgafos 168, LDPE / 1.4-diphenyl-1.3-butadieen (DPBD), HDPE / Chimassorb 81 / Uvitex OB, PP / Irganox 1076 / Irgafos 168, HIPS, 1% minerale olie / styreen, PA 6 / caprolactam). De haalbaarheid van CRM productie voor deze 6 materialen werd onderzocht tijdens een certificatie test waaraan 4 laboratoria deelnamen. Alle 6 materialen bleken geschikt voor productie als CRMs. De in dit onderzoek bepaalde diffusiecoefficiënten werden gebruikt ter vergelijking van experimentele en voorspelde migratie, gebaseerd op resultaten van dezelfde materialen. In vergelijking met de experimentele gegevens vertoonde de helft van de voorspelde waarden een marge van maximaal 10%, terwijl alle voorspelde waarden in een marge van maximaal 40% resulteerden. Binnen het onderzoek werden meerdere analytische methodes ontwikkeld voor de bepaling van de certificatie parameters. Als

ontwikkeld voor de bepaling van de certificatie parameters. Als voorbeelden zijn de methodes voor de bepaling van nylon 12 monomeer, cyclisch dimeer en trimeer beschreven. HPLC met ultraviolet detectie (HPLC-UV) en massa spectrometrische detectie (HPLC-MS) zijn allebei geschikt bevonden om monomeer, cyclisch dimeer en trimeer ver onder de grenswaarde voor specifieke migratie van laurolactam (5 mg/kg) te identificeren en te kwantificeren. Gas chromatografie met vlamionisatie detectie (GC-FID) werkte goed voor de detectie van laurolactam in waterige en vethoudende voedselsimulanten. Voor het bepalen van laurolactam in olijfolie werd eerst een zuiveringsmethode toegepast m.b.v. gel permeatie chromatografie (GPC). De nieuwe methodes werden gebruikt in een studie, waar de migratie van laurolactam en het cyclisch dimeer en trimeer van nylon 12 werd geëvalueerd met drie verschillende films en vijf voedsel simulanten (olijfolie, iso-octaan, 95% alcohol, 50% alcohol en water). De testvoorwaarden voor migratie volgens de EU richtlijn EC/97/48 werden toegepast. De resultaten toonden aan dat het gebruik van 95% en 50% alcohol als simulanten de migratie in olijfolie overschatte, terwijl iso-octaan de migratie onderschatte. Water bleek het beste olijfolie substituut te zijn aangezien de migratie van laurolactam in water en olijfolie gelijkwaardige resultaten gaf. Bovendien werd de diffusiekinetiek van laurolactam in nylon 12 met behulp van iso-octaan en olijfolie geëvalueerd. Diffusiecoëfficiënten die met iso-octaan werden bepaald waren signifikant hoger dan die met olijfolie bepaald. Aangetoond werd dat iso-octaan het polymeer penetreerde, terwijl de olijfolie voor het polymeer inert was (d.w.z. niet penetreerde). Een in de literatuur en officiële regelingen gebruikte parameter is de zogenaamde polymeer-coëfficiënt Ap. Het is een empirische parameter die een polymeer-specifieke maat is voor de diffusie-coëfficiënt. Deze polymeer coëfficiënt A<sub>P</sub> was voor nylon 12 en oliifolie 0,3 tot 0,6.

Nylon verpakking wordt vaak als kunstdarm gebruikt voor worst, als gevolg waarvan het polymeer aan één kant aan vetrijk voedsel en aan de andere kant aan kokend water wordt blootgesteld. Om deze situatie te simuleren werd een speciale migratiecel geconstrueerd . De cel werd daartoe aan één kant van het polymeer met olijfolie en aan de andere kant met water gevuld. Een nylon 6 film werd aan de hierboven beschreven omstandigheden blootgesteld. Na 2 uren bij 100°C bleek de totale massa van het monomeer caprolactam in de waterfase te zijn overgegaan. Het nylon 12 materiaal gaf gelijkwaardige hoeveelheden van zijn monomeer aan de waterige en olie fase af. Een bestaand computer model werd aangepast om deze situatie van gelijktijdige tweezijdige migratie te simuleren. Het model werd gevalideerd met experimentele gegevens.

Ook zijn de gevolgen van ioniserende straling op polymeren, de daarin aanwezige additieven en monomeren onderzocht. De veranderingen van de oorspronkelijke concentraties van bepaalde additieven en monomeren, een verandering in hun specifieke migratie alsook de daardoor veroorzaakte sensorische veranderingen t.a.v. geur van de polymeren werden onderzocht. Polymeer stabilisatoren zoals Irganox 1076 en Irgafos 168 in LDPE en HDPE werden door de ioniserende straling afgebroken. De lage concentraties van stabilisatoren in polyolefinen leidden tot lagere specifieke migratie, echter niet tot lagere globale migratie. De concentratie van Irganox 1076 in PS veranderde niet tot stralingsdoses van 54 kGy. De geur van LDPE, HDPE, PA6 en PA12 werd slechter, terwijl de geur van PS met stijgende stralingsdoses verbeterde.

Tenslotte zijn de resultaten van dit proefstuk kritisch met resultaten uit de literatuur vergeleken en is implementatie van de resultaten besproken. Een integratie van de nieuw bepaalde diffusiecoëfficiënten in de migratie modelleringsmethode die momenteel door de EU wordt toegepast wordt voorgesteld.

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# **Curriculum Vitae**

Niels Holger Stoffers was born 31 July 1972 in Hattem, The Netherlands as the second son of Ellen and Alle Stoffers. Only one year later, Niels and his parents moved to Bookholzberg in Germany, where he lived until 1992 when graduating from the *Gymnasium Ganderkesse*. After one year of mechanical engineering studies at *Universität Hannover*, he enrolled the environmental sciences programme at the *Van Hall Instituut* in Groningen. Niels completed two internships dealing with environmental technology issues at *Oregon State University* in Corvallis and again at *Universität Hannover*. In 1997, he moved to Vienna, Austria working at the *Institute for Agrobiotechnology* in Tulln, where he did his research project needed to finish his studies. In 1998, after graduation, he was offered a job as Laboratory Manager in the mycotoxin-testing laboratory of *Biomin Laboratory Singapore Pte. Ltd.* where he worked for 15 months, followed by 18 months of work as Area Sales Manager for *Romer Labs, Inc.* located in Union, near St. Louis, Missouri.

The work for this thesis was conducted at *Fraunhofer Institute for Process Engineering and Packaging*, in Freising, near Munich where Niels has been working in the Department of Product Safety and Analysis since early 2001 mainly being involved with the EU funded project "Specific Migration", a feasibility study for the development of certified reference materials for food packaging specific migration testing.

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