Angela Schaefer

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Dissertation

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Contents

Abstract (english)

The quantity of foodstuffs packed in coated cans and laminates increased in the last decades due to their advantages like long storage time and low costs. A mass transfer of metal ions is prevented by the plastic-based coating or the plastic film in the laminate but a mass transfer (migration) of plastic components exists instead. Two legal regulations focus on the amount of migrating substances from these types of packaging. Firstly, the Council of Europe imposes a limit of 10 mg/dm^2 packaging for the sum of migrating substances (overall or total migrate) which can be determined by the gravimetric reference method. Secondly, the Directive 2002/16/EC limits the migration of bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether as well as their hydroylsis and hydrochlorination derivatives to 1 mg/kg foodstuff.

Most previously published investigations based on methods for the surveillance of these legal regulations. However, another decisive point is the fact that the Scientific Committee on Food differentiates the migrants into the substances with a molecular weight < 1000 Da, potentially being absorbable by the gastro-intestinal tract, and the less toxicologically relevant species > 1000 Da. This thesis is the first approach to develop applicable methods for the elucidation of the total migrate < 1000 Da. Finally, a mass balance of migration was achieved by comparing the sum of the single results for these migrate components with the value determined for the total migrate < 1000 Da. This concept was tested for an epoxy and a polyester coating.

Therefore, an alternative method for the simultaneous determination of the global migrate and the migrants < 1000 Da based upon separation by size exclusion chromatography (SEC) followed by evaporative light scattering detection (ELSD) was developed. A statistical comparison of both methods (the gravimetric method is also extended in order to determine the part < 1000 Da) revealed that the SEC-ELSD method is not an equivalent substitute for the gravimetric reference method due to significantly lower results considering the overall migrate. However, the SEC-ELSD method is the tool of choice due to better reproducibility and lower limit of detection analysing the part < 1000 Da for which a reference method does not exist.

About 50 % of the total migrate < 1000 Da in 95 % ethanol (for both coating types about 1.3 mg/dm^2) were elucidated as resin-related substances, mainly residual oligomers and reaction products with other constituents of the lacquer. A screening gradient was developed on a reverse phase in order to achieve highest resolution for all resin-related substances < 1000 Da. The migrants of epoxy resin were identified using electrospray ionisation-mass selective detection (ESI-MSD) and were confirmed by micro-syntheses of monomeric and dimeric BADGE with solvents and phenols. The sensitive fluorescence detection and the commercially available BADGE standard was used for the quantification of all migrants < 1000 Da including a bisphenol A (BPA)-backbone as BADGE-equivalents, since the fluorescence response relies on the amount of BPA-moiety in the respective molecule. The quantification could be extended by combining fluorescence response and structural information gained from the mass spectra, which provides more accurate results for each single migrant. The calculation is based on the calibration of the BPA-chromophore content of the molecule.

For the regulated BADGE-derivatives a specific method using a ternary gradient, reference standards for each substance and an internal standard was developed and fully validated for surveillance purposes.

The structural elucidation of polyester migrants required an identification of the monomeric polyvalent acids and polyols after hydrolysis by specific methods. With the knowledge of the polyester monomers it was possible to - at least tentatively - identify the main components in the migrate as cyclic oligoesters by ESI-MSD based on terephthalic acid (TPA) and isophthalic acid (IPA) as well as several polyols. The amount of the migrating oligoesters was determined using an UV_{232nm} calibration of a commercially available TPA/IPA-ester (bis-hydroxyethylene terephthalate, BHET) and the number of IPA/TPA moieties/ molecule gained from the mass spectra.

The specific method used for the identification of polyester monomers also enabled the identification and quantification of residual trimellitic acid (about 0.08 mg/dm^2) in the 95 % ethanol migrate used as anhydride hardener in the epoxy coating.

Migrating lubricants made up another part of about 20 % of the total migrate < 1000 Da in 95 % ethanol for the tested epoxy coating. A normal-phase liquid chromatography method was developed for the identification and quantitative estimation of mainly used lubricants which were separated on a diol phase by their polarity into twelve lipid classes (paraffins, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amide). Since the detector response of the used ELSD depends on the chain length and the degree of saturation, the quantification of a lipid class with unknown composition is only semi-quantitative.

In conclusion, about 75 % of the total migrate < 1000 Da in 95 % ethanol from the epoxy coating have been elucidated. The fact that nearly 60 % of the migrate < 1000 Da in 95 % ethanol of the polyester coating remained unknown is dissatisfying. A reasonable uncertainty of the determined values especially due to the substance depending response of the ELS-detector (determination of the total migrate < 1000 Da and of the lubricants) has to be considered when discussing the amount of unknowns. Moreover this analysis schema should be enlarged by the determination of hardener components (especially for coatings cured with phenol- or aminoplasts) and of other additives like pigments, catalysts, defoamers and flow aids in order to enhance the comprehensiveness. Nevertheless, this is one of the first approaches to identify the actual migrating substances from coatings < 1000 Da.

Abstract (german)

Die Menge an Lebensmitteln, die in beschichteten Konservendosen oder beschichteten Leichtmetallbehältern verpackt wird, ist in den letzten Jahrzehnten aufgrund ihrer Vorzüge wie lange Haltbarkeit und geringe Kosten angestiegen. Ein Übergang von Metallionen wird durch eine Lackierung oder Kunststoffbeschichtung vermieden, an dessen Stelle tritt jedoch der Stofftransfer (Migration) von Bestandteilen aus den Beschichtungen. Derzeit beziehen sich nur zwei rechtliche Regelungen betreffend die Menge der migrierenden Substanzen auf diese Verpackungsarten. Einerseits legte der Europäische Rat einen Grenzwert von 10 mg/dm^2 Verpackung für die Summe aller migrierenden Substanzen (Gesamtmigrat) fest, andererseits wird der Übergang von Bisphenol A Diglycidylether (BADGE), Bisphenol F Diglycidylether und deren Hydrolyse- und Hydrochlorierungsprodukten in Lebensmittel auf 1 mg/kg beschränkt.

Die meisten der zuvor publizierten Arbeiten beschreiben Methoden zur Überwachung der genannten Grenzwerte. Ein weiterer, entscheidender Punkt zur Beurteilung der Migration ist das Molekulargewicht der migrierenden Substanzen und damit verbunden ihre Absorption im menschlichen Gastro-Intestinaltrakt. Der „Scientific Committee on Food“ bezeichnet Substanzen mit einem Molekulargewicht < 1000 Da als potentiell absorbierbar und damit toxikologisch relevant, während den Substanzen mit einem Molekulargewicht > 1000 Da eine geringere Bedeutung beigemessen wird. Die vorliegende Arbeit stellt einen ersten Versuch dar, geeignete Methoden zu entwickeln, mit denen die Zusammensetzung des Gesamtmigrates < 1000 Da aufgeklärt werden kann. Abschließend wurde die Summe der Einzelergebnisse für die Migratkomponenten mit dem Wert für das Gesamtmigrat < 1000 Da verglichen, um eine Massenbilanz zu erhalten. Dieses Konzept wurde an einem Epoxy- und einem Polyestercoating getestet.

Hierzu wurde zuerst eine alternative Methode zur simultanen Bestimmung des Gesamtmigrates und des Anteil < 1000 Da unter der Verwendung der Größenauss chlußchromatographie (SEC) gekoppelt mit einem Verdampfungslichtstreudetektor (ELSD) entwickelt. Ein statistischer Vergleich beider Methoden (die Gravimetrie wurde zur Bestimmung des Anteils < 1000 Da erweitert) ergab, dass die SEC-ELSD-

Methode in Hinblick auf die Bestimmung des Gesamtmigrates kein äquivalenter Ersatz für die Gravimetrie ist, da sie signifikant niedrigere Ergebnisse liefert. Jedoch zur Bestimmung des Anteils < 1000 Da, wofür keine Referenzmethode existiert, ist die SEC-ELSD-Methode die Methode der Wahl, da nur mit dieser aufgrund der besseren Reproduzierbarkeit und der niedrigeren Nachweisgrenze geringe Migratgehalte meßbar sind.

Etwa 50 % des Gesamtmigrates < 1000 Da in 95 % Ethanol (bestimmt als ca. 1.3 mg/dm^2 für beide Coating-Typen) konnten als harzverwandte Substanzen, hauptsächlich Oligomere und Reaktionsprodukte mit weiteren Coating-Inhaltsstoffen, aufgeklärt werden. Ein Screening-Gradient wurde dafür auf einer Umkehrphase entwickelt, der eine hohe Auflösung für alle harzverwandten Substanzen < 1000 Da erreichte. Die Identifizierung der Bisphenol A (BPA)-verwandten Substanzen aus dem Epoxycoating erfolgte nach Elektrosprayionisation mit massenselektiver Detektion (ESI-MSD) und wurde durch Mikrosynthesen von monomerem und dimerem BADGE mit Solventien und Phenolen abgesichert. Die sensitive Fluoreszenzdetektion und ein kommerziell erhältlicher BADGE-Referenzstandard wurden zur Quantifizierung aller BPA-verwandten Substanzen < 1000 Da als BADGE-Äquivalente verwendet, da die Fluoreszenzresponse der Substanzen auf dem BPA-Gerüst beruht.

Eine akkuratere Quantifizierung der Einzelsubstanzen konnte durch die Kombination der Fluoreszenzresponse mit der Strukturinformation (erhalten aus den Massenspektren) erreicht werden. Die Berechnung erfolgte über die Kalibrierung des BPA-Chromophoranteils im Molekül.

Für die rechtlich geregelten BADGE-Derivate wurde eine weitere, spezifischere Methode unter Verwendung eines ternären Gradienten, synthetisierten Referenzstandards für alle Derivate und eines internen Standards entwickelt sowie in Hinblick auf eine Verwendung für Überwachungszwecke vollständig validiert.
Die Identifizierung der Polyester-verwandten Substanzen erforderte die Bestimmung der Polyestermonomere mit spezifischen Methoden nach deren Freisetzung durch eine hydrolytische Spaltung. Erst mit Hilfe der Information über die enthaltenen Monomere konnten sich die migrierenden Substanzen als cyclische Oligoester auf

Basis von Terephthalsäure (TPA) und Isophthalsäure (IPA) sowie mehreren Polyolen mittels ESI-MSD identifizieren lassen. Die Menge der einzelnen, migrierenden Ester wurde unter Verwendung der UV-Kalibration eines kommerziell erhältlichen TPA/IPA-Ester (z.B. Bis-hydroxyethylenterephthalat) und der Information über die Anzahl der TPA/IPA-Einheiten im Molekül bestimmt. Die spezifische Methode zur Analyse der Säurekomponenten im Polyester ermöglichte weiterhin die Identifizierung und Quantifizierung eines Rückstandes der Trimellithsäure im Epoxycoating-Migrat (95 % Ethanol, ca. 0.08 mg/dm^2), die als Monomer im Anhydridhärter verwendet wird.

Ein weiterer Anteil von ca. 20 % des Gesamtmigrates < 1000 Da in 95 % Ethanol aus dem Epoxycoating konnte migrierenden Schmierstoffen zugeschrieben werden. Eine flüssigchromatographische Methode mit einer Diolphase wurde zur Identifizierung und quantitativen Abschätzung der hauptsächlich verwendeten Schmierstoffe entwickelt, die deren Trennung nach dem polaren Molekülrest in zwölf Lipidklassen (Paraffin, Wachsester, Cholesterinester, Fettsäuremethylester, Triacylglyceride, Fettalkohole, freie Fettsäuren, Cholesterin, 1,3-Diacylglyceride, 1,2-Diacylglyceride, Monoacylglyceride und Fettsäureamide) ermöglichte. Da die Detektorresponse des verwendeten ELSDs von der Kettenlänge und dem Grad der Sättigung abhängig ist, ist die Quantifizierung von Lipidklassen mit unbekannter Zusammensetzung nur semi-quantitativ.

Mit allen entwickelten Methoden konnten ca. 75 % des Gesamtmigrates < 1000 Da in 95 % Ethanol des Epoxycoatings aufgeklärt werden. Die Tatsache, daß noch ca. 60 % des Gesamtmigrates < 1000 Da in 95 % Ethanol vom Polyestercoating unbekannt sind, ist nicht zufriedenstellend. Bei der Diskussion des unbekannten Anteils ist jedoch die beträchtliche Unsicherheit der Werte, besonders der mit dem ELSD gemessenen Werte, zu berücksichtigen. Weiterhin sollte dieses Analysenkonzept durch Methoden zur Bestimmung von weiteren Härterkomponenten (besonders für Phenol- und Aminhärter) oder von anderen Additiven wie Pigmenten, Katalysatoren, Entschäumern oder Fließhilfen erweitert werden. Es bleibt jedoch hervorzuheben, daß diese Arbeit einer der ersten Versuche ist, die Identität und Menge der migrierenden Substanzen < 1000 Da aufzuklären.

Symbols and abbreviations

AP	atmospheric pressure (in combination with CI or ESI)
ATR	attenuated total reflection
BADGE	bisphenol A diglycidyl ether
BADGE (n=1)	BADGE dimer
BADGE (n=2)	BADGE trimer
BADGE·2H_2O	dihydrolysis product of BADGE
BADGE·2HCl	dihydrochlorination product of BADGE
BADGE·2x	BADGE reacted on both oxirane rings with e.g. alcohols
BADGE·H_2O	monohydrolysis product of BADGE
BADGE·HCl	monohydrochlorination product of BADGE
BADGE·x	BADGE reacted on one oxirane ring with e.g. an alcohol
BADGE·x·y	BADGE reacted on both oxirane rings e.g. with different alcohols
BADHPE	bisphenol A di-3-hydroxypropyl ether
BFDGE	bisphenol F diglycidyl ether
BHEI	bis-hydroxyethylene isophthalate
BHET	bis-hydroxyethylene terephthalate
BPA	bisphenol A
BuEtOH	butoxyethanol
BuOH	butanol
CE	cholesterol ester
CEN	European Committee for Standardisation
CHOL	cholesterol

CI	chemical ionisation
CN	cyanopropyl, normal phase material
COEI	cyclic oligoethylene isophthalate
CV	coefficient of variance
cyclo-DiBADGE	cyclic BADGE dimer
D	diffusion coefficient
DA	dicarboxylic acid
1,2-DAG	1,2-diacyl glycerol
1,3-DAG	1,3-diacyl glycerol
diol	dihydroxylpropyl, normal phase material
DL	detection limit
DMF	diemethyl formamide
EG	ethylene glycol
EI	electron impact ionisation
ELSD	evaporative light scattering detector
1EPO	BPA-related derivatives containing one epoxy group
2EPO	BPA-related oligomers containing two epoxy groups
ESI	electrospray ionisation
EtEtOH	ethoxyethanol
F	foodstuff
FAA	fatty acid amide
FAME	fatty acid methyl ester
FDA	Food and Drug Administration
FFA	free fatty acid
FID	flame ionisation detector
FLD	fluorescence detector

FOH	fatty alcohol
FT	fourier transformation
GC	gas chromatography
HPLC	high performance liquid chromatography
IPA	isophthalic acid
IR	infrared
$K_{P,F}$	partition coefficient
LD_{50}	letal dose for 50 % of the tested organism
MAG	monoacyl glycerol
MeCN	acetonitrile
MeEtOH	methoxyethanol
MSD	mass selective detector
MTBE	tert-butyl methyl ether
NEPO	BPA-related derivatives without epoxy groups
NMR	nuclear magnetic resonance
NOGE	novolac diglycidyl ether
NP	normal phase
NPG	neopentyl glycol
P	packaging
PAR	paraffin
PET	polyethylene terephthalate
Ph	phenol
PO	polyol
PP	polypropylene
PrOH	propanol
PTFE	poly tetrafluor ethylene

PVC	polyvinyl chloride
QM (t)	quantitative maximum
RID	refractive index detector
RP	reversed phase
RR	relative retention
RSD	relative standard deviation
SCF	Scientific Committee on Food, substances limited by SCF and 2002/16/EC
SD	standard deviation
SEC	size exclusion chromatography
SECanalyt	analytical SEC
SECprep	preparative SEC
SFC	superfluid chromatography
Si	silicagel, normal phase material
SIM	single ion monitoring
SML	specific migration limit
SPE	solid phase extraction
TAG	triacyl glycerol
tBuPH	tert-butylphenol
TIC	total ion current
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TPA	terephthalic acid
UVD	ultraviolet detector
WE	wax ester

Hazard chemicals

Chemical	Hazard Signs	R Phrase	S Phrase
Acetic acid	C	10-35	23.2-26-45
Acetone	F, Xi	11-36-66-67	9-16-26
Acetonitrile	Xn, F	11-20/21/22-36	16-36/37
Acrylic acid	C	10-34	26-36-45
Adipic acid	Xi	36	-
Ammonia solution, 25 %ig	C, N	34-50	26-36/37/39-45-61
Bis-hydroxyethylene isophthalate	substance is not evaluated		
1,4-Bis-(hydroxymethyl)-cyclohexane	-	-	24/25
Bisphenol A	Xi	36/37/38-41	26-36
Bisphenol A di-3-hydroxypropyl ether	substance is not evaluated		
Bisphenol A (2,3-dihydroxypropyl)-diglycidyl ether	Xi	36/38-43	28-37/39
Bisphenol A (3-chloro-2-hydroxypropyl) diglycidyl ether	Xi	36/38-43	28-37/39
Bisphenol A (3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) diglycidyl ether	Xi	36/37/38	26-36
Bisphenol A bis (2,3-dihydroxypropyl)-diglycidyl ether	Xi	36/38-43	28-37/39
Bisphenol A –bis (3-chloro-2-hydroxypropyl) diglycidyl ether	Xi	36/37/38	26-36
Bisphenol A diglycidyl ether	Xi	36/38-43	28.1-37/39
Bisphenol A diglycidyl ether; reacted on both sides with butoxyethanol	substance is not evaluated		
1-Butanol	Xn	10-22-37/38-41-67	7/9-13-26-37/39-46

Butoxyethanol	Xn	20/21/22-36/38	36/37-46
Calcium oxide	Xi	41	22-24-26-39
Chloroform deut. + 1 % trimethylsilane	Xn	22-38-40-48/20/22	36/37
3-Chlor-1-propanol	Xn	22-36	26
Cyclic oligoethylene isophthalate	substance is not evaluated		
Dichloromethane	Xn	40	23.2-24/25-36/37
Diethyl ether	F+, Xn	12-19-22-66-67	9-16-29-33
Dimethyl formamid	T	61-E20/21-36	53-45
2,2-Dimethyl-1,3-propanediol	Xi	41	26-39
1,4-Dioxane	F, Xn	11-19-36/37-40	16-36/37
Epichlorhydrin	T	45.3-10-23/24/25- 34-43	53-9-44
Ethanol, 99,8 %ig	F	11	7-16
Ethoxyethanol	T	60-61-10-E20/21/22	53-45
Ethyl acetate	F, Xi	11-36-66-67	16-26-33
Ethylene glycol	Xn	22	-
Formic acid	C	35	23.2-26-45
Hydrochloric acid, 37 %	C	34-37	26-36/37/39-45
Hydrogen	F+	12	9-16-33
Isooctane	F	11	9-16-29-33
Methanol	F, T	11-23/24/25-39/23/ 24/25	7-16-36/37-45
Methoxyethanol	T	60-61-10-E20/21/22	53-45
n-Heptane	F, Xn, N	11-38-50/53-65-67	9-16-29-33-60-61-62
Oxygen	O	8	17
Phenol	T	24/25-34	28.6-45
Phosphoric acid, 85 %	C	34	26-36/37/39-45

Phthalic acid	Xi	36/37/38	-
1-Propanol	F, Xi	11-41-67	7-16-24-26-39
2-Propanol	F	11	7-16
Pyromellitic acid	Xi	36	24/25
Sodium hydroxide	C	35	26-37/39-45
Sulphuric acid	C	35	26-30-45
tert-Butylphenol	Xn	36/37/38-42/43	22-24-26-37-45
tert-Methyl butyl ether	F	11	9-16-29-43.3
Tetrahydrofurane	F, Xi	11-19-36/37	16-29-33
Tetraisopropyl-ortho-titanate	Xi	10-36	26
Toluol	Xn, F	11-20-47	16-25-29-33-53
Trifluoroacetic acid	C	20-35	9-26-27-28.1-45
2,2,4-Trimethylpentane-1,3-diol	-	-	24/25

1

Introduction and aims

1.1 Food packaging

Food has been preserved and packed for centuries in order to enable a longer storage time and safe transport. Foodstuffs have to be protected against microbiological attack, oxygen, moisture and light, because these parameters are major reasons for decreasing the inherent safety, sensory quality or deterioration of the foodstuffs. Different types of packaging materials accomplish these requirements: paper or cardboard, glass, ceramic, plastic-based materials, coated metal cans and closures as well as non-coated tinplate cans and light weight containers based on laminated aluminium. Besides product preservation there are other arguments like costs, environmental impact or consumer lifestyle which influence the choice of packaging material for each application. Cans are commonly used as packaging materials for foodstuffs and beverages because of their low costs, sterilisation stability and their "hard shell" as well as their proven track record for safe long term storage of foodstuffs particularly in hostile environments and in regions where frozen foods are unavailable. Laminated light weight containers are popular, too, since their low weight is an advantage (Turner 2001).

1.2 Metals

1.2.1 Tinplate

Tinplates used for food cans are based on cold rolled steel sheets (malleable Fe-C-alloy with less than 2.1 % C). Both sides of the sheets are cleansed with sulphuric acid, tinned using acidic ($SnSO_4$ or $Sn(BF_4)_2$) or basic ($Na_2[Sn(OH)_6]$) electrolysis and finally lubricated. Since the last of the four layers (0.2-0.3 mm Fe, 0.1 µm $FeSn_2$, 0.4-1.5 µm Sn and 0.002 µm SnO_2) does not provide good adhesion for polymeric coatings the sheets are coated with Cr/Cr_2O_3 (0.001 µm). Recently, due to decreasing tin resources and increasing costs, tin-free steels are used wherever possible. They are coated only with a Cr/Cr_2O_3 layer. A weakness of the tinplate is the corrosion which can be traced back to local elements. The presence of fruit or vegetable juice (electrolytes) and of two metals (Sn, Fe) induce this electrochemical process. As a

consequence of the released container metals ions the container may either swell or perforate resulting in commercial spoilage. Nitrate, oxygen or sulphur-containing substances react as corrosion accelerators (depolariser) whereas the sulphur-containing substances form iron and tin sulphides changing the colour and flavour of the canned foodstuff.

For organoleptic reasons (flavour, odour, texture and colour stability) some white products (asparagus, mushrooms) require “available tin” since the residual oxygen in the can is absorbed on the can surface by acting as depolariser **(**Piringer 1993, Montag 1997, Turner 1998, Turner 2001).

1.2.2 Aluminium

Aluminium is obtained from Bauxite (60 % Al_2O_3, 30 % Fe_2O_3) using the Bayer-process for isolating Al_2O_3 under pressure and subsequent enamel electrolysis with Na_3AlF_6 (2 Al_2O_3 + 3 C → 4 Al + 3 CO_2). Pure aluminium forms immediately with oxygen a grey oxide-layer. In order to enhance this corrosion stability the oxide-layer is increased by industrial anodisation. Alternatively, many grades of aluminium used for food cans or food can components undergo a pre-treatment, which is today normally chromium ion based. Aluminium bands with 0.02 – 0.35 mm thickness are used for beverage cans which are formed in the deep-drawn process. Plastic laminated foils (< 0.02 mm, alloys with magnesia, manganese or iron) provide also excellent packaging materials (Piringer 1993, Montag 1997, Turner 2001).

1.3 Polymers

With regard to corrosion most tinplate- and aluminium-based food containers are coated internally in order to protect the metal from their aggressive contents and to avoid contamination of the product by metal ions. External coatings are used for decoration and product identification and form a barrier to external corrosion (depending on the nature of the metal) or abrasion. Like paints can

coatings mainly consist of a binder-hardener system forming a three-dimensional network (crosslinked structure) which provides adhesion to the metal, chemical resistance and pack resistance and enables subsequent can manufacture. Epoxy-, polyester- and organosol-based formulations are commonly used. The liquid lacquer composition is applied to the sheets in a film thickness from about 1 to 10 µm and cured (crosslinked) under high temperature at about 180 to 220 °C for period of time of 5 to 15 min. Commonly solvents used for the „wet" coating are glycols (e.g. butoxyethanol) or aromatic hydrocarbons (e.g. naphthalene). They all evaporate during the curing process and are not present in the final cured (dried) film (Turner 1998, Turner 2001).

Laminates are based on aluminium foils and plastic (normally polypropylene) films, which are stuck together with an adhesive. The combination of these two materials prevents the food from contact with aluminium and leads otherwise to a synergy of advantages like gas tightness or stability (e.g. against sterilisation or deep-drawing process). Polyester-urethane-systems, in some case blended with epoxy resins, are commonly used as two-components adhesives for laminates. The solvent of the adhesive evaporate from laminated foils by passing through a drying oven. The complete hardening and cross-linking process occurs during the storage of the laminates for 5 to 7 days at 40 to 50 °C (Sielaff 1996, Pocius 2002).

1.3.1 Binder-hardener systems

Epoxy-based systems. Epoxy resins based on bisphenol A (BPA) are a major component of can coatings and adhesives intended to come into contact with foodstuffs. The monomer bisphenol A diglycidyl ether (BADGE, see figure 1.1) is formed during the reaction of BPA with excessive epichlorhydrin. Two synthesis-pathways are employed for the production of high molecular BPA-based epoxy-resin: the Taffy process using the same reaction as for the monomer with a smaller surplus of epichlorhydin (chain structure with n = 0, 1, 2, 3..., see figure 1.1) or the fusion process using BPA and monomeric BADGE as starting materials (chain structure with n = 0, 2, 4, 6..., see figure 1.1). The chain reaction can be terminated by using phenols like tert-butylphenol as chain-stoppers.

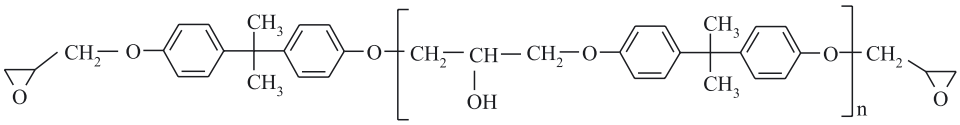

Figure 1.1. Structure of BPA-based epoxy resin (monomer n = 0)

Curing depends on the reaction with the epoxy groups and the secondary hydroxyl group, whereas acid anhydrides, amino resins (aminoplasts) or phenolic resins (phenolplasts) are commonly used as hardeners.

Curing by anhydrides requires high temperature and catalysts (tertiary amines). Typically anhydride hardeners consist of trimellitic acid, phthalic acid or 1,2-cyclohexanedicarboxylic acid. Anhydride cured resins have superior resistance properties, especially good acid resistance and do not impart an undesirable taste to foods.

"Gold lacquers" are obtained by curing with phenolplasts (phenol-formaldehyde adducts). The reactive methylol groups undergo a condensation with the hydroxyl groups of the epoxy backbone, thereby generating the characteristic gold coloration. The benefit for the food can industry is their high degree of flexibility, adhesion and chemical resistance over a wide range of acidic and non-acidic foods. They can also be used in a pigmented form (aluminium powder or zinc carbonate) to mask or absorb sulphide staining.

Due to their high chemical resistance, nearly colourless and low stoving temperature aminoplasts are used for beverage cans. Dimethylol urea (butylated) and methylolated melamine are used commonly (Lee and Neville 1967, May and Tanaka 1988, Oldring 1996, Ciba 1997, Stoye and Freitag 1998, Muskopf and McCollister 1999).

Polyester-based systems. In contrast to plastic food contact materials based on polyethylene terephthalate (PET), coating and adhesive polyesters (as example see figure 1.2) are produced from a variety of different hydroxylic monomers (e.g. ethylene glycol, neopentyl glycol, propanediol-1,2, cyclohexyldimethanol,

trimethylolpropane, glycerol, diethylene glycol, butanediol-1,4, hexanediol-1,6) and acid monomers (maleic acid, adipic acid, tetrahydrophthalic acid, phthalic acid, isophthalic acid terephthalic acid, trimellitic anhydride). Generally polyesters consisting of several polycarboxylic acids and polyalcohols are used since homopolymers with phthalic acid isomers exhibit poor solubility and elasticity. Polyester resins are cured with phenolplasts, aminoplasts and polyisocyanates. The curing with polyisocyanates relies on the high reactivity of isocyanate groups with compounds containing acidic hydrogen atoms (e.g. hydroxylgroups). Monomers are typically aliphatic isocyanates like hexamethylene diisocyanate or aromatic isocyanates like toluene diisocyanate. Since the monomeric isocyanates have a high volatility and hence toxicity the use of blocked isocyanates and prepolymers has been increased. Blocked isocyanates are obtained by chemical addition of ketones, whereby the isocyanate group is not reactive at room temperature but can be regenerated by heating (stoving) to eliminate the blocking agent. Furthermore, due to the toxicity concerns surrounding the use of aromatic isocyanates, or polyisocyanates based upon an aromatic monomer, aliphatic polyisocyanates are typically used for food contact applications or they are replacing their aromatic counterparts (Kittel 1998, Stoye and Freitag 1998, Thomas 1998, Turner 1998).

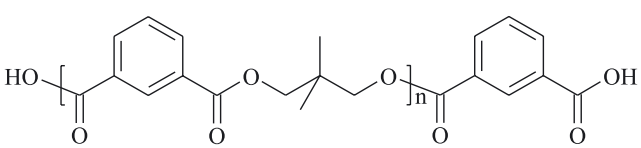

Figure 1.2. Structure of a polyester resin (based on isophthalic acid and neopentyl glycol)

Organosol-based systems. Organosols are polyvinyl chloride (see figure 1.3) dispersions in plasticisers with hydrocarbon solvents that also contain extenders, pigments and processing agents as well as an additional solvent-soluble binder promoting adhesion to metal (polyester or acrylic resins). In addition it is necessary to scavenge any eliminated free hydrochloric acid formed through dehydrochlorination by the use epoxidised phenols or oils. BADGE and BPA-based epoxy resins have been traditionally used as HCl-scavengers. Furthermore, bisphenol F-based and

novolac-based epoxy resins have also been used. Novolac resin is a condensation product of phenol and formaldehyde containing at least two rings per molecule. This can be subsequently epoxidised forming NOGE (Novolac Glycidyl Ether). Advantages of these systems are the absence of flavour, high flexibility, abrasion stability and chemical resistance to oils, alcohols, alkalis, dilute mineral acids. A disadvantage is staining due to the absorption of food colorants (Turner 2001).

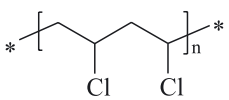

Figure 1.3. Structure of a polyvinyl chloride resin

1.3.2 Lubricants

Lubricants are used as additives for the production of coated or laminated packaging for different reasons. Most metal-forming processes require some form of external lubrication because the surface of the material is rigid and abrasive and it would damage the tooling, required to form the can or can component. They may also be applied to the surface of the packaging in order to minimise the adhesion of food components to the packaging ("meat release"). Internal lubricants are added to liquid lacquer-compositions and are partially compatible with the polymer matrix. The Van der Waal's forces between the polymer chains are reduced in order to enhance the cold flow and to allow elasticity which is required for the deep-drawing process. Slip additives as another group of internal lubricants are added to plastic and coating formulations where they gradually tend to migrate to the surface preventing the adhesion between films by reducing friction. Useful lubricants for both applications are waxes, paraffins, fats and oils as well as partial acyl glycerols or fatty acid amides (Cooper and Tice 1995, Turner 2001, Oleofine Organics 2002, Specialchem 2002).

1.3.3 Additives

Different types of additives like pigments, fillers, wetting and flow aids as well as defoamer are integrated into liquid coating formulation.

Typical pigments are white (TiO_2) and silver pigments (aluminium powder). $BaSO_4$ is commonly used as filler. Wetting aids are used to improve the wetting of the substrate (metal) by the coating improving the coverage and integrity of the film. A 'wet' film must cover the substrate properly in order to become an integral and coherent dried film. Defoamers are used because either during manufacture or application by roller coater the agitation can cause excessive foaming of the coating. During application this can lead to bubbles and subsequently formed pinholes, which are sites for potential perforation and hence microbiological contamination and spoilage. Flow aids are used to ensure that the 'wet' film flows to give uniform coverage before it is cured. They are active during the evaporation of the solvent and the initial stages of curing, when the components of the film still have some mobility. Examples of wetting aids and defoamers are silicones and flow agents include acrylic resins (Stoye and Freitag 1998).

1.4 Migration

The food packaging is not a passive material since multiple interactions are possible: transition of water vapour or oxygen through the plastic-based packaging into the foodstuff (permeation) or mass transfer of food ingredients into the packaging as well as mass transfer from packaging ingredients into the foodstuff (migration). Food ingredients like fat or water can swell the packaging material and thus enhance the migration of packaging components, particularly if the food is heat processed in its packaging. Furthermore, migration depends on several other factors: contact time and temperature, type of packaging material, its film thickness and molecular weight of the migrating species.

From the physical point of view the underlying key parameters of migration are the diffusion coefficient D of the migrant (migrating substances) in the packaging material (P) as well as the partition coefficient $K_{P,F}$ of the migrant between the packaging material and the foodstuff (F) or simulants. The diffusion equation (Equation 1.1) – also known as Fick's 2nd equation – and the partition equation (Equation 1.2) describe the migration process.

$$\frac{\delta c}{\delta t} = D \cdot \frac{\delta^2 c}{\delta x^2}$$

c concentration of the migrant in P
t time
x thickness of packaging material

Equation 1.1 Fick's 2nd equation

$$K_{P,F} = \frac{c_{P,\infty}}{c_{F,\infty}} \frac{\rho_P}{\rho_F}$$

$c_{P,\infty}/c_{F,\infty}$ equibrilium concentration of the migrant in P
$\rho_{P/F}$ density of P/F

Equation 1.2. Partition equation

Global migration (also termed as total or overall migration) is the total mass transfer of substances from the packaging material into food during contact time. Specific migration is the mass transfer of a single substance or a substance group from the packaging material into food during their contact time (Piringer 1988, Piringer 1993, European Commission 2003a).

In order to compare the amount of migrating substances the values are referred to the packaging surface (mg/dm^2) or to the packed mass of foodstuff (mg/kg). Since the surface-to-volume-ratio differs from packaging to packaging the amounts can be related to the standard cube of food of 6 dm^2/dm^3 with average density of 1 kg/dm^3 (European Directive 2002/72/EC, European Commission 2002a).

1.5 Legal regulations and toxicology

1.5.1 Legal regulations for global and specific migration

Actual European legislation regulates the application of monomers and additives as well as their residue in packaging and their migration from the packaging for the

production of plastic packagings intended to come into food contact by the Directive 2002/72/EC (European Commission 2002a). This directive compromises only plastic food contact materials but no coatings or light weight containers. For coatings the resolution AP(96)5 (Council of Europe 1996) was released which adopted the list of approved starting materials and additives in Directive 90/128/EEC (European Commission 1990, preceding directive of 2002/72/EC). The resolution limits the sum of migrating substances (**global, overall or total migration**) to 10 mg/dm^2 (or 60 mg/kg) and includes a positive list for approved starting materials and specific limits for some substances. Whilst this Resolution has no legal status within the EU member states, it can be used to demonstrate 'due diligence'. Furthermore some EU member states adopt Council of Europe resolutions into their national regulations for areas not covered by an EU Directive, such as coatings.

Concerning the **specific migration** of starting materials and their reaction products from can coatings, ten derivatives of BADGE and bisphenol F diglycidyl ether (BFDGE) (dihydrolysis derivatives are only included for aqueous media) are limited to 1 mg/kg food by Directive 2002/16/EC (European Commission 2002b).

However, most starting materials for lacquers are already (pre-)polymers or substance mixtures and react during the curing process with other constituents of the lacquer. Consequently, migrating substances from coatings are only to a minor extent monomers from the positive list mainly being their polymerisation or reaction products. The Scientific Committee on Food (SCF, European Commission 2001) emphasised the necessity to determine the identity of all chemicals which actually migrate into food, their quantities in the total diet and their toxicological profile. Thereby only the **fraction with a molecular weight below 1000 Da** is of toxicological concern, because only this fraction is regarded as potentially absorbable in the gastro-intestinal tract.

Recently, the 1000 Da boarder was introduced to Directive 2002/16/EC (European Commission 2002b) for NOGE-derivatives: the use of NOGE is practically excluded by restricting the presence of all derivatives below 1000 Da containing at least one epoxy- or hydrochlorination group to 0.2 mg/kg in the material. Hitherto, a legal

limit either for the sum of all migrating substances below 1000 Da (total migrate below 1000 Da) or for the sum of migrating epoxy resin-related substances below 1000 Da except for NOGE does not exist.

Introduced by the Food and Drug Administration (FDA) in the USA a **threshold of regulation for individual migrating substances** of 0.5 µg/kg in the diet exists for the application of new substances below which a toxicological evaluation of these components is not required (Begley 1997, U.S. FDA 2002). A consumption factor of 0.17 is defined for canned food resulting in a threshold concentration of 3 µg/kg (U.S. FDA 2002). However, no such threshold exists in European legislation. There, in the case of migration below 0.05 mg/kg food (irrespective of the diet) only three mutagenicity tests are needed (European Commission 2003b). Based on these definitions and technical feasibility Grob *et al.* (1999) suggested a value of 30 µg/kg (which corresponds between to 1 and 5 µg/dm^2 depending on the size of the can) for individual compounds in migrates of can coatings as the limit for identification. For the sum of unknown/untested migrants below 1000 Da the authors proposed a maximum of 300 µg/kg (according to 10 to 50 µg/dm^2).

1.5.2 Legal regulations for simulation of migration

Due to technical or analytical reasons analysis of migration into foodstuffs is not feasible in most cases. Therefore, European regulation allows the use of approved food simulants. The simulants or simulant mixtures recommended by Directive 85/572/EEC (European Commission 1985) represent four different groups of foodstuffs (see table 1.1). Since olive oil or sunflower oil cannot be analysed by reverse phase or size exclusion chromatography systems without prior sample preparation and an extraction of analytes into solvents could be incomplete, substitutes (95 % ethanol and isooctane) could be used instead.

Table 1.1. Simulants and treatment (simulating a treatment of foodstuffs between 30 and 60 min at 121 °C) according to 97/48/EC amending 82/711/EEC and 85/572/EEC

Simulant	shortcut	treatment	Simulated food
Bidistilled water	A	1 h at 121 °C	aqueous food, with pH above 4.5
3 % Acetic acid	B	1 h at 121 °C	aqueous food, with pH below 4.5
10 % Ethanol	C	1 h at 121 °C	alcoholic food or beverages
olive or sunflower oil	D	1 h at 121 °C	fatty food
95 % Ethanol	Da	4 h at 60 °C	substitute for the simulant D
Isooctane	Db	2 h at 60 °C	substitute for the simulant D

Approved treatment temperatures and times are regulated by Directive 97/48/EC amending Directive 82/711/EEC (European Commission 1982, 1997) depending on the sterilisation time and temperature of the simulated foodstuff.

1.5.3 Toxicology of epoxy-containing substances

Epoxy resins exhibit a low acute toxicity. The letal dose for 50 % of the tested rats (LD_{50} rats) of resins with an average molecular weight of 380 g/mol (mainly monomer) was reported with 11.4 g/kg body weight whereas an average molecular weight of monomers of 900 g/mol (mainly trimer) does not lead to a classical LD_{50}. The application of 30 g resin per kg body weight led to a rate of 50 % rats with health defects (May and Tanaka 1988). However, allergenic reactions were reported after contact with epoxy resins (Kanerva *et al.* 1991).

The toxicology of monomeric BADGE is not completely elucidated. The identification of adducts with human DNA (Vanhoutte *et al.* 1995) led to the assumption of teratogenic and mutagenic effects which were verified by Ames *Salmonella* assay (Sueiro *et al.* 2001). The mono-hydrolysis product of BADGE is by a factor of 10 less mutagenic and no mutagenic activity could be shown for the bisdiol and –dihydrochlorination products (Sueiro *et al.* 2001). This effect was explained by fast hydrolysis of BADGE in the presence of epoxide hydrolases of mouse liver and skin (Bentley *et al.* 1989, Sueiro *et al.* 2001).

1.6 Previous work

1.6.1 Overall migration and migrating species below 1000 Da

Methods of the European Committee for Standardisation (CEN) approved for plastic materials (EN 1186, CEN 2002a) are available for the gravimetric determination of the global migrate. An equivalent method for polymeric coatings on metal substrates is drafted (ENV****-1, CEN 2001). Due to the long duration and poor precision these gravimetric methods are not suitable for rapid surveillance in industrial laboratories developing new products. A new, time-saving method is required. One way to handle the analysis of contaminants is the application of evaluation procedures based on theoretical prediction of migration (Piringer 1988).

The use of size exclusion chromatography (SEC) with fluorescence detection as a tool for the quantitative estimation of the global migrate (calculated from the total area) as well as the part below 1000 Da from epoxy-based coatings was introduced by Bronz *et al.* (1998). A final draft method-description (prCEN/TS 14577, CEN 2002b) is also based on the application of SEC with a suitable detector or subsequent gravimetry for the determination of the mass fraction of a polymeric additive below 1000 Da. To include non-fluorescent and non-UV-active migrants into this technique a non-specific detector like an evaporative light scattering (ELSD) is suitable.

1.6.2 Specific migration

Single substances. Numerous methods are published for the identification and quantification of regulated BADGE-derivatives from can coatings (Summerfield *et al.* 1998, Biedermann *et al.* 1999, Biles *et al.* 1999, Simoneau *et al.* 1999, Theobald *et al.* 1999, Hammarling *et al.* 2000, Lintschinger and Rauter 2000, Uematsu *et al.* 2001). The validation of analytical procedures, however, was not carried out consistently. Prior to February 2000, not all standard substances were commercially available. Thus, analyses were carried out using a mixture of synthesised substances for the identification of analytes and using the external standard calibration of BADGE for all relevant substances based on the assumption of identical fluorescence activity for all analytes.

Since NOGE, (which contains BFDGE as its lowest oligomers) has been used as substitute for BADGE in organosols BFDGE and NOGE are also regulated by the Directive 2002/16/EC (European Commission 2002) some published methods include these derivatives (Biedermann *et al.* 1999, Lintschinger and Rauter 2000, Uematsu *et al.* 2001).

Substance groups. The migration from can coatings produced with BPA-based epoxy resins is not limited to monomeric BADGE or its regulated derivatives. Migration of oligomers up to BADGE-pentamer (1476 Da) was found by Biedermann and Grob (1998). Migrating BADGE-adducts with chain stoppers or solvents were already analysed by Biedermann *et al.* (1998) and Berger *et al.* (2001). However, contradictory methods for quantification of these BADGE-derivatives were published either assuming the same fluorescence-response for all derivatives (Biedermann *et al.* 1998), because fluorescence activity is solely based on the BPA-backbone, or applying different response factors for monomeric, dimeric and trimeric derivatives, which were determined after isolation of small amounts of the oligomers (Berger *et al.* 2001).

While Biedermann *et al.* (1998) considered the absorption of the gastro-intestinal tract by isolation of the migrants below 1000 Da using size exclusion chromatography Berger *et al.* (2001) identified and quantified substances with a molecular weight up to 2000 Da.

To date, methods for identification and quantification of migrating substances from polyester-based can coatings have not been published. Referring to PET bottles homologous series (n = 3-7) of cyclic oligomers based on terephthalic acid and monoethylene glycol were found (Begley *et al.* 1990 and Castle *et al.* 1989). The first quantification of the sum of migrating cyclic oligoesters was carried out by ester hydrolysis, subsequent methylation of free terephthalic acid and determination of dimethyl terephthalate by GC-MSD (Castle *et al.* 1989). An HPLC determination of the cyclic trimer, tetramer and pentamer in food was developed by Begley *et al.* (1990) using a cyclic PET trimer as an external standard. In contrast to PET, coating polyesters are produced from a variety of different monomers (acids and polyols). Therefore, many homologous series of migratable oligoesters are possible.

Concerning the group of migrating lubricants examinations have mostly been carried out in order to determine external lubricants like paraffin and waxes using gas chromatography (Jickells *et al.* 1994), coupled liquid and gas chromatography (Grob *et al.* 1991, Simal Gandara and Sarria Vidal 2000) and superfluid chromatography (Mellor *et al.* 1993, Kühn *et al.* 1996).

The residues of different individual slip additives like erucylamide were analysed by gas chromatography in food simulants (Cooper and Tice 1995). Non-specific separation methods for lipid classes have been developed by several authors for different analytical tasks, e.g. control of the production of technical partial glycerols (Bruns 1988) or observing degradation of acylglycerides after enzymatic cleavage (Foglia and Jones 1997). Biedermann-Brem *et al.* (2001) determined migrating epoxidised oil such as soybean and linseed oil and its reaction products by gas chromatography with mass selective detection since these substances are used as stabilisers for organosols.

1.7 Aims

The introduction has given an overview about the technology of metal-/ polymer-based packaging materials, definitions, legal regulations and toxicological potentials of migrants as well as previously published investigations for the identification and quantification of the migrating substances. The following chapters of this thesis describe the first approach to develop applicable methods with regard to legal regulations (global migration limit, specific migration limits, absorption barrier of 1000 Da and threshold of regulation) for the elucidation of the total migrate below 1000 Da. Finally, the quantitative results are going to be combined in order to assess the comprehensiveness of the presented analysis concept (see figure 1.4) whereby the main focus is on epoxy- (only BPA-type) and polyester-based coatings and adhesives. Only migrants from epoxy-stabilisers are analysed from organosol coatings.

The following questions and figure 1.4 represent the main individual tasks for this overall analytical concept:

What is the quantity of the total migrate and what part is potentially absorbable by the gastro-intestinal tract (migrating substances below 1000 Da)? ***(Chapter 2)***

What amount of this total migrate below 1000 Da can be explained by components of the binder-system (epoxy- and polyester binders) and what is their identity? ***(Chapter 3 and 4)***

How much of this total migrate below 1000 Da can be traced back to hardener components (only for epoxy-anhydride systems)? ***(Chapter 3)***

What is the identity and quantity of the lipophilic additives? ***(Chapter 5)***

What percentage of the total migrate below 1000 Da is until now unknown? ***(Chapter 7)***

How can the regulated specific BPA-related migrants be confirmed by a valid method for surveillance institutes
Figure 1.4. Example for aspired composition of the total migrate ***(Chapter 6)***

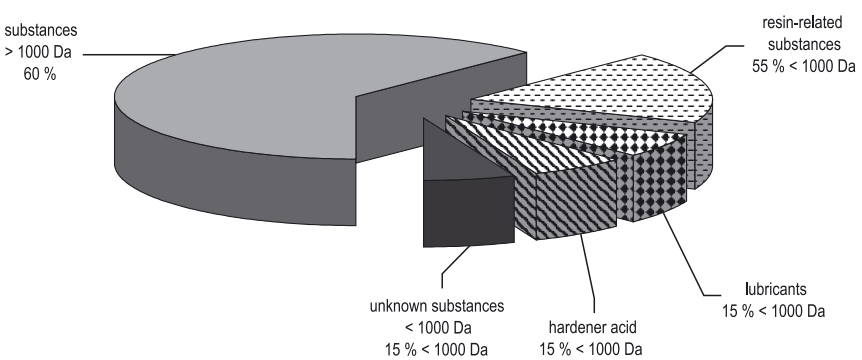

Figure 1.4. Example for aspired composition of the total migrate

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Migration from can coating: Part 1. size-exclusion chromatographic method for the simultaneous determination of overall migration and migrating substances below 1000 Da

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Migration from can coatings: Part 1. A size-exclusion chromatographic method for the simultaneous determination of overall migration and migrating substances below 1000 Da

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The Council of Europe Resolution on coatings suggests a limit of 10 mg dm^{-2} for the sum of substances migrating into food simulants from an internal can coating. The Scientific Committee on Food differentiates the migrants into the substances with a molecular weight below 1000 Da, potentially being of toxicological concern, and the less toxicologically relevant species above 1000 Da. Hitherto, the determination of overall migration was based on a gravimetric method. A new method is described for the simultaneous determination of both overall migration and the migration of substances below 1000 Da based on separation by size exclusion chromatography (SEC) followed by ultraviolet detection (UVD) and evaporative light scattering detection (ELSD). The method is suitable for all volatile extraction media and simulants recommended by the European Union. For statistical comparison of both methods, the slightly modified reference method was validated in-house and extended to an additional gravimetric measurement of the migrants below 1000 Da. For the determination of the overall migration, both methods provided similar reproducibility (validated gravimetry: standard deviation (SD) = 0.16 mg dm^{-2}; SEC-ELSD/UVD: SD = 0.12 mg dm^{-2}) but significantly better results were obtained by the SEC-ELSD/UVD method. For migrating substances below 1000 Da, the gravimetric determination provides a poor sensitivity (limit of detection = 0.35 mg dm^{-2}) compared with the SEC-ELSD/UVD method (limit of detection = 0.04 mg dm^{-2}). The new method offers a lower limit of detection and higher precision as well as being less time consuming and easier to use.

Keywords: can coatings, overall migration, migrating substances below 1000 Da, gravimetry, size exclusion chromatography, evaporative light scattering detector

Introduction

Most cans are internally coated with an organic coating to protect the food against metal ions as well as the tinplate from aggressive food ingredients. In some instances, cans are coated to enable them to be manufactured. These coatings usually contain a reactive (thermoset) resin (e.g. epoxy or polyester type) or a thermoplastic (like polyvinyl chloride) resin as the primary component. Typically, the coating composition contains hardeners like phenolics, anhydrides, isocyanates or amines to develop a stable network during the curing process as well as other additives. During and after the sterilization process residual mono- and oligomers and free additives can potentially migrate into the packed foodstuff.

European legislation regulates the application and concentration limits of monomers and additives for the production of plastic packaging intended to come into food contact with Directive 2002/72/EC (European Commission 2002). Those directives, however, are not directly applicable to coatings used for cans and lids. With Resolution AP (96) 5 (Council of Europe 1996), the Council of Europe

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passed a resolution for surface coatings in contact with foodstuffs. The resolution limits the transfer of constituents to a maximum of $10\,mg\,dm^{-2}$ (overall migration limit). Additionally, this resolution provides a positive list for approved starting materials and specific migration limits for some substances.

Generally, migrates do consist not only of the starting monomers and additives, but also of unknown reaction products (Grob *et al.* 1999, Fordham *et al.* 2001). Within the determination of the migration of bisphenol A diglycidyl ether (BADGE), Biedermann and Grob (1998) found that all the BADGE oligomers made up less than 10% of the BADGE-related migrants below 1000 Da.

Consequently, considering the discussion about the overall migration limit, the determination of the overall migrate can only be the first step. Methods of the European Committee for Standardization (CEN) approved for plastic materials (EN 1186, CEN 2002a) are available for the gravimetric determination of the overall migration. An equivalent method for polymeric coatings on metal substrates is drafted (ENV****-1, CEN 2001). Since 2000, a certified reference plastic material for measurement of the overall migration was available for proving accuracy and variances during in-house validation (Lund *et al.* 2000). However, the approved gravimetric procedure is laborious, time consuming and exhibits a relatively high detection limit of $3\,mg\,dm^{-2}$ (threefold standard deviation). Therefore, Reeves (1997) proposed a method for the spectrophotometric measurement of overall migration based on the ultraviolet (UV) activity of aromatic polyester packaging using the first derivative of absorbance. The idea of using chromatography for the determination of the overall migration by summation of the components separated on columns was first postulated by Lawson *et al.* (1994).

Recently, the toxicological interest of the Scientific Committee on Food (SCF) (European Commission 2001) mainly focused on the fraction with a molecular weight below 1000 Da because this fraction is supposed to be absorbed in the gastrointestinal tract to a reasonable extent. However, there is no 1000 Da barrier in the gastrointestinal tract of man. With reference to clinical permeability studies (Parlesak *et al.* 1994, Bjarnason *et al.* 1995, Öman *et al.* 1995, Kim 1996, Ghandehari *et al.* 1997, Nejdfors *et al.* 2000), absorption in the gastrointestinal tract depends on several factors, e.g. absorption pathway (trans- or paracellular), molecular weight and shape, region of the gastrointestinal tract or type of mammal. Parlesak *et al.* (1994) reported a decrease of absorption for administered polyethylene glycols from 34% ($M_r = 400$) over 1.1% ($M_r = 1500$) to 0.049% ($M_r = 4.000$) by determination of these substances in the urine of humans. To date, there is no general accepted model that describes the relationship between properties of a molecule and permeability. Most authors suggested a correlation between permeability and molecular shape using the smallest effective diameter by Bjarnason *et al.* (1995) or molecular diameter (related to the cube root of relative molecular weight mass) by Parlesak *et al.* (1994). With regard to this physiological point of view, there is no fixed 1000 Da border, but rather a shift of absorption rate depending on the molecular diameter and the polarity of the molecules. Roughly, the absorption of molecules above 1000 Da is on average less than 1%. Thus, the SCF assessed the migrating species below 1000 Da as particularly relevant.

A final draft method description is based on the application of size exclusion chromatography (SEC) for the determination of the mass fraction of a polymeric additive below 1000 Da (prCEN/TS 14577, CEN 2002b). The molecular mass calibration is recommended either with commercial standards or by the use of an on- or off-line coupled mass-selective detector (mass spectrometry or multi-angle light scattering).

The use of analytical SEC as a tool for the quantitative estimation of the overall migration as well as the part below 1000 Da was introduced by Bronz *et al.* (1998). They determined migrates with a structural relationship to BADGE, bisphenol F diglycidyl ether (BFDGE) or novolac diglycidyl ether (NOGE) using fluorescence detection. Migrates from coatings without lubricants led to a recovery rate of 90% compared with gravimetry and verified that resin-related substances are the main part of the migrating substances. To include non-fluorescent and non-UV active migrants into this technique, a non-specific detector like an evaporative light scattering (ELSD) is required in addition to an UV detector (UVD). The final draft prCEN/TS 14577 (CEN 2002b) proposes either a gravimetric determination of the SEC fractions or the integration of a detector signal, each with the part below and above 1000 Da.

The aims of this work were the development of a SEC-ELSD/UVD method for simultaneous determination of the overall migrate and the migrants below 1000 Da that could be used as faster substitute for

the gravimetric method. Besides applicability, both methods for the overall migrate should also be compared critically in consideration of accuracy and precision, followed by the comparison of the equivalent methods for the determination of migrating substances below 1000 Da.

Materials and methods

Materials

Resins and coatings were analysed. The resin is the binding component in a lacquer (coating) composition forming together with the hardener component the three-dimensional network. Besides the binder and hardener, the coating formulation also covers pigments, lubricants and other additives. Two different epoxy resins, a polyester resin and a commercial polystyrene standard were used for SEC calibration: epoxy resin 1 (solid, high molecular weight, component of in epoxy coating 1), epoxy resin 2 (solid, low molecular weight), polyester resin (solid, high molecular weight, component of polyester coating 1) and a polystyrene standard (polystyrene test mixture for SEC columns of 1000 Å; Polymer Standards Service, Mainz, Germany).

Test strips were provided in the size of 1×25 cm, single-side coated and cured at conditions equivalent to the commercial process (5 to 15 min at 180 to 220°C depending on the coating type and film thickness). The strips were coated with an epoxy coating 1 (commercial epoxy-anhydride, two different batches: one for validation and another one for method comparison and application), epoxy coating 2 (commercial epoxy-phenolic), epoxy coating 3 (not commercial test epoxy-anhydride), polyester coating 1 (commercial polyester-urethane), polyester coating 2 (commercial polyester-phenolic), and organosol coating (not commercial test organosol).

Apparatus

The separation was performed on an HP1100 (Agilent, Waldbronn, Germany) system equipped with an autosampler (G1313A), an automatic degasser (G1322A), a binary pump (G1312A), a column oven (G1316A), a variable wavelength detector (G1314A) and Chemstation® software. The ELSD (Sedex 75) and a special nebulizer chamber for supercritical fluid chromatography were purchased from Sedere (Alfortville, France). The ELSD parameters were 0.8 bar nitrogen pressure, 30°C heated nebulizer chamber and 45°C vaporizer. A stainless steel in-line filter (0.5 μm mesh) was inserted between the column and the detector to avoid spikes produced by particles from a bleeding column. Sterilizations were performed in an autoclave (Sanoclav, Geislingen, Germany). SPSS® software was used for the statistical evaluation of the SEC-ELSD/UVD method. Filtrations were carried out with folded paper filters from Schleicher & Schüll (Dassel, Germany).

Identification and confirmation experiments were performed on a similar HP1100 system equipped with a diode array detector (G1315A), an additional mass selective detector (G1946A) and Chemstation software. For mass selective detection, electrospray ionization in the positive mode was used (capillary voltage, 4000 V; nebulizer pressure, 80 psig; dry gas flow, 10.0 l min^{-1}; dry gas temperature, 350°C; fragmentation voltage, 100 V).

Reagents

All used solvents were high-performance liquid chromatography (HPLC) grade except tetrahydrofuran (THF) and dioxane, which were of analytical grade and freshly distilled each day over sodium hydroxide (NaOH) and a vigreux column.

Methods

Simulation and extraction conditions. Coated tinplate strips (1×25 cm) were folded like a concertina and migration experiments were performed using approved European Union simulants and acetonitrile as an extraction solvent (table 1). The use of simulating oil is not suitable since a re-extraction into a solvent would not be complete and extracted oil components would interfere with the non-specific UV and ELSD. The number of strips used for one test depends on the expected amount of migrate and the technique used. Four to eight strips were usually extracted with 50 ml simulant for SEC-ELSD/UVD experiments. A 5-ml aliquot of the 95% ethanol migrate and the acetonitrile extract, respectively,

Table 1. Simulants/extraction media and their treatment according to 97/48/EC (European Commission 1997), amending 82/711/EEC (European Commission 1982) and 85/572/EEC (European Commission 1985).

Simulant	Treatment	Simulated food
Bidistilled water	1 h at 121°C	aqueous food, with pH above 4.5
3% Acetic acid	1 h at 121°C	aqueous food, with pH below 4.5
10% Ethanol	1 h at 121°C	alcoholic food or beverages
95% Ethanol	4 h at 60°C	substitute of the simulant oil for fatty food
Isooctane	2 h at 60°C	substitute of the simulant oil for fatty food
Acetonitrile	24 h	extraction media

were brought to dryness by a nitrogen stream and dissolved in 500 µl dioxane. A total of 25 ml of the aqueous simulants and isooctane were concentrated by a rotary evaporator and likewise dissolved in 500 µl dioxane. Twenty strips (5 dm^2) were normally used for gravimetry and completely covered with 200 ml simulant. Up to 60 strips were extracted with 200 ml simulant in the case of coatings and simulants with low migration, so that the residue exceeded the limit of determination. If coated strips were not available, empty cans were extracted using the highest surface–volume ratios. A worst-case simulation, a heat treatment of food for 1 h at 121°C, led to sterilization conditions as indicated in table 1. If rust or flaking coating pieces were present, the extracts were then filtrated through folded paper filters.

CEN method. EN 1186 (CEN 2002a) was performed for the determination of overall migration from various coatings into several recommended simulants and extraction media. Modifications are indicated in the Results.

Size exclusion chromatography (SEC, analytical and preparative). The system was equipped with a preparative SEC column (SECprep), SDV® 1000 Å, 5 µ, 20 × 300 mm (Polymer Standards Service) for the gravimetric determination of the migrating substances below 1000 Da. The column was heated to 30°C, 100 µl were injected, the eluent was THF (5 ml min^{-1}) and the UVD was set to 275 nm as the specific wavelength for epoxy substances (also suitable for polyester components containing an aromatic moiety).

Analytical separations were performed on two analytical SEC columns (SECanalyt, 8 × 300 mm) in series obtained from Polymer Standards Service filled with the same polymer, but with two different porous sizes 100 and 1000 Å. The columns were heated to 50°C, 20 µl were injected and the eluent was dioxane (1 ml min^{-1}).

Results and discussion

Overall migration

Gravimetric method (slightly modified EN 1186). The EN 1186 (CEN 2002a) method was slightly modified according to laboratory facilities. CEN demands the examination of 1 dm^2 per 100 ml solvent and weighing until constancy (maximum difference of 0.5 mg) on a scale with an accuracy of 0.1 mg. In order to gain a residue exceeding the limit of determination, especially taking into account the amount of migrate below 1000 Da, 5 dm^2 were extracted in 200 ml solvent. Taring was performed until the differences between the last two steps were less than 0.2 mg on a scale with an accuracy of 0.05 mg. As recommended by CEN, stainless steel vessels were used with a weight less than 100 g and a diameter between 50 and 90 mm (here 60 mm, 40 g). CEN recommends a concentration by a rotary evaporator and a drying process at 105–110°C. After recognizing a browning of the migrate (reaction with oxygen, etc.), a vacuum drying oven at 40°C and 80 mbar was used instead.

Validation. Validation parameters for acetonitrile extracts were determined as indicated in table 2.

Ruggedness. Since 100 ml or more of solvent/simulant are brought to dryness, the dry residue of

Table 2. Validation data: gravimetric determination of the overall migrate for acetonitrile extracts.

Coating	*n*	Mean ($mg\,dm^{-2}$)	SD[1] ($mg\,dm^{-2}$)	RSD[2] (%)	Assessed mean ($mg\,dm^{-2}$)	Assessed SD ($mg\,dm^{-2}$)	RSD_{total} (%)	LD[3] ($mg\,dm^{-2}$)
Epoxy coating 1	3	7.23	0.22	3.00	5.16	0.16	3.15	0.35
Epoxy coating 2	2	4.89	0.06	1.30				
Polyester coating 1	3	2.53	0.09	3.67				
Polyester coating 2	2	5.99	0.28	4.61				

[1] Standard deviation, [2] relative standard deviation, [3] detection limit.

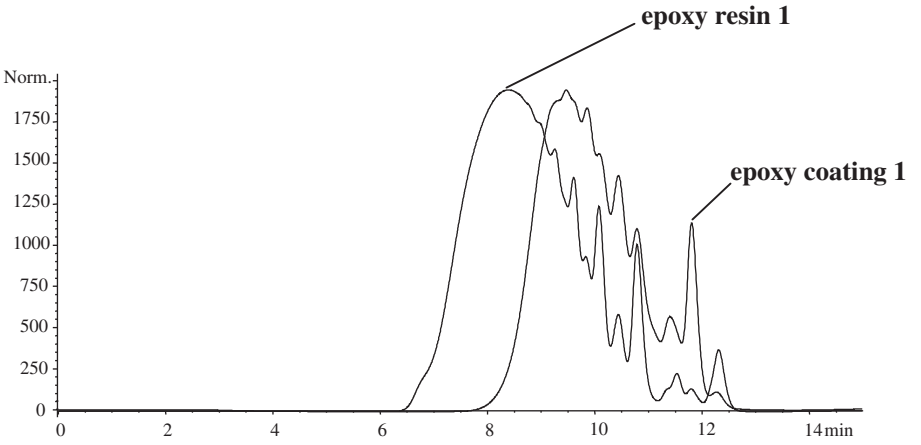

Figure 1. Overlay of SECprep chromatograms (UV detection, normalized) of epoxy resin 1 and epoxy coating 1, acetonitrile extract.

the solvents has to be determined as blank values. If the solvent residue exceeds the limit of detection, the content has to be subtracted as a blank. Before drying, the extraction solution should be filtered from flaking coating pieces and rust. Tice (1994) also mentioned this as one of the unsolved problems of the CEN method when applied to acidic aqueous migrates of plastic coatings on metals. Generally, the gravimetric method is rugged against variations of the drying temperature ($\pm 10^{\circ}C$) and pressure ($\pm 50\,mbar$).

SEC-ELSD/UVD method. Epoxy resin 2 was used as a standard substance for the calibration of the ELSD because resin components were expected as the main migrating substances in coating extracts and migrates. The overlay chromatogram (figure 1) of an epoxy resin 1 solution and an acetonitrile extract of the corresponding commercial epoxy coating 1 shows that the dominant pattern of the resin is also present in the coating extract. This confirms the results of Bronz *et al.* (1998) who recognized that 90% of the migrants from epoxy coating extracts (from special coatings without lubricants) were BADGE derivatives.

The application of a second-grade regression (figure 2) produced a satisfactory fit ($r = 0.9978$) for a suitable working range. The basis of computation for validation of the second-grade regression was obtained from Funk *et al.* (1992). Trathnigg *et al.* (1999) used exponential regression, but did not calculate validation data.

The coefficient of variation of 1.99% proves the high repeatability of this detector. However, a coefficient of variation $> 7.5\%$ (calculated over 8 days) was observed in day-to-day variances, when the apparatus had been switched off. Thus, a three- to four-point calibration was performed every day.

Validation. Validation parameters of ELSD were determined as indicated in table 3. The reproducibility was tested in an extensive statistical

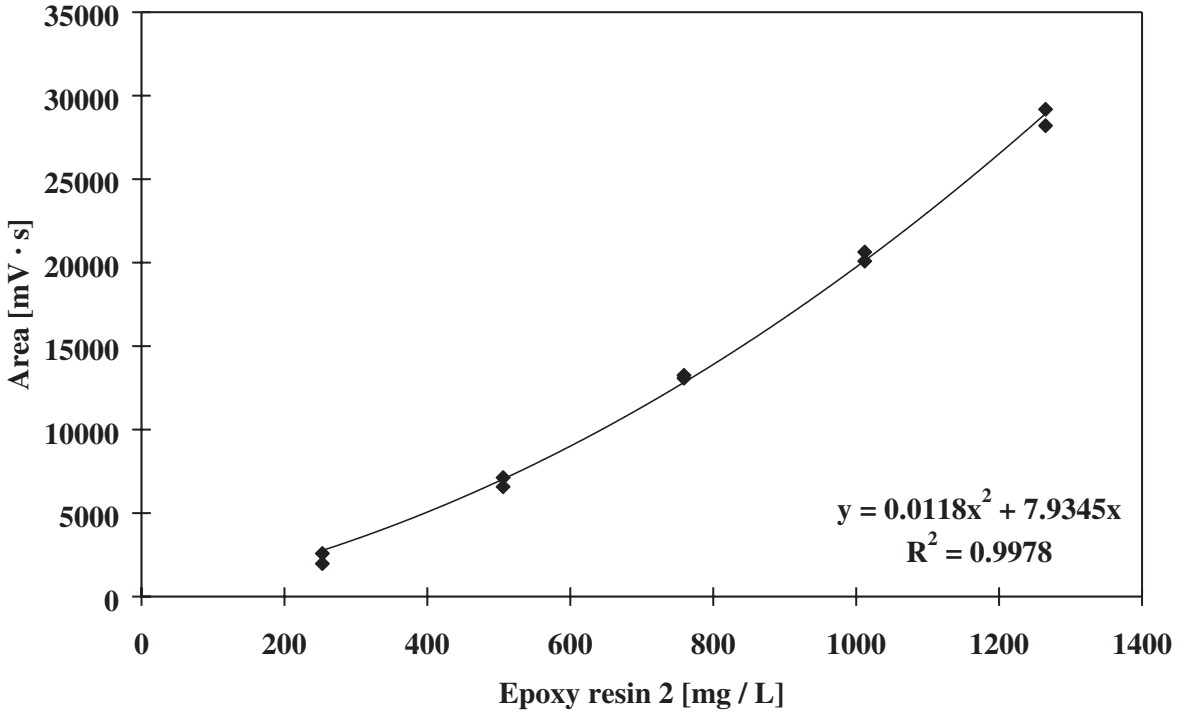

Figure 2. Calibration curve of the ELSD signal with epoxy resin 2 (five levels, triple injections, 15.1 mg l^{-1} standard deviation, 1.99% coefficient of variation).

Table 3. Validation data: SEC-ELSD determination of the overall migrate of an acetonitrile extract of coatings 1 and 3.

Coating	Chemist	*n*	Mean (mg dm^{-2})	SD (mg dm^{-2})	RSD (%)	Assessed mean (mg dm^{-2})	Assessed SD (mg dm^{-2})	RSD_{total} (%)	SD_{lab} (mg dm^{-2})	DL* (mg dm^{-2})
Epoxy coating 1	1	5	4.18	0.12	2.87	4.15	0.11	2.53		
Epoxy coating 3	1	5	4.11	0.09	2.19					
Epoxy coating 1	2	5	4.23	0.11	2.60	4.22	0.12	2.84	0.12	0.04
Epoxy coating 3	2	5	4.20	0.12	2.86					
Epoxy coating 1	3	5	4.29	0.14	3.26	4.22	0.14	3.33		
Epoxy coating 3	3	5	4.14	0.13	3.14					

* DL estimated by the detector signal-to-noise ratio of 3, calculated with 15 mg l^{-1}, which corresponds to 0.04 mg dm^{-2} for a coating extract of 2 dm^2/50 ml and a concentration factor of 10.

study performed by three different chemists analysing acetonitrile extracts of two different coatings with five replicates (including extraction) on each coating.

Ruggedness. The ELSD, using the mentioned apparatus and highly volatile solvents, is susceptible to fluctuating room temperature. The single glass-wall chamber was replaced by a thermostable double glass-wall chamber (nebulizer chamber for super fluid chromatography). The use of this heated nebulizer was essential because otherwise an inconstant room temperature (± 5°C) led to signal differences of up to 300% (data not shown). In addition to this heated chamber, the commonly used eluent THF was substituted by the less volatile eluent dioxane in order to minimize variances in response. Nevertheless, a calibration (three to four points) had to be done after each restart of the ELSD.

To verify whether the quantification was independent of the resin type, the responses of two different resin

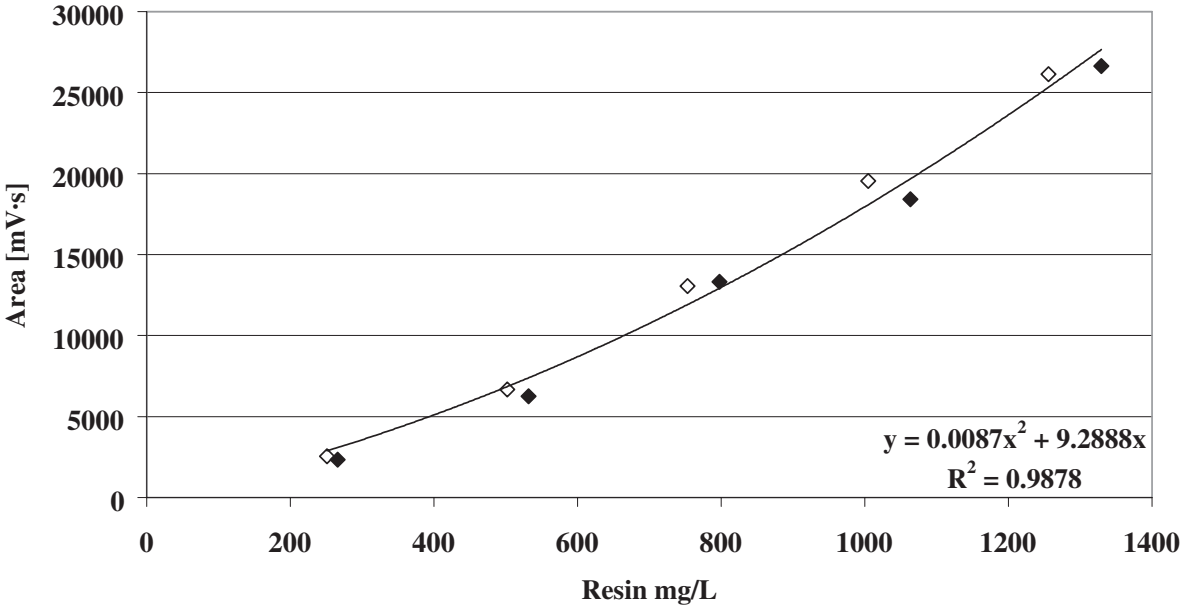

Figure 3. Calibration of the total amount of dissolved epoxy 2 resin (black symbols) and polyester resin (white symbols) by SEC-ELSD.

types (polyester resin, nearly Gaussian distributed — chromatogram not shown; epoxy resin, typical substructure; figure 1) were compared (figure 3). No significant deviations were observed and different resins could be used for calibration. Strictly, since the response was not linear, only Gaussian-distributed peaks without a substructure were allowed to be integrated as a single peak. A possible remedy could provide the linearization of the detector signal before integration by transformation of the signal values applying an exponential function, e.g. $y' = y^{1/1.5}$.

Problem of accuracy. The determination of the 'overall migrate' by gravimetry owns reference status. It has to be taken into account that volatiles are not included into the overall migrate. The reference method (EN 1186, CEN 2002a) and the presented SEC-ELSD/UVD are compared and discussed with consideration of the total amount of migrating substances. Figure 4 shows all data based on acetonitrile extracts.

For all coatings, significant lower values (65–90% of the gravimetrically determined values) were obtained for the overall migrate determined by SEC-ELSD/UVD analyses compared with gravimetry, whereas no significant differences were observable between ELSD and UV detection (t-test, $\alpha < 0.05$).

The ELSD exhibits no or a minor response for volatile substances boiling point ($< 200°C$), which are also lost during the gravimetric determination according to EN 1186 (CEN 2002a). Furthermore, the ELSD response might also depend on the structural features of the analytes (surface activity, solubility, etc.) (Schaefer *et al*. 2003) and may further depend on the type of ELSD used. Nevertheless, the ELSD responses of epoxy resin 2 and the polyester resin were comparable (figure 3). This is important information since the overall migrate mainly consists of binder resin components, while lubricants, hardeners and catalysts are minor components of the migrate. On the other hand, gravimetry (EN 1186, CEN 2002a) gives too high values due to the inclusion of solvents (shown for a solution of epoxy resin 2 in THF/95% ethanol — 60/40, v/v: 8% weight increase was measured after the drying process). Neither the EN 1186 (CEN 2002a) nor the presented SEC-ELSD procedure enables a determination of the 'total migrating substances' since both omit volatile migrants. This might be acceptable for the analysis of coating migrates since most of the volatiles are evaporated during the curing process. In conclusion, the gravimetric method owns the reference status. The SEC-ELSD/UVD method produces significantly lower results for the overall migrate which is not acceptable in the surveillance of regulatory migration

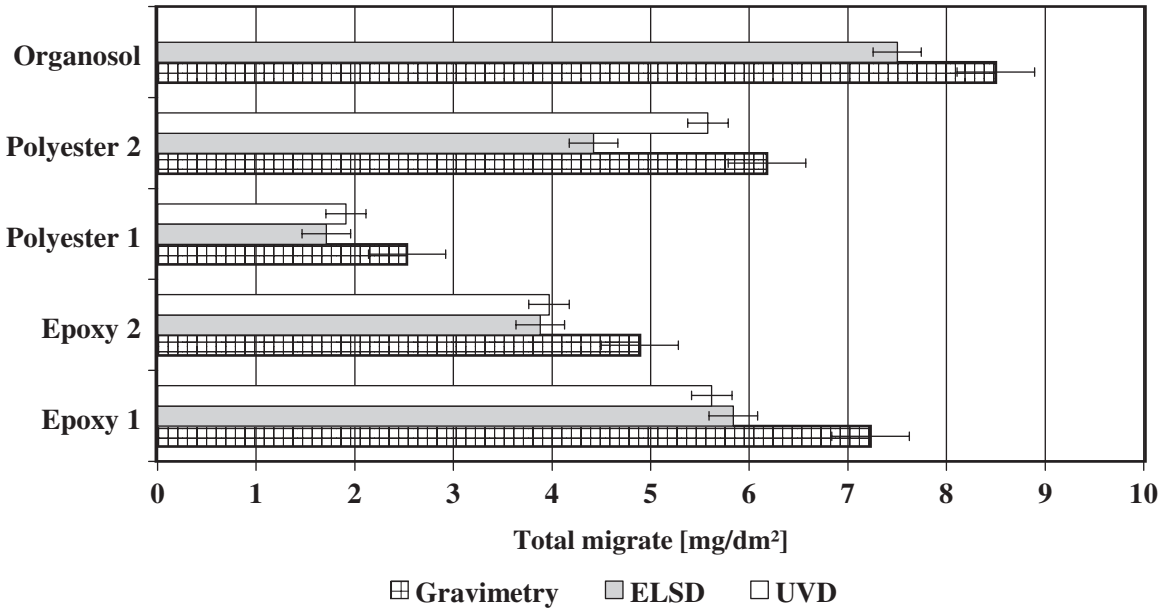

Figure 4. Comparison of both methods applied on acetonitrile extracts of five coatings. Confidence intervals ($\alpha = 0.05$) are indicated and determined with $a_{grav} = 0.392\,mg\,dm^{-2}$, $a_{ELSD} = 0.246\,mg\,dm^{-2}$ and $a_{UVD} = 0.205\,mg\,dm^{-2}$.

limits. However, the following parameters and effects argue for the chromatographic method: time, limit of detection and better ruggedness. The SEC-ELSD method still provides coherent results for coatings of similar chemistry and provides a helpful tool for coating development and optimization.

Migrating substances with a molecular weight below 1000 Da

Molecular weight calibration. SEC columns are packed with porous particles and separate molecules based on the hydrodynamic volume. Owing to the variable sizes of molecules with identical molecular weight (e.g. linear or cyclic), the columns were calibrated with different kinds of polymeric resins to determine the specific cut for each type.

Figure 5 shows the separation of an authentic epoxy resin standard (epoxy resin 2) on the SECprep column. The calibration was performed by off-line coupled mass-selective detection as recommended by CEN (2002b). All significant peaks were fractionated and identified as linear oligomers of BADGE by HPLC-electrospray ionization-mass selective detection (HPLC-ESI-MSD, data not shown).

As an authentic low molecular weight polyester standard was not available, so an extract of polyester coating 1 was used for molecular weight calibration. Four significant prominent peaks (figure 6) were fractionated and identified as cyclic oligoesters of terephthalic and/or isophthalic acid with different polyols by HPLC-ESI-MSD.

The logarithmic regression between molecular mass and retention time was used for molecular weight calibration (figure 7). The 1000 Da cut for the epoxy resin 2 was determined at 10.76 min when using SECprep and at 15.69 min when using SECanalyt. The 1000 Da borders obtained from polyester coating 1 extract were at 11.08 min (SECprep) and 16.51 min (SECanalyt), respectively. The examination of additional resins confirmed these results. Every cut set for one definite molecular weight (in this case 1000 Da) may exclude some substances below 1000 Da and may include some substances above 1000 Da since all peaks suffer on-peak broadening. It is assumed that this error is compensated by substances with a molecular weight fairly above and below 1000 Da.

Figure 7 shows a difference of 0.8 min for the retention time of components below 1000 Da for the important epoxy and polyester resin types in coatings.

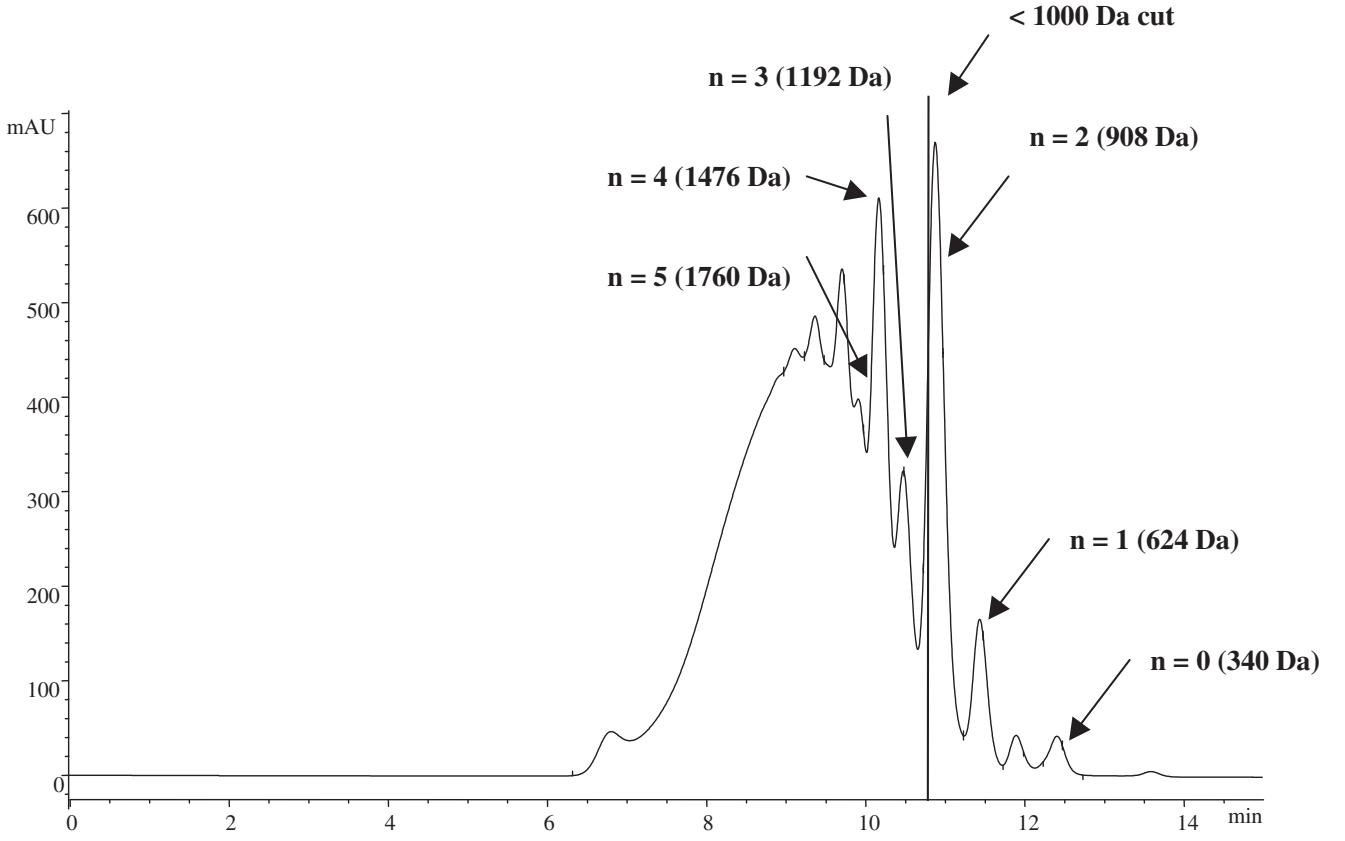

Figure 5. Chromatogram of epoxy resin 2, SECprep, UV detection, THF 5 ml min^{-1}, column temperature 30°C.

This difference can presumably be traced back to the linear shape of the epoxy resin compounds and the mostly cyclic structure of the polyester migrants. However, 0.8 min difference in the exclusion border results in significantly deviating amounts of migrating substances below 1000 Da (figure 6). Additionally, Brem and Grob (2000) found a strong shift to shorter retention times for hydrolysed and hydrochlorinated NOGE-derivatives compared with NOGE, which can presumably be traced back to polar interactions with column material.

Thus, Runyon *et al.* (2002) calibrated the SEC separation with triarachin, which has a molecular weight near 1000 Da and proved to exhibit the shortest retention time per unit molecular weight in SEC compared with different oligomer types. Migrating substances with a molecular weight up to 2000 Da are wrongly included in the below 1000 Da part, but those below 1000 Da are never excluded. This technique leads to a most conservative estimation of the amount of migrating substances below 1000 Da. In order to gain a high accuracy for the coatings investigated, the exclusion border determined from the corresponding resin types was applied to the migrates (figure 7). However, this procedure either needs reference standards for each main coating type or as presented in this investigation a fractionation with subsequent determination of the most abundant molecular weight of the binder type resin.

Gravimetric method. The dry residue of the acetonitrile extract was resolved in 1.2 ml THF for the gravimetric determination of the migrating substances below 1000 Da. This solution was injected into the SECprep in 10 portions of 100 µl each using a 1000 Da cut depending on the coating type (polyester or epoxy), the collected fractions were concentrated and dried in the vacuum oven, and the residual weight was corrected by a factor of 1.2. The addition of the determined parts below and above 1000 Da results in the overall migrate after SEC.

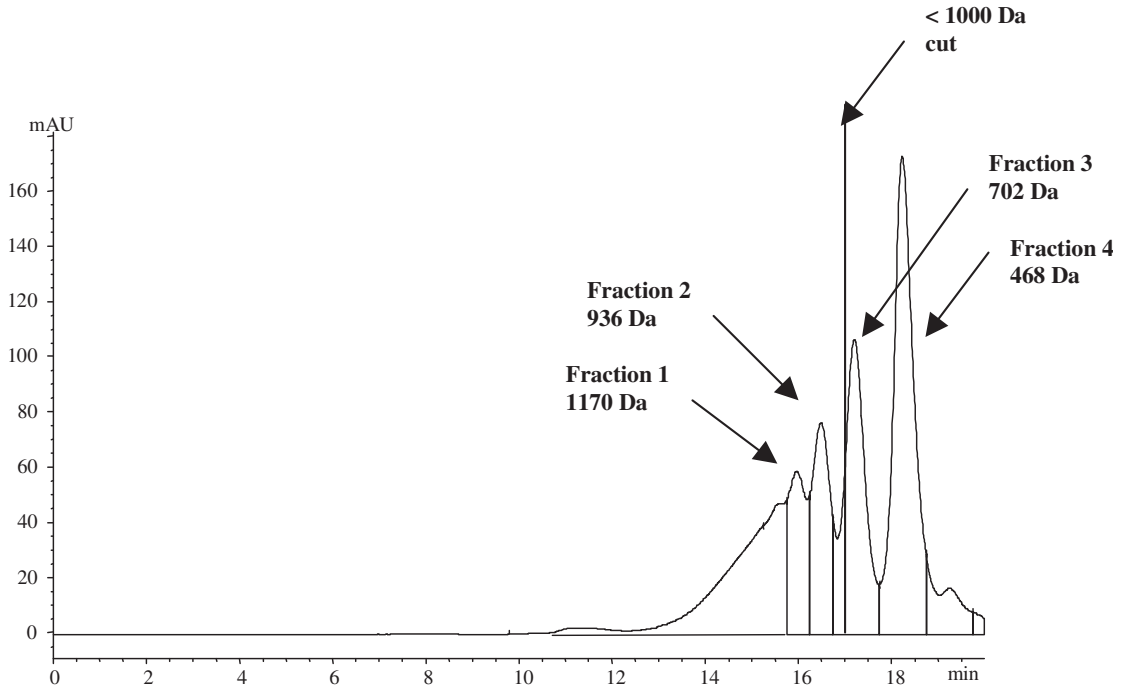

Figure 6. Chromatogram of polyester 1 coating, acetonitrile extract, SECanalyt, UV detection, dioxane 1 ml min^{-1}, column temperature 50°C.

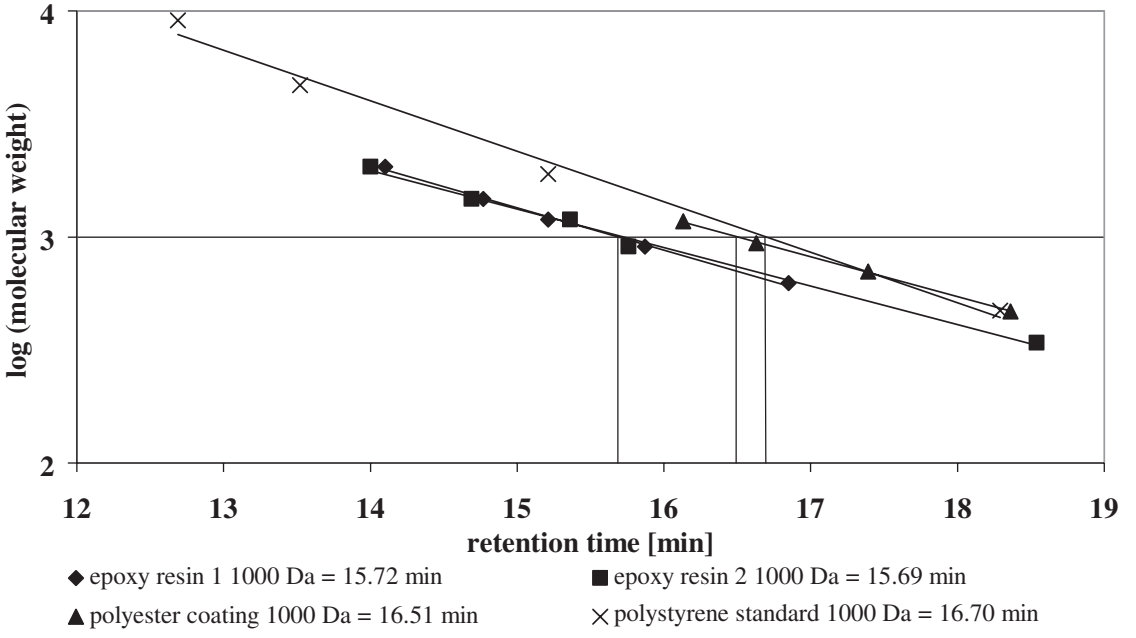

Figure 7. Comparison of four molecular weight calibration curves obtained from epoxy as well as polyester and polystyrene oligomers (single data points for epoxy and polyester resins are oligomers as indicated in figures 5 and 6; the identity of polystyrene oligomers was obtained from the supplier of this calibration standard).

Table 4. Validation data: gravimetric determination of the migrating substances below 1000 Da for acetonitrile extracts.

Coating	n	Mean ($mg\,dm^{-2}$)	SD ($mg\,dm^{-2}$)	RSD (%)	Assessed mean ($mg\,dm^{-2}$)	Assessed SD ($mg\,dm^{-2}$)	RSD_{total} (%)	DL ($mg\,dm^{-2}$)
Epoxy coating 1	3	1.54	0.14	9.03	1.49	0.10	6.71	0.35
Epoxy coating 2	2	1.85	0.12	6.51				
Polyester coating 1	2	1.09	0.04	3.89				
Polyester coating 2	1	1.05	–	–				

Table 5. Validation data: SEC-ELSD determination of the migrating substances below 1000 Da for acetonitrile extracts.

Coating	Chemist	n	Mean ($mg\,dm^{-2}$)	SD ($mg\,dm^{-2}$)	RSD (%)	Assessed mean ($mg\,dm^{-2}$)	Assessed SD ($mg\,dm^{-2}$)	RSD_{total} (%)	SD_{lab} ($mg\,dm^{-2}$)	DL* ($mg\,dm^{-2}$)
Epoxy coating 1	1	5	1.48	0.054	3.65	1.12	0.035	3.13		
Epoxy coating 3	1	5	0.76	0.016	2.11					
Epoxy coating 1	2	5	1.52	0.046	3.03	1.17	0.034	2.91	0.035	0.04
Epoxy coating 3	2	5	0.81	0.025	3.09					
Epoxy coating 1	3	5	1.53	0.046	3.01	1.17	0.037	3.16		
Epoxy coating 3	3	5	0.80	0.027	3.38					

* DL was estimated by the detector signal-to-noise-ratio of 3, calculated with 15 $mg\,l^{-1}$, which corresponds to 0.04 $mg\,dm^{-2}$ for a coating extract of 2 dm^2/50 ml and a concentration factor of 10.

Validation. Validation parameters were determined as indicated in table 4.

Accuracy. In order to evaluate the systematic error of the SECprep procedure, epoxy resin 2 was dissolved in THF and injected into the SECprep UV apparatus. The total eluate was collected and dried. Five concentration levels (range of 3.54–25.10 mg for the absolute amount of resin 2) were injected and each level was done in triplicate. The coefficient of variation for the recovery function (abscissa: theoretically injected amount of resin 2; ordinate: resulting weight after drying the eluate) was 3.0%, corresponding to a standard deviation of 0.34 mg. The linear equation of the recovery function ($y = 1.10x - 0.27$) features a positive proportional and a small negative constant error. The slope indicates a proportional error of 10% that could not be explained by dry residue from the THF or bleeding of the column because these mistakes would lead to constant additional residue. As already stated, inclusion of the solvent or volumetric errors (pipettes, autosampler) could be a reason for the small proportional error.

SEC-ELSD/UVD method. In contrast to the gravimetric method, the migrating substances with a molecular weight below 1000 Da and the overall migrate were determined simultaneously. The total area of the SECanalyt-ELSD signal was split into two using the 1000 Da cut depending on the coating type (figures 5 and 6).

Validation. Validation parameters of the ELSD were determined for acetonitrile extracts as indicated in table 5.

Problem of accuracy. More important, however, is the comparison of both methods applied to coating extracts, which also contain internal lubricants and other additives besides resin-related substances. Data are shown in figure 8. No significant difference is observed for the gravimetric determination of the overall migrate of the epoxy coatings before and after the fractionation by the SECprep (t-test, $\alpha = 0.05$; CEN 1976).

Concerning the absolute amount of migrants below 1000 Da significantly ($\alpha = 0.05$), lower results (except for polyester 1) were observed for SEC-UVD and -ELSD analyses compared with gravimetry. No signi-

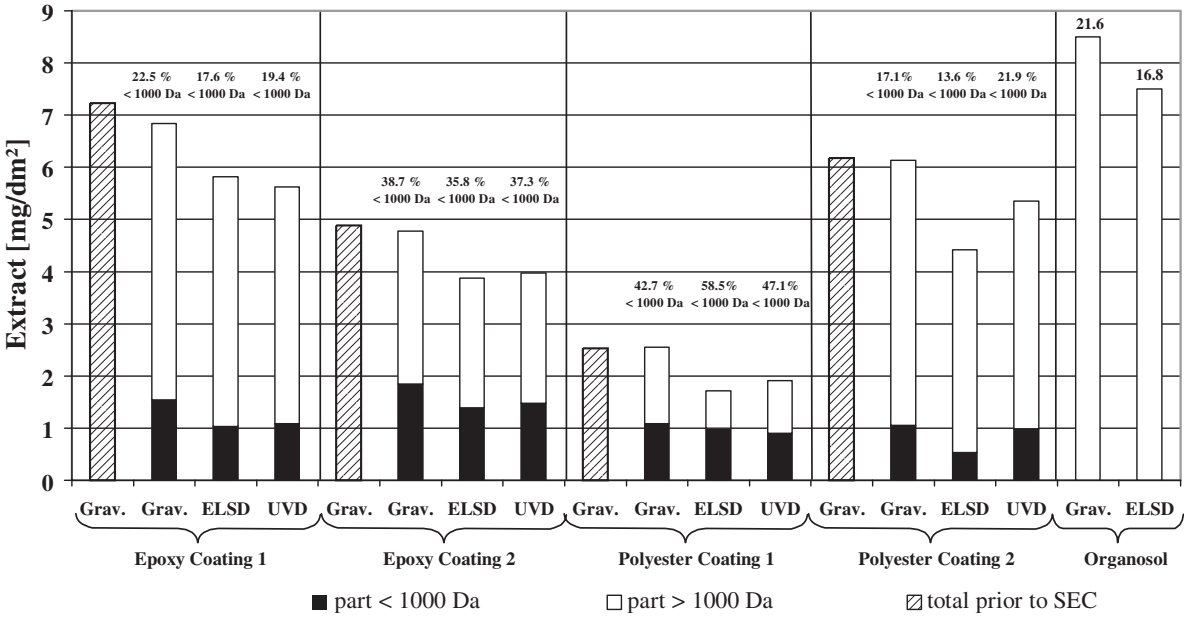

Figure 8. Comparison of all methods applied on acetonitrile extracts of five different coatings, confidence intervals (a) for part below 1000 Da were determined with $a_{grav} = 0.278\,mg\,dm^{-2}$, $a_{ELSD} = 0.082\,mg\,dm^{-2}$ *and* $a_{UVD} = 0.062\,mg\,dm^{-2}$.

ficant differences were observable between ELSD and UV detection (except polyester 2).

The new extended gravimetric method for the part below 1000 Da is time consuming and provides a relatively high standard deviation compared with the SEC-ELSD/UVD method. The limitation of gravimetry is its high limit of detection ($0.35\,mg\,dm^{-2}$ in-house validated for strips and $3\,mg\,dm^{-2}$ — threefold standard deviation (SD) — of CEN method), especially when the procedure is applied for polar simulants. The SEC-ELSD/UVD method is faster because the overall migrate and substances below 1000 Da are determined simultaneously.

Comparison of both methods (epoxy coating 1, different simulants)

Both techniques were applied to commercial standard epoxy coating 1 extracted with all applicable simulants (table 1) and the extraction solvent acetonitrile. Simulant oils (sunflower, olive or synthetic oils) are not applicable to the SEC-ELSD/UVD method since re-extraction of the migrants into a solvent would lead to a loss of analytes and co-extraction of oil components.

Overall, migration reaches only 5% of the values for 95% ethanol or acetonitrile (figure 9b) for aqueous simulants and isooctane (figure 9a). Migration into the latter simulants is below the limit of detection (LD) of the gravimetric method (CEN 2002a), indicating the need for a more sensitive method like the introduced SEC-ELSD. Almost all migrants in the polar simulants have a molecular weight below 1000 Da. These results can be explained by the low solubility of most coating ingredients and their reaction products in these simulants. A noticeable point is the difference of a factor of 25 between migration into 95% ethanol and isooctane since both are officially used as substitutes for fatty simulants for plastic materials. A reason could be a poor penetration capability of the isooctane into the epoxy-hardener network. This is confirmed by Czerniawski and Pogorzelska (1997), who demonstrated that isooctane can be suitable as a substitute for the examination of non-polar plastics, while 95% ethanol should be used for polar plastics. De Kruijf and Rijk (1997) found similar results for non-polar food contact materials like polypropylene, polyethylene or polystyrene by migration into olive oil or isooctane, respectively. Goulas (2001) verified these results by determining a correlation between film thickness and migration into isooctane, suggesting a complete penetration of non-polar plastic films by isooctane.

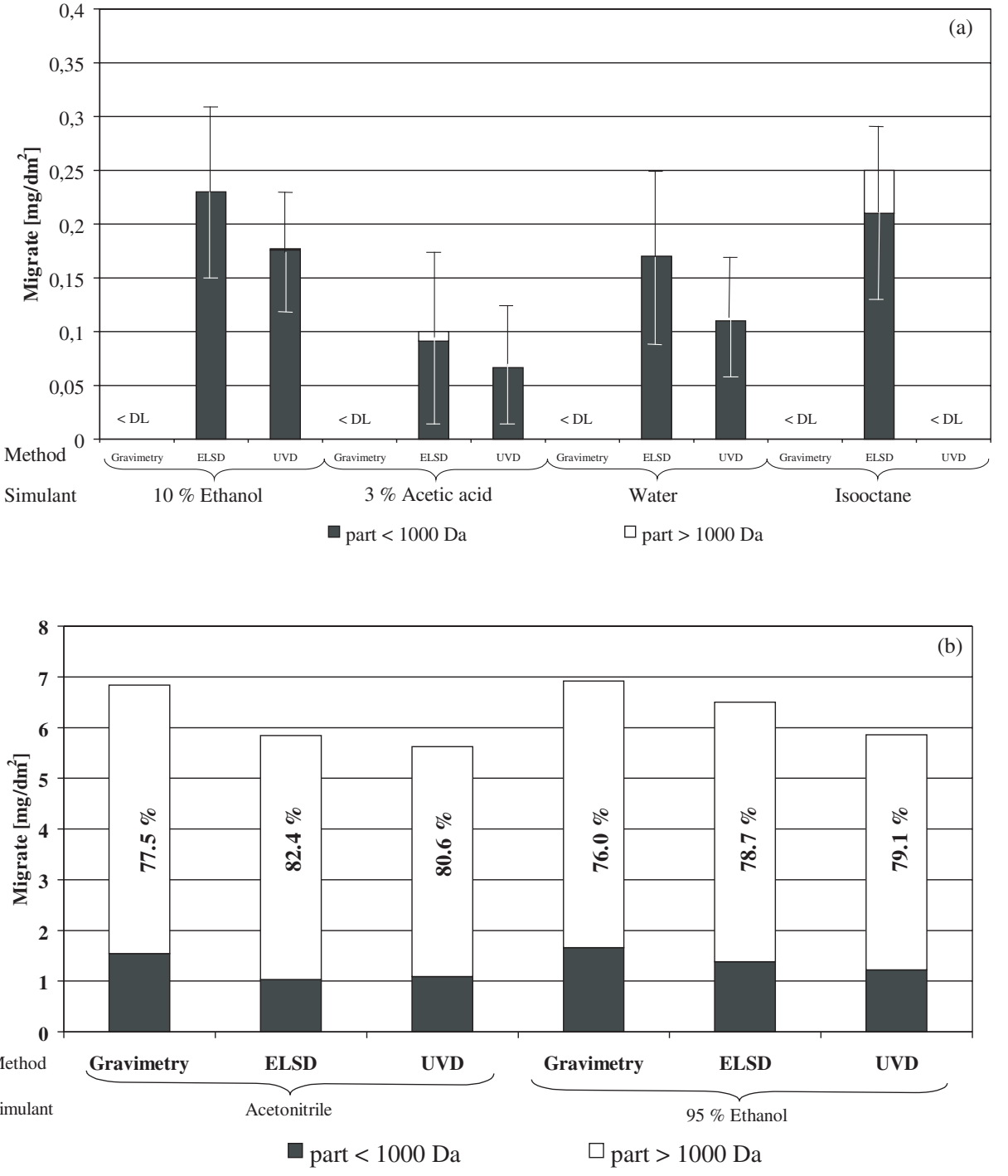

Figure 9. Comparison of migration into different simulants and extraction into acetonitrile applied for epoxy coating 1. (a) Polar simulants and isooctane. Confidence intervals ($\alpha = 0.05$) for the part below 1000 Da are indicated; (b) 95% ethanol and acetonitrile. The parts below 1000 Da are indicated by the black parts of the bars. The percentages of the parts above 1000 Da are given.

Conclusions

An SEC-ELSD/UVD and a SEC/gravimetric method were established and critically compared for the determination of the overall migrate and migrating substances below 1000 Da in various approved food simulants. The SEC-ELSD/UVD procedure provides a simultaneous determination of both the overall migrate and the part below 1000 Da exhibiting a better precision than the gravimetric measurement. The determination by SEC-ELSD/UVD is suitable even for low amounts of migrants (below 1 mg dm^{-2}). However, the overall migrate of samples from five different coating types gave significantly lower values when determined by SEC-ELSD/UVD compared with the official gravimetric method (EN 1186). Similar deviating results were found for the part below 1000 Da, but here, the gravimetric method is time consuming and not applicable to polar simulants due to its high detection limit.

Acknowledgements

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Migration from can coatings: Part 2. Identification and quantification of migrating cyclic oligoesters below 1000 Da

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Migration from can coatings: Part 2. Identification and quantification of migrating cyclic oligoesters below 1000 Da

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Metal cans for food use can be coated with lacquers based on polyester resins. Recent research has focussed on the identification and quantification of migrants released by coatings that are potentially absorbable (below 1000 Da). The presented method describes a procedure that was optimized to hydrolyse the polyester migrants into their monomers, polyvalent acids and polyols. The polyols were identified by gas chromatography with flame ionization detection GC-FID and the acids by high-performance liquid chromatography (HPLC) coupled with an ultraviolet and an electrospray ionization-mass selective detector (HPLC-ESI-MSD/UVD), respectively. With the knowledge of the polyester monomers, it was possible — at least tentatively — to identify the main components in the migrate as cyclic oligoesters by HPLC-ESI-MSD/UVD. A cyclic oligomer, CYCLO [3IPA (isophthalic acid) 3EG (ethylene glycol)] was synthesized and characterized by infrared, nuclear magnetic resonance and mass spectrometry as well as by elementary analysis for further confirmation. To determine the amount of migrating cyclic oligoesters, the response of the migrating substances was compared using different detectors, UVD, MSD and evaporative light scattering detector (ELSD). The response of the ELSD was dependent on the molecular weight of the analytes that reduced the accuracy of this detection type. The wavelength with the same absorption coefficient for IPA and terephthalic acid (TPA) was obtained at 232 nm. The UV_{232nm} response of an oligoester is proportional to the number of its IPA/TPA moieties, which was verified for several TPA/IPA esters. The amount of the migrating oligoesters was determined using an UV_{232nm} calibration of a commercially available TPA ester and the number of IPA/TPA moieties molecules gained from the ESI-MSD spectra. According to this method, the amount of migrating oligoesters below 1000 Da in the 95% ethanol migrate varied from 0.1 to 0.6 mg dm^{-2} (0.6–3.6 mg kg^{-1} food) in the examined coatings. The determined amounts account for about 50% of the total migrate below 1000 Da.

Keywords: food packaging, can coatings, migration, cyclic oligoesters, polyester hydrolysis, HPLC-ESI-MSD/UVD, HPLC-ELSD, GC-FID

Introduction

Cans are coated internally with a polyester coating in order to protect the food against metal ions as well as to protect the tinplate from aggressive food ingredients. The application of this coating type was examined as an alternative for epoxy-based coatings that release reactive intermediates like bisphenol A diglycidyl ether (BADGE) or bisphenol F diglycidyl ether and their derivatives into the packed food. These substances have been a cause of concern for public health (Paseiro Losada *et al.* 1991, Biedermann and Grob 1998).

However, only little information is available about the low molecular weight fraction in polyester resins. These substances are mainly cyclic oligoesters, present in all polyester resins due to ring-chain equilibrium at a level up to 1–2% (Semlyen 2000). As a consequence of their cyclic structure, they are not integrated into the polymer network during the curing process of the plastic material. Briggs (1986), Ahjopalo *et al.* (2000) and Laine *et al.* (2001) identified the non-bonded

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cyclic oligoesters as the reason for negative alteration of polyester surfaces ('blooming'). These cyclic oligomers can migrate from polyester-based packaging into the food. Those oligoesters with a molecular weight below 1000 Da are important from the toxicological point of view for the Scientific Committee on Food (SCF), since they are likely to be absorbed in the gastrointestinal tract (European Commission 2001).

During the introduction of poly(ethylene terephthalate) (PET) bottles into the beverage market, extensive studies have been carried out concerning the analysis of migratable and/or extractable components. Most investigations have been done to identify the migrating oligomers in polyester extracts (Milon 1991, Barnes *et al*. 1995, Harrison *et al*. 1997), mineral water (Buiarelli *et al*. 1993) and food (Castle *et al*. 1989, 1990, Begley *et al*. 1990). High-performance liquid chromatography (HPLC) coupled with an ultraviolet detector (UVD) was used by Begley *et al*. (1990) and Castle *et al*. (1990) or coupled with a mass selective detector (MSD) by Milon (1991), Barnes *et al*. (1995) and Harrison *et al*. (1997) as well as gas chromatography (GC) coupled with MSD was used by Castle *et al*. (1989) and Buiarelli *et al*. (1993). Most found a homologous series ($n = 3$–7) of oligomers based on terephthalic acid and monoethylene glycol. Barnes *et al*. (1995) also identified oligomers based on diethylene glycol, a degradation product of ethylene glycol. The first quantification of the sum of migrating cyclic oligoesters in food, beverages and simulating olive oil was carried out by ester hydrolysis, subsequent methylation of free terephthalic acid and determination of dimethyl terephthalate by GC-MSD (Castle *et al*. 1989). As an external standard, they used a dried chloroform extract from PET. An HPLC determination of the cyclic trimer, tetramer and pentamer in food was developed by Begley *et al*. (1990) and Castle *et al*. (1990) using a cyclic PET trimer as an external standard.

To the best of the authors' knowledge, methods for the identification and quantification of migrating cyclic oligoesters from polyester coatings have not been published to date. In contrast to PET, coating polyesters are produced from a variety of different monomers (acids and polyols) (table 1). Therefore, many homologous series of migratable oligoesters are possible.

The aim of this investigation was the development of an analytical concept for the identification and quantification of the various polyester migrants below 1000 Da. Analyses based on single-quadrupole MSD do not provide unequivocal results for their identification (position isomers of acids and alcohols cannot be differentiated). Therefore, an adequate hydrolysis of the polyester migrate, in accordance to the PET hydrolysis by Castle *et al*. (1989), should release the alcohols and acids to enable the identification of polyester monomer composition by specific methods. A simple HPLC-MSD/UVD screening method should be developed for the identification of the migrating cyclic oligoesters using the additional information about the monomers. Furthermore, a practicable solution for their quantification should be investigated using a commercially available standard.

Table 1. Commonly used monomers for polyester resins.

Polyols	Polyvalent carboxylic acid
1. Ethylene glycol	11. Trimellitic acid
2. 1,2-Propanediol	12. Phthalic acid
3. 1,3-Propanediol	13. Adipic acid
4. 1,3-Butanediol	14. Terephthalic acid
5. 2-Methyl-1,3-propanediol	15. Isophthalic acid
6. 2,2,-Dimethyl-1,3-propandiol (neopentyl glycol)	
7. 1,6-Hexanediol	
8. 2,2,4-Trimethylpentane-1,3-diol	
9. 1,1,1-Tris(hydroxymethyl)propane (trimethylolpropane)	
10. 1,4-*Bis*(hydroxy-methyl)-cyclohexane (cyclohexyldimethanol)	

Materials and methods

Samples

Can coating samples were provided by Valspar Corporation as single-side coated tinplate-strips (1×25 cm). The strips were coated with polyester coating 1 (polyester-urethane type), polyester coating 2 or 3 (both polyester-phenolic type), respectively.

Chromatography

The identification of the diols was performed on a gas chromatograph (8A, Shimadzu, Columbia,

MD, USA) equipped with a flame ionization detector (FID), a capillary column (fused silica, CP-Pora-Plot Q-HT, 12.5 m × 0.32 mm i.d., 10 µm film thickness, max temperature 290°C, Varian, Darmstadt, Germany) and an integrator (D-2500, Merck-Hitachi, VWR, Darmstadt, Germany). The HPLC analyses were performed on an HP1100 system (Agilent, Haldbronn, Germany) equipped with an autosampler (G1313A), an automatic degasser (G1322A), a binary pump (G1312A), a column oven (G1316A), a diode array detector (G1315A), a mass selective detector (G1946A) using electrospray ionization (ESI, positive mode, capillary voltage 4000 V, nebulizer pressure 40 psig, dry gas flow 10 l min^{-1}, dry gas temperature 350°C) and a reverse phase column (Multospher® 100 5C18, 250 × 3 mm or Phenomenex® Aqua 125 5C18, 250 × 3 mm) or on an equivalent system with a variable wavelength detector (G1314A) and an evaporative light scattering detector (ELSD; Sedex 75, Sedere, Alfortville, France, 3.3 bar nitrogen pressure, 25°C nebulizer chamber temperature, 38°C vaporizer temperature). The ELSD was equipped with a special nebulizer chamber for supercritical fluid chromatography and a stainless steel in-line filter (0.5 µm mesh) was inserted between column and the ELSD to avoid spikes produced by particles from a bleeding column. Data were assessed by Chemstation® software (Rev. A 08.03). Synthesized products were purified on a preparative HPLC apparatus equipped with a preparative pump (Nova Prep 200®, Merck-Hitachi), variable wavelength detector (LaChrom L-7400, Merck-Hitachi), a 5 ml sample loop and a reverse-phase column (LiChrospher® 100 5C18, 250 × 25 mm). Data were assessed by LC-Responder® (R&S Technologies, Version 1.15) software.

Materials

Unless otherwise stated, reagents were of analytical grade. Reference standards for HPLC, adipic acid, isophthalic acid (IPA), phthalic acid, terephthalic acid (TPA) and trimellitic acid were purchased from Merck (Darmstadt, Germany), whereas dimethyl isophthalate was purchased from Acros (Geel, Belgium) and *bis*-hydroxyethylene terephthalate (BHET) from TCI (Tokyo, Japan). Reference standards for GC, 1,3-butanediol, 1,2-ethandiol (ethylene glycol, EG), 1,6-hexanediol, 2,2-dimethyl-1,3-propanediol (neopentyl glycol, NPG), 1,2-propanediol, 1,3-propanediol, 1,1,1-*tris*(hydroxymethyl)propane (trimethylolpropane) were purchased from Merck, 2,2,4-trimethylpentane-1,3-diol and 1,4-*bis*(hydroxymethyl)-cyclohexane (cyclohexyldimethanol) from Acros and 2-methyl-1,3-propanediol from Fluka (Buchs, Switzerland). Acetonitrile was HPLC gradient grade (Acros). Purified water was obtained by bidistillation (Heraeus, Hanover, Germany). All solutions were filtered through a membrane filter (Spartan 0.2 µm RC, Schleicher & Schüll, Dassel, Germany) in case of precipitations. For cation exchange clean-up, Dowex 50 W (HCR-2W, Sigma, Deisenhofen, Germany, 1.81 m eq. g^{-1}, 100–200 dry mesh, cross-linkage 4%) was used. The swollen exchange resin was converted into H^+ form with 20 ml hydrochloric acid (5%, v/v) and washed with water until the eluate was free of chloride ions. Solid-phase extraction (SPE) was carried out using Bondelut® C18ec (0.5 g, Varian) cartridges. Ammonium formate buffers (5 mM, pH 3.9 or 1 mM, pH 3) were prepared by diluting 500 or 100 ml 10 mM ammonia solution (0.75 ml concentrated ammonia solution diluted to 1000 ml) to 1000 ml water and adjusting the pH to 3.9 or 3.0 with formic acid.

Synthesis of bis-*hydroxyethylene isophthalate (BHEI) according to Zahn and Krizikalla (1955).* A total of 2.5 g (12.9 mmol) dimethyl isophthalate was added to 32 g (0.52 mol) ethylene glycol. The solution was heated to 140°C under stirring. A total of 0.09 g (1.6 mmol) of the catalysing reagent CaO was added. The temperature was raised to 180°C and the mixture stirred for 2 h under reflux. The water in the reflux cooler was not allowed to circulate in order to let the methanol evaporate, which is generated during the transesterification. After cooling, the residual ethylene glycol and other by-products were removed by preparative HPLC with acetonitrile/water (20/80, v/v) eluent at a flow rate of 35 ml min^{-1}. The product-containing fractions were combined and concentrated on a rotary evaporator. Residual water was removed by lyophyllization. The product (figure 1a) was a white powder with 99% purity (HPLC, 220 nm).

Characteristics of BHEI. Yield: A total of 1.0 g (32.3%); elementary analysis ($C_{12}H_{14}O_6$, 254.24 g mol^{-1}): 56.73% C (calculated 56.69%), 5.55% H (calculated 5.51%); UV (acetonitrile): 229.0 (maximum), 274.0 (maximum), 280.3 (maximum), 288.1 (maximum) nm; IR (KBr): 731s, 1022m, 1075s, 1111s, 1154s, 1244s, 1319s, 1375m, 1452m, 1723ss,

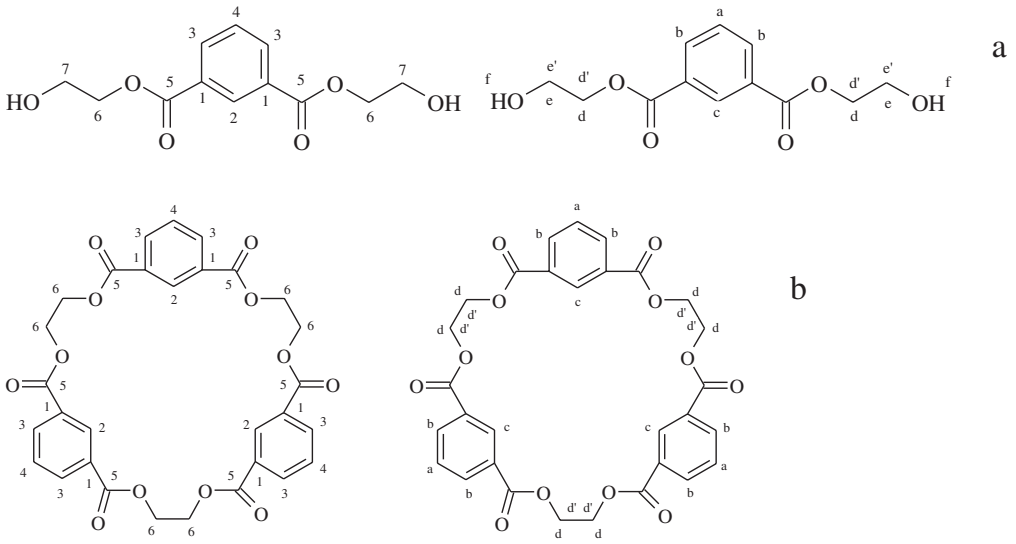

Figure 1. Numeration of carbon and hydrogen atoms for BHEI (a) and CYCLO (3IPA 3EG) (b).

2933m, 3307ss cm^{-1}; ^{13}C-NMR ($CDCl_3$): $\delta = 166.0$ (C-5), 134.2 (C-3), 130.9 (C-3), 130.4 (C-1), 128.8 (C-4), 67,0 (C-6), 61.3 (C-7) ppm; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 8.71$ (H-c, s, 1H), 8.26 (H-b, d, 2H), 7.55 (H-a, t, 1H), 4.51 (H-d/d′, t, 4H), 3.99 (H-e/e′, q, 4H), 2.12 (H-f, s, 2H) ppm.

Synthesis of cyclic oligoethylene isophthalate (COEI) according to Burch et al. *(2000)*. The synthesis of COEI was performed in a special high temperature (maximum temperature 400°C) air-heated bath (Präzitherm, Störk- Tronic, Stuttgart, Germany) using stone wool for better insulation. BHEI (0.65 g, 2.56 mmol) was added to 20 g (88.4 mmol) hexadecane and 0.86 ml tetra-isopropyltitanate (0.1% (v/v) in hexadecane, prepared under nitrogen) and heated for 30 min at 370°C and atmospheric pressure in the insulated air-heated bath. Meanwhile, 10 ml azeotrop of hexadecane and ethylene glycol were distilled. Cyclic products were separated from linear products by decanting the hot hexadecane solution. The cyclic oligoesters crystallize in long, white needles, which are dissolved in acetonitrile/water (50/50) and separated by preparative HPLC with acetonitrile/water (55/45, v/v) at a flow rate of 35 ml min^{-1}. The fractions containing CYCLO (3IPA 3EG) were concentrated using a rotary evaporator and residual water is removed by lyophyllization. CYCLO (3IPA 3EG) (figure 1b) is a white powder with 95% purity and 5% CYCLO (2 IPA 2 EG) as impurity (HPLC, 220 nm).

Characteristics of CYCLO (3IPA 3EG). Yield: A total of 2.6%; UV (acetonitrile): 229.0 (maximum), 280.1 (maximum), 288.0 (maximum) nm; IR (KBr): 729s, 1077m, 1100s, 1136s, 1240ss, 1306s, 1344s, 1371m, 1611s, 1723ss, 2961m, 3447ss cm^{-1}; ^{13}C-NMR ($CDCl_3$): $\delta = 167.0$ (C-5), 130.9 (C-2), 130.4 (C-1), 134.0 (C-3), 128.3 (C-4), 65.9 (C-6), 61.3 (C-7) ppm; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 8.6$ (H-c, s, 3H), 8.2 (H-b, d, 6H), 7.5 (H-a, t, 3H), 4.7 (H-d/d′, t, 12H) ppm.

Methods

Simulation of migration. Coated tinplate strips (1 × 25 cm) were folded like a concertina and migration experiments were performed using the approved substitute for the EU-simulant olive oil, 95% ethanol. The use of simulating oil was not suitable since a re-extraction into a solvent would not be complete and extracted oil components would interfere the non-specific UV detection. The approved oil substitute iso-octane is also not suitable for migration testing of polyester-based coatings due to the poor solubility of the polyesters migrants in this solvent as similarly shown for epoxy-based migrants exhibiting same polarity (Schaefer and Simat 2003). A sample of eight strips (according to 2 dm^2) was usually extracted with 50 ml simulant. In case coated strips are not

available, empty cans are extracted by using highest surface–volume ratios. In order to simulate a worst case scenario (canned fatty food sterilized for 1 h at 121°C), the strips were treated with 95% ethanol for 4 h at 60°C. Migration experiments were performed in a sterilizator, Sanoclav, Wolf, Geislingen, Germany.

Optimized hydrolysis. A total of 25 ml migrate was concentrated by using a rotary evaporator. The liquid was transferred to a 4 ml brown glass vial and the solvent was further evaporated under a nitrogen stream. The residue was dissolved in 2 ml ethanol and 50 µl bidistilled water and after the addition of 150 µl 0.5 M NaOH hydrolysed in the closed vial for 1 h at 60°C in a vacuum drying oven (Heraeus, Hanau, Germany). The solution was acidified with one drop of 85% phosphoric acid after cooling for the determination of the polyvalent carboxylic acids. In order to identify the polyols by GC, the sodium ions were removed by a strong acidic cation exchange resin. Therefore, 500 µl of the swollen resin were converted to H^+ form and added to the alkaline hydrolysis solution. After stirring, the resin was removed by membrane filtration. If lubricants are expected in the migrate, a clean-up should be performed on a C18-SPE column. The SPE column was washed with ethanol and brought to dryness. The complete sample (including the exchange resin) was loaded onto the column and filtrated completely. Prior to the injection into the GC, the eluate was concentrated under nitrogen stream to approximate 100 µl. In order to avoid an evaporation of the glycols, the solution should not be brought to dryness.

Identification of polyols by GC-FID. A reference solution of 10 commonly used polyols (table 1) was analysed by gas chromatography (figure 2). A good separation was obtained using following chromatographic conditions: nitrogen was used as a carrier gas and a mixture of oxygen and hydrogen was used as the flame gas. The injector and detector were heated to 280°C. Separation started at 160°C column temperature and ended at 270°C with a heating rate of $6°C\,min^{-1}$.

Identification of dicarboxylic acids by HPLC-ESI-MSD/UVD. A reference solution of five commonly used polyvalent carboxylic acids (table 1) was analysed by HPLC (figure 3). A good separation was obtained using the following chromatographic conditions: gradient elution was performed on a Phenomenex® Aqua column with (A) 5 mM ammonium formate in water (pH 3.9) and (B) acetonitrile from 95% A to 60% A in 25 min, followed by a 10 min rinsing of the stationary phase (5% A) and 10 min equilibration to 95% A at 30°C and a flow rate of $0.5\,ml\,min^{-1}$. Detection of the analytes was achieved with UVD (232 nm). Mass selective detection (60 V fragmentor voltage, single-ion monitoring

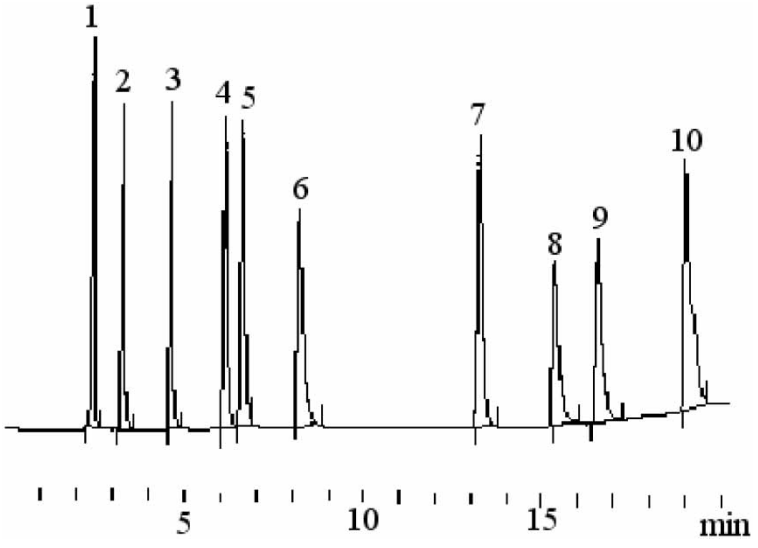

Figure 2. GC-chromatogram of a reference mixture of 10 polyols used for polyesters, each $2\,g\,l^{-1}$, GC-FID. See the Materials and methods for chromatographic conditions; see table 1 for substance identification.

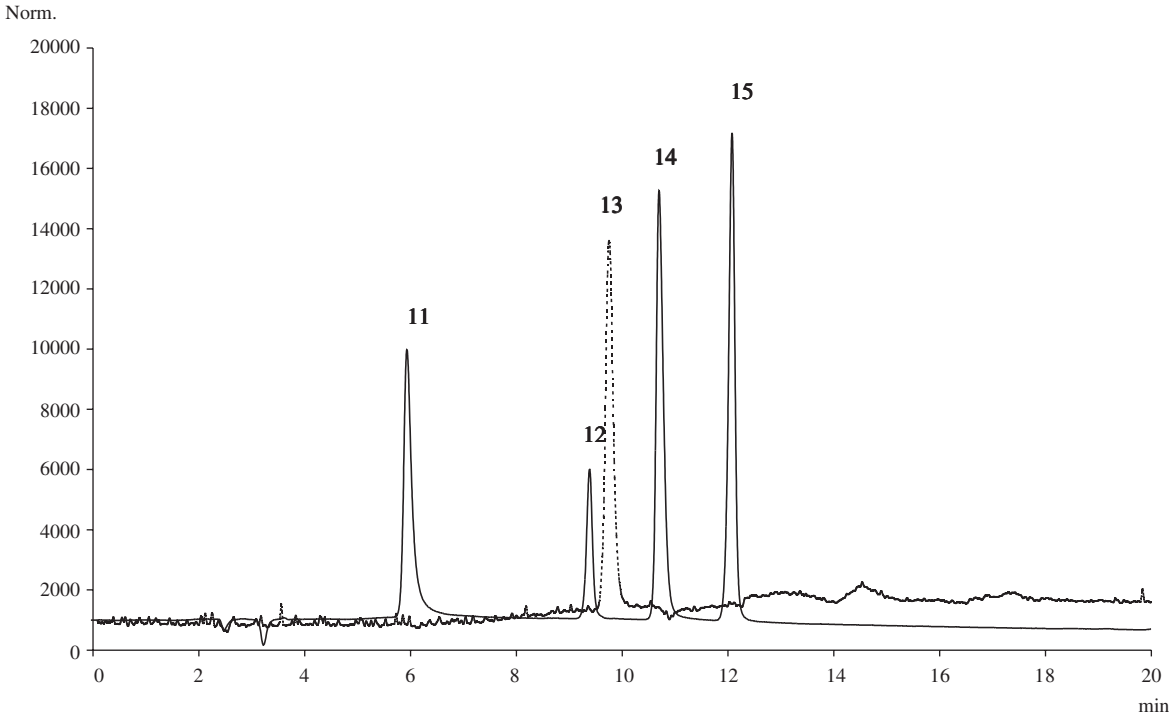

Figure 3. Standard chromatogram of five relevant polyvalent carboxylic acid for polyesters, each 10 mg l^{-1}, HPLC-ESI-MSD/UVD$_{232nm}$, dotted line is single ion trace (m/z A47) for adipic acid. See the Materials and methods for chromatographic conditions; see table 1 for substance identification.

of the characteristic ion for adipic acid 147 (M + 1)) was especially useful for the identification of the poor UV-active adipic acid.

Identification and quantification of cyclic oligoesters by HPLC-ESI-MSD/UVD and HPLC-ELSD/UVD. Migrating cyclic oligoesters were identified and quantified by HPLC (figure 5) under the following chromatographic conditions: gradient elution was performed on a Multospher® column with (A) 1 mM ammonium formate in water (pH 3) for MSD or bidistilled water for ELS detection and (B) acetonitrile from 50% A to 5% A in 45 min, followed by 25 min rinsing of the stationary phase (5% A) and 10 min equilibration with 50% A at 25°C and a flow rate of 0.5 ml min^{-1}. Detection of the analytes was done by UV (232 nm), mass selective (150 V fragmentor voltage, scan *m/z* 80–1000) and ELS detection. Therefore aliquots (4 ml) of the migrates of all three polyester coatings were dried under a nitrogen stream, resolved in 200 µl *N*,*N*-dimethylformamide, 100 µl acetonitrile and 100 µl water and injected into the HPLC system.

Results and discussion

Identification of migrants from polyester-based can coatings

In order to identify migrants from polyester-based can coatings, two methods were used: the migrants were analysed as such (after solvent exchange) or they were allowed to react with sodium hydroxide, which cleaved the ester bonds.

Hydrolysis of polyester migrates for identification of polyvalent carboxylic acids and polyols. Migrates of three different polyester coatings were hydrolysed as described above. Isophthalic acid and terephthalic acid were identified by HPLC-ESI-MSD/UVD in all polyester coatings and six different polyols were identified by GC-FID by comparison of their retention time with those of the standard solution. Polyesters of coating 1 and 3 were made up of three different alcohols and the polyester of coating 2 of two different alcohols (table 2). Owing to contracted

confidentiality on the polyester compositions, the identity of the polyols was coded.

Identification of migrating cyclic oligoesters by HPLC-ESI-MSD/UVD. All major peaks with a molecular weight below 1000 Da were tentatively identified (table 2) by using the calculated molecular weight (from the detected H^+-, Na^+-, K^+-clusters and fragments) (e.g. figure 4) and the information about the monomers used in the polyesters. In some cases, MS detection measured the same molecular weight for three or more peaks (figure 5). This can be explained by cyclic oligomers consisting of different phthalic acid isomers and diastereomers caused by chiral polyols. They cannot be differentiated by MSD, but they give different retention on the reverse phase.

Table 2. Identified dicarboxylic acids, polyols and cyclic oligoesters in polyester coatings 1–3.

	Polyester coating 1	Polyester coating 2	Polyester coating 3
Dicarboxylic acids (DA)	IPA, TPA	IPA, TPA	IPA, TPA
Polyols (PO)	PO-1, PO-2, PO-3	PO-1, PO-4	PO-3, PO-5, PO-6
Cyclic oligoesters	a. CYCLO (2DA 2PO-1) b. CYCLO (2DA 1PO-1 1PO-2) c. CYCLO (2DA 2PO-2) d. CYCLO (3DA 1PO-1 2PO-2) e. CYCLO (2DA 2PO-2 1PO-3) f. CYCLO (3DA 3PO-2) g. CYCLO (4DA 1PO-1 3PO-2) h. CYCLO (3DA 2PO-2 1PO-3) i. CYCLO (4DA 4PO-3)	CYCLO (2DA 2PO-1) CYCLO (3DA 3PO-1) CYCLO (2DA 1PO-1 1PO-4) CYCLO (3DA 2PO-1 1PO-4) CYCLO (2DA 2PO-4) CYCLO (3DA 1PO-1 2PO 4)	CYCLO (2DA 1PO-5 1PO-6) CYCLO (2DA 2PO-5) CYCLO (2DA 1PO-3 1PO-5) CYCLO (3DA 1PO-3 2PO-5)

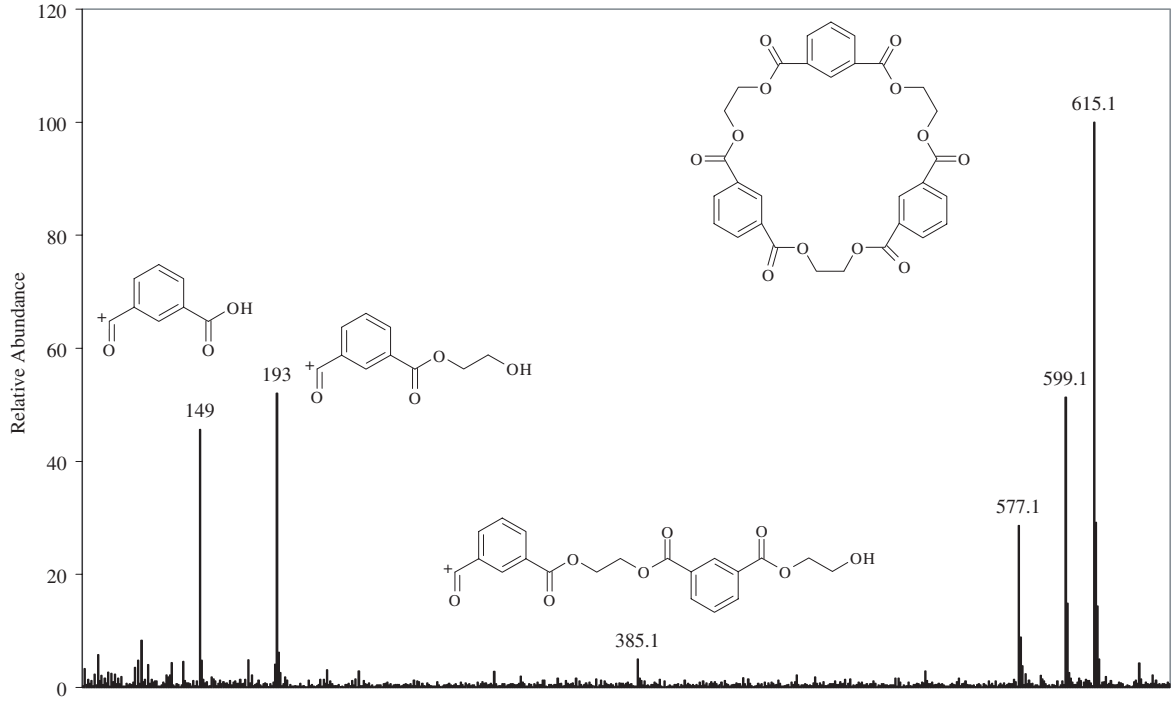

Figure 4. MS-spectrum of CYCLO (3IPA 3EG), qualifiers: H^+-cluster (1577 Da), Na^+-cluster (1599 Da) and K^+-cluster (1615 Da) and characteristic fragments.

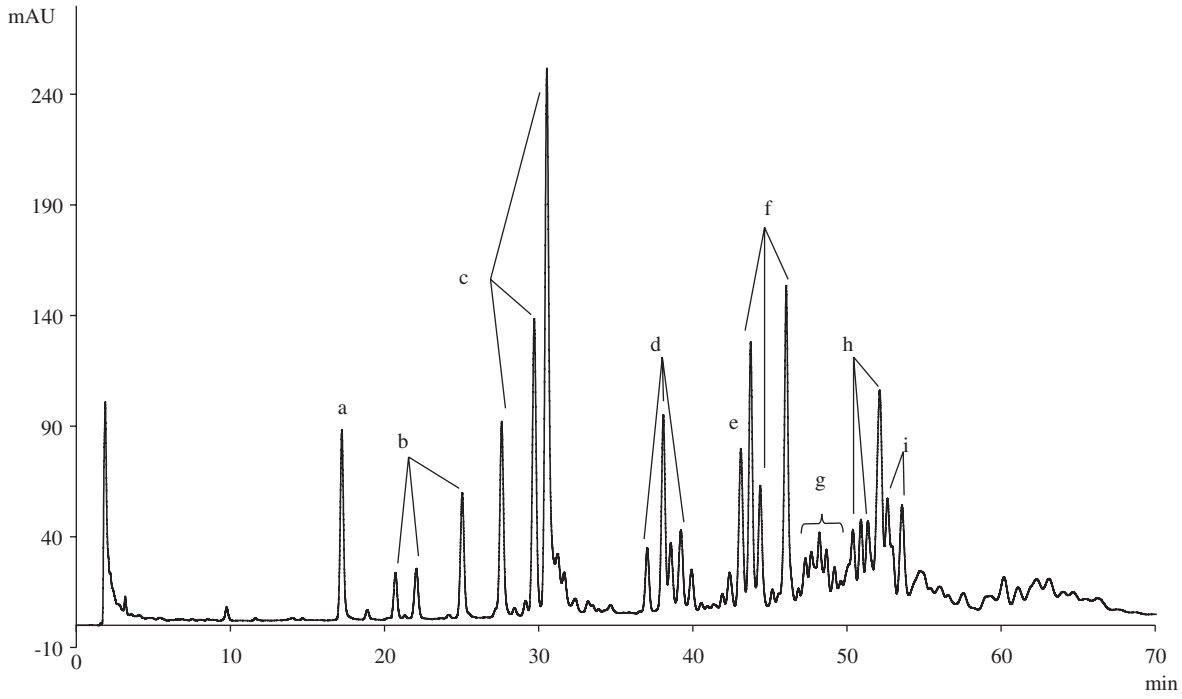

Figure 5. HPLC-UVD$_{232nm}$ chromatogram, migration of polyester coating 1. See the Materials and methods for chromatographic conditions; see table 2 for the legend.

Estimation of the amount of cyclic oligoesters by HPLC-ESI-MSD/UVD and HPLC-ELSD/UVD

The quantification of cyclic oligoesters differs from the quantification of specific migrants (e.g. 2002/16/EC-regulated BADGE derivatives) since more than 50 substance peaks were structurally identified in only three polyester coating migrates, which are traced back to 20 cyclic oligoesters and their isomers (table 2). There were no individual standards commercially available for any of them. Moreover, in contrast to BADGE- or PET-related migrants, the substances are attributed to high number of monomers. Begley *et al.* (1990) and Biedermann *et al.* (1998), respectively, quantified all BADGE derivatives and cyclic PET oligomers by using a single, commercially available standard (BADGE or cyclic PET-trimer, respectively) assuming the same response factor in fluorescence or UV for all migrants. Considering the different absorption spectra of IPA and TPA, a wavelength with the same molar absorption coefficient for both acids had to be identified for the quantification of different oligoesters by UVD. Two similar esters of isophthalic and terephthalic acid, commercially available BHET and BHEI, synthesized by transesterification of dimethyl isophthalate with ethylene glycol (Zahn and Krizikalla 1955), were used for the determination of this special wavelength. The measured absorption was transformed into molar absorption coefficient applying the Lambert–Beer law. An overlay of the absorption spectra (figure 6) indicated a wavelength of about 228 nm with identical molar absorption coefficients of both substances. A final adjustment by comparing the areas of the HPLC-UVD chromatograms for 227–234 nm elucidated 232 nm as wavelength for equal response of BHEI and BHET.

Considering the variety of polyols used for can coatings, the UV-active part in the molecule and the UVD response, respectively, depended on the molecular weight of the polyol contained in the oligoester. For example, BHEI (Mw/n = molecular weight/number of phenyl moieties/molecule: $254/1 = 254$) has got a lower relative UV active content than CYCLO (3IPA 3EG) ($576/3 = 192$) or CYCLO (2IPA 2NPG) ($468/2 = 234$). Therefore, the quantification of oligoesters applying UVD calibrated with CYCLO (3IPA 3EG) using substance concentration is not accurate.

The situation concerning the different UV-active content could be improved by using a calibration

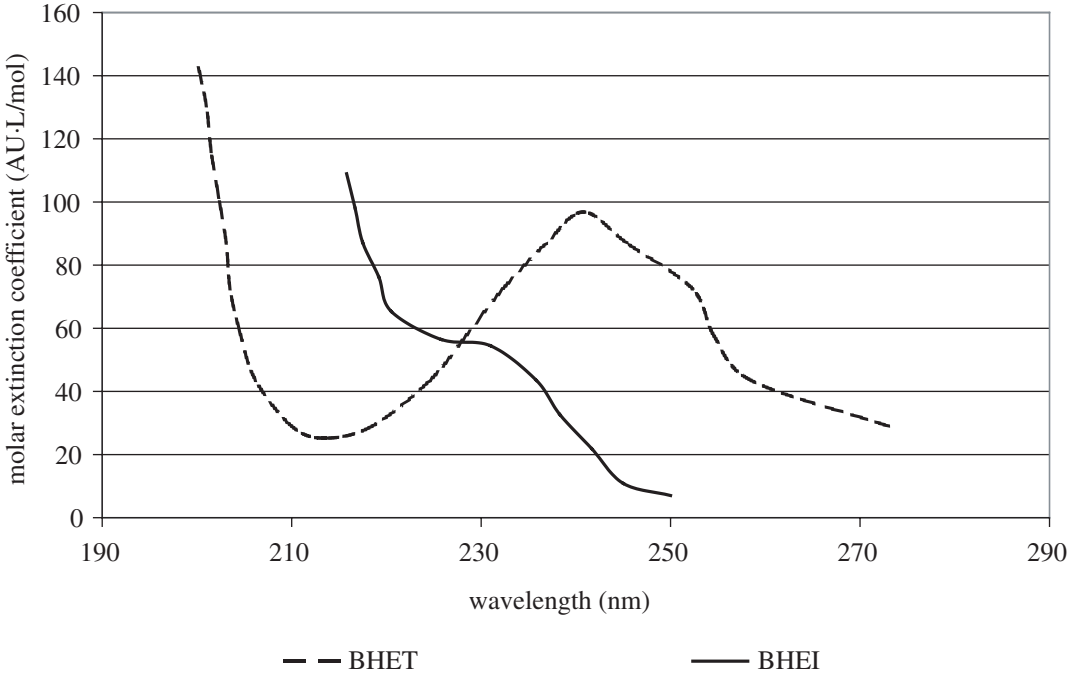

Figure 6. Overlay of absorption spectra (sectors) of BHEI and BHET.

with chromophore concentration instead of substance concentration since UVD can be combined with MSD, which provides information about the molecular weight and number of phenyl moieties. Therefore, the substance concentration is transformed by multiplication with the number of benzoic rings subsequent division with the molecular weight:

$$c_c = c_s \cdot \frac{n}{Mw},$$

where c_c is the chromophore concentration ($\mu mol\,l^{-1}$), c_s is the substance concentration ($\mu g\,l^{-1}$), n is the number of benzoic rings and Mw is the molecular weight of substance ($g\,mol^{-1}$).

Different esters were used for response comparison in order to confirm the accuracy of the calibration by chromophore concentration. Since the cyclic PET-trimer used by Begley *et al.* (1990) was not available, a cyclic oligoester had to be synthesized. Bryant and Semlyen (1997), Brunelle *et al.* (1998), Liu *et al.* (1999), Burch *et al.* (2000) and Hall *et al.* (2000) described the production of cyclic oligoesters, which were used technologically for continuous ring-opening polyester synthesis. As isophthalic acid and ethylene glycol are commonly used polyester monomers, the synthesis of COEI was preferred. Burch *et al.* (2000) reported a synthesis of COEI consisting of 70% cyclic trimer, CYCLO (3IPA 3EG) and 30% dimer, CYCLO (2IPA 2EG) from BHEI catalysed by tetra-isopropyl titanate. Several changes of the reaction conditions (temperature, time, pressure) could not improve the poor yield of less than 5% CYCLO (3IPA 3EG). However, the obtained amount of 25 mg substance enabled a full structural confirmation.

As shown in figure 7a, the slope of the calibration curves based on substance concentration strongly depends on the ester. The difference is much attenuated when the peak area is plotted against chromophore concentration (figure 7b). This technique can be applied for the quantification of all cyclic structurally identified oligoesters containing at least one benzoic group in the molecule whereas it is not suitable for polyesters consisting only of aliphatic monomers.

Concerning quantification, another option was the use of an ELSD, which can detect all substances less volatile than the eluent, including also polyester migrants based only on aliphatic monomers. The ELSD was calibrated with CYCLO (3IPA 3EG) in a concentration range from 2.5 to 25 $mg\,l^{-1}$ using second-grade regression for ELSD. Quantification of oligomers obtained by UVD using substance concentration and ELSD was compared directly for 10 cyclic

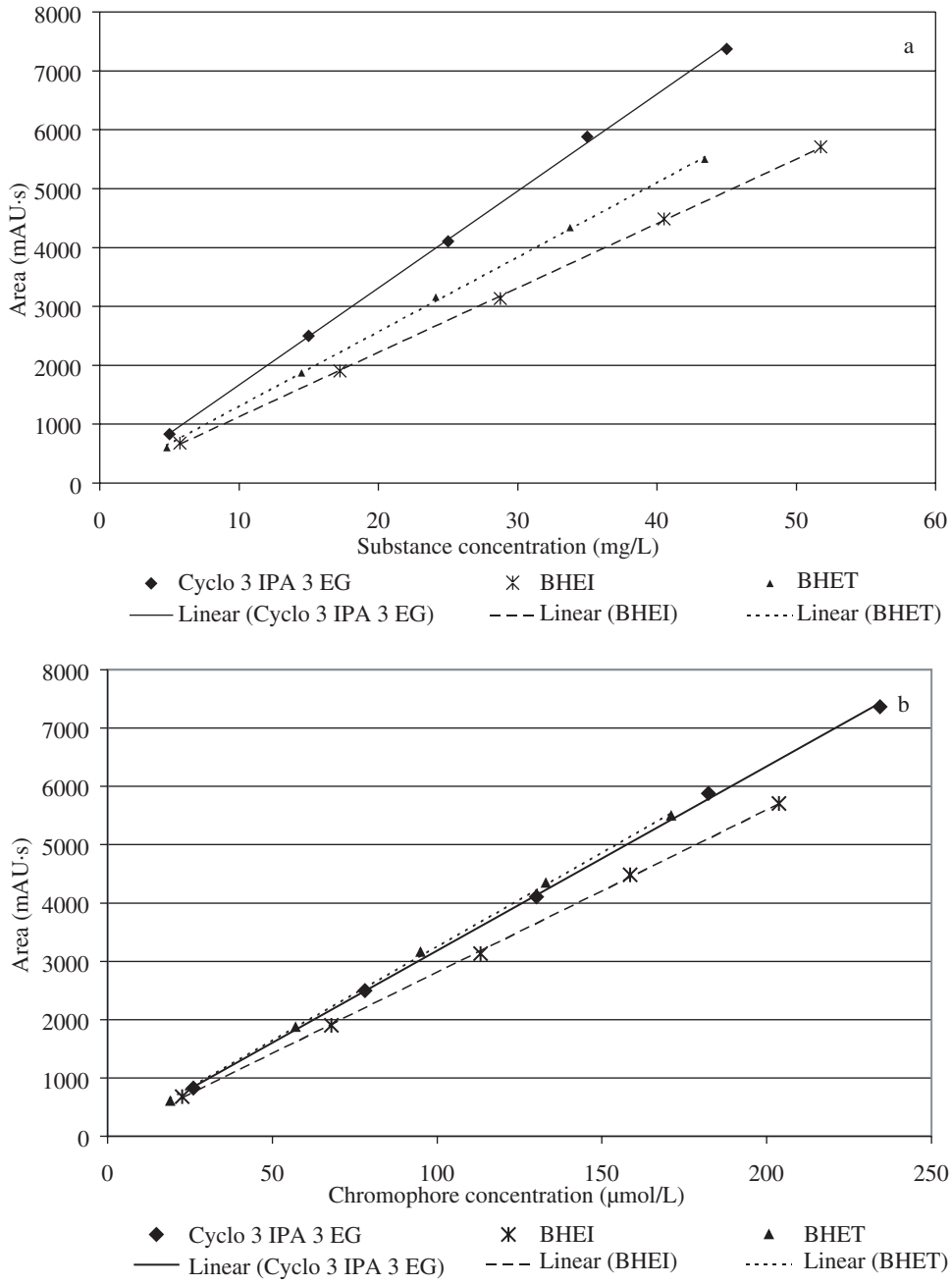

Figure 7. Comparison of curves (BHEI, BHET and CYCLO (3IPA 3EG)) calibrated by HPLC-UVD$_{232nm}$ and substance concentration (a) or chromophore concentration (b).

oligomers from polyester coating 1 (figure 8). The quotient of ELSD and UVD response indicated that the ELSD overestimates most oligoesters with a higher molecular weight than CYCLO (3IPA 3EG), since the masses of the polyols rise from PO-1 to PO-3. Because these substances are considered to be less volatile than the standard CYCLO (3IPA 3EG), they might be detected more sensitively. However, the differences might also be explained by an underestimation of all substances containing one or more

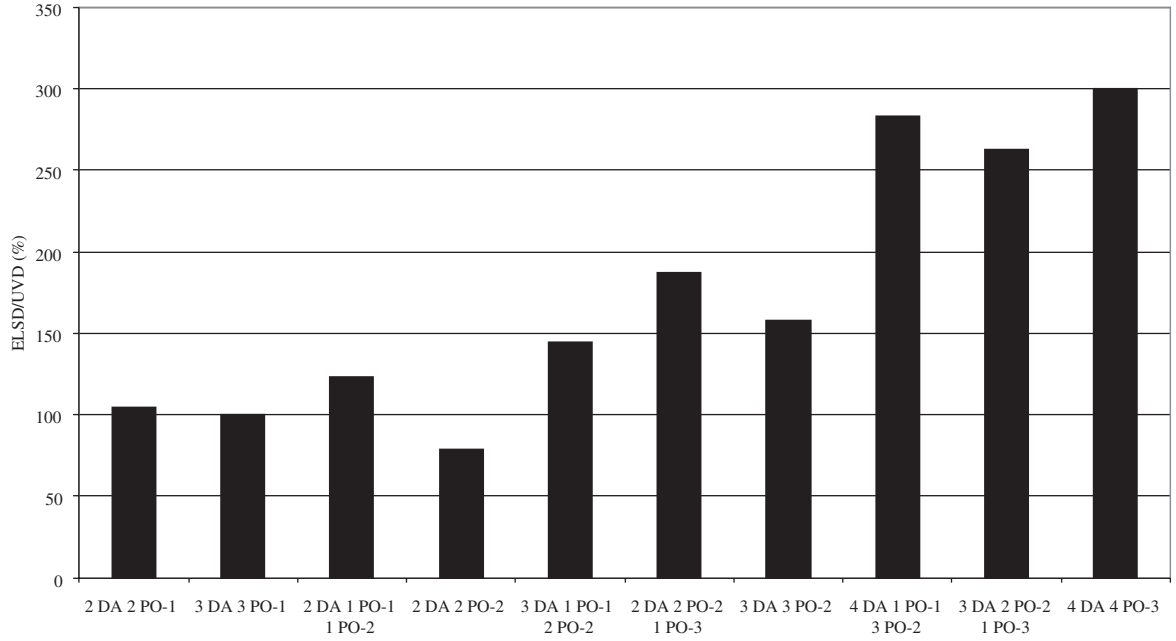

Figure 8. Direct comparison of the response quotient ($ELSD/UVD_{232nm}$) of different cyclic oligoesters contained in polyester coating 1.

polyols with a higher molecular weight than EG by UVD due to the relative UV active content (phenyl moiety) depending on the structure as mentioned above.

Determination of polyester-related migrants below 1000 Da

All substance peaks exceeding the 10-fold noise height (estimated as a quantification limit of 0.01 mg dm^{-2} for UVD_{232nm}) and a molecular weight below 1000 Da were structurally identified by MS detection for three different coatings. All identified substances below 1000 Da were quantified applying three different techniques: ELSD calibrated with CYCLO (3IPA 3EG), UVD_{232nm} calibrated with CYCLO (3IPA 3EG) using substance concentration and UVD_{232nm} calibrated with CYCLO (3IPA 3EG) or the commercially available BHET using chromophore concentration according the above equation. All values of the substances below 1000 Da were added and the sum compared (figure 9). It indicates that the UVD_{232nm} using chromophore concentration gives higher results than those obtained by UVD_{232nm} using substance concentration, but still lower than the results of ELSD. This result confirms the conclusion of figure 8.

As a result, UVD_{232nm} using chromophore concentration is most accurate but very laborious since all individual migrants have to be identified by HPLC-MSD before. Nevertheless, UVD_{232nm} using substance concentration may give sufficient values for the amount of migrating oligoesters below 1000 Da unless the polyester does not contain aliphatic dicarboxylic acids.

To estimate the amount of these cyclic oligoester of the total migrate below 1000 Da, the migrates were analysed by size exclusion chromatography coupled with an ELSD as published by Schaefer *et al.* (2003). The migrate of coating 1 contains 1.0 mg dm^{-2}, migrate of coating 2 contains 0.88 mg dm^{-2} and migrate of coating 3 contains 0.58 mg dm^{-2} substances below 1000 Da. The new method can explain about 50% (*m*/*m*) of the total migrate below 1000 Da as components of the basic resin of polyester coating 1 and 2. For polyester coating 3, only 20% (*m*/*m*) of the total migrate below 1000 Da can be explained. Since not all of the UV-active peaks could be

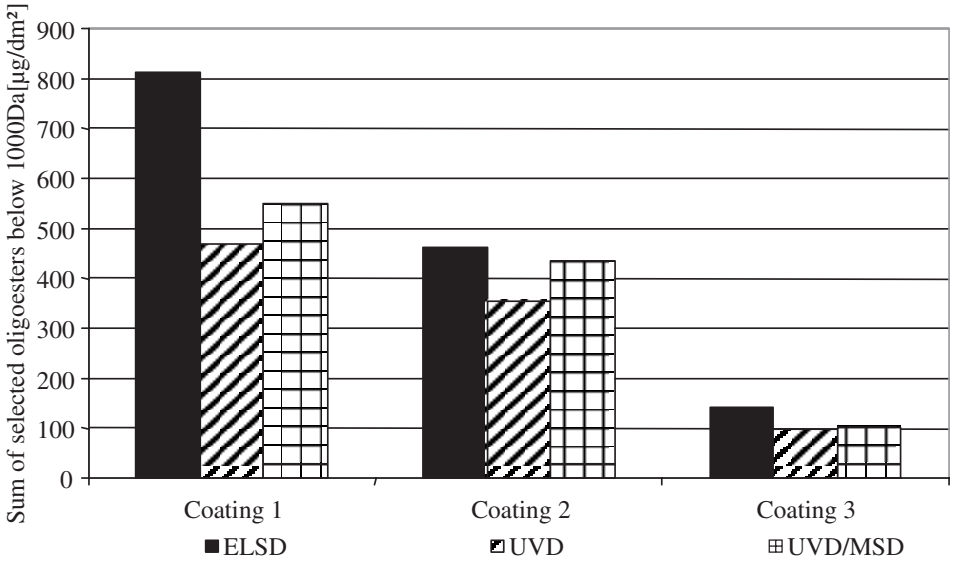

Figure 9. Comparison of results obtained by HPLC-ELSD, HPLC-UVD_{232nm} using substance concentration and HPLC-UVD_{232nm} using the chromophore concentration for polyester coatings 1–3 (migrates in 95% ethanol) calculated for all identified peaks below 1000 Da.

structurally identified for this coating, the amount of polyester resin-based substances of total migrate below 1000 Da might be higher.

Conclusions

Polyester coatings are being introduced to the food can market as alternatives to epoxy coatings. In accordance with studies about extractable substances from PET, cyclic oligomers of the initial polyester monomers were identified as migrants from can coatings based on polyester resins.

The variety of polyester monomers, especially of the polyols, in use for coatings renders a quantification of the migrating oligomers much more difficult than for migrants from PET materials. The monomer identification in the migrate in addition to the combination of UV detection and structural elucidation of cyclic oligomers by HPLC-MSD provides a costly but effective solution for the quantification of all migrants below 1000 Da containing at least one TPA or IPA group in the molecule. BHET or any other commercial oligoester of TPA or IPA is effective as the external standard. The migration of the potentially absorbable oligomers (molecular weight below 1000 Da) from three polyester coatings into a fatty food simulant added up to 0.1–0.8 mg dm^{-2}, which is comparable with the amount of bisphenol A-related compounds below 1000 Da migrating from recent epoxy coatings into food. Consequently, the migrate below 1000 Da mainly (about 50%) consists of components of the basic resin of the respective coating.

To date, nothing is known about the gastrointestinal hydrolysis, absorption and physiological effects of these cyclic oligomers. For linear poly(1,2-propylene adipate), Hamdani *et al.* (2002) showed partial hydrolysis in intestinal-simulating solutions but not under saliva and gastric conditions.

Acknowledgements

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Migration from can coatings: Part 3. Synthesis, identification and quantification of migrating epoxy-based substances below 1000 Da

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Migration from can coatings: Part 3. Synthesis, identification and quantification of migrating epoxy-based substances below 1000 Da

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Bisphenol A-derived glycidyl ethers as well as its reaction products with other lacquer components can migrate into the packed food from epoxy-based can coatings. A sensitive and selective method is presented using high-performance liquid chromatography coupled with ultraviolet light, fluorescence and electrospray ionization-mass selective detection for the identification and quantification of all migrants with a bisphenol A backbone and a molecular weight below 1000 Da, an estimated boundary for the absorption in the gastrointestinal tract. The identification of migrants was confirmed by microreactions of technical bisphenol A diglycidyl ether with solvents and phenols, which provided the fragmentation pattern of the mass selective detection and relative retentions of 42 different bisphenol A-related substances. It was shown by calibration of different isolated and synthesized bisphenol A derivatives that the fluorescence response relies on the amount of bisphenol A moiety in the respective molecule. Therefore, all migrating bisphenol A-related substances below 1000 Da were determined as bisphenol A diglycidyl ether equivalents using a calibration (fluorescence detection) of the commercially available bisphenol A diglycidyl ether monomer. The limit of quantification was set at 5 μg bisphenol A diglycidyl ether equivalents kg^{-1} (or 0.8 μg dm^{-2}). This method was validated for epoxy coatings (0.1 μg dm^{-2} limit of detection and 24 μg bisphenol A-related substances below 1000 Da dm^{-2} standard deviation, corresponding to 4.4% relative standard deviation). The quantification could be extended by combining the fluorescence response and structural information gained from the mass spectra, which provides more accurate results for each migrant. The calculation is based on the calibration of the bisphenol A chromophore content of the molecule. According to this method, the amount of migrating bisphenol A-related substances below 1000 Da in the acetonitrile extract (assuming a worst case) varied from about 0.4 to 0.7 mg dm^{-2} in the examined coatings. The determined amounts comply with about 50% of the total migrate below 1000 Da.

Keywords: food packaging, can coatings, migration, epoxy-based substances, high-performance liquid chromatography-electrospray ionization-mass selective detection (HPLC-ESI-MSD)/ultraviolet detection (UVD)/fluorescence detection (FLD), size exclusion chromatography (SEC)-UVD

Introduction

Most food cans are internally coated in order to protect the food from metal ions as well the metal from aggressive food ingredients. Lacquers based on bisphenol A (BPA)-type epoxy resins are frequently used for these internal can coatings and have been in use since the 1950s/60s (May and Tanaka 1988, Oldring 1996). Epoxy resins in can coatings are typically cured with anhydrides, amino resins (aminoplasts) or phenolic resins (phenolplasts) (Oldring 1996) in order to form a three-dimensional network (cross-linked structure) that provides chemical resistance and pack resistance and enables subsequent can manufacture.

More than 10 years ago the epoxy monomer bisphenol A-diglycidyl ether (BADGE) has been found to be extractable from epoxy resins (Paseiro Losada *et al.* 1991a) and migrating BADGE was found in food

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simulants (Paseiro Losada *et al.* 1991b). As a consequence of the instability of BADGE in aqueous media, the determination was extended to mono- and dihydrolysis products (Paseiro Losada *et al.* 1993, Philo *et al.* 1997). Biedermann *et al.* (1996) first reported the determination of BADGE in fatty foods. Because monomeric BADGE and epoxy resins were also used as stabilizers for organsol coatings in order to scavenge residual hydrochloric acid. Biedermann *et al.* (1997) modified their analysis procedure to determine mono- and dihydrochlorination products of BADGE.

In 1996, the Scientific Committee on Food (SCF) (European Commission 1996) introduced a specific migration limit of $1\,mg\,kg^{-1}$ (or $0.17\,mg\,dm^{-2}$) food or simulants for BADGE itself and its monohydrolysis ($BADGE \cdot H_2O$) and dihydrolysis ($BADGE \cdot 2H_2O$, in aqueous simulants only) products, and included (SCF, European Commission 1999) the mono- ($BADGE \cdot HCl$) and dihydrochlorination ($BADGE \cdot 2HCl$) products as well as the mixed adduct ($BADGE \cdot H_2O \cdot HCl$). These five (six, including $BADGE \cdot 2H_2O$ for aqueous simulants) specific migrants are now regulated by Directive 2002/16/EC (European Commission 2002) demanding the same specific migration limit as the SCF in 1999. Since the migration of these substances has been a cause of concern for public surveillance, the publication of analytical methods increased (Sharman *et al.* 1995, Roubtsova *et al.* 1997, Summerfield *et al.* 1998, Biles *et al.* 1999, Simoneau *et al.* 1999, Theobald *et al.* 1999, Hammarling *et al.* 2000, Lintschinger and Rauter 2000, Uematsu *et al.* 2001, Petersen *et al.* 2003). All previously mentioned authors used normal phase- or reverse phase-high performance liquid chromatography (NP- or RP-HPLC) with a fluorescence detector (FLD) for identification and quantification of the BADGE derivatives. For structural confirmation of the migrants, a mass selective detector (MSD) coupled with gas chromatography (GC) was applied by Biedermann *et al.* (1996), Summerfield *et al.* (1998), Biles *et al.* (1999), Theobald *et al.* (1999) and Uematsu *et al.* (2001), or coupled with LC by Philo *et al.* (1997), Roubtsova *et al.* (1997) and Petersen *et al.* (2003).

The migration from can coatings based on epoxy resins is not limited to monomeric BADGE or its regulated derivatives. Migration of oligomers of up to BADGE tetramer (1192 Da) was found by Biedermann *et al.* (1997). The predominant peak in the chromatograms was not a linear oligomer but a cyclic BADGE dimer, namely cyclo-DiBADGE, forming a characteristic double peak due to its two stereo isomers (Biedermann and Grob 1998). The cyclo-DiBADGE is not integrated (cross-linked) into the polymer network and therefore it can migrate easily.

Beside these epoxy oligomers, migrants from epoxy coatings also contain BADGE adducts with chain stoppers (e.g. *tert*-butylphenol [tBuPh], as constituent of the basic epoxy resin) or reaction products of either solvents (alcohols and glycols) or phenolic monomers, both of which can be formed during the curing process. Some have already been analysed by Biedermann *et al.* (1998), Berger *et al.* (2001) and Theobald *et al.* (2002). These different BADGE derivatives were identified either by GC-MSD (Biedermann *et al.* 1998, Theobald *et al.* 2002) or by LC-MSD (Berger *et al.* 2001). Contradictory methods were published for quantification of these BADGE derivatives: either assuming the same fluorescence response for all derivatives (Biedermann *et al.* 1998), because fluorescence activity is solely based on the BPA backbone, or applying different response factors for mono-, di- and trimeric derivatives, which were determined after isolation of small amounts of the oligomers (Berger *et al.* 2001).

All derivatives of BADGE oligomers do not have the same level of concern from the toxicological point of view. Recently, the interest of the SCF (European Commission 2001) mainly focused on the fraction with a molecular weight below 1000 Da because only this fraction is regarded as potentially absorbable in the gastrointestinal tract. Biedermann *et al.* (1998) considered this restriction by isolation of the migrants below 1000 Da using size exclusion chromatography (SEC). Berger *et al.* (2001) identified and quantified substances with a molecular weight up to 2000 Da.

A threshold of regulation for individual migrating substances of $0.5\,\mu g\,kg^{-1}$ in the diet is recommended for non-regulated substances by the US Food and Drug Administration (Begley 1997) above which toxicological evaluation of components is required. A consumption factor of 0.17 is defined for canned food resulting in a threshold concentration of $3\,\mu g\,kg^{-1}$. Since the threshold must compromise between requirements for food safety and technical feasibility, Grob *et al.* (1999) proposed a 10 times higher value of $30\,\mu g\,kg^{-1}$ (corresponding to 1 and $5\,\mu g\,dm^{-2}$ depending on the size of the can).

No such threshold exists in current European Union regulations.

The aim of the present investigation was the development of a method for the identification and quantification of all BADGE-related substances below 1000 Da and above the threshold of concern ($0.5\,\mu g\,kg^{-1}$ in the daily diet) using HPLC coupled with an ultraviolet light (UVD), FLD and an electrospray ionization (ESI) MSD. A small database of monomeric and dimeric BADGE adducts with frequently used solvents and chain stoppers should be established for confirmation of the structural identity of migrants containing their relative retentions as well as molecule and fragment ions detected by MSD. Therefore, microsyntheses of these adducts are necessary. It should be clarified whether the fluorescence activity of different BADGE derivatives was similar as presumed by Biedermann *et al.* (1998). Therefore, BADGE derivatives were synthesized and isolated and their fluorescence activity compared.

Materials and methods

Samples

Samples were provided by Valspar Corporation as single-side coated tinplate strips (1×25 cm). The strips were coated with epoxy coating 1 (epoxy anhydride), epoxy coating 2 (epoxy anhydride based on same recipe as coating 1, but different epoxy resin) and epoxy coating 3 (epoxy phenolic).

Chromatography

The HPLC analyses were performed on an HP1100 (Agilent, Waldbronn, Germany) system equipped with an autosampler (G1313A), an automatic degasser (G1322A), a binary pump (G1312A), a column oven (G1316A), a fluorescence detector (G1321A), a diode array detector (G1315A) and a mass selective detector (G1946A). Parameters for electrospray ionization were: positive mode, capillary voltage 4000 V, nebulizer pressure 40 psig, dry gas flow $10\,l\,min^{-1}$ and dry gas temperature 350°C. The separation was performed on a reverse-phase column (Multospher® 100 5C18, 250×3 mm, CS Chromatographie Service, Langer Wehe, Germany). Data were assessed by Chemstation® software (Rev. A 08.03). Isolation of the BADGE oligomers was performed on an equivalent system equipped with an variable wavelength detector (G1314A) and a preparative SEC column (SDV® 1000 Å, 5 µ, 20×300 mm; Polymer Standard Service, Mainz, Germany). Synthesis and isolation products were purified on a preparative HPLC system equipped with a preparative pump (Nova Prep 200®; Merck-Hitachi, VWR, Darmstadt, Germany), variable wavelength detector (LaChrom L-7400, Merck-Hitachi), 5 ml sample loop, a reverse phase column (LiChrospher® 100-RP 18, 5 µm, 250×25 mm; Merck) and LC-Responder® (R&S Technologies, VWR) software.

Materials

Unless otherwise stated, reagents were of analytical grade. Technical BADGE (Araldit GY 250) was provided by Valspar Corporation. BADGE (p.a.) was purchased from Fluka. Acetonitrile was gradient grade (Acros, Geel, Belgium). Purified water was obtained by bidistillation (Destamat Bi 18 E; Heraeus, Hanau, Germany). The solutions were filtered before injection in the case of precipitations (Spartan 0.2 µm RC; Schleicher & Schüll, Dassel, Germany). Ammonium formate buffer was prepared by mixing 100 ml 10 mM ammonia solution (0.75 ml concentrated ammonia solution diluted to 1000 ml) with 400 ml water and adjusting the pH to 3 with formic acid. This mixture was filled up to 1000 ml with water.

Synthesis of BADGE reacted on both oxirane rings with butoxyethanol (BADGE·2BuEtOH). A total of 0.30 g (0.9 mmol) technical BADGE was dissolved in 15 ml (115 mmol) butoxyethanol. The solution was heated to 70°C under stirring for 24 h. After cooling, the reaction mixture was diluted 1:5 with acetonitrile and the residual butoxyethanol and other by-products removed by preparative HPLC with acetonitrile/water (75/25, v/v) at a flow rate of $35\,ml\,min^{-1}$. The product containing fractions was combined and concentrated by a rotary evaporator. The purification step with preparative HPLC was repeated. Residual water was removed by lyophyllization. The product (figure 1) was a clear syrup with 96.6% purity. Impurities were determined as 1.0% BADGE·H_2O·BuEtOH, 0.75% BADGE ($n = 1$)·BuEtOH and 1.7% BADGE ($n = 1$)·2BuEtOH (percentages given for UV_{220nm} response).

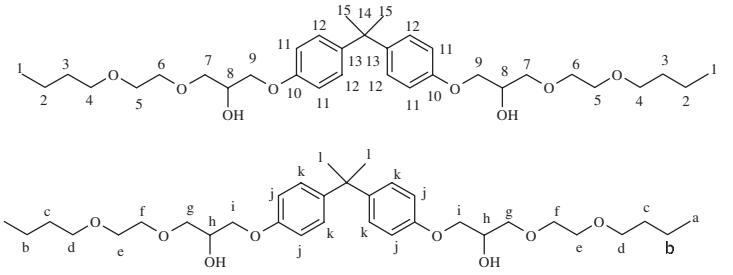

Figure 1. BADGE · 2BuEtOH: enumeration of carbon and hydrogen atoms.

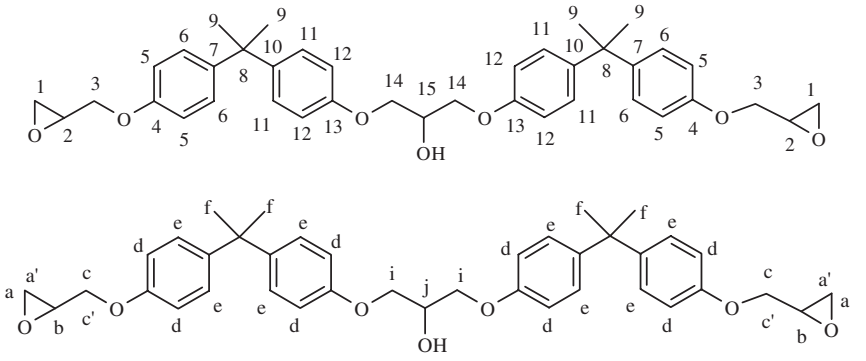

Figure 2. BADGE (n = 1): enumeration of carbon and hydrogen atoms.

Characteristics of BADGE · 2BuEtOH. Yield: 48 mg (10%); elementary analysis ($C_{33}H_{52}O_8$, 576.78 g mol^{-1}): 67.32% C (calculated 68.72%), 8.95% H (calculated 9.09%); UV (acetonitrile): 284.4 (maximum), 282.0 (minimum), 277.2 (maximum), 251.2 (minimum), 229.2 (maximum) nm; IR (KBr): 557(m), 574(m), 737(w), 769(w), 829(s), 954(w), 1041(s), 1119(ss), 1183(s), 1251(ss), 1298(m), 1362(w), 1382(w), 1414(w), 1464(m), 1509(s), 1582(w), 1609(s), 2872(s), 2932(s), 2960(s), 3038(w) cm^{-1}; ^{13}C-NMR ($CDCl_3$): $\delta = 156.5.0$ (C-10), 143.5 (C-13), 127.7 (C-12), 113.9 (C-11), 72.4 (C-9), 71.3 (C-7), 70.2 (C-5/6), 69.2 (C-8), 68.8 (C-4), 41.7 (C-14), 31.7 (C-3), 31.0 (C-15), 19.3 (C-2), 13.9 (C-1) ppm; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 7.12$ (H – *k*, m, 4H), 8.81 (H – *j*, m, 4H), 4.15 (H – *h*, m, 2H), 4.00 (H – *i*, d, 4H), 3.71 (H – *g*′, dd, 2H), 3.69 (H – *f*, t, 4H), 3.63 (H – *g*, dd, 2H), 3.58 (H – *e*, m, 4H), 3.46 (H – *d*, t, 4H), 1.63 (H – *l*, s, 6H), 1.57 (H – *c*, m, 4H), 1.36 (H – *b*, m, 4H), 0.91 (H – a, t, 6H) ppm.

Isolation of BADGE dimer (n = 1) and BADGE trimer (n = 2). A total of 15 g epoxy resin with minimal free BADGE (provided by Valspar Corporation) was dissolved in 100 ml tetrahydrofuran (THF). Substances with a high molecular weight (above 2000 Da) were precipitated by the addition of 350 ml ethanol and 50 ml water. The supernatant was filtrated and brought to dryness by a rotary evaporator. The residue was redissolved in 8 ml THF. A total of 100 µl of the solution was injected 75 times into preparative SEC for fractionation of the oligomers (THF as eluent, 5 ml min^{-1}, UVD). The solvent of the collected fractions of BADGE ($n = 1$ and 2, respectively) was removed by a rotary evaporator. The residue of the fractions was dissolved in 5 ml THF and 20 ml acetonitrile. Other oligomers were removed by preparative HPLC with acetonitrile/water (80/20 as well as 90/10, v/v) at a flow rate of 35 ml min^{-1}. The oligomer-containing fractions were combined and concentrated on a rotary evaporator. Residual water was removed by lyophyllization. The isolated BADGE ($n = 1$) (figure 2) is a white powder with 91.4% purity. Impurities were determined as 1.7% BADGE ($n = 1$) · H_2O, 0.6% cyclo-DiBADGE, 0.9% BADGE ($n = 1$) · HCl and 0.8% BADGE ($n = 2$). The isolated BADGE ($n = 2$) (figure 3) was a white powder with 77.9% purity.

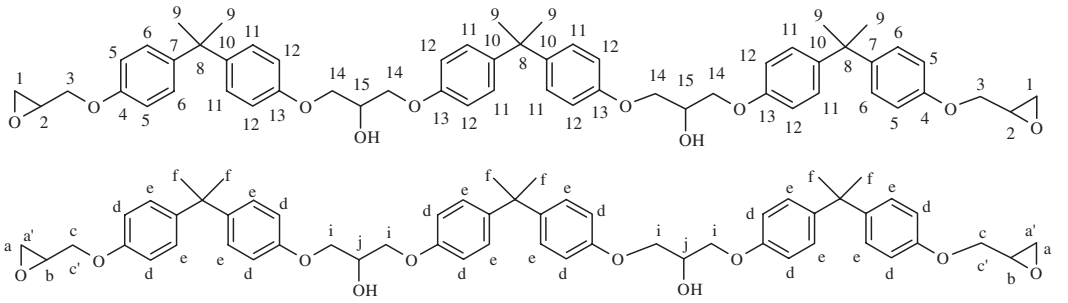

Figure 3. BADGE (n = 2): enumeration of carbon and hydrogen atoms.

Impurities were determined as 1.3% BADGE ($n = 1$) · H_2O, 5.2% BADGE ($n = 1$), 5.1% BADGE ($n = 1$) · BPA and 4.3% BADGE ($n = 3$) (percentages given for UV_{220nm} response).

Characteristics of BADGE (n = 1). Yield: 55 mg (0.37%); elementary analysis ($C_{39}H_{44}O_7$, 624.78 g mol^{-1}): 74.92% C (calculated 74.98%), 7.21% H (calculated 7.10%); UV (acetonitrile): 284.0 (maximum), 281.6 (minimum), 276.8 (maximum), 250.8 (minimum), 229.6 (maximum) nm; IR (KBr): 554(m), 773(w), 829(s), 915(w), 1037(s), 1107(w), 1183(s), 1247(ss), 1298(m), 1362(w), 1384(w), 1413(w), 1458(m), 1510(ss), 1582(w), 1608(s), 1719(w), 2872(m), 2923(m), 2965(m), 3037(w), 3056(w) cm^{-1}; ^{13}C-NMR ($CDCl_3$): $\delta = 156.3$ (C-4/13), 143.7 (C-7/10), 127.8 (C-6/11), 114.0 (C-5/12), 68.8 (C-15/14/3), 50.2 (C-2), 44.8 (C-1), 41.8 (C-8), 31.0 (C-9) ppm; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 7.13$ (H − *e*, m, 8H), 6.82 (H − *d*, m, 8H), 4.35 (H − *j*, m, 1H), 4.18, (H − *c*′, dd, 2H), 4.11 (H − *i*, m, 4H), 3.95 (H − *c*, dd, 2H), 3.34 (H − *b*, m, 2H), 2.90 (H − *a*′, dd, 2H), 2.73 (H − *a*, dd, 2H), 1.63 (H − *f*, s, 12H) ppm.

Characteristics of BADGE (n = 2). Yield: 82 mg (0.55%); elementary analysis ($C_{57}H_{64}O_{10}$, 909.14 g mol^{-1}): 72.55% C (calculated 75.31%), 7.22% H (calculated 7.10%); UV (acetonitrile): 284.0 (maximum), 281.6 (minimum), 277.2 (maximum), 251.2 (minimum), 229.6 (maximum) nm; IR (KBr): 557(m), 772(w), 828(s), 914(w), 1038(s), 1106(w), 1182(s), 1246(ss), 1298(m), 1362(w), 1384(w), 1413(w), 1459(m), 1510(ss), 1582(w), 1608(s), 1720(w), 2872(m), 2919(m), 2965(m), 3037(w), 3058(w) cm^{-1}; ^{13}C-NMR ($CDCl_3$): $\delta = 156.3$ (C-4/13), 143.7 (C-7/10), 127.8 (C-6/11), 114.0 (C-5/12), 68.8 (C-15/14/3), 50.2 (C-2), 44.8 (C-1), 41.8 (C-8), 31.0 (C-9) ppm; ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 7.13$ (H − *e*, m, 12H), 6.82 (H − *d*, m, 12H), 4.35 (H − *j*, m, 2H), 4.18, (H − *c*′, dd, 2H), 4.12 (H − *i*, m, 8H), 3.95 (H − *c*, dd, 2H), 3.34 (H − *b*, m, 2H), 2.90 (H − *a*′, dd, 2H), 2.73 (H − *a*, dd, 2H), 1.63 (H − *f*, s, 18H) ppm.

Reaction mixtures for confirmation of solvent, phenolic and chain stopper adducts. A total of 20 mg technical BADGE (containing about 86% monomer, 12% dimer and 2% trimer) were dissolved in 5 ml solvent, propanol (PrOH), butanol (BuOH), methoxyethanol (MeEtOH), ethoxyethanol (EtEtOH) or butoxyethanol (BuEtOH) and acidified by one to three drops of concentrated phosphoric acid. About 20 mg technical BADGE, 200 mg phenol (Ph) or tBuPh and one pellet of sodium hydroxide were added to 5 ml dioxane. After dispersing the solutions in a supersonic bath, the mixtures were stirred for 2 days at 50°C. A total of 100 µl of each solution were added to 400 µl acetonitrile and 500 µl water before analysing with HPLC-ESI-MSD/UVD/FLD.

Methods

Coating extraction. Four coating strips (1 × 25 cm) were folded like a concertina and extracted with 50 ml of the extraction solvent acetonitrile for 24 h at room temperature. A total of 4 ml of the extracts were evaporated to dryness under a nitrogen stream and dissolved in 200 µl acetonitrile and 200 µl water for the identification of fluorescence-active substances with HPLC-ESI-MSD/UVD/FLD. In the case of precipitation occurring, the extracts were filtrated through a membrane filter before injection.

For quantitative purposes, 500 µl of the extracts were diluted with 500 µl water without the preceding concentration step.

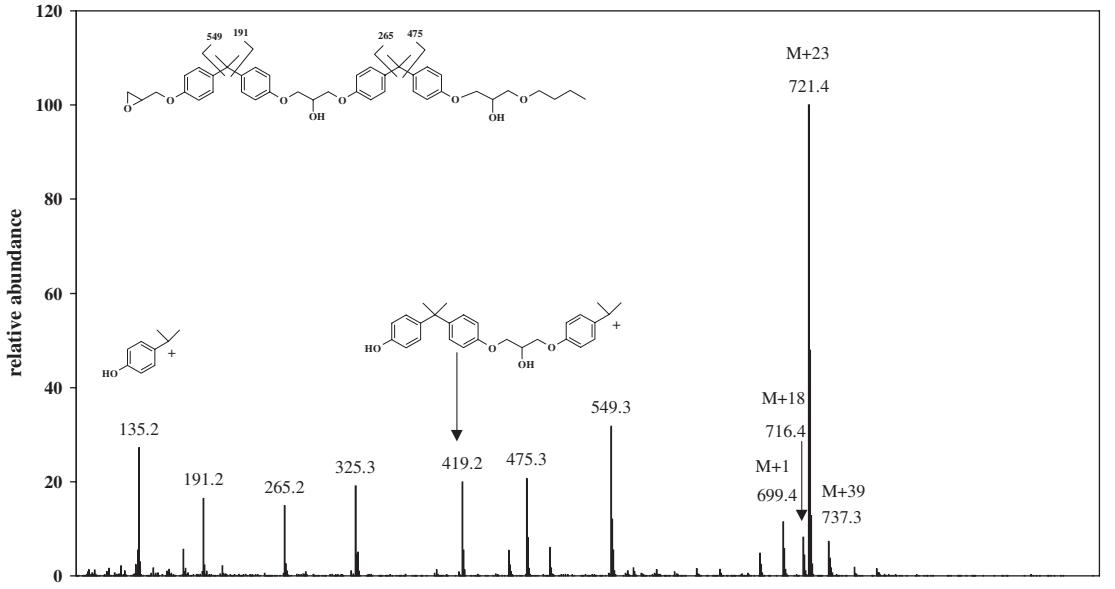

*Figure 4. MS spectrum (150 V fragmentor voltage) of BADGE (*n = *1) · BuOH (butanol) with characteristic fragments.*

Identification and quantification of BPA-related substances below 1000 Da by HPLC-ESI-MSD/UVD/FLD. Migrating BADGE-related substances were identified and quantified by HPLC-ESI-MSD/UVD/FLD (figure 4) under the following chromatographic conditions: gradient elution was performed on a Multospher® column with (A) 1 mM ammonium formate in water (pH 3) and (B) acetonitrile from 50% A to 5% A in 45 min, followed by 25 min rinsing of the stationary phase (5% A) and 10 min equilibration to 50% A at 25°C and a flow rate of 0.5 ml min^{-1}. Detection of the analytes was performed by UV (275 nm), FL (excitation 275 nm, emission 305 nm) and selected masses (100 or 150 V fragmentor voltage, scan *m/z* 80–1000). All fluorescence-active peaks with an area equivalent to 5 µg BADGE kg^{-1} (or 0.8 µg dm^{-2}) or more were quantified as BADGE equivalents by HPLC-FLD. This calculation limit was traced back to the threshold of concern (0.5 µg kg^{-1}) and the consumption factor (0.17) under consideration of the consumption of canned foodstuffs.

Statistical analyses. Statistical evaluations were performed with the statistical software package SPSS® 10.0.7 (SPSS, Inc., Cary, NC, USA).

Results and discussion

Structural information about various BADGE solvent and chain stopper adducts

Reactions with frequently used solvents and chain stoppers were performed in accordance with the microsyntheses as described by Biedermann *et al.* (1998). Some syntheses were repeated, since the information from published tables with identified fragments obtained by GC-EI-MSD (electron ionization) is not suitable for interpretation of LC-ESI-MSD spectra (electrospray ionization). Additionally, the mass spectra of the dimer adducts as well as the relative retentions of all substances (calculated relative to BADGE monomer, dimer and trimer) were integrated into a small database (table 1).

In contrast to the EI, the ESI provides stable molecule clusters with either a proton (M + 1) or ammonium (M + 18), sodium (M + 23) or potassium (M + 39) ions. The combination of these clusters can be used for the unequivocal determination of the molecular weight of an unknown substance peak. The intensity of the ammonium cluster was increased by using an ammonium formate buffer instead of water as eluent (previously tested for BADGE, BADGE hydrolysis

Table 1. ESI-mass spectra and relative retentions (RR) to BADGE monomer (n = 0), dimer (n = 1) and trimer (n = 2) of BADGE derivatives generated by microsynthesis with propanol (PrOH), butanol (BuOH), methoxyethanol (MeEtOH), ethoxyethanol (EtEtOH), butoxyethanol (BuEtOH), phenol (Ph) and tert-*butylphenol (tBuPh).*

	M + 1	NH_4^+-, Na^+-, K^+-cluster	Fragments (100 V)	RR to BADGE (n = 0)	RR to BADGE (n = 1)	RR to BADGE (n = 2)
Monomer derivatives						
BADGE · PrOH	401	418, 423, 439	191, 251	1.104	0.617	0.485
BADGE · 2PrOH	461	478, 483, 499	251	1.210	0.676	0.532
BADGE · H_2O · PrOH	419	436, 441, 457	209, 251	0.481	0.269	0.211
BADGE · BuOH	415	432, 437, 453	191, 265	1.318	0.736	0.579
BADGE · 2BuOH	489	506, 511, 527	265	1.642	0.917	0.722
BADGE · H_2O · BuOH	433	450, 455, 471	209, 265	0.631	0.352	0.279
BADGE · MeEtOH	417	434, 439, 455	191, 267	0.686	0.383	0.301
BADGE · 2MeEtOH	493	510, 515, 531	267	0.445	0.249	0.196
BADGE · H_2O · MeEtOH	435	452, 457, 473	209, 267	0.272	0.152	0.119
BADGE · EtEtOH	431	448, 453, 469	191, 281	0.828	0.463	0.364
BADGE · 2EtEtOH	521	538, 543, 559	281	0.675	0.377	0.296
BADGE · H_2O · EtEtOH	449	466, 471, 487	209, 281	0.334	0.192	0.147
BADGE · BuEtOH	459	476, 481, 497	191, 309	1.117	0.625	0.493
BADGE · 2BuEtOH	577	594, 599, 615	309	1.492	0.833	0.655
BADGE · H_2O · BuEtOH	477	494, 499, 515	209, 309	0.586	0.327	0.257
BADGE · Ph	435	452, 457, 473	191, 285	1.275	0.712	0.561
BADGE · 2Ph	529	546, 551, 567	285	1.516	0.847	0.667
BADGE · H_2O · Ph	453	470, 475, 491	209, 285	0.630	0.352	0.277
BADGE · tBuPh	491	509, 513, 529	191, 341	1.895	1.091	0.832
BADGE · 2tBuPh	641	658, 663, 679	341	2.516	1.405	1.105
BADGE · H_2O · 2tBuPh	509	526, 531, 547	209, 341	1.212	0.677	0.533
Dimer derivatives						
BADGE (n = 1) · PrOH	685	702, 705, 721	191, 251, 475, 535	1.882	1.051	0.826
BADGE (n = 1) · 2PrOH	745	762, 767, 783	251, 535	1.975	1.103	0.867
BADGE (n = 1) · H_2O · PrOH	703	720, 725, 741	209, 251, 493, 535	1.257	0.702	0.552
BADGE (n = 1) · BuOH	699	716, 721, 737	191, 265, 475, 549	2.041	1.140	0.897
BADGE (n = 1) · 2BuOH	773	790, 795, 811	265, 549	2.276	1.271	1.000
BADGE (n = 1) · H_2O · BuOH	717	734, 739, 755	209, 265, 493, 549	1.441	0.806	0.635
BADGE (n = 1) · MeEtOH	701	718, 723, 739	191, 267, 475, 551	1.502	0.838	0.660
BADGE (n = 1) · 2MeEtOH	777	794, 799, 815	267, 551	1.201	0.672	0.528
BADGE (n = 1) · H_2O · MeEtOH	719	736, 741, 757	209, 267, 493, 551	0.875	0.489	0.385
BADGE (n = 1) · EtEtOH	715	732, 737, 753	191, 281, 475, 565	1.640	0.916	0.720
BADGE (n = 1) · 2EtEtOH	805	822, 827, 843	281, 565	1.484	0.829	0.652
BADGE (n = 1) · H_2O · EtEtOH	733	750, 755, 771	209, 281, 493, 565	1.000	0.558	0.439
BADGE (n = 1) · BuEtOH	743	760, 765, 781	191, 309, 475, 593	1.655	0.926	0.730
BADGE (n = 1) · 2BuEtOH	861	878, 883, 899	309, 593	2.161	1.207	0.951
BADGE (n = 1) · H_2O · BuEtOH	761	778, 783, 799	209, 309, 493, 593	1.373	0.767	0.603
BADGE (n = 1) · Ph	719	736, 741, 757	191, 285, 475, 569	1.960	1.095	0.862
BADGE (n = 1) · 2Ph	813	830, 835, 851	285, 569	2.096	1.173	0.923
BADGE (n = 1) · H_2O · PH	737	754, 759, 775	209, 285, 493, 569	1.392	0.779	0.614
BADGE (n = 1) · tBuPh	775	792, 797, 813	191, 341, 475, 625	2.389	1.334	1.049
BADGE (n = 1) · 2tBuPh	925	942, 947, 963	341, 625	2.798	1.562	1.229
BADGE (n = 1) · H_2O · tBuPh	793	810, 815, 831	209, 341, 493, 625	n.d.	n.d.	n.d.

and hydrochlorination products by Petersen *et al.* 2003). Chemical ionization (CI), as used by Berger and Oehme (2000), was also tested with the above described system, but it exhibited lower sensitivity (Petersen *et al.* 2003). The CI also provided proton adducts (M + 1) and ammonium clusters (M + 18), but did not lead to the formation of sodium or potassium clusters. Instead of these clusters, some

BADGE derivatives formed clusters with acetonitrile and a proton (M + 42), which is also suitable for the determination of the molecular weight of an unknown substance.

The fragmentation pattern in the ESI is similar to that obtained by CI (Berger and Oehme 2000, Petersen *et al.* 2003) and is much more simple than that obtained by GC-EI-MS spectra (Biedermann *et al.* 1998). The fragmentation relies on the formation of stable carbenium ions. In BPA-based substances, the C-14 atom (figure 1) can generate a stable carbenium ion at a fragmentation voltage above 40 V. Therefore, the BADGE fragment with an oxirane ring has a molecular weight of 191. In the case of BADGE adducts, the molecular weight of this main fragment can be calculated by addition of 191 and the molecular weight of this respective solvent or chain stopper (e.g. BADGE · BuOH 191 + 74 = 265). Analogously, the main fragments of the BADGE dimer at a fragmentor voltage above 120 V are *m*/*z* 475 and 191, respectively. The spectra of dimer adducts can be interpreted as explained for the above monomeric BADGE adducts (figure 4). The fragments 135 and 419 are characteristic for the BPA backbone.

Identification of BADGE-related substances in coating extracts

The RP-HPLC separation was optimized for the highest resolution of BADGE adducts below 1000 Da in an acceptable analysis time (maximum 80 min). The most polar BADGE derivative BADGE · $2H_2O$ (1) eluted at about 4 min and the most non-polar derivative BADGE ($n = 2$) · PrOH (19) at 40 min. This gradient slope of 1% min^{-1} provided less co-elution of migrants than the rapid separation (gradient slope 3.3% min^{-1}) as described by Berger and Oehme (2000), who analysed BADGE derivatives up to 2000 Da. In order to protect the column from high molecular weight components, the concentrated extracts were dissolved in acetonitrile/water (50/50, v/v). These solutions were only used for identification purposes since it could not be excluded that some components below 1000 Da were also precipitated. This technique could avoid a time-consuming SEC preseparation of the migrate below 1000 Da before HPLC analysis (Biedermann *et al.* 1998, Bronz *et al.* 1998). However, for the quantitative analysis, the coating extracts were not concentrated (but diluted, see the Materials and methods) and then no precipitation of migrants occurred.

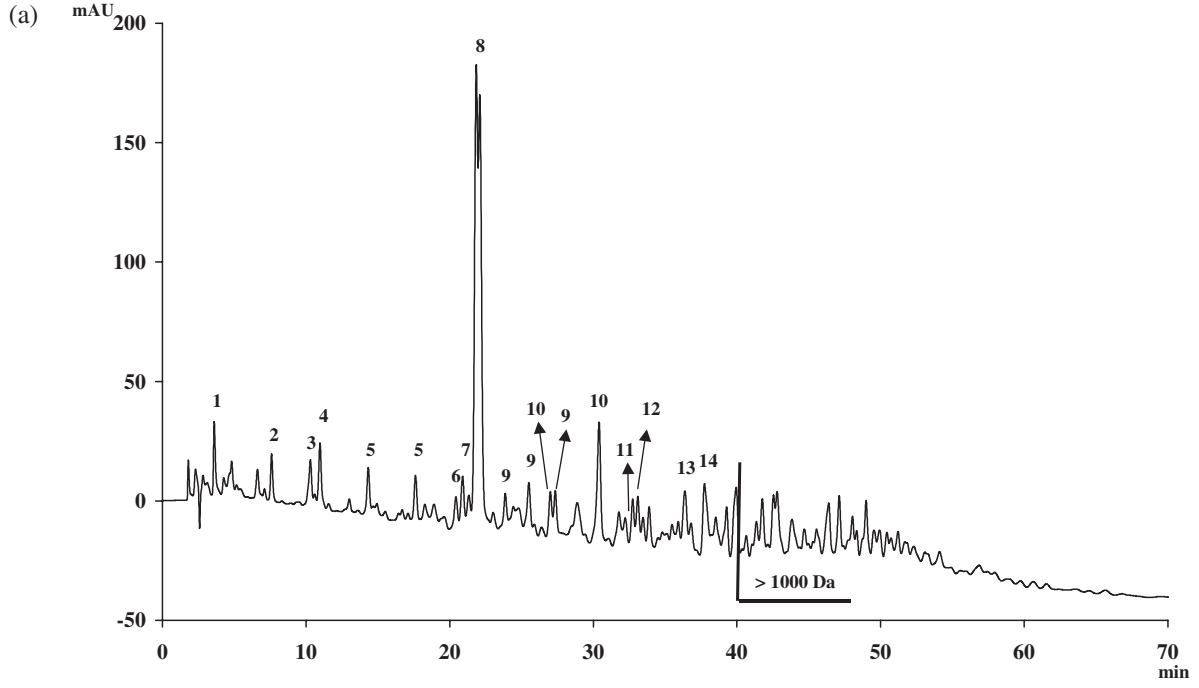

Figure 5. UVD_{220nm} chromatograms of acetonitrile extracts (10-fold concentrated) of coating 1 (a), 2 (b) and 3 (c); see the Materials and methods for chromatographic conditions; see table 2 for substance identification.

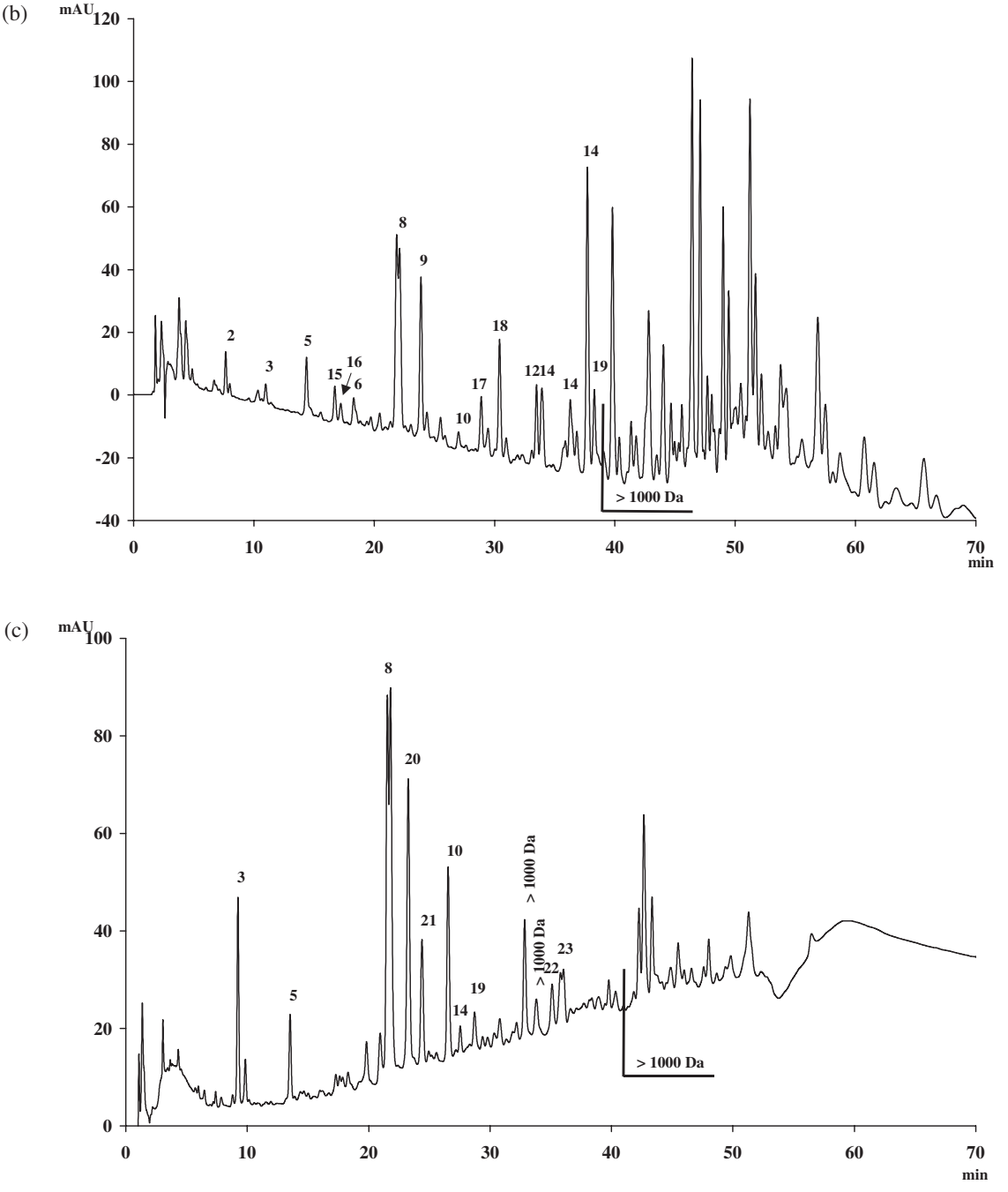

Figure 5. Continued.

The analysis of three different coatings (figure 5a–c) led to an identification of 23 different BADGE derivatives below 1000 Da (table 2). As also observed by Biedermann and Grob (1998), cyclo-DiBADGE (8) was one of the predominant peaks in the migrate since it cannot be integrated into the network due to its ring structure and hence its lack of potential functional sites for cross-linking. The spectrum of substance (9) showed the same molecular mass as cyclo-DiBADGE ($M + 1 = 569$), but in contrast

Table 2. Structurally identified and classified substance peaks of epoxy coatings 1–3.

	Classification	M + 1	Substance
1	(SCF)	377	BADGE · $2H_2O$
2	SCF	359	BADGE · H_2O
3	NEPO	477	BADGE · H_2O · BuEtOH
4	NEPO	453	BADGE · H_2O · Ph
5	NEPO	587	BADGE · H_2O · BPA
6	1EPO	459	BADGE · BuEtOH
7	1EPO	435	BADGE · Ph
8	NEPO	569	Cyclo-DiBADGE
9	1EPO	569	BADGE · BPA
10	NEPO	687	BADGE · BPA · BuEtOH
11	1EPO	743	BADGE ($n = 1$) · BuEtOH
12	1EPO	853	BADGE ($n = 1$) · BPA
13	NEPO	971	BADGE ($n = 1$) · BPA · BuEtOH
14	2EPO	909	BADGE ($n = 2$)
15	SCF	341	BADGE
16	1EPO	401	BADGE · PrOH
17	NEPO	797	BADGE · 2BPA
18	1EPO	927	BADGE ($n = 2$) · H_2O
19	1EPO	969	BADGE ($n = 2$) · PrOH
20	NEPO	577	BADGE · 2BuEtOH
21	NEPO	533	BADGE · BuEtOH · BuOH
22	NEPO	861	BADGE ($n = 1$) · 2BuEtOH
23	NEPO	817	BADGE ($n = 1$) · BuEtOH · BuOH

SCF, substances limited by SCF and 2002/16/EC and including BADGE · $2H_2O$; 2EPO, oligomers containing two oxiran rings; 1EPO, derivatives containing one oxiran ring; NEPO, derivatives without an oxiran ring.

to cyclo-DiBADGE, this substance formed the characteristic fragment of a linear BPA adduct (191 + 228 = 419). Some peaks were labelled with the same number because their MS spectra showed the same molecule ions and the same fragmentation (5, 9, 10, 14). For this phenomenon, different explanations are possible: (1) the epoxy resins did not exclusively consist of the *p*,*p*-isomers, but also contained *o*,*o*- or *o*,*p*-isomers as impurities that was also observed for commercial BADGE monomer by Cottier *et al.* (1997); (2) Jost *et al.* (1999) and Biedermann *et al.* (1998) described the formation of regio-isomers due to addition of the hydroxyl group to position 2 of the glycidyl group instead of position 3, during the reaction of BADGE the phenols and solvents; or (3) BADGE monoreaction products might also form cyclic structures by addition of the hydroxyl group to the epoxy group at the other end of the molecule (Biedermann *et al.* 1998).

Since the exact structural identity of the isomers is not relevant for the main aim of this investigation (an accurate quantification of all BADGE derivatives below 1000 Da), further confirmation was not carried out.

The fragmentation patterns and relative retentions could be confirmed by microsyntheses for all identified substances except the BPA adducts and the above-mentioned isomers. Microsynthesis of BADGE with BPA was not possible under the chosen conditions. However, they were tentatively identified by analysis of commercially available epoxy resins by this HPLC-ESI-MSD/UVD method.

During the last 3 years, more than 30 epoxy-based coatings were analysed using this method and more than 50 different extractable oligomer derivatives were identified by HPLC-ESI-MSD/UVD/FLD.

Quantification of BADGE-related substances below 1000 Da

Since fluorescence detection provided the highest sensitivity and selectivity compared with UV detection, this detection technique is mostly used for quantification. The fluorescence activity depends on the BPA moiety in the molecule; therefore, the fluorescence response for all BADGE derivatives was assumed (Biedermann *et al.* 1998, Bronz *et al.* 1998). In contrast, Berger *et al.* (2001) found a decreasing response factor for increasing molecular weight of BADGE oligomers after isolation of 500 µg dimer and 43 µg trimer by semipreparative HPLC. Owing to the small isolated amount, a full characterization of the substances was impossible. In order to clarify the contradictory results about fluorescence activity, BADGE dimer and trimer were isolated in higher amounts (55 mg dimer, 82 mg trimer) and additionally BADGE · 2BuEtOH (48 mg) was synthesized. After full characterization of these substances by nuclear magnetic resonance (NMR), infrared (IR), UV and MS spectrometry as well as by elementary analysis, the assumption of Biedermann *et al.* (1998) could be confirmed. The difference of FLD response between mono-, di- and trimer is less than 10% (figure 6). The concentrations of the synthesized standards used for the calibrations are corrected by their degree of purity as measured by HPLC-UVD_{220nm} (see the Materials and methods). However, the same tendency to a lower response for oligomers with higher molecular weight (Berger *et al.* 2001) does exist. This could be interpreted by an interaction of the BPA groups or by

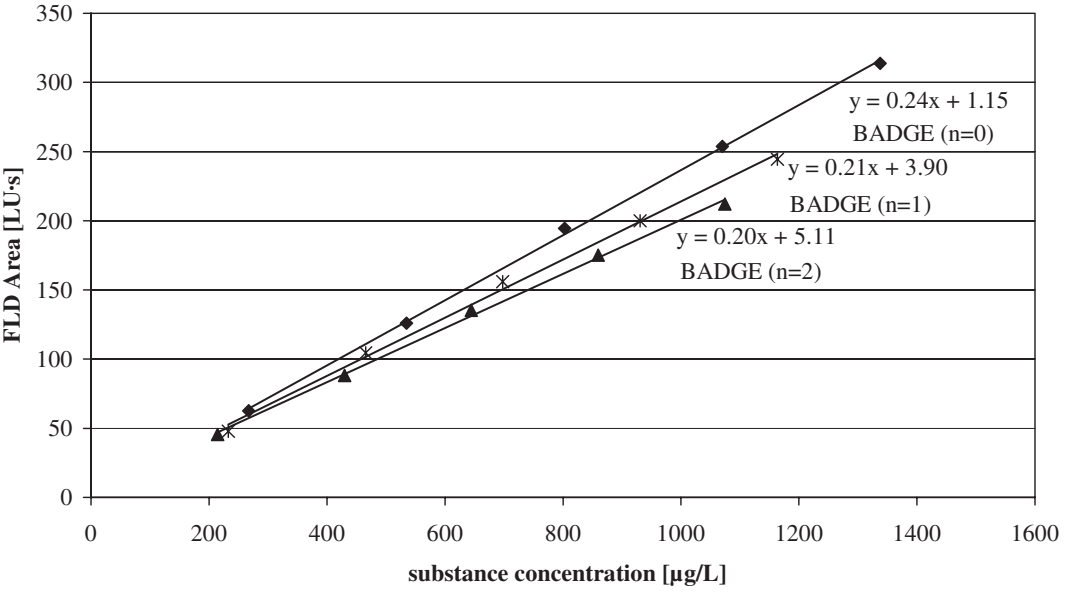

*Figure 6. Comparison of FLD responses of three oligomers BADGE (*n = *0, 1 and 3) (concentrations were corrected by the determined purity of the substances).*

an inaccurate determination of the purities (solvent residues).

The proportion of the fluorescence active BPA moiety in BADGE adducts varies from 36% in BADGE · 2tBuPh to 86% in BADGE · 2BPA. Hence, the comparison of the calibration curves of BADGE and synthesized BADGE · 2BuEtOH elucidate that the quantification of BADGE adducts as BADGE equivalents irrespective of the single substance is not accurate.

As already published for the quantification of polyester-based migrants (Schaefer *et al.* 2004a), the difference of the proportion of the fluorescence-active moiety can be eliminated by using a calibration of the chromophore concentration instead of the substance concentration (figures 7a and b). However, the structural information (molecular weight and number of BPA moieties/molecule) has to be obtained by MSD beforehand. Therefore, the substance concentration is transformed by multiplication with the number of BPA moieties in the molecule and by division with the molecular weight. The calibration of the chromophore concentration is done with BADGE standards before using equation (1):

$$c_c = c_s \cdot \frac{n}{MW} \quad (1)$$

where c_c is the chromophore concentration ($\mu mol\,l^{-1}$), c_s is the substance concentration ($\mu g\,l^{-1}$), n is the number of BPA moieties and MW is the molecular weight of the substance ($g\,mol^{-1}$).

Equation (1): calculation of chromophore concentration from substance concentration

Likewise, Berger *et al.* (2001) proposed a calculation using the response factors of three reference standards: BADGE, BADGE ($n = 1$) and BADGE ($n = 2$), and a molecular weight correction.

All peaks below 1000 Da and above the calculation limit of $5\,\mu g\,BADGE\,kg^{-1}$ were quantified as BADGE equivalents. Furthermore, the sum of substances below 1000 Da was also calculated using the structural information and chromophore calibration as described above. The values of the combined method are a little bit lower because many of the identified BADGE derivatives have a higher proportion of the fluorescence active moiety in the molecule than monomeric BADGE (e.g. cyclo-DiBADGE, BADGE · BPA and BADGE · 2BPA) (figure 8).

The identified substances below 1000 Da are classified according to the number of epoxy groups in the

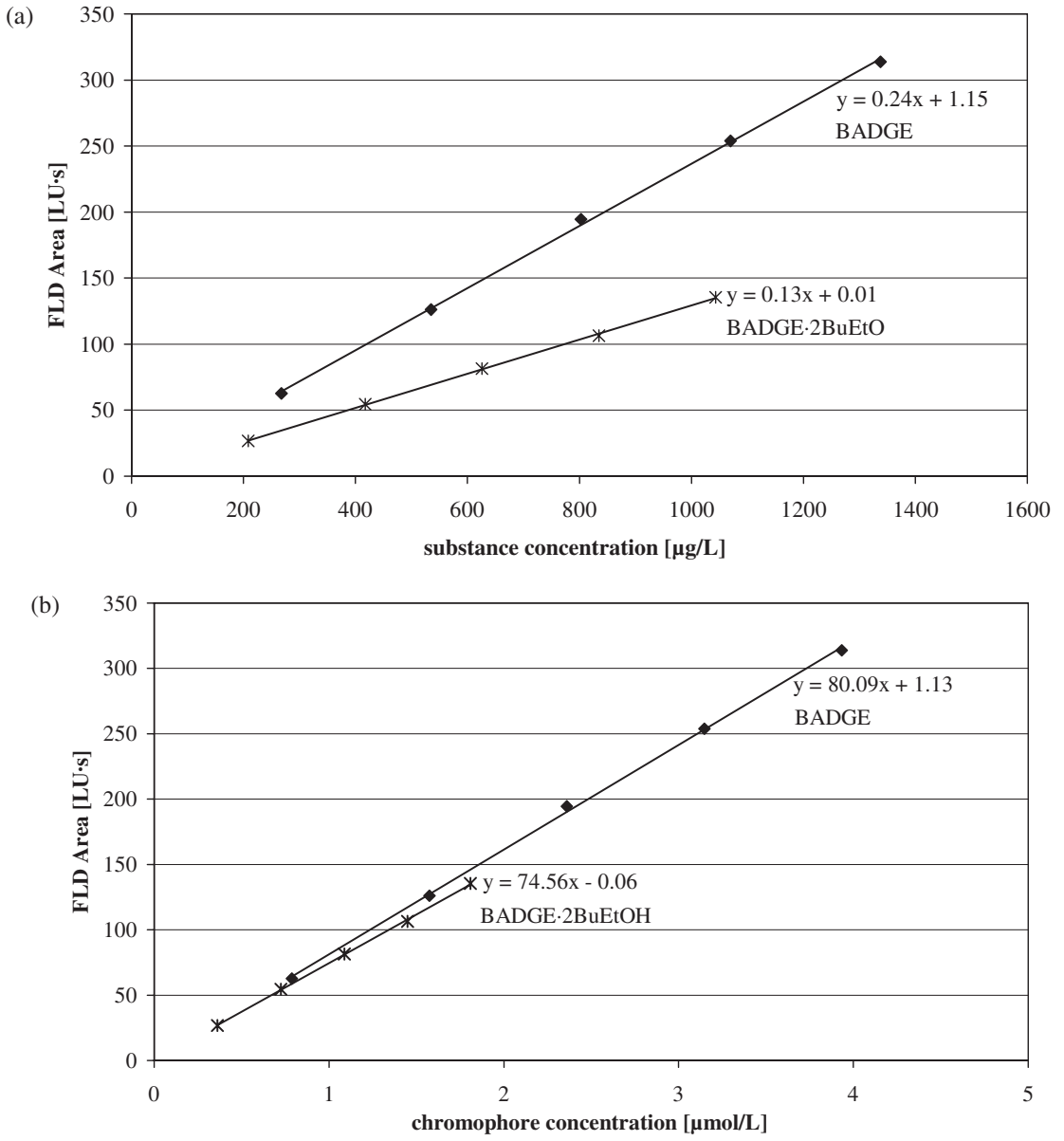

Figure 7. Comparison of FLD responses of BADGE and BADGE·2BuEtOH calculated from (a) substance concentration or (b) chromophore concentration (concentrations were corrected by the determined purity of the substances).

molecule (table 2). To date, the SCF has only regulated BADGE and its derivatives ('SCF class', here also including BADGE · $2H_2O$) in 2002/16/EC (European Commission 2002) with a specific migration limit of 1 mg kg^{-1} (or 0.17 mg dm^{-2}). Since the SCF regarded substances with an intact oxirane ring as potentially toxic (SCF, European Commission 1997), all other derivatives with at least one oxirane ring (Class 2EPO and 1EPO) and a molecular weight below 1000 Da have been determined. Owing to the lack of an toxicological profile, Directive 2002/16/EC (European Commission 2002) limits novolac diglycidyl ether (NOGE) and all its derivatives below 1000 Da with at least one epoxy group to a total quantitative maximum QM(t) of 0.2 mg/6 dm^2 (or 0.03 mg dm^{-2}).

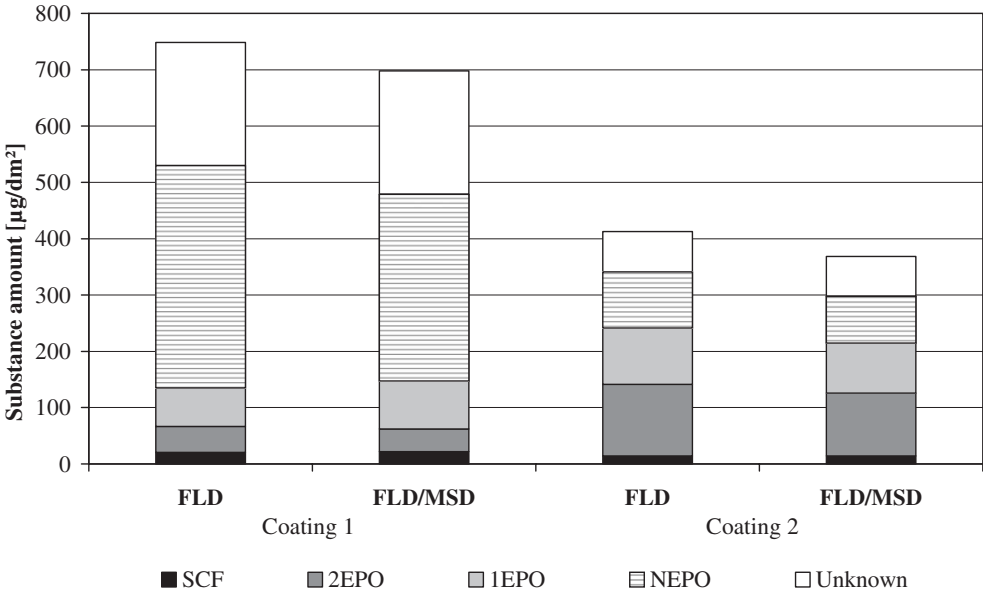

Figure 8. Comparison of the sum of BADGE-related substances below 1000 Da (classified as SCF, 2EPO, 1EPO, NEPO and unknown, calculation limit 5 µg kg^{-1} or 0.8 µg dm^{-2}) calculated as BADGE equivalents (FLD) and after chromophore proportion correction (FLD/MSD), respectively.

Table 3. Validation data: HPLC-FLD determination of the sum of migrating BADGE-related substances below 1000 Da as uncorrected BADGE equivalents.

Coating	Chemist	*n*	Mean (µg dm^{-2})	SD (µg dm^{-2})	RSD (%)	Assessed mean (µg dm^{-2})	Assessed SD (µg dm^{-2})	RSD_{total} (%)	SD_{lab} (µg dm^{-2})
Epoxy coating 1	1	5	731	13	1.78	578	31	5.36	
Epoxy coating 2	1	5	426	49	11.50				
Epoxy coating 1	2	5	620	35	5.65	502	23	4.58	24.3
Epoxy coating 2	2	5	384	11	2.86				
Epoxy coating 1	3	5	713	22	3.08	564	19	3.37	
Epoxy coating 2	3	5	414	16	3.86				

The sum of the SCF, 2EPO and 1EPO classes in the examined coatings was determined as 0.15 mg dm^{-2} for coating 1 and 0.25 mg dm^{-2} for coating 2, respectively.

In order to estimate the amount of these BPA-related substances in the total migrate below 1000 Da, the migrates were analysed by SEC with evaporated light scattering detection (Schaefer *et al.* 2004b). The migrate of coating 1 contained 1.5 mg dm^{-2} substances below 1000 Da and that of coating 2 0.8 mg dm^{-2}, respectively. The presented method elucidated about 50% (m/m) of the total migrate below 1000 Da as BPA-related components originated from the epoxy resin. Biedermann *et al.* (1998) found only 13–20% (m/m) of the migrate below 1000 Da to be resin related. However, they quantified predominantly monomeric BADGE adducts and the cyclo-DiBADGE, but not BADGE-dimer and -trimer derivatives.

Validation of the quantification of BADGE-related substances below 1000 Da

The reproducibility was tested in an extensive statistical study performed by three different chemists analysing two different coatings with five replicates (including extraction) on each coating. BADGE monomer was calibrated in the working range of 10–1250 µg l^{-1} (6–750 µg kg^{-1} food or 1–125 µg dm^{-2})

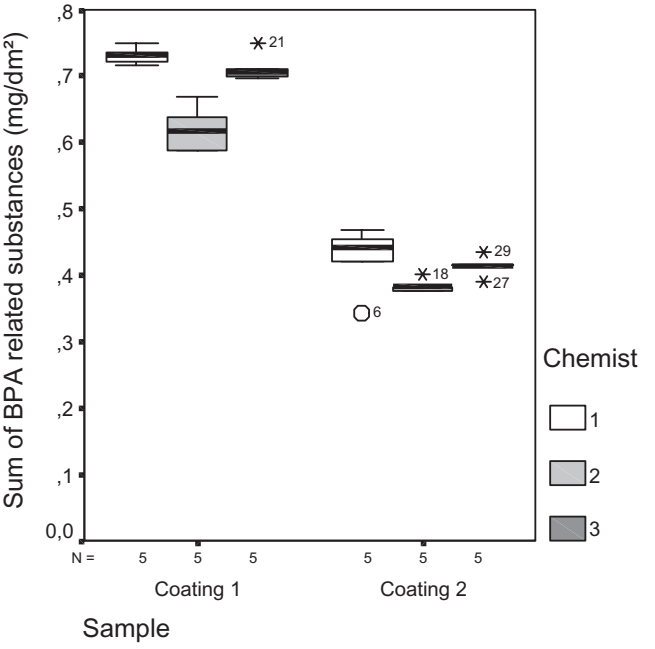

*Figure 9. Box plot for multifactorial analysis of variances (medium, quartiles, * outliers, ○ extremes); the sum was calculated from BADGE equivalents without correction.*

in eight levels with double injections provided 9.4 $\mu g\,l^{-1}$ (5.64 $\mu g\,kg^{-1}$ food or 0.94 $\mu g\,dm^{-2}$) standard deviation, 1.2% coefficient of variation and a limit of detection of 1.0 $\mu g\,l^{-1}$ (0.6 $\mu g\,kg^{-1}$ food or 0.1 $\mu g\,dm^{-2}$) estimated by a signal-to-noise ratio of 3. All validation parameters are summarized in table 3.

Analysis of variance of the results indicated that there were significant differences of the determined values between the three chemists. A Tukey post-hoc test revealed chemist 2 to have significantly different results from the other two chemists (figure 9). This difference could be elucidated by divergent manual peak integration. Chemist 2 did not draw a horizontal basis line for unseparated peaks.

Conclusions

In Europe, the safety evaluation of coatings is based on the approval of monomers, pre-polymers and additives of which at least mutagenic activity was tested. For reaction products generated in pre-products (resins) or during the processing of the coating, no regulation exists except for the 'BADGE, BFDGE and NOGE Directive' (2002/16/EC European Commission 2002). Likewise, Biedermann *et al.* (1998) stated that migrates from epoxy coatings hardly contain any of the approved monomers.

In the present investigation, all BADGE-based derivatives from several epoxy coatings were identified and quantified considering the potentially absorbable part below 1000 Da as well as the threshold of toxicological concern (calculation limit) for the first time. The described calculation method using the chromophore proportion correction was confirmed by isolation and synthesis of reference standards and provided accurate results. About 50% (m/m) of the total migrate below 1000 Da consisted of BPA-related compounds. Monomeric BADGE represented less than 3% (w/w) of the BPA-related compounds, whilst 15–60% (w/w) consisted of compounds below 1000 Da with at least one oxirane ring. Consideration should be given to assess the relevance of these oxirane-containing species less than 1000 Da, since to the best of the authors' knowledge, no toxicological information about these substances exists other than that obtained by extrapolation of the toxicological data on BADGE.

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Migration of lubricants from food packagings: Screening for lipid classes and quantitative estimation using normal-phase liquid chromatographic separation with evaporative light scattering-detection

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Migration of lubricants from food packagings Screening for lipid classes and quantitative estimation using normal-phase liquid chromatographic separation with evaporative light scattering detection

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Abstract

A normal-phase high-performance liquid chromatography (NP-HPLC) method is introduced for the identification and quantitative estimation of 12 lipid classes (paraffin, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amide) used as lubricantsin food packaging materials. The HPLC separation is carried out on a LiChrospher® Diol (100 Å, 5 μm, 125 mm × 3 mm) column with gradient elution (isooctane/0.1% acetic acid in *tert*-butyl methyl ether) and evaporative light scattering detection (ELSD). The method has been calibrated with representatives of each class in working ranges of about 5–150 mg/l, depending on the lipid class. Intra-day variance for all representatives range from 1.9 to 5.1%, inter-day variances from 7.0 to 26.5% and the limits of detection from 0.79 to 3.65 mg/l (except for two classes). A simple sample preparation could be established for the determination of migrating lubricants obtained from packaging materials containing external or internal lubricants. Since the detector response depends on the chain length and the degree of saturation, the quantification of a lipid class with unknown composition is only semi-quantitative. The amount of migrating lubricants from an epoxy-based can coating could be estimated with 0.3 mg/dm^2 and from a light weight container with 5.5 mg/dm^2.

Keywords: Evaporative light scattering detection; Food packaging; Migration; Lipids; Lubricants

1. Introduction

1.1. Presence of different lipid classes as lubricants in food packagings

Lubricants are used as additives for the production of coated or laminated packaging for different reasons. External lubricants may be applied to the surface of

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the finished packaging in order to enable the forming process and to minimise the adhesion of food components to the packaging. Internal lubricants are added to liquid lacquer-compositions in order to enhance the elasticity, which is required for the deep-drawing process [1]. Slip additives used for plastic films belong to this group of internal lubricants. They are added to plastic formulations where they gradually tend to migrate to the surface preventing the adhesion between films by reducing friction [2,3].

Useful substance classes for internal and external applications are waxes, paraffins, fats and oils as well as partial acyl glycerols or fatty acid amides. Lubricants are mostly technical mixtures of isomers and analogues of one chemical class (e.g. paraffin) or natural products of diverse compositions (e.g. waxes or oils). Natural waxes like carnauba wax can also contain long-chain aliphatic alcohols, free fatty acids, cholesterol or cholesterol esters beside the main class fatty acid ester [4]. Partial acyl glycerols can also include fatty acid methyl esters [5]. In consequence, 12 lipid classes (Table 1) are expected to be found in migrates of food packagings. Their identification and quantitative estimation will enable the elucidation of the composition of the total migrate. Concerning the variety of possible components in each class a chromatographic separation method has to be developed for the determination of these classes avoiding a specific separation into the single substances forming these classes.

Table 1
Investigated lipid classes and standard substances as representatives

	Lipid class	Abbreviation	Representative
1	Paraffin	PAR	Paraffin (liquid)
2	Wax ester	WE	*n*-Hexadecyl palmitate
3	Cholesterol ester	CE	Cholesteryl palmitate
4	Fatty acid methyl ester	FAME	Stearic acid methyl ester
5	Triacyl glycerol	TAG	Glycerol tripalmitate
6	Fatty alcohol	FOH	Hexadecyl alcohol
7	Free fatty acid	FFA	Stearic acid
8	Cholesterol	CHOL	Cholesterol
9	1,3-Diacyl glycerol	1,3-DAG	Glycerol-1,3-dipalmitate
10	1,2-Diacyl glycerol	1,2-DAG	Glycerol-1,2-dipalmitate
11	Monoacyl glycerol	MAG	Glycerol monopalmitate
12	Fatty acid amide	FAA	Erucylamide

1.2. Previous work

Hitherto, examinations have mostly been carried out in order to determine external lubricants like paraffin and waxes using GC–FID [6], LC–GC–FID-coupling [7,8] and SFC–FID-techniques [9,10]. The presence of different slip additives like erucylamide was analysed by GC–FID in food simulants [3]. Non-specific separation methods for lipids have been developed by several authors for different analytical tasks. Bruns [5] determined five lipid classes (FAME, TAG, 1,3-DAG, 1,2-DAG and MAG) on a cyanopropyl-phase in order to control the production of technical partial glycerols. El-Hamdy and Christie [11] analysed the influence of the stationary phase (cyanopropyl-phase) and the eluent on the liquid chromatographic separation of seven lipid classes (CE, TAG, FFA, CHOL, 1,3-DAG, 1,2-DAG and MAG). Based on the optimized method of El-Hamdy and Christie, Foglia and Jones [12] extended the separated lipid classes by FAME and applied this technique to acylglycerides after enzymatic cleavage and subsequent esterification. Nine of the 12 lipid classes mentioned in Table 1 were determined by Elfman-Börjesson and Härröd [13] on a dihydroxypropyl-phase using unusual flash chromatography with a high flow (3 ml/min for a column diameter of 4 mm) and several gradient steps. All of the referenced authors combined the liquid chromatograph with an evaporative light scattering detector (ELSD) to enable the detection of UV-inactive analytes. Additionally, the necessity of gradient elution does not allow the use of a refractive index detector (RID).

The aim of this work was the development of a chromatographic separation for all 12 lipid classes with subsequent ELS-detection using representatives of each class. Within these classes four unpolar lipid classes (PAR, WE, CE and FAME), the medium polar TAG and eight polar lipid classes (FOH, FFA, CHOL, 1,3-DAG, 1,2-DAG, MAG and FAA) are included. The ELS-detection had to be optimized in order to achieve a reasonable precision and low detection limits for all lipid classes. Furthermore, a procedure for the extraction and subsequent purification of both

internal and external lubricants from packagings had to be established.

2. Experimental

2.1. Chemicals

Glycerol monopalmitate (MAG), glycerol-1,3-dipalmitate (1,3-DAG), glycerol-1,2-dipalmitate (1,2-DAG) and glycerol-tripalmitate were obtained from Fluka, Buchs, Switzerland. Cetyl alcohol (FOH), cholesterol (CHOL) and stearic acid (FFA) were obtained from Merck, Darmstadt, Germany whereas cholesteryl palmitate (CE) and *n*-hexadecyl palmitate (WE) were obtained from Acros, Geel, Belgium and paraffin (liquid, PAR) from Riedel-de Haen, Hannover, Germany. Ethanol (99.5%, p.a.) used as simulant and acetic acid were obtained from Merck, Darmstadt, Germany. Isooctane (Biosolve, Valkenswaard, Germany) used as simulant and for chromatography as well as *tert*-butyl methyl ether (MTBE), 2-propanol and *n*-heptane (Merck, Darmstadt, Germany) used for chromatography were HPLC grade and were freshly distilled over NaOH and a vigreux column. The water used was bidistilled. All other chemicals were of analytical grade.

2.2. Samples

Tinplate strips (1 cm × 25 cm) coated with a commercial epoxy-anhydride coating containing carnauba wax and a partial acyl glycerol as internal lubricants were obtained from the Valspar Corporation, Grüningen, Switzerland. Aluminium light weight container prepared with an unknown external lubricant were obtained from the Federal Institute of Military Technique and the Supply of the German Armed Forces.

2.3. Apparatus

Separation was performed on an HP1100 (Agilent, Waldbronn, Germany) system equipped with an autosampler (G1313A), an automatic degasser (G1322A), a binary pump (G1312A), a column oven (G1316A) and a variable wavelength detector (G1314A). Data were assessed by Chemstation® software (Rev. A 08.03). Chromatographic separation was carried out at 25 °C on different HPLC columns, a LiChrospher® Diol (100 Å, 5 μm, 125 mm × 3 mm, Merck, Darmstadt, Germany), a Spherisorb® Si (80 Å, 3 μm, 125 mm × 4 mm, Waters, Eschborn, Germany), a Spherisorb® CN (80 Å, 5 μm, 125 mm × 3 mm, Waters, Eschborn, Germany) and Kromasil® RP1 (100 Å, 5 μm, 125 mm × 3 mm, Eka Nobel, Bohus, Sweden). The ELSD (Sedex 75) and a thermostatisable nebulizer chamber were from Sedere, Alfortville, France. A stainless steel in-line filter (0.5 μm mesh) was inserted between the column and detector. Sterilizations were performed in an autoclave Sanoclav (Wolf, Geislingen, Germany) and centrifuged in a Sigma 3K 30 (Sigma Laboratory, Osterode am Harz, Germany).

2.4. Simulation of migration

For migration experiments, approved EU-simulants and simulation conditions (97/48/EC amending 82/711/EEC, 85/572/EEC) were used. The assumed worst case, a sterilization of fatty food for 1 h at 121 °C, has to be performed using the following solvents (approved substitutes for the fatty simulant olive oil) and conditions: 95% ethanol (4 h at 60 °C) and isooctane (2 h at 60 °C). Therefore, coated tinplate strips were folded like a concertina and a sample of four strips (ca. 1 dm^2) was extracted with 50 ml simulant. In case coated strips are not available, empty cans and light weight containers can be extracted. However, it is important to achieve a high surface–volume ratio.

2.5. Optimized sample preparation and chromatographic conditions

2.5.1. Sample preparation for external lubricants

Four milliliters of isooctane migrate (see Section 2.4) was evaporated to dryness under nitrogen stream. The residue was redissolved in 400 μl isooctane and injected directly into the HPLC.

2.5.2. Sample preparation for internal lubricants

Four milliliters of 95% ethanol migrate (see Section 2.4) was brought to dryness under a nitrogen stream. The residue was dissolved in 400 μl ethanol. Subsequently 400 μl isooctane and 1600 μl water were added. The mixture was stirred vigorously. Centrifugation (2 min, 5000 g) was performed to enhance the

separation of two layers. The upper isooctane layer was used for HPLC analysis.

2.5.3. Chromatographic conditions

The separation of the lipid classes was performed on a LiChrospher® Diol (100 Å, 5 μm, 125 mm × 3 mm) column at 25 °C and a flow rate of 0.5 ml/min with (A) isooctane and (B) MTBE/acetic acid (99.9/0.1, v/v) using following gradient: 100% A held for 1 min, linear decrease to 86% A in 24 min and then linear decrease to 40% A in 10 min, followed by 10 min rinsing of the stationary phase (40% A) and 10 min equilibration to 100% A. Detection of the analytes was done by ELS (20 °C nebulizer, 35 °C vaporizer, 3.5 bar nitrogen and gain 8).

3. Results and discussion

3.1. Development of a lipid class-specific normal-phase (NP)-HPLC separation

As reported in the literature, reversed phases enable the separation of lipid classes mainly by the number of carbon atoms and the degree of saturation [14–19]. In contrast normal-phase chromatography is based on the separation by the polar part of the molecule neglecting mostly the unpolar side chain. Different normal phases were used for separation of the lipid classes: silicagel (Si)- [20–23], alumina- [24], cyanopropyl (CN)- [5,11,12,25,26] and dihydroxypropyl (diol)-phase [13,27]. Due to the long equilibration time and the poor reproducibility of retention times of unmodified phases chemically bonded phases were preferred [5,11,12,26]. PAR exhibited no retention on any of the examined normal phases (Si, CN, diol). The diol-phase gave best results especially for the non-polar lipids (PAR, WE, CE and FAME) and, as the only phase, produced a sufficient selectivity between PAR and WE.

For the separation of the non-polar lipid classes, the choice of the non-polar component in the eluent is important. Isooctane [5,22], *n*-heptane [13] and *n*-hexane [12,20,21,23–27] were used previously, whereas some of the mentioned authors modified the non-polar eluent with up to 1% tetrahydrofuran or acetic acid. Only the use of pure isooctane without modifier provided a baseline separation for all four non-polar lipid classes.

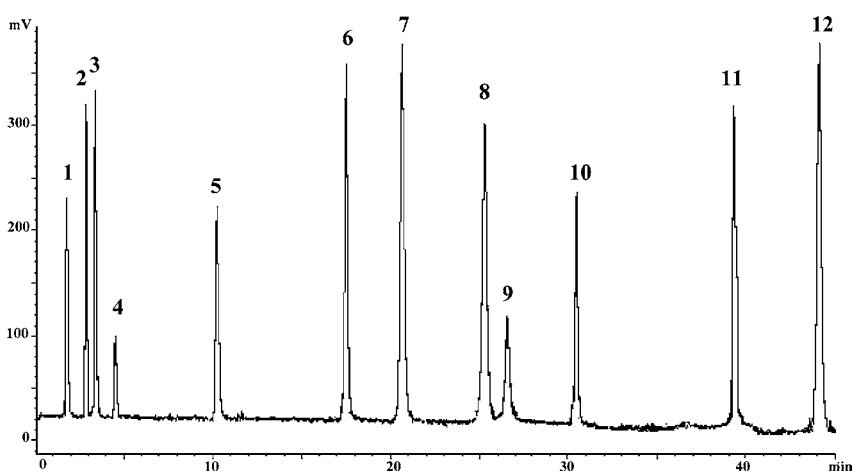

Fig. 1. Separation of lipid classes representatives (1) PAR (paraffin, liquid), (2) WE (*n*-hexyldecyl palmitate), (3) CE (cholesteryl palmitate), (4) FAME (stearic acid methyl ester), (5) TAG (glycerol tripalmitate), (6) FOH (hexadecyl alcohol), (7) FFA (stearic acid), (8) CHOL (cholesterol), (9) 1,3-DAG (glycerol-1,3-dipalmitate), (10) 1,2-DAG (glycerol-1,2-dipalmitate), (11) MAG (glycerol monopalmitate) and (12) FAA (erucylamide), chromatographic conditions see Section 2.5, concentrations 20–50 mg/l, except CHOL 100 mg/l.

A separation for TAG and the seven polar lipid classes required 2-propanol [5,13,21,23,25,27] or MTBE [11,12,25,26] as the polar component in the eluent. We found that very small changes of the eluent composition (99.4/0.6 to 98.8/1.2, isooctane/2-propanol, v/v) led to a coelution of four of five originally baseline separated classes having similar polar properties (FOH, FFA, CHOL, 1,3-DAG, 1,2-DAG). Thus, a development of a gradient with isooctane and 2-propanol failed for not reproducible resolution. The use of MTBE, because of its moderate polarity, enabled the separation of all 12 lipid classes by gradient elution (see Section 2.5 and Fig. 1).

However, the influence of the MTBE content in the mobile phase on the separation is high. The change of the isocratic eluent composition from 92.5/7.5 (isooctane/MTBE, v/v) to 85/15 (isooctane/MTBE, v/v) changed the order of elution of FFA and FOH as well as CHOL and 1,3-DAG.

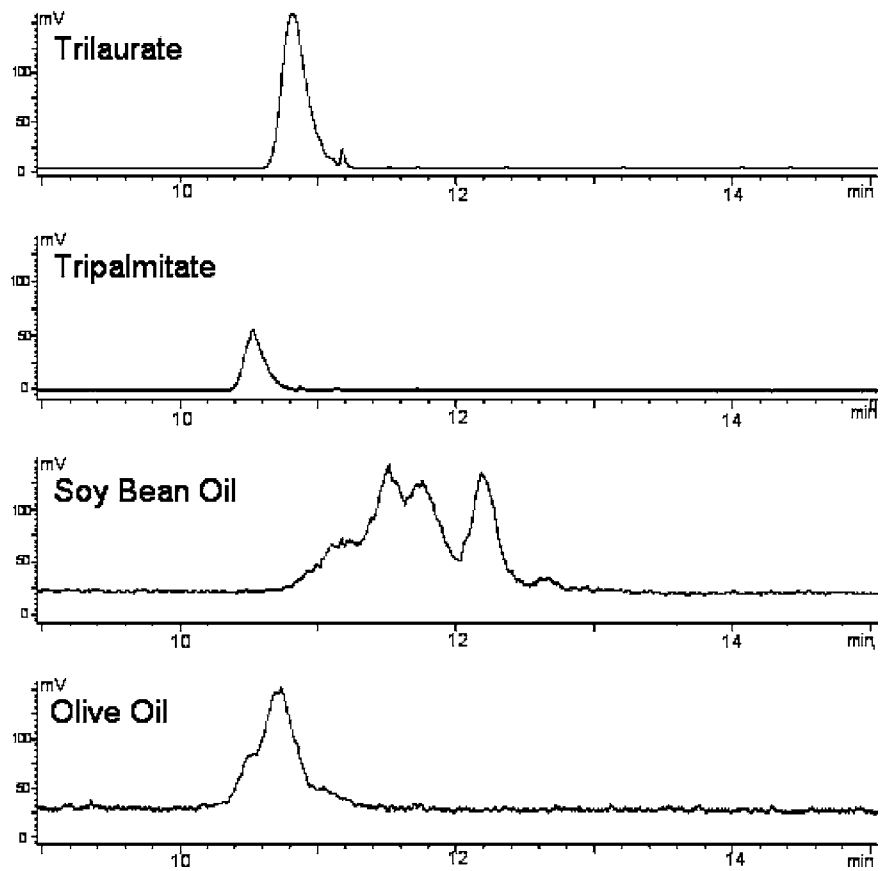

Fig. 2. Peak-splitting of TAGs from soy bean and olive oil compared to defined TAGs (trilaurate and tripalmitate), chromatographic conditions see Section 2.5.

Additionally, a C1-reversed phase was tested in order to achieve a retention of PAR. Even the C1-phase separated by the number of carbon atoms in the molecule and PAR, as a complex mixture of alkanes, appeared as a "hump".

Peak-splitting of lipid classes was also observed on the diol-phase when comparing defined TAGs and two natural oils (see Fig. 2). As proved for four FAME standards (C18:0, C18:1, C18:2, C18:3), the splitting mostly occurred due to the different degrees of saturation.

3.2. Optimization of ELS-detection regarding highest sensitivity

All three parameters of the ELSD (N_2-pressure, nebulizer temperature and vaporizer temperature) were optimized in order to enhance the sensitivity for all lipid representatives. The optimized conditions were found to be: a nebulizer temperature of 20 °C, a vaporizer temperature of 35 °C and a nitrogen pressure of 3.5 bar. Since the ELSD-signal does not linearly increase with substance concentration second order regression (see Fig. 3a) was used to compute the correlation between concentration and detector response. The slope of the calibration curve for all representatives, calculated by the first deviation on a fixed concentration (here 50 mg/l), provides information about the sensitivity of the detector for these substances.

Fig. 3b shows varying sensitivities for all lipid classes whereas Fig. 3c compares the sensitivities of different TAGs with different chain lengths and different degrees of saturation. Sensitivity decreases with decreasing chain length and increasing degree of saturation.

The method has been calibrated with representatives of each class in working ranges of about 5–75 mg/l (except FAME, FOH and CHOL 10–150 mg/l), depending on the lipid class. Coefficients of intra-day variance range from 1.88 to 5.09% and the limits of detection from 0.79 to 3.65 mg/l (except FOH, FAME) as indicated in Table 2. With regard to the substance specific response even within one lipid class, the accuracy of quantification is limited. Without additional information, or other more specific methods, the amounts of migrating species can only be estimated.

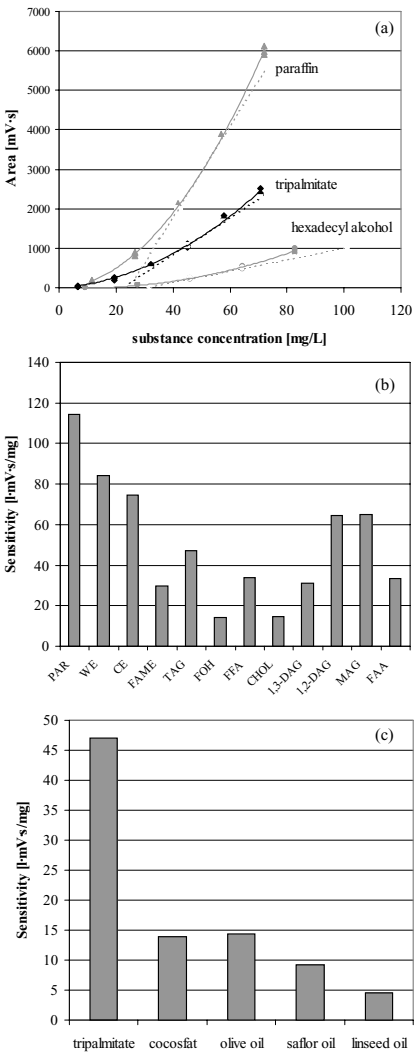

Fig. 3. Determination of the sensitivity of the ELS-detection (a) exemplary second grade regression for the detector responses of PAR, TAG and FOH, sensitivity at 50 mg/l is illustrated by the dashed tangent, (b) comparison of the sensitivity obtained for representatives of 12 different lipid classes at 50 mg/l (c) and for various TAGs (fatty oils and the representative glycerol tripalmitate) at 50 mg/l.

Table 2
Calibration data for representatives of all lipid classes

	Intra-day variances[a] (%)	Inter-day variances[b] (%)	Detection limit[c] (mg/l)	Sensitivity (l mV s/mg)
PAR	4.1	9.9	1	115
WE	3.1	8.6	1	84
CE	4.6	24.7	2	74
FAME	1.9	–[d]	6	30
TAG	4.1	12.9	2	47
FOH	5.1	15.8	6	14
FFA	2.7	7.0	2	34
CHOL	4.7	8.0	4	15
1,3-DAG	5.0	14.3	3	31
1,2-DAG	4.3	19.5	1	64
MAG	3.2	26.5	1	65
FAA	2.4	–[d]	2	33

[a] Intra-day variances are calculated from the standard deviation of the 18-point calibration.
[b] Inter-day variances are calculated from the Sheward control chart (recording one standard with a concentration of the center of the working range at 14 days during 1 month).
[c] Signal-to-noise ratio of 3 is used for the estimation of the detection limit. The respective height is transformed to concentration using second grade standard calibrations (obtained by peaks heights) for every lipid class.
[d] Not determined.

The variances of retention times and the ELSD reponse of the lipid class representatives (except of FAME and FAA) were recorded by Sheward control charts. Retention times for non-polar as well as polar substances varied less than 0.2 min during 1 month. The inter-day variances during this month varied from 7.0 to 26.5% as indicated in Table 2. Although these values are high, they are acceptable for an ELS-detection which normally requires new calibration each day. Time depending deterioration of MTBE developing within 1 week after distillation led to an increasing noise which especially disturbed the quantification of the later eluting components like MAG. The high inter-day variance of MAG can be explained by this effect.

3.3. Sample preparation for food packagings

The sample preparation for migrants from external lubricants differs from the sample preparation for migrants from internal lubricants (see Section 2.5). Being located on the inner surface of the packaging, external lubricants migrate directly (e.g. aluminium laminated with PP) into the simulant isooctane and can be analysed after concentration. While no migration of internal lubricants from coatings into isooctane was measurable, TAG and 1,2-DAG as well as 1,3-DAG could be detected in the 95% ethanol migrate. This result may be traced back to an enhanced partition coefficient between the lacquer and ethanol compared to isooctane. Furthermore, migration of ethanol into the lacquer swells the polymer network and enhances the diffusion of the internal lubricants. Therefore, 95% ethanol was chosen as simulant for internal lubricants in coatings.

However, the direct injection of the 95% ethanol extract leads to loss of resolution of the early eluting non-polar lipid classes. After evaporation of ethanol a dissolution of the residue in isooctane is not possible. Obviously extracted resin components soluble in 95% EtOH but not in isooctane enclose the lubricants in a fixed resin-like structure. Therefore, the residue is dissolved in ethanol with subsequent addition of isooctane. A phase separation was achieved by the addition of water. For the polar lipid classes, especially MAG and FAA, the concentration of the water must be high enough to enable the transfer of these substances to isooctane phase. By using varying amounts of added water (400–1600 μl) it was shown for standards that at least 70% water is necessary for highest recoveries ($62 \pm 3\%$) of MAG and FAA. For standards of less polar lipid classes (tested for 1,2-DAG, 1,3-DAG, TAG and WE) the recovery is for all different amounts of added water $100 \pm 3\%$.

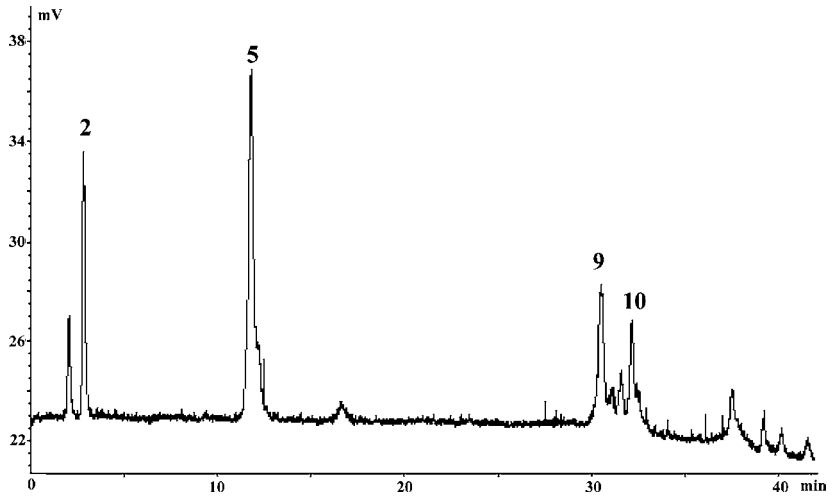

Fig. 4. Chromatogram of the 95% ethanol migrate of a commercial epoxy-anhydride coating, (2) WE (0.05 mg/dm^2), (5) TAG (0.1 mg/dm^2), (9) 1,3-DAG (0.1 mg/dm^2), (10) 1,2-DAG (0.05 mg/dm^2), chromatographic conditions see Section 2.5.

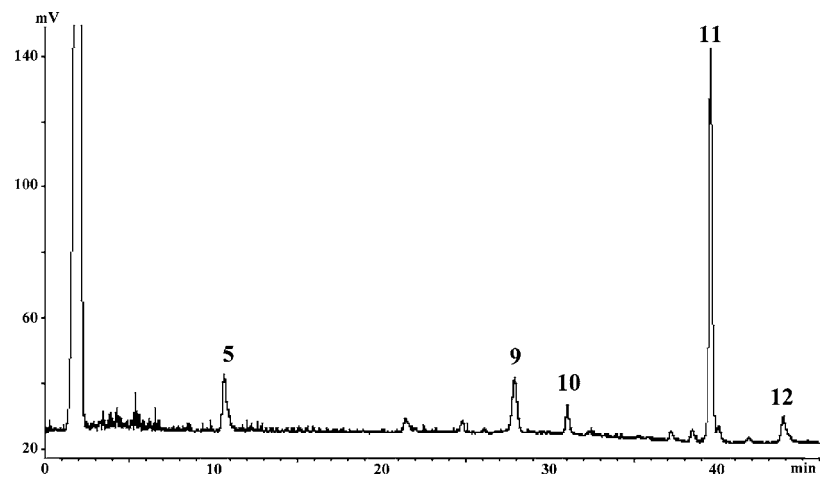

Fig. 5. Chromatogram of the isooctane migrate of a commercial lid made of PP-aluminum laminate, (5) TAG (0.8 mg/dm^2), (9) 1,3-DAG (1.4 mg/dm^2), (10) 1,2-DAG (0.5 mg/dm^2), (11) MAG (2.3 mg/dm^2), (12) FAA (0.5 mg/dm^2), chromatographic conditions see Section 2.5.

The extraction procedure was verified with a coating migrate to which a standard mixture (WE, TAG, 1,2-DAG, 1,3-DAG and MAG) was spiked. Similarly the more polar lipid classes exhibit a smaller recovery than the non-polar classes. However, due to an altered phase separation the recovery rates amounted to about 100 and 130%.

3.4. Application to food packagings

The developed method was applied to a commercial can coating (epoxy-anhydride) including carnauba wax and a partial acyl glycerol as internal lubricants. Consequently, WE, TAG, 1,3-DAG and 1,2-DAG were identified (see Fig. 4) while MAG was not detectable. It is assumed that MAG reacted quantitatively with the epoxy groups of the resin under formation of MAG-resin ethers. The initial peak is possibly PAR, but confirmation should be obtained by specific GC methods for paraffin as described in [6]. The identified migrating lubricants were estimated as 0.3 mg/dm^2.

Fig. 5 shows the chromatogram of a migrate from a laminated commercial light weight container with external and internal lubrication of the polypropylene film. The container was extracted with 100 ml isooctane, which covered a surface of 1.2 dm^2. A partial acylglycerol mixture containing TAG (5), 1,3-DAG (9), 1,2-DAG (10) and MAG (11) used as external lubricant as well as FAA (12) used as slip additive were detected. As already stated for the can coating migrate, the identity of the initial peak must be confirmed by specific method for hydrocarbons. The sum of the identified migrating lubricants was estimated as 5.5 mg/dm^2.

4. Conclusion

A simple screening method of 12 lipid classes commonly used in lubricant formulations for packaging materials was established. The chromatographic system was optimized in order to obtain a separation for all lipid classes whilst minimising the separation of the analogues within one class. However, due to the varying sensitivity of ELSD, especially for different representatives of one lubricant class, the method provides only an approximate quantitative estimation. To ensure identification and provide valid determination of single components in samples with unknown lubricant composition specific methods have to be applied. The presented method is useful in order to confirm the presence of lubricants in the total migrate of packaging materials as well as to estimate their amount.

Acknowledgements

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Determination of bisphenol A diglycidyl ether (BADGE) and its derivatives in food:

Identification and quantification by internal standard

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ORIGINAL PAPER

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Determination of bisphenol A diglycidyl ether (BADGE) and its derivatives in food: identification and quantification by internal standard

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Abstract Bisphenol A diglycidyl ether (BADGE) and its reaction products with water and hydrochloric acid have recently been subject to new regulations concerning their migration from food packaging into foodstuff. A method for the simultaneous identification and quantification of these substances and their precursor bisphenol A in food is described introducing bisphenol A di-(3-hydroxypropyl)ether as an internal standard.

Analysis was carried out using RP-HPLC gradient elution with fluorescence detection. Additional information in the case of suspect samples was obtained using RP-HPLC with mass selective detection. The described method is validated for the analysis of foodstuffs as well as fatty food simulants. The limits of detection were between 10 and 30 µg/kg of food; recovery experiments gave identical behaviour for all analytes and the internal standard. The enforcement of the specific migration limit set by regulatory standards of the European Union for BADGE and its hydrolysis and hydrochlorination products is possible for producers as well as food quality surveillance institutions.

Keywords Bisphenol A diglycidyl ether and derivatives · Migration · Food packaging · Canned food

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Introduction

Epoxy monomers for can coatings

Coated cans and lids are used as food packaging for a great variety of foodstuffs, such coating being carried out to prevent corrosion and migration of metals into food during heat stabilisation and storage of canned food preserves. Bisphenol A diglycidyl ether (BADGE) (Fig. 1) is used both as a monomer for epoxy resins and epoxy-based polymers, and as an additive for the elimination of surplus hydrochloric acid in the production of PVC organosols [1, 2].

Both coating types contain residual BADGE monomer; moreover PVC organosols contain BADGE·HCl and BADGE·2HCl (Fig. 1) as hydrochlorination products of BADGE which are formed during the production process. Due to their lipophilic character, these substances tend to migrate from the coating into fat-containing foodstuffs. In foodstuffs, various reactions take place. Most likely, the remaining epoxy groups are hydrolysed, forming BADGE·H_2O, BADGE·$2H_2O$ and BADGE·HCl·H_2O (Fig. 1). Furthermore, hydrochlorination products may be formed in the presence of sodium chloride under slightly acidic conditions [3].

BADGE and its derivatives as health hazards

It is not only their technological properties but also their low acute toxicity which makes epoxy resins useful for food packaging. The LD_{50} (rats) of resins formed of monomers with an average molecular weight of 380 g/mol was reported with 11.4 g/kg body weight, whereas an average molecular weight of monomers of 900 g/mol does not lead to a classical LD_{50}. The application of 30 g resin/kg body weight led to a rate of 50% rats with health defects [4].

The toxicology of monomeric BADGE, however, is not completely elucidated. The identification of adducts with human DNA [5] led to the assumption of teratogen-

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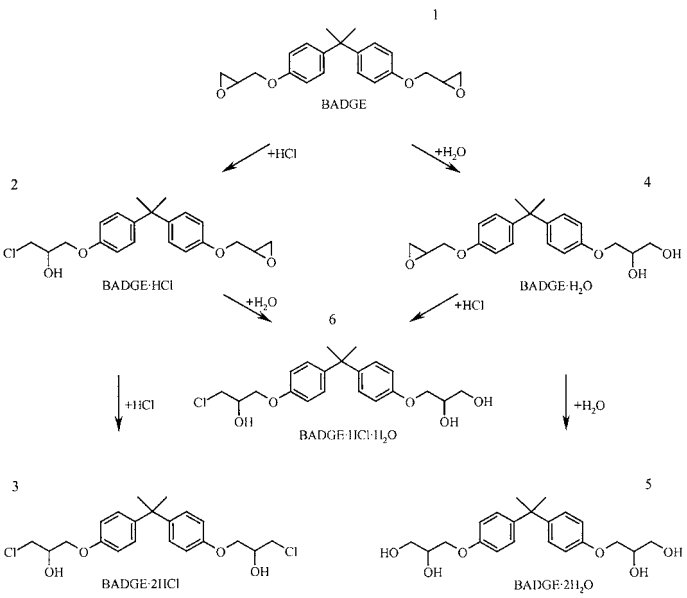
Fig. 1 Bisphenol A diglycidyl ether (*BADGE*) and its reaction products



ic and mutagenic effects. Taking into consideration that formation of those adducts was observed in vitro and that BADGE undergoes fast hydrolysis in the presence of epoxide hydrolases of mouse liver and skin [6], chronic toxicity of BADGE is estimated to be low.

Regulatory standards in the European Union

In order to minimise possible health risks for the consumer and to define the technological state of the art, the concentration of residual BADGE monomer and the migration of BADGE and its hydrolysis and hydrochlorination products into foodstuffs have already been subject to quantitative restrictions for some time.

The application and concentration limits of monomers and additives for the production of plastics for food packaging were regulated by the European Union with the Directive 90/128/EEC [7]. Those regulations, however, were not related to varnishes and coatings for cans and lids for food contact.

In order to elucidate the toxicological risks of BADGE the Scientific Committee on Food of the European Commission has evaluated numerous investigations and set up a temporary specific migration limit (SML) of 1.0 mg (total)/kg of food for BADGE and its hydrolysis products in 1996 [8]. The SML was confirmed in 1999 [9] and included BADGE and its derivatives (Fig. 1). The recommended value was adopted by European legislation [10].

Most recently, the regulations for epoxy monomers were removed from their place in the Directive 90/128/EEC and taken into a specific directive regulating the use of epoxy monomers in most kinds of food contact materials [11]. Thus, although the SML for BADGE and its derivatives remained unchanged, the regulations now comprised all types of packaging relevant for the production of foodstuffs for consumers.

The continued extensive use of BADGE and the described regulatory standards demand an analytical procedure capable of the identification and quantification of BADGE and its relevant derivatives in both food simulants and foodstuffs.

Method development in the past

After first reports about the migration of BADGE into food [12], method development focused on the determination of BADGE in aqueous food simulants, including its hydrolysis products [1, 13, 14, 15]. A first survey of the content of BADGE in foodstuffs was carried out in 1995, taking up earlier results [12] by examining various microwave-heated foodstuffs [16]. The results of Swiss authorities' examinations of lightweight containers [2] led to the development of analytical methodologies capable of the quantification of BADGE and its hydrolysis and hydrochlorination derivatives.

Most valuable results were published by Biedermann et al. [17], who developed a normal phase HPLC-fluo-

rescence detection (FLD) method for the identification and quantification of BADGE and its derivatives. Subsequently, various methods for the determination of BADGE and its derivatives were published [18, 19, 20, 21].

The validation of analytical procedures, however, was not carried out consistently. Prior to February 2000, not all standard substances were commercially available. Thus, it was necessary to use a mixture of synthesised substances for the identification of analytes [21, 22, 23]. Quantification was carried out based on the assumption of identical fluorescence activity for all analytes using the external standard calibration of BADGE for all relevant substances [17, 20, 21, 22, 23].

Our aim was the development of a method which enables the supervision of the restrictions imposed by the European Commission. It was important to construct a validated analytical system with a rapid and effective sample preparation followed by HPLC analysis with a detection system which would be available for most analytical laboratories.

Materials and methods

Reference substances. Bisphenol A (BPA) was obtained from Sigma, Germany. Standards of BADGE, BADGE·2HCl and BADGE·2H_2O were purchased from Fluka, Switzerland. Prior to commercial availability, BADGE·HCl, BADGE·HCl·H_2O, BADGE·H_2O were synthesised. Later, they were purchased from Fluka, Switzerland.

Reagents. Unless otherwise stated, reagents were of analytical grade. Epichlorohydrine was obtained from Fluka, Switzerland. 3-Chloropropan-1-ol, silica gel (Kieselgel 60, 230–400 mesh, diameter 0.040–0.063 nm) and kieselguhr were from Merck, Germany. Methanol and acetonitrile were HPLC gradient grade (Merck). Purified water was obtained from a Millipore water purification system (Millipore, Italy) or by bidistillation (Heraeus, Germany).

For anion exchange clean-up, Diaion SA21A gel (Supelco, Germany) was used (16–50mesh, Cl^-, 3.1 meq/g). Solid phase extraction (SPE) was carried out using Chromabond C18ec (Macherey & Nagel, Germany). Ammonium formate buffer was prepared by mixing 100 ml of 50 mmol/l ammonia solution with 400 ml of water and adjusting the pH value to 3 with formic acid. This mixture was filled up to 1 l with water.

Apparatus. Sample clean-up for analysis was performed using glass columns with PTFE stopcocks (300×30 mm). HPLC analysis was done using a Hewlett Packard 1100 system with automatic degasser, binary pump, autosampler, column oven and fluorescence detector or mass selective detector with atmospheric pressure (AP)-electrospray ionisation (ESI) and AP-chemical ionisation (CI) interfaces, respectively.

Stationary phases. The evaluation of the selectivity of stationary phases was carried out on Nucleosil 120 3-C18ec 250×4 mm, Superspher 100 4-C18 250×4.6 mm, Multospher 100 5-C18ec 250×4 mm, Multospher AQ 120 5-C18ec 250×4 mm, Multospher FBS 100 5-C18ec 250×4 mm and LiChrospher 100 5-C18 125×4 mm, respectively.

Synthesis of BADGE·HCl. To 5 g (22 mmol) BPA, dissolved in 48 ml (440 mmol) epichlorohydrine, 15 ml of 2.9M NaOH were added during 60 min under constant stirring. The mixture was stirred at 40 °C for 3 days. After cooling, residual epichlorohydrine was removed at 60 °C under vacuum. The residual liquid was extracted twice with toluene. The combined extracts were washed

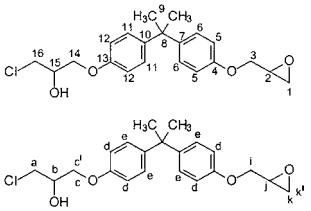


Fig. 2 BADGE·HCl – enumeration of carbon and hydrogen atoms

three times with water. Residual water was removed with sodium sulphate. After evaporation to dryness, the residue was dissolved in methanol/water (10/1 v/v). A first clean-up step was carried out on an anion exchange resin (conditioned with 1 M NaOH and washed with methanol/water, 10/1) to remove the intermediate bisphenol A monoglycidyl ether and BPA, followed by purification on silica gel (200 g) using dichloromethane/acetone (60/1) in order to remove the by-product BADGE·2HCl. Fractions of 10 ml eluent [dichloromethane/acetone (60/1 v/v)] were collected and analysed for the presence of the product (HPLC-UVD 280 nm, acetonitrile/water, 50/50). The fractions containing pure product were combined and evaporated to dryness. Residual solvent was removed under vacuum for 24 h. The product (Fig. 2) was a light yellow syrup with 99% purity (HPLC, 280 nm).

Characteristics: yield: 4.2 g (51%); $C_{21}H_{25}O_4Cl$ (376.88 g/mol), 67.22% C (calc. 66.93%), 6.64% H (calc. 6.69%); UV (acetonitrile/water): 227.8 nm (max), 276.0 nm(max); IR (KBr): 831 s, 1040 s, 1185 s, 1250 s, 1510 s, 1608 m, 2966 m cm^{-1}; ^{13}C-NMR (400 MHz, $CDCl_3$): δ=156.4 (C-4), 156.0 (C-13), 144.0 (C-10), 143.6 (C-7), 127.9 (C-11), 127.8 (C-6), 114.0 (C-5), 113.9 (C-12), 69.9 (C-15), 68.8 (C-3), 68.4 (C-14), 50.2 (C-2), 46.0 (C-16), 44.8 (C-1), 41.8 (C-8), 31.0 ppm(C-9); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.15 (H-e, m, 4H), 6.83 (H-d, m, 4H), 4.22 (H-b, m, 1H), 4.19 (H-c', m, 1H), 4.07 (H-i, m, 2H), 3.96 (H-c, m, 1H), 3.76 (H-a, m, 2H), 3.35 (H-j, m, 1H), 2.91 (H-k', dd=t, 1H), 2.76 (H-k, dd, 1H), 1.64 (CH_3, s, 6H) ppm, $J_{a/b}$ 5.6, $J_{b/c}$ 5.6, $J_{d/e}$ 3.1, $J_{k/j}$ 2.5, $J_{k/k'}$ 5.1, $J_{i/j}$ 3.6 Hz

Synthesis of BADGE·HCl·H2O. BADGE·HCl (1.6 g, 4.2 mmol) was dissolved in 14 ml acetone/water (4/1), 0.5 ml of 1 M sulphuric acid was added and the mixture was stirred at room temperature for 3 days. The reaction broth was brought to neutral pH with an aqueous solution of 10% sodium bicarbonate and extracted three times with diethyl ether. The combined extracts were washed twice with water. Residual water was removed with sodium sulphate. After evaporation to dryness, the residue was dissolved in ethyl acetate/toluene (3/1, v/v). Clean-up was carried out on a silica gel column (40 g) to remove traces of BADGE·HCl. Fractions of 10 ml eluent [ethyl acetate/toluene (3/1, v/v)] were collected and analysed for the presence of the product (HPLC-UVD 280 nm, acetonitrile/water, 50/50). The fractions containing pure product were combined and evaporated to dryness. Residual solvent was removed under vacuum for 24 h. The product was a white solid with 98.4% purity (HPLC, 280 nm).

Characteristics: yield: 0.8 g (47%); m.p.: 54 °C, $C_{21}H_{27}O_5Cl$ (394.89 g/mol), 63.12% C (calc. 63.87%), 6.68% H (calc. 6.89%); UV (acetonitrile/water): 226.8 nm (max), 277.9 nm (max); IR (KBr): 827 s, 1041 s, 1180 s, 1254 s, 1511 s, 1609 m, 2872 m cm^{-1}, 2964 m; ^{13}C-NMR (400 MHz, $CDCl_3$): δ=156.2 (C-4), 156.1 (C-13), 143.9 (C-10), 143.7 (C-7), 127.9 (C-11), 127.8 (C-6), 114.0 (C-5), 113.9 (C-12), 70.4 (C-3), 69.9 (C-2), 69.2 (C-15), 68.4 (C-14), 63.7 (C-1), 46.0 (C-16), 41.8 (C-8), 31.0 ppm (C-9); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.13 (H-e, m, 4H), 6.81 (H-d, m, 4H), 4.19 (H-b, m, 1H), 4.09 (H-j, m, 1H), 4.05 (H-c, m, 2H), 4.01 (H-i, m, 2H), 3.78 (H-k, m, 1H), 3.73 (H-a, m, 2H), 1.63 ppm (CH_3, s, 6H), $J_{a/b}$ 5.1, $J_{b/c}$ 5.1, $J_{d/e}$ 3.1, $J_{k/j}$ 2.5, $J_{i/j}$ 2.5 Hz

Enumeration of atoms for NMR analysis corresponds to Fig. 2 (lower numbers bisdiol side chain).

Synthesis of BADGE·H2O. BADGE (700 mg, 2 mmol) was dissolved in 18 ml acetone/water (5/1). Sulphuric acid (1 ml of 1 M) was added and the mixture stirred at room temperature for 24 h. The reaction broth was brought to neutral pH with an aqueous solution of 10% sodium bicarbonate and extracted three times with diethyl ether. The combined extracts were washed twice with water. Residual water was removed with sodium sulphate. After evaporation to dryness, the residue was dissolved in ethyl acetate/methanol (60/1 v/v). Clean-up was carried out on a column filled with silica gel (40 g) to remove traces of BADGE and BADGE·$2H_2O$. Fractions of 10 ml eluent [ethyl acetate/methanol, (60/1, v/v)] were collected and analysed for the presence of the product (HPLC-UVD 280 nm, acetonitrile/water 60/40). The fractions containing pure product were combined and evaporated to dryness. Residual solvent was removed under vacuum for 24 h. The product was a white solid with 98.4% purity (HPLC, 280 nm).

Characteristics: yield: 0.2 g (28%); m.p.: 40 °C, $C_{21}H_{26}O_5$ (358.34 g/mol), 69.62% C (calc. 70.37%), 7.28% H (calc. 7.31%); UV (acetonitrile/water): 226.5 nm (max), 277.1 nm (max); IR (KBr): 832 s, 1038 s, 1183 s, 1250 s, 1510 s, 1608 m, 2872 m cm^{-1}, 2967 m; ^{13}C-NMR (400 MHz, $CDCl_3$): δ=156.4 (C-4), 156.2 (C-13), 143.8 (C-10), 143.6 (C-7), 127.8 (C-11), 127.8 (C-6), 114.0 (C-5), 113.9 (C-12), 70.4 (C-15), 69.2 (C-14), 68.8 (C-3), 63.8 (C-16), 50.2 (C-2), 44.8 (C-1), 46.0 (C-16), 41.8 (C-8), 31.0 ppm (C-9); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.13 (H-e, m, 4H), 6.81 (H-d, m, 4H), 4.18 (H-c', dd, 1H), 4.09 (H-j, m, 1H), 4.02 (H-i, m, 2H), 3.94 (H-c, dd, 1H), 3.83 (H-k', m, 1H), 3.74 (H-k, m, 1H), 3.34 (H-b, m, 1H), 2.89 (H-a', dd, 1H), 2.74 (H-a, dd, 1H), 1.63 ppm (CH_3, s, 6H), $J_{a/b}$ 2.0, $J_{a'/b}$ 4.1, $J_{b/c'}$ 3.1, $J_{d/e}$ 3.1 Hz

Enumeration of atoms for NMR analysis corresponds to Fig. 2 (lower numbers epoxy side chain).

Synthesis of bisphenol A di-3-hydroxypropyl ether. 3-Chloropropan-1-ol [1.04 g (0.92 ml or 11 mmol)] was added to 1.14 g (5 mmol) BPA, dissolved in 20 ml propanol. During a period of 30 min, 5 ml of 2.2 M NaOH were added slowly under constant stirring. The mixture was stirred under reflux at 60 °C for 3 days. After cooling, the reaction broth was extracted three times with toluene. The combined toluene extracts were washed three times with water. Residual water was removed with sodium sulphate. After evaporation to dryness, the residue was dissolved in ethyl acetate/toluene (3/1, v/v). Clean-up was carried out on a silica gel column (40 g) in order to remove traces of BPA and the intermediate bisphenol A 3-hydroxypropyl ether. Fractions of 10 ml eluent [ethyl acetate/toluene (3/1,v/v)] were collected and analysed for the presence of the product (HPLC-UVD 280 nm; acetonitrile/water, 60/40). The fractions containing pure product were combined and evaporated to dryness. Residual solvent was removed under vacuum for 24 h. The product (Fig. 3) was a white solid with 99.2% purity (HPLC, 280 nm).

Characteristics: yield: 0.8 g (47%); m.p.: 40 °C; $C_{21}H_{28}O_4$ (344.45 g/mol), 73.38% C (calc. 73.23%), 8.26% H (calc. 8.19%); UV (acetonitrile/water): 227.4 nm (max), 276.0 nm (max); IR (KBr): 828 s, 1066 s, 1182 s, 1253 s, 1513 s, 1610 m, 2873 m, 2961 m cm^{-1}; ^{13}C-NMR (400 MHz, $CDCl_3$): δ=156.6 (C-4), 143.4 (C-7), 127.8 (C-6), 113.8 (C-5), 65.8 (C-3), 60.7 (C-1), 41.7 (C-8), 32.0 (C-2), 31.1 ppm (C-9); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.13 (H-e, m, 4H), 6.80 (H-d, m, 4H), 4.10 (H-c, t, 4H) 3.85 (H-a, t, 4H), 2.03 (H-b, m, 4H), 1.63 ppm (CH_3, s, 6H), $J_{b/c}$ 6.1, $J_{b/a}$ 5.6, $J_{d/e}$ 3.6 Hz

Optimised HPLC conditions. Separation of the analytes was carried out on a Multospher 100 5-C18ec 250×4 mm column. Gradient elution was performed with 5 mM ammonium formate in water (pH 3) (A) and acetonitrile/methanol (1/2, v/v) (B) from 40% A to 25% A in 30 min, followed by 10 min rinsing of the stationary phase (5% A) and 10 min equilibration to 40% A at 30 °C and a flow rate of 0.8 ml/min. Detection of the analytes was done with fluorescence (275/305 nm) or mass selective [AP-ESI positive, 100 V fragmentor voltage, 4000 V capillary voltage, 40 psig nebuliser pressure, 10 l/min drying gas with 350 °C; scan *m/z* 100–450 or single ion monitoring (SIM) of characteristic ion tracks] detection.

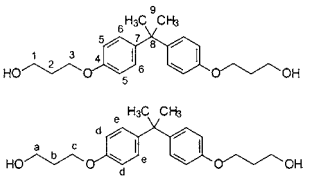


Fig. 3 Bisphenol A di-3-hydroxypropyl ether – enumeration of carbon and hydrogen atoms

Optimised sample preparation. Homogenised food samples (9.5 g) were spiked with 0.5 g olive oil containing 7.6 mg/kg of the internal standard, giving a concentration of 400 µg/kg foodstuff. The mixture was homogenised thoroughly. Homogenate (10.0 g) was weighed into a mortar and ground with approximately the same amount of kieselguhr. The result should be a free-flowing powder. The powder was transferred into a chromatographic separating column and extracted with 100 ml diethyl ether. The extract was evaporated to dryness resulting in the fatty phase of the foodstuff. The lipoid fraction containing the analytes was extracted with 5 ml acetonitrile under vigorous shaking.

Fatty food simulants were spiked identically. Samples (10.0 g) of this mixture were extracted directly with 5 ml acetonitrile.

Acetonitrile extracts (2 ml) were purified through a C18ec SPE phase (500 mg) which had previously been conditioned with 2 ml of acetonitrile. The column was rinsed with 2 ml acetonitrile/water (90/10, v/v). The combined eluates were filled up to 5 ml with acetonitrile. For HPLC analysis, the filtrate was diluted with water in a ratio of 1:1. In case BPA was detected, 200 µl of the purified extract were evaporated to dryness. After dissolving the residue in 50 µl pyridine, 50 µl acetic anhydride was added for the acetylation of the phenol. The mixture was kept at room temperature for 5 min and the reagent removed by a nitrogen stream. The residue was dissolved in 200 µl acetonitrile/water (50/50 v/v) and used for confirmation by HPLC-mass selective detection (MSD).

Results

Synthesis of reference substances

All missing BADGE derivatives were synthesised in purities and yields allowing their use as reference substances. The extensive characterisation proved the identities of the proposed structures.

In order to prove the assumptions of previous investigations with regard to equal fluorescence intensities of all analytes [17, 20, 21, 22, 23], the fluorescence responses of all BADGE derivatives were examined using both 225 nm and 275 nm as excitation wavelengths. With minor variations, equal properties were observed for all reference substances, suggesting sufficient precision of results when calibrating all substances as BADGE.

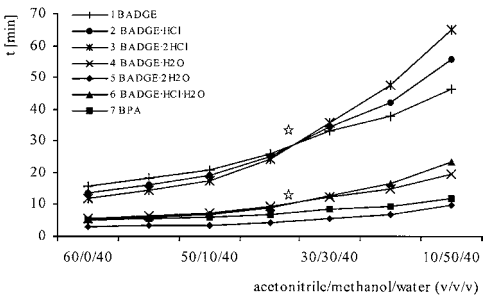


Fig. 4 Retention times with different isocratic elutions (acetonitrile/methanol/water). *Star* Change of elution order

Study of chromatographic variables

Our investigations were based on a method proposal published by the European Committee for Standardisation for the determination of BADGE in food simulants [24] by RP-HPLC with isocratic elution [acetonitrile/water (60/40, v/v)] followed by FLD (228/305 nm).

Since the chromatographic system was optimised for BADGE, it was only partly suitable for the separation of a standard mixture containing all seven analytes. Besides some interference with the food matrix at retention times up to 5 min, it was impossible to resolve the coelution of BPA and BADGE·HCl·H_2O. The reduction of the share of the organic modifier acetonitrile led to high retention times for the hydrochlorinated derivatives, while the polar substances still eluted prior to 6 min and the separation of BPA and BADGE·HCl·H_2O was not improved.

These results suggested the necessity of a more subtle differentiation of polarity. Thus, a third, moderately polar component was introduced. While the aqueous phase of the eluent persisted at 40%, acetonitrile was subsequently replaced by methanol (Fig. 4).

Increasing selectivity, especially focusing on BPA and BADGE·HCl·H_2O, was achieved. Furthermore, the separation of BADGE·2H_2O from the foodstuff matrix improved significantly. Additionally, a change in elution order was found with increasing methanol for BADGE·H_2O and BADGE·HCl·H_2O on the one hand and for BADGE, BADGE·HCl and BADGE·2HCl on the other hand (Fig. 4).

To limit the analysis to a reasonable duration without increasing retention times for the polar analytes, gradient elution was required. The best separation of reference substances was achieved by a linear gradient with an increase of the organic modifier (acetonitrile/methanol 1/2) from 60% to 75% in 30 min.

Evaluation of ruggedness of the chromatographic system

The development of a method suitable for routine analysis in different analytical laboratories demanded extensive optimisation studies.

The temperature of the analytical column does not influence the separation of analytes in the range 20–50 °C. Retention times decreased slightly with increasing temperature. Furthermore, selectivity depended neither on the use of buffer reagents in the aqueous phase (0.1% TFA, 5 mM ammonium formate, 5 mM ammonium acetate or no buffer) nor on the variation of pH values ranging from pH 2 to pH 8.

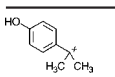
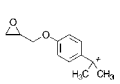
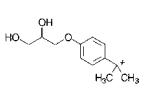
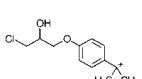
Additionally, the applicability of various stationary phases was examined (see Materials and methods). Selectivity was not influenced by the different reversed phase materials. However, as shown above, the retention times of the analytes strongly depended on the proportions of methanol and acetonitrile in the organic modifier and on the gradient parameters.

Thus, an easy transfer of this method into the conditions of routine analysis in different laboratories is possible. The following experiments were carried out on a Multospher 100 5-C18ec 250×4 mm column.

Confirmation of HPLC-FLD results by HPLC-AP-ESI-MSD

Further confirmation of results was achieved by MSD with the determination of the specific molecule clusters and fragment ions via SIM. Using positive ionisation mode the ion M+18 (ammonium adduct, Table 1) formed the main cluster. Furthermore, the fragmentation of all BADGE-related compounds led to group-related and substance-related fragments. Thus, a characteristic pattern of ions for each substance was detected which enabled a highly specific identification of the analytes. Table 1 summarises the most important group-related fragment ions.

Table 1 Group–related fragment ions of Bisphenol A diglycidyl ether (*BADGE*) and its derivatives

Fragment	*m/z*	Occurs	*m/z* of ammonium adducts
	135	All BADGE derivatives and BPA	
	191	BADGE BADGE·H_2O BADGE·HCl	358 376 394
	209	BADGE·H_2O BADGE·2H_2O BADGE·HCl·H_2O	376 394 412
	227/229	B ADGE·HCl BADGE·2HCl BADGE·HCl·H_2O	394 430 412

360

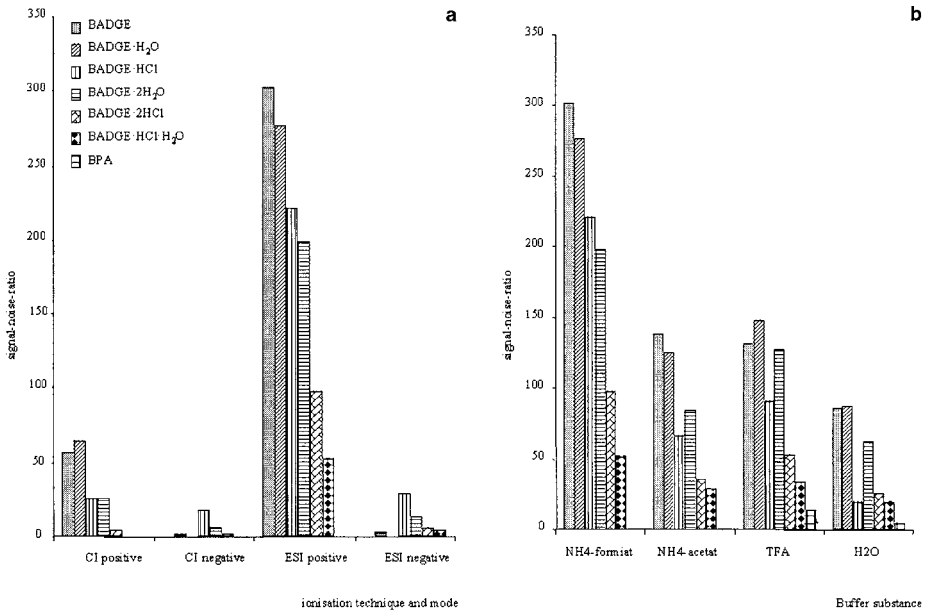


Fig. 5a, b Signal-noise-ratio (total ion current); chromatographic conditions cf. Materials and methods. **a**: 5mM-ammonium formate buffer, depending on ionisation technique and mode. **b** Electrospray ionisation positive, depending on buffer

The ionisation technique exhibited strong effects on the detection of the analytes. The lipophilic structure of BPA as the basic structural element of all analytes allows both ESI and CI. Depending on the ionisation mode, positive or negative ions are formed.

As regards the signal to noise-ratio, CI is significantly outnumbered by ESI (Fig. 5a). Moreover, MSD response is markedly reduced using negative ionisation mode. Additionally, LC eluent composition exerts considerable influence on the ESI-MSD response of the analytes [25]. In positive mode, responses decreased in the order ammonium formate>trifluoroacetic acid≈ammonium acetate>water. Results suggest the use of ammonium formate in order to achieve low detection limits (Fig. 5b).

Under the conditions examined, the MSD of BPA was only possible after acetylation with acetic anhydride in pyridine [20]. The disappearance of BPA and the occurrence of a new substance peak proved the quantitative derivatisation. MSD confirmed the proposed structure of bisphenol A diacetate.

Optimisation of the clean-up procedure

The use of RP-HPLC demands an extraction of analytes from food or food simulant matrix. The general preparation scheme for lipophilic analytes includes drying, extraction of lipids and separation of lipids and lipoids including analytes. This scheme has been used by previous investigations [18, 26]. The single steps, however, were performed with wide variations. Our investigations were based on the conditions of a proficiency testing scheme [27]. Water was removed by homogenisation of food with kieselguhr, followed by lipid extraction with *n*-pentane/diethyl ether (50/50 v/v). The extract was evaporated to dryness and lipoids were extracted with acetonitrile. Residual lipids were removed using C18-SPE.

Sample preparation improvement followed a retro-analytical scheme. Optimisation started with the final clean-up step and ended with the extraction of lipids from the foodstuff matrix.

A complete recovery of all analytes was achieved by elution of the C18-SPE column with a mixture of acetonitrile and water (90/10, v/v). Addition of water was necessary for the extraction of polar substances, especially BADGE·$2H_2O$.

The extraction of BADGE derivatives from food lipids was optimised to give high and equal extraction yields for all analytes. Various solvents with different polarities were examined, and it was found that the extraction with acetonitrile from *n*-hexane dissolved oil [26] gave the best results, with yields of nearly 90%. The liquid-liquid extraction, however, is time- and solvent-consuming and requires the additional evaporation of the acetonitrile extract. This step was avoided by a single direct extraction of the oil with acetonitrile, giving homog-

enous yields of about 80% for all analytes. It was decided that the additional step was not justified by slightly higher recovery rates.

For the evaluation of different lipid extraction conditions, Jagdwurst (a German sausage speciality with 16.8% fat, 15.5% protein and 64.0% water) was used as blind food matrix and spiked with a standard mixture. Since the initial extraction solvent *n*-pentane/diethyl ether gave poor extraction yields for the polar substances, especially BADGE·2H_2O, several more polar solvents were checked in order to achieve a uniform recovery of all standard substances. Best results were found using diethyl ether, giving homogenous extraction yields of 75%±10% (n=3) for all analytes.

Introduction of an internal standard

The introduction of an internal standard enables the compensation of poor analyte recovery due to losses during sample preparation as well as preparation imprecision depending on varying food matrices. A structural relationship to the analytes is preferred in order to find similar behaviour in sample processing. Consequently, it was necessary to establish an internal standard with BPA structure which is detectable by both FLD and MSD without being present in migrating material.

In the course of our investigations, bisphenol A di-3-hydroxypropyl ether (BADHPE) turned out to be the most suitable substance. BADHPE gave extraction yields similar to the analytes, is detectable via FLD and MSD and, furthermore, shows no coelution with standards or matrix components (Fig. 6).

Validation of the optimised method

Initially, standard calibration was carried out using multi-component standards of all seven analytes between 10 and 800 µg/l, resulting in linear correlation (correlation coefficient>0.999 for all analytes) with variation coefficients between 2.5% and 5% and calculated detection limits ranging from 15 µg/l to 25 µg/l (according to DIN 32645).

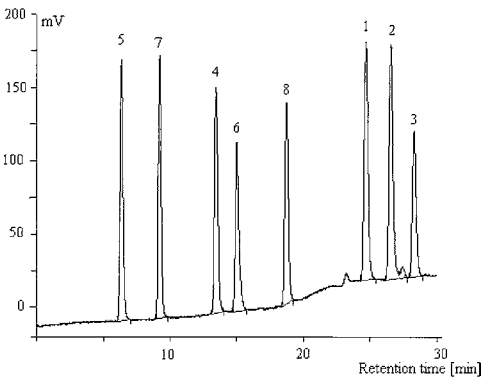


Fig. 6 Separation of a standard mixture including internal standard, RP-HPLC-FLD, conditions cf. Materials and methods. *1* BADGE, *2* BADGE·HCl, *3* BADGE·2HCl, *4* BADGE·H_2O, *5* BADGE·2H_2O, *6* BADGE·HCl·H_2O, *7* BPA, *8* internal standard

Subsequently, the analytical procedure was applied to different food matrices and validated. Canned pork, sweet corn and tuna in water were chosen as fatty, carbohydrate-rich and protein-rich foodstuffs, respectively. All matrices were checked thoroughly for the presence of the analytes before the standard mixture was added.

Since all sample preparation steps result in some loss of analytes and coelution with matrix components cannot be prevented totally, the calculated calibration data do not match the basic calibration. With 70% from canned pork (vs. 82% from sweet corn and 80% from tuna), extraction yields of BADGE and its derivatives were significantly lower in fatty foodstuffs. The precision of matrix calibration data was nearly identical for all examined matrices. The recovery rates of the internal standard were similar to the analytes and were determined with 68% for canned pork and 87% (sweet corn) or 83% (tuna), respectively. Thus, the internal standard calculation is suitable to eliminate losses in analyte recovery and for the enhancement of result precision (Table 2, data for canned pork as an example).

Table 2 Characteristic validation data, matrix calibration in canned pork, corrected with internal standard (matrix spiked with six different levels of concentration, analysed in triplicate)

Analyte	Working range µg/kg	Procedural standard deviation µg/kg	Variation coefficient %	Coefficient of linear correlation	Detection limit, calc. DIN 32645 µg/kg	Detection limit signal/noise>3 µg/kg
BADGE·2H_2O	50–800	46.4	12.6	0.9879	105.8	7.8
BPA	50–800	46.0	12.3	0.9885	104.8	21.6
BADGE·H_2O	50–800	35.4	9.8	0.9927	80.8	11.0
BADGE·HCl·H_2O	50–800	26.6	9.5	0.9931	60.7	17.6
BADGE	50–800	23.3	6.3	0.9969	53.2	19.8
BADGE·HCl	50–800	51.4	11.6	0.9898	117.1	12.9
BADGE·2HCl	50–800	42.9	14.3	0.9845	97.9	23.7

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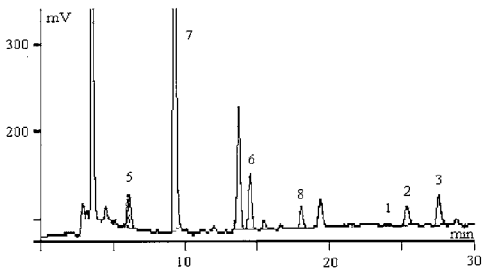

Fig. 7 BADGE and its derivatives in 'goulash and potatoes'; HPLC-FLD, conditions cf. Materials and methods. *1*: BADGE *2* BADGE·HCl *3* BADGE·2HCl *5* BADGE·2H_2O, *6* BADGE·HCl·H_2O *7* BPA, *8* internal standard

Table 3 BADGE and its derivatives determined in four different lots of 'goulash and potatoes' in lightweight containers (n=6)

Analyte	A µg/kg	B µg/kg	C µg/kg	D µg/kg
BADGE·2H_2O	590	493	523	458
BADGE·HCl·H_2O	1073	1002	1085	780
BADGE	45	113	95	25
BADGE·HCl	477	404	378	400
BADGE·2HCl	740	704	751	476
Sum of derivatives	2925	2716	2832	2139
Sum without BADGE·2H_2O	2335	2223	2309	1681

Considerable analytical uncertainty remains in the quantification of BADGE·2H_2O because of possible co-elution with matrix components. This is supposed to be acceptable because this analyte is not included in the cumulative limitation for BADGE and its derivatives in food. The average corrected recovery amounts up to 98.9% after internal standard calculation.

Previously reported losses of BADGE after spiking of tuna in water [3] were also found during our investigations. No relevant effect, however, was observed when sample preparation was started immediately after spiking the homogenised tuna. Analyte recoveries ranged from 91% (BADGE) to 108% (BADGE·2H_2O) or 93% (epoxy containing derivatives) to 95% (derivatives without epoxy group), respectively, after internal standard correction.

Homogeneity of contamination of food preserves

In order to elucidate the homogeneity of migration in production lots as well as deviations between different lots, products from lightweight containers designed for long-term storage were analysed for the presence of migrated BADGE and its derivatives. Four production lots of lightweight containers filled with goulash and potatoes had been manufactured within 1 month. No information was available as to whether the lightweight containers used for the filling were of one lot. The contents of six containers of each lot were analysed.

BADGE and most of its relevant derivatives and BPA were found in all containers (Fig. 7). Since the containers were not coated but consisted of a laminate of aluminum foil und a polyolefin film it was proposed that the detected migration resulted from the application of an epoxy adhesive.

The average amount of BADGE and its derivatives of all investigated lots (1680-2340 µg/kg) exceeded the regulatory limit of 1 mg/kg (Table 3). Statistical evaluation proved the homogeneity of variances of all analytes within the lots. The standard deviation of the analytes ranged from 30 to 90 µg/kg, which indicates a homogenous migration of the BADGE derivates into the food matrix within a single lot. Therefore, a single or few analyses are sufficient to characterise the migration into food within a production lot. However, migration of single derivatives was significantly different (α<0.05) in the lots investigated, indicating that the containers used for the lots did not have the same migration properties, or that processing parameters (sterilisation, storage) were different.

Discussion

A chromatographic system for the separation of BADGE and all relevant derivatives including BPA and an internal standard was established. Previously published methods have either focused on BADGE [1, 2, 13, 18, 19, 26] or BADGE and its hydrolysis products [14, 15, 21] and BADGE and its chlorohydroxy derivatives [17], respectively. Recently, methods including all relevant derivatives have been published [23, 28]. Both these studies included some bisphenol F derivatives but included neither BPA nor an internal standard.

Although the fluorescence intensity of all derivatives was shown to be equal and previous assumptions proved to be valid, the synthesis and use of individual reference substances represents a significant enhancement of the validity of the developed method. Moreover, FLD was done with 275 nm as excitation wavelength in order to minimise the analytical background because a lesser number of matrix components exhibit fluorescence compared to 225 nm [23, 28].

While most methods utilised analysis by reversed phase HPLC, Swiss authorities mainly used normal phase (NP) HPLC [2, 17] or reversed phase HPLC with an additional NP separation after acetylation of the analytes as confirmation [28]. NP-HPLC allows a rapid sample preparation when analysing fatty food simulants or foodstuffs for lesser polar analytes because the dissolved fatty phase is injected directly.

For the intended enhancement of identification via HPLC-MSD, solvents for NP-HPLC show an important disadvantage. MSD requires protic solvents to achieve sufficient ionisation, whereas the solvents used [28] do

not possess such properties. Consequently, NP-HPLC was not considered suitable for our analytical approach.

In this study, we present a baseline separation of seven BADGE-related analytes using RP-HPLC with gradient elution. High and equal recovery rates for all analytes over a broad range of polarity were achieved by the removal of water prior to lipid extraction with diethyl ether as a moderately polar solvent.

Using RP-HPLC, Biedermann et al. achieved comparable selectivity with ethanol as the organic modifier [28]. The simplified sample preparation of solid foodstuffs by direct extraction of the analytes with a mixture of ethanol and water followed by centrifugation, however, led to unacceptable losses in recovery of epoxy-containing derivatives. It was assumed that during the long period between the beginning of sample preparation and HPLC analysis, epoxy groups are hydrolysed or degraded by other reactions [28]. In our opinion, the formation of ethyl ethers by addition of ethanol might also occur under those conditions. Consequently, it was concluded that this method was not applicable for the quantification of BADGE in solid foodstuffs.

Lintschinger and Rauter [23] did not achieve a separation of all BADGE derivatives by using binary systems consisting of methanol/water or acetonitrile/water, respectively. For the resolution of BADGE·H_2O and BADGE·HCl·H_2O, they introduced a second chromatographic separation under isocratic conditions [23]. No results, however, were given for the separation of the other derivatives. Additionally, the resulting retention times for BADGE·H_2O and BADGE·HCl·H_2O lead to the assumption that the retention times especially for the hydrochlorinated derivatives are increasing significantly, thus resulting in unreasonably long duration of HPLC analysis. Under the conditions given, two different chromatographic systems have to be applied to each sample in order to achieve valid results. Furthermore, sample preparation by fat extraction with methyl-tert. butyl ether and subsequent isolation of analytes by extraction with methanol led to reduced recoveries for the polar derivatives [23]. The enhancement of homogeneity was only partly successful with the changes made during method development.

The introduction of an internal standard leads to the elimination of losses in analyte recovery and the enhancement of result precision. BADHPE does not occur in migrating material and is detectable by FLD as well as by MSD. The internal standard Bisphenol F diglycidyl ether (BFDGE) used elsewhere [26] is not appropriate because this substance is a component of Novolac glycidyl ether, which can be used as BADGE substitute in coating materials [20]. Thus, BFDGE might occur in migrating material from can coatings. Besides, BFDGE is a mixture of three isomers, giving three single peaks under the chromatographic conditions used.

Since different foodstuffs were analysed as examples of fatty, carbohydrate-rich and protein-rich matrices, the analytical procedure was validated for a great variety of foodstuffs. The extensive investigations led to a simple, rapid and valid method for the identification and quantification of BADGE and its derivatives in various food matrices. Thus, the enforcement of legal restrictions is possible for both producers and surveillance institutions. The introduction of HPLC-MSD for additional identification and an internal standard for the enhancement of method precision and elimination of analyte losses constitute the most important improvements compared to previously published methods.

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7

Migration from can coatings: Part 4. An approach to elucidate the overall migrate from can coatings

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food packaging; can coatings; migration;
HPLC-ESI-MSD/UVD/FLD, SEC-ELSD/UVD

7.1 Abstract

Migration of polymeric components was observed during the investigation of food cans internally coated with epoxy-, polyester- and organosol-based lacquers, whereby the migrating substances with a molecular weight below 1000 Da are regarded as potentially absorbable in the gastro-intestinal tract.

Different methods of analysis (for determination of: overall migrate and part below 1000 Da, bisphenol A-related substances, polyester cyclic oligomers, lubricants and specific carboxylic acids) were applied to 10 % and 95 % ethanol migrates from epoxy- and polyester-based coatings. Their results were combined in order to obtain a mass balance of migration.

The potentially absorbable substances below 1000 Da in the 95 % ethanol migrate of two investigated coatings (an epoxy and a polyester coating) amounted to about 1.3 mg/dm^2. Of this 50 % (epoxy coating) and 40 % (polyester coating), respectively, could be allocated to resin-related substances. Another 30 % of the epoxy migrate below 1000 Da were identified as lubricants and free trimellitic acid, whereas 60 % of the polyester migrate below 1000 Da remained unknown. The amounts of migrating substances in 10 % ethanol were much lower (0.23 mg/dm^2 for epoxy and 0.05 mg/dm^2 for polyester coating). These migrates consisted only of substances below 1000 Da of which 70 % (epoxy coating) and 95 % (polyester coating) could be explained as resin-related substances and trimellitic acid. Isooctane was shown not to be a suitable substitute for the migration of resin related substances into oil as simulant for fatty food stuff, since no detectable amounts of resin-related substances were found in these isooctane migrates.

7.2 Introduction

Most food cans are internally coated in order to protect the food against metal ions as well as to prevent metal corrosion occurring with aggressive food ingredients. The lacquers used for these applications are commonly based on binder resins as their main components (epoxy resins, polyester resins and organosols), hardeners, lubricants and other additives. Epoxy resins for can coatings are prepared from bisphenol A (BPA), novolac resins or other phenols, respectively. Frequently used monomers for polyester resins are phthalic acid isomers and polyvalent aliphatic or cyclo-aliphatic polyfunctional (di or tri) alcohols. Organosols consist of polyvinylchloride-resins normally stabilised by additives containing epoxy-functionality.

7.2.1 Legal regulations

In Europe, only a few regulations are specifically applicable to can coatings. Directive 2002/72/EC (European Commission 2002a) provides a positive list of starting materials and additives, with restrictions for some substances, and a limit for the sum of all migrating substances. In the scope of this directive are plastic food contact materials but coatings and metal containers are excluded. The resolution AP(96)5 (Council of Europe 1996) was published for coatings, but it has no legal status within Europe except for those member states which have adopted into their national regulations for coatings and to demonstrate due diligence. The resolution limits the sum of migrating substances (overall, global or total migration) to 10 mg/dm^2 and includes a positive list for approved starting materials and specific limits for some substances. Recently, the specific migration of ten monomeric bisphenol A-digylcidyl ether (BADGE)- and bisphenol F-diglycidyl ether (BFDGE)- derivatives (the dihydrolysis products BADGE·2H_2O or BFDGE·2H_2O are only included for aqueous media) is limited to 1 mg/kg food by Directive 2002/16/EC (European Commission 2002b).

However, most starting materials for lacquers are already (pre-)polymers or substance mixtures and they react during the curing with other constituents of the

lacquer. Consequently, migrating substances from coatings are to a minor extent monomers from the positive list, the major proportion being their polymerisation or reaction products. The Scientific Committee on Food (SCF, European Commission 2001) emphasised the necessity to determine the identity of all chemicals which actually migrate into food, their quantities in the total diet and their toxicological profile. Thereby only the fraction with a molecular weight below 1000 Da is of toxicological interest, because only this fraction is regarded as potentially absorbable in the gastro-intestinal-tract.

Recently, the 1000 Da border was introduced to the regulation 2002/16/EC (European Commission 2002) for novolac diglycidyl ether (NOGE)-derivatives: the use of NOGE is nearly excluded by limiting the presence of all derivatives below 1000 Da containing at least one epoxy- or hydrochlorination group to a quantitative maximum QM(t) of 0.2 mg/kg in the material. Consequently, the can supply chain voluntarily agreed not to use NOGE for canned foodstuffs entering Europe. However, this material is acceptable under §175.300 of 21 CFR (Code of Federal Regulations, FDA 2004). To date a legal limit either for the sum of all migrating substances below 1000 Da (overall migrate below 1000 Da) or for the sum of migration of epoxy resin-related substances below 1000 Da except for NOGE does not exist.

For application of new substances in the USA the Food and Drug Administration (FDA) established a threshold of regulation for individual migrating substances of 0.5 µg/kg in the diet below which a toxicological evaluation of this components is not required (Begley 1997). A consumption factor of 0.17 is defined for canned food resulting in a threshold concentration of 3 µg/kg (U.S. FDA 2002). However, no such thresholds exists in the European legislation. There, in case of migration below 0.05 mg/kg food (irrespective of the diet) only three mutagenicity tests are needed (Note for Guidance, European Commission 2003a). Based on these definitions and technical feasibility Grob *et al.* (1999) suggested a value of 30 µg/kg (corresponding to 1 – 5 µg/dm^2 depending on the size of the can) for individual compounds in the migrate of can coatings as limit for identification. For the sum of unknown/untested migrants below 1000 Da the same authors proposed a maximum of 300 µg/kg (corresponding to 10 - 50 µg/dm^2).

7.2.2 Previous work

Many methods are published for the identification and quantification of the regulated specific BADGE-/BFDGE-based migrants from can coatings (Roubtsova *et al.* 1997, Summerfield *et al.* 1998, Biedermann *et al.* 1999, Biles *et al.* 1999, Simoneau *et al.* 1999, Theobald *et al.* 1999, Hammarling *et al.* 2000, Lintschinger and Rauter 2000, Uematsu *et al.* 2001, Petersen *et al.* 2003). Concerning epoxy-related migrants below 1000 Da other than the regulated derivatives, methods for identification and quantification were published by Biedermann *et al.* (1998), Berger *et al.* (2001), Theobald *et al.* (2002) and Schaefer and Simat (2004) as well as a method for identification and quantification of migrants from organsols which are stabilised with NOGE was published by Biedermann und Grob (1998). An equivalent method for identification and quantification of all polyester-based migrants below 1000 Da was published by Schaefer *et al.* (2004b).

Methods using size exclusion chromatography (SEC) were published by Bronz *et al.* (1998) and Schaefer *et al.* (2004a) for the determination of the toxicologically relevant part below 1000 Da in the overall migrate.

The comparison of the determined part below 1000 Da and the determined individual resin-related substances resulted in an explanation of the overall migration below 1000 Da of between 20 % (Biedermann *et al.* 1998) and 40-50 % (Schaefer *et al.* 2004b, Schaefer and Simat 2004). The unknown remainder can presumably be traced back to the migration of hardener components (e.g. free carboxylic acids from anhydride hardener, method published by Schaefer *et al.* 2004b), lubricants (published by Schaefer *et al.* 2003) or other additives.

The aim of this investigation has been the application of several substance-specific and screening methods to migrates from different types of coatings (epoxy and polyester) in order to achieve a mass balance for known and unknown migrants. Results of the overall migrate as well as the part below 1000 Da and the sum of migrating resin-related substances obtained from an epoxy-based coating should be compared for different EU-approved simulants and the extraction medium acetonitrile. Since all results were based on the analysis

of lab-made coated strips, the comparability with industrial made cans and migrants in oily food filled in those cans needed to be proven.

7.3 Experimental

7.3.1 Materials

Lab-made single-side coated tinplate-strips (1 x 25 cm) cured at conditions equivalent to the commercial process (5 to 15 min at 180 to 220 °C depending on the coating type and film thickness of 5 to 15 g/m^2) were provided by the Valspar Corporation. The strips were coated with an epoxy coating (epoxy-anhydride), a polyester coating (polyester-urethane) and an organosol coating (stabilised with an epoxy resin). Industrial made empty cans and cans filled with tuna in oil were internally coated with the same organosol (same film thickness and curing conditions) that was applied onto the strips mentioned above.

7.3.2 Apparatus

The high performance liquid chromatography (HPLC) analyses were performed on two different HP1100 (Agilent, Waldbronn, Germany) systems both equipped with an autosampler (G1313A), an automatic degasser (G1322A), a binary pump (G1312A) and a column oven (G1316A). One system consists additionally of a fluorescence detector (FLD, G1321A), a diode array detector (UVD, G1315A) and a mass selective detector (MSD, G1946A) using electrospray ionisation (ESI, positive mode, capillary voltage 4000 V, nebuliser pressure 40 psig, dry gas flow 10 L/min, dry gas temperature 350 °C). The second system contains a variable wavelength detector (UVD, G1314A) and an evaporative light scattering detector (ELSD, Sedex 75, Sedere, Alfortville, France). Data were assessed by Agilent Chemstation® software (Rev. A 08.03).

7.3.3 Methods

Simulation of migration. Coated tinplate strips folded like a concertina were used for migration experiments. A sample of 8 strips (according to 2 dm^2) was usually extracted with 50 mL simulant. Empty cans are extracted by using highest surface-volume-ratios. In order to simulate a migration into canned aqueous as well as fatty food (sterilised for 1 h at 121 °C) the strips were treated as indicated in table 7.1. Migration experiments were performed in a sterilisor, Sanoclav, Wolf, Geislingen, Germany.

Table 7.1. Simulants / Extraction media and their treatment according to 97/48/EC amending 82/711/EEC and 85/572/EEC

Simulant	treatment	simulated food
Bidistilled water	1 h at 121 °C	aqueous food, with pH above 4.5
3 % Acetic acid	1 h at 121 °C	aqueous food, with pH below 4.5
10 % Ethanol	1 h at 121 °C	alcoholic food or beverages
sunflower oil	1 h at 121 °C	fatty food
95 % Ethanol	4 h at 60 °C	substitute of the simulant oil for fatty food
Isooctane	2 h at 60 °C	substitute of the simulant oil for fatty food
Acetonitrile	24 h at room temperature	extraction media

Sample preparation. The coating migrates obtained by simulants and food were prepared as shown in table 7.2.

Table 7.2. Sample preparation for all different chromatographic techniques ([1]acetonitrile, [2]dimethyl formamide)

Migrate	preparation	dissolvation/ dilution	analysis method	analytes	detailed description
simulants of table 1 (except the oil)	evaporation of 4 mL to dryness	400 µL dioxane	SEC-ELSD/UVD	quantification: overall migrate + < 1000 Da part	Schaefer *et al.* (2004a)
		200 µL MeCN[1] + 200 µL H_2O	RP-HPLC-ESI-MSD/UVD	identification: epoxy- and polyester-based substances <1000 Da	Schaefer *et al.* (2004b) + Schaefer and Simat (2004)
		200 µL DMF[2] + 100 µL MeCN + 100 µL H_2O		quantification: polyester-based substances < 1000 Da	Schaefer *et al.* (2004b)
		200 µL DMF + 200 µL H_2O		quantification: monomeric acids	Schaefer *et al.* (2004b)
		liq.-liq.-extraction 400 µL ethanol + 400 µL isooctane + 1600 µL H_2O	NP-HPLC-ELSD	quantification: unpolar substances	Schaefer *et al.* (2003)
simulants of table 1 (except the oil)	–	dilution with 500 µL H_2O	RP-HPLC-FLD	quantification: epoxy-based substances < 1000 Da	Schaefer and Simat (2004)
Simulanting oil + food	lipid extraction with diethyl ether lipid-lipoid-extraction with MeCN clean-up with C18-SPE			quantification: specific BADGE-/ BFDGE-derivatives (regulated by 2002/17/EC)	Petersen *et al.* (2003)

Quantification of migrating substances below 1000 Da by SEC-ELSD/UVD. Overall migrate and part below 1000 Da were separated by SEC using isocratic elution on two columns (100 Å and 1000 Å) with dioxane. Detection of the analytes was done by UV (220 nm) and ELS (30 °C nebuliser, 45 °C vaporiser and 0.8 bar nitrogen). The detection signal was calibrated using an epoxy or polyester resin and second grade regression. The molecular weight calibration was done using a low molecular weight epoxy resin or the migrate itself for polyester coatings. For detailed description see Schaefer *et al.* (2004a).

Identification and quantification of epoxy-related substances below 1000 Da by RP-HPLC-ESI-MSD/UVD/FLD. Migrating epoxy-related substances were identified and quantified by HPLC using gradient elution (50/50 –95/5 % acetonitrile/ ammonium formate, 1 mM pH 3.0) on a reverse phase (RP-18) column. Detection of the analytes was done by UV (220 and 275 nm) FL (ex: 275 nm, em: 305 nm) and MS (150 V fragmentor voltage, scan m/z 80 – 1000). The system was calibrated with BADGE using chromophore concentration ($c_c = c_s \cdot n \cdot MW^{-1}$, c_c= chromophore concentration (µmol/L), c_s= substance concentration in (µg/L), n = number of BPA-moieties and MW = molecular weight ($g \cdot mol^{-1}$)) and substance concentration. For detailed description see Schaefer and Simat (2004).

Identification and quantification of polyester-related substances below 1000 Da by RP-HPLC-ESI-MSD/UVD. Migrating cyclic oligoesters were identified and quantified by HPLC using gradient elution (50/50 –95/5 % acetonitrile/ ammonium formate, 1 mM pH 3.0) on a RP-18 column. Detection of the analytes was done by UV (232 nm), and MS (150 V fragmentor voltage, scan m/z 80 – 1000). The system was calibrated with an oligoester based on terephthalic or isophthalic acid using chromophore concentration ($c_c = c_s \cdot n \cdot MW^{-1}$, c_c= chromophore concentration (µmol/L), c_s= substance concentration in (µg/L), n = number of phenyl moieties and MW = molecular weight ($g \cdot mol^{-1}$)). For detailed description see Schaefer *et al.* (2004b).

Identification and quantification of migrating lubricants below 1000 Da by NP-HPLC-ELSD/UVD. Migrating lubricants were identified and quantified by HPLC using gradient elution (isooctane/tert-butyl methyl ether/acetic acid) on a normal phase (NP-Diol) column. Detection of the analytes was done by UV (275 nm) and ELS (20 °C nebuliser, 35 °C vaporiser and 3.5 bar nitrogen). For detailed description see Schaefer *et al.* (2003).

Identification and quantification of free carboxylic acids by RP-HPLC-ESI-MSD/UVD. Five commonly used acids (trimellitic acid, adipic acid, terephthalic acid, isophthalic acid, phthalic acid) were identified and quantified by HPLC using gradient elution (95/5 – 60/40 % acetonitrile/ammonium formate, 5 mM

pH 3.9) on a RP-18 column. Detection of the analytes was done by UV (232 nm) or MS (40 V fragmentor voltage, selected ion monitoring of characteristic ions (M+1): 147 for the poor UV-active adipic acid). For detailed description see Schaefer *et al.* (2004b).

7.4 Results and Discussion

7.4.1 Verification of the simulation/extraction with measurements of migration into canned food using an organosol coating stabilised with an epoxy-resin

Since most migration experiments were carried out using lab-made, coated tinplate-strips, the transfer of the results to real cans had to be verified. One difference between strips and cans is the fact that the strips exhibit cut edges. A better penetration of the simulant through the edges of the coating may be possible. In order to prove the applicability of these strips, empty cans produced with the same coating (an epoxy-stabilised organosol), the same film thickness and the same curing conditions were provided by the Valspar Corporation. The sum of migrating BPA-related substances below 1000 Da were compared. The migrate was dominated by mono- and dihydrochlorination products of mono-, di- and trimeric BADGE. The migrating species were classified according to their toxicological relevance into substances regulated by SCF and 2002/16/EC (SCF), as oligomers containing two oxirane rings (2EPO), as derivatives containing one oxirane ring (1EPO) and as derivatives without any oxirane rings (NEPO, see figure 7.1).

No significant difference between strips and empty cans was found for the amount of BPA-related substances in sunflower oil migrates and acetonitrile extracts. Thus, the existence of cut edges did not have a significant influence on the amount of migrating substances (see figure 7.1).

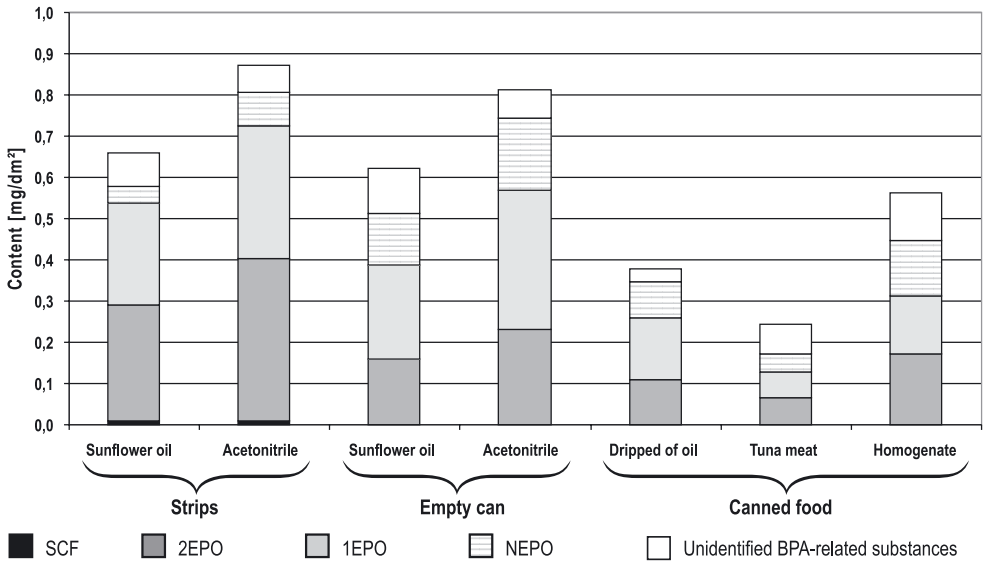


Figure 7.1. Comparison of amount of migrating BPA-related substances into simulating oil and acetonitrile from test-strips and empty cans coated with organosol as well into food from cans coated with organosol (tuna in oil) classified due to their toxicological cause of concern into SCF (substances regulated by SCF and 2002/16/EC), 2EPO (oligomers containing two epoxy groups), 1EPO (derivatives containing one oxiran ring) and NEPO (derivatives without oxiran ring)

In order to compare also the results of the sunflower oil (121 °C, 60 min) with processed oily food the Valspar Corporation provided canned tuna in oil, where the coating was the same as that for the empty cans and strips, although not from the same batch of coating. The simulant sunflower oil contained about the same amount of migrating BPA-related species below 1000 Da as did the homogenised tuna in oil. Additionally, the tuna in oil was analysed for the content of BPA-related substances in the drained oil and the tuna meat separately. Addition of both values agreed with the result of the homogenised food. More than 50 % of contaminants were found in the oil, which is not always considered as edible by the consumer.

7.4.2 Balance of migration

Numerous methods have been established for single starting substances with respect to their legal restrictions. However, little work has been done about the composition of coating migrates, in particular the potentially absorbable part with a molecular weight below 1000 Da.

For a comprehensive elucidation of migrates from an epoxy-anhydride and a polyester-urethane coating, respectively, analytical methods for the determination of binder components (Schaefer *et al.* 2004b, Schaefer and Simat 2004), hardeners (carboxylic acids, Schaefer *et al.* 2004b) and lubricants (Schaefer *et al.* 2003) have been combined. The sum of the single results for these migrate components were verified by comparison with the value determined for the sum of migrants below 1000 Da by SEC-ELSD (balance of the migration below 1000 Da).

7.4.3 Epoxy-anhydride coating

Balance of migration for 95 % and 10 % ethanol-migrates. The overall migrate and the potentially absorbable part below 1000 Da were determined simultaneously by SEC-ELSD (Schaefer *et al.* 2004a). The overall migrate was 6.50 mg/dm^2 for the 95 % ethanol (see figure 7.2a) and 0.23 mg/dm^2 for the 10 % ethanol migrate (see figure 7.2b). Of the overall migrate about 20 % (1.3 mg/dm^2) in case of 95 % ethanol and 100 % (0.23 mg/dm^2) in case of 10 % ethanol consisted of substances below 1000 Da.

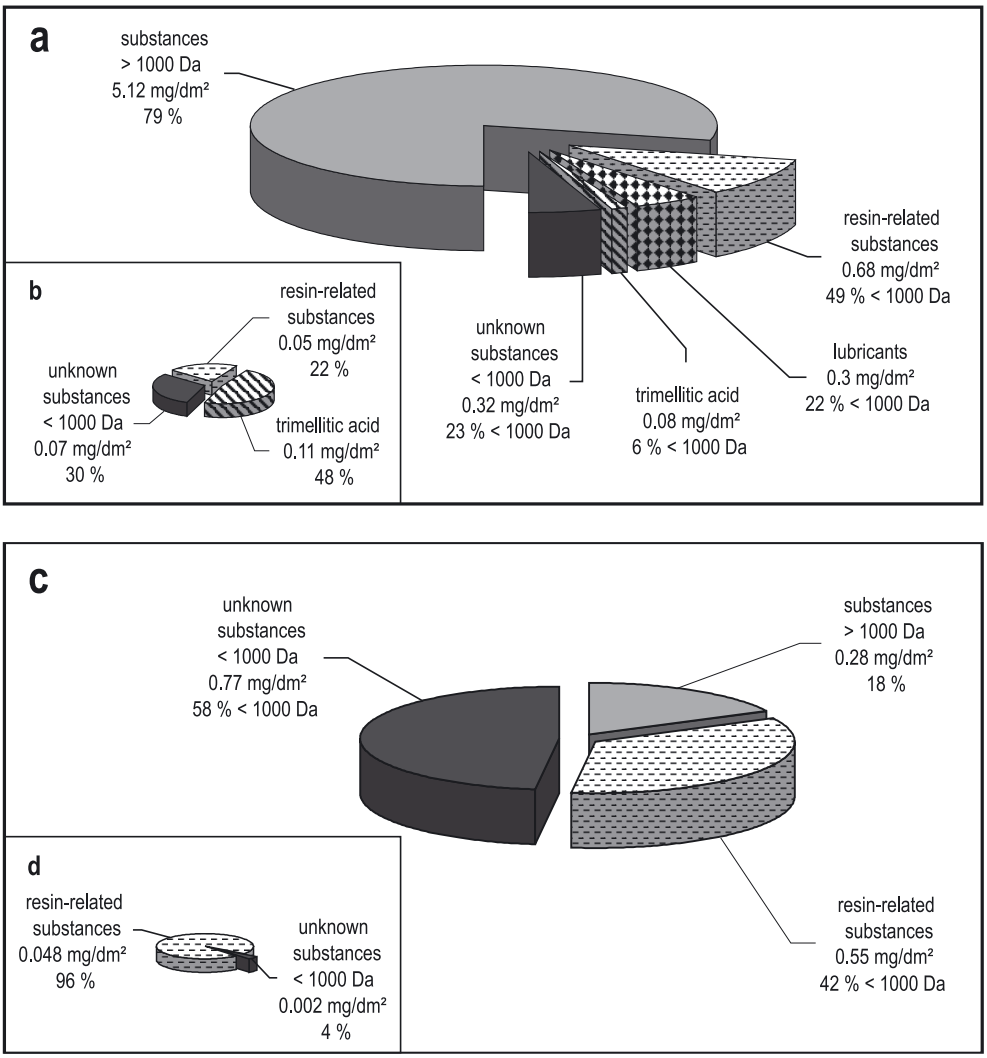


Figure 7.2. Mass balance for the total migrate (indicated as total are of the disc, 'percentages' below 1000 Da referred to the sum of migrating substances below 1000 Da) in

a) 95 % ethanol (6.50 mg/dm^2) of the epoxy coating, in
b) 10 % ethanol (0.23 mg/dm^2, complete below 1000 Da) of the epoxy coating, in
c) 95 % ethanol (1.60 mg/dm^2) of the polyester coating and in
d) 10 % ethanol (0.05 mg/dm^2, complete below 1000 Da) of the polyester coating.

The BPA-based migrating binder components were identified and quantified as BADGE-equivalents by RP-HPLC-ESI-MSD/FLD (Schaefer and Simat 2004).

About 50 % (0.68 mg/dm^2) of the overall migrate below 1000 Da in 95 % ethanol were identified as BPA-related substances: derivatives regulated by Directive 2002/16/EC (5 %), oligomers (5 %), reaction products with solvents and chain stoppers (60 %) and unidentified BPA-related substances (30 %). Non-polar additives like internal and external lubricants were identified and semi-quantitatively estimated by a NP-HPLC-ELSD screening method. Thus, another 20 % (0.3 mg/dm^2) of the 95 % ethanol migrate below 1000 Da were assessed as lubricants (wax esters, di- and triacyl glycerols). A residue of the anhydride hardener, migrating free trimellitic acid, was determined by a RP-HPLC-ESI-MSD/UVD method. The determined amount accounted for 6 % (0.08 mg/dm^2) of the overall migrate below 1000 Da in 95 % ethanol.

The overall migrate in 10 % ethanol exclusively consisted of substances below 1000 Da. Thereof 20 % (0.05 mg/dm^2) could be explained as BPA-related substances, 50 % (0.11 mg/dm^2) as trimellitic acid, whereas lubricants were not detectable (< 0.05 mg/dm^2).

In summary, about 20 % (0.32 mg/dm^2) of the overall migrate < 1000 Da in 95 % ethanol and about 30 % (0.07 mg/dm^2) in the 10 % ethanol migrate still need to be elucidated.

However, for the discussion of the validity of the balances the variances of the methods for overall migrate below 1000 Da (standard deviation SD = 0.035 mg/dm^2), for the sum of BPA-related substances below 1000 Da (SD = 0.004 mg/dm^2) and for the lubricants (coefficient of variance (CV) up to 200 % since the ELSD-response depends on chain length and degree of saturation of the detected substances) have to be considered.

Therefore, the calculated amount of unknowns for the overall migrate below 1000 Da in 95 % ethanol may be within the uncertainty of the applied methods, especially because of the high CV of lubricant determination. Otherwise the amount of unknowns could be twice as high. For the overall migrate below 1000 Da in 10 % ethanol a significant unknown residue was calculated, but can be estimated below 0.1 mg/dm^2. Thus, in both migrates unidentified single components up to 0.05 mg/dm^2 are still expected.

Comparison of the overall migrate and its part below 1000 Da for various simulants. All approved aqueous EU-simulants, approved substitutes for the simulant oil (95 % ethanol and isooctane) as well as acetonitrile were used to investigate the epoxy coating (see figure 7.3). Simulant oils (sunflower oil, olive oil or synthetic oils) are not applicable to the SEC-ELSD/UVD method, since a complete re-extraction of the migrants from the oil without co-extraction of oil components is impossible.

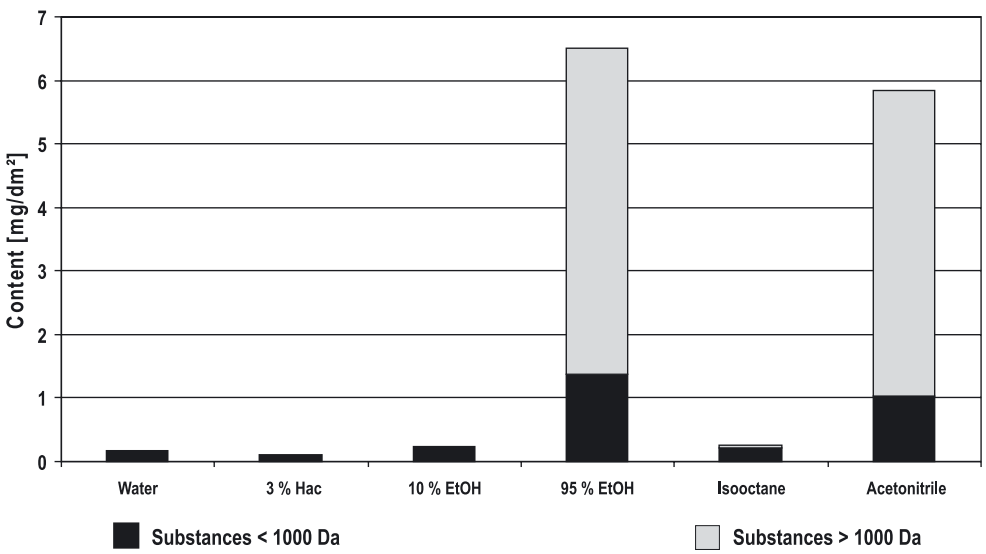


Figure 7.3. Comparison of the overall migrate and its part below 1000 Da obtained from the epoxy coating using different approved simulants and acetonitrile

The overall migrate in aqueous simulants was more than 20 times lower than in 95 % ethanol or acetonitrile. In the aqueous simulants all migrants had a molecular weight below 1000 Da. Although only about 20 % of the overall migrate in 95 % ethanol and acetonitrile had a molecular weight below 1000 Da, the migrate below 1000 Da in aqueous simulants was more than 5 times lower than in 95 % ethanol or acetonitrile. Isooctane gives more than 10 times lower migration values which may be explained by an enhanced partition coefficient between the lacquer and ethanol compared to isooctane. Furthermore, migration of ethanol into the lacquer could swell the polymer network which enhances the diffusion of the migrable substances.

Comparison of BPA-related substances below 1000 Da in various simulants. The migrating BPA-related substances below 1000 Da were classified by their toxicological relevance as described above (see figure 7.4). Their distribution was compared for all approved simulants and substitutes as well as for acetonitrile. The sum of migrating BPA-related substances in aqueous simulants was about 10 times lower than that in sunflower oil, 95 % ethanol or acetonitrile. In the aqueous simulants all migrants belonged to the SCF-class, which contains monomers up to 400 Da and also includes the dihydrolysis product of BADGE.

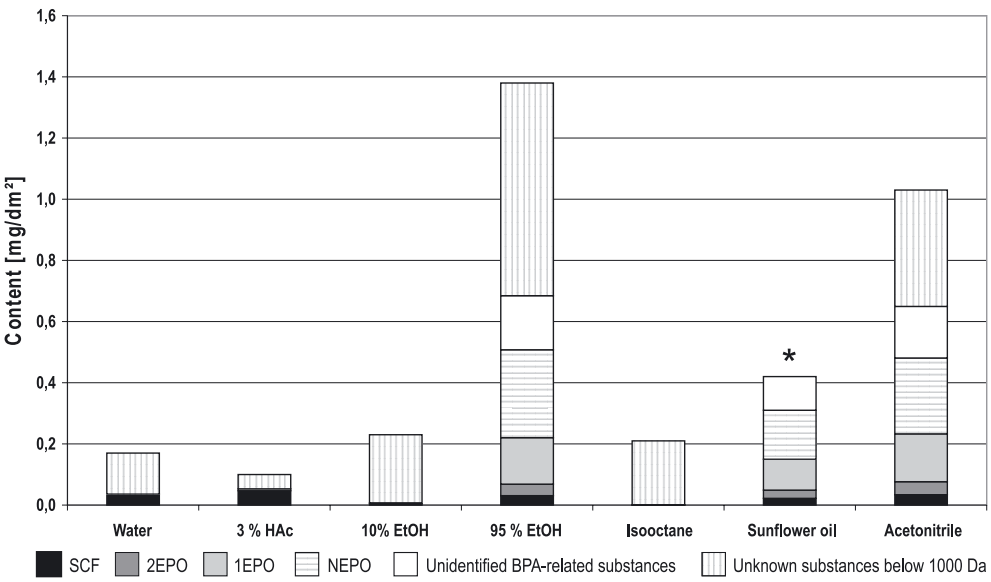


Figure 7.4. Comparison of the amount of BPA-related substances in the part below 1000 Da obtained from migration into different approved simulants and acetonitrile classified due to their toxicological relevance into SCF (substances regulated by SCF and 2002/16/EC), 2EPO (oligomers containing two oxiran rings), 1EPO (derivatives containing one oxiran ring) and NEPO (derivatives without oxiran rings),
*amount of overall migrate below 1000 Da is unknown

The migration into the substitute simulant 95 % ethanol was 60 % higher than in the oily simulant as well as nearly the same as into the solvent acetonitrile and is therefore suitable for worst case simulation. The pattern of the five migrant classes was the same for sunflower oil, 95 % ethanol and acetonitrile. Since no BPA-related

substances were detectable in the isooctane migrate, it is not suitable as substitute for the simulant oil in the case of these coatings. A possible explanation is given in the paragraph above. Since both isooctane and 95 % ethanol are not suitable as substitute simulants Biedermann *et al.* (2003) described an innovative solvent mixture of 30 % butylacetate in isooctane as alternative.

7.4.4 Polyester-urethane coating

Balance of migration for 95 % and 10 % ethanol-migrates. The overall migrate was determined as 1.60 mg/dm^2 (see figure 7.2c) for the 95 % ethanol and 0.05 mg/dm^2 (see figure 7.2d) for the 10 % ethanol migrate of the polyester coating by SEC-ELSD. Although the overall migrate of the polyester coating in 95 % ethanol was about 4 times lower than the overall migrate of the epoxy coating the toxicological relevant part below 1000 Da was comparable for both coatings (1.3 mg/dm^2). However, polyester oligomers might be hydrolysed during their passage through gastro-intestinal tract. Hamdani *et al.* (2002) described a partial hydrolysis of poly(1,2-propylene adipate) in intestinal simulating solutions. Therefore, the amount of substances below 1000 Da may be underestimated since molecules with a molecular weight higher than 1000 Da could be absorbed after hydrolysis. The overall migrate in 10 % ethanol contained likewise the epoxy coating only substances with a molecular weight below 1000 Da.

The screening method for resin-related substances enabled the explanation of 40 % (0.55 mg/dm^2) of overall migrate below 1000 Da in 95 % ethanol as cyclic oligoesters (Schaefer *et al.* 2004b), whereas, nearly the complete 10 % ethanol migrate (0.048 mg/dm^2) was identified as cyclic and linear (hydrolysed) oligoesters. Free residual carboxylic acids were neither detectable in the 95 % nor in the 10 % ethanol migrate (< 0.001 mg/dm^2). No lubricants could be detected in both migrates of the polyester coating. However, in the 95 % ethanol migrate a peak in the dead volume coeluting with paraffin occurred that was not identified and not calculated.

In summary, about 60 % (0.77 mg/dm^2) of the 95 % ethanol migrate below 1000 Da are still unknown. As prospect, the injection peak of the NP-HPLC-ELSD should be identified and quantified by special GC-methods for substances with low polarity like hydrocarbons.

7.4.5 Comparison of the presented concept with other approaches and future prospects

To date legislation and most analytical work focus on the amount of migrating starting substances that have already been evaluated during their approval as food contact materials. However, especially in case of coatings and recycling materials other substances than the approved ones contribute to the migrate of the packaging to a reasonable extent. That is why analytical screening concepts are needed for the substance groups frequently used for coatings. Due to analytical feasibility and to concentrate on the toxicological relevant part of the migrate the current concepts mainly focus on migrating substances below 1000 Da (see figure 7.5).

The approach of the Kantonal Laboratory in Zürich (Grob 2004) starts with a separation of the migrants below 1000 Da using triarachin as standard for the 1000 Da border. Subsequently the major migrants are isolated and identified with final evaluation of the amount of unknowns.

The authors' concept consists of 3 screening methods including a possibility of quantification or estimation of substance groups (BPA-related, oligoesters, lubricants) and an approach for the quantification of the sum of migrants below 1000 Da.

However, the 1000 Da concept has to be considered carefully, because: The migrants could be hydrolysed during their passage through the human gastro-intestinal-tract and the amount of migrants below 1000 Da could be theoretically increased. Therefore **hydrolysable migrants** have to be tested for decomposition in saliva, gastric and intestinal fluids (Hamdani *et al.* 2002, European Commission 2001).

The application of our three developed screening methods has been more or less satisfying concerning the amount of elucidated migrants. Further work should

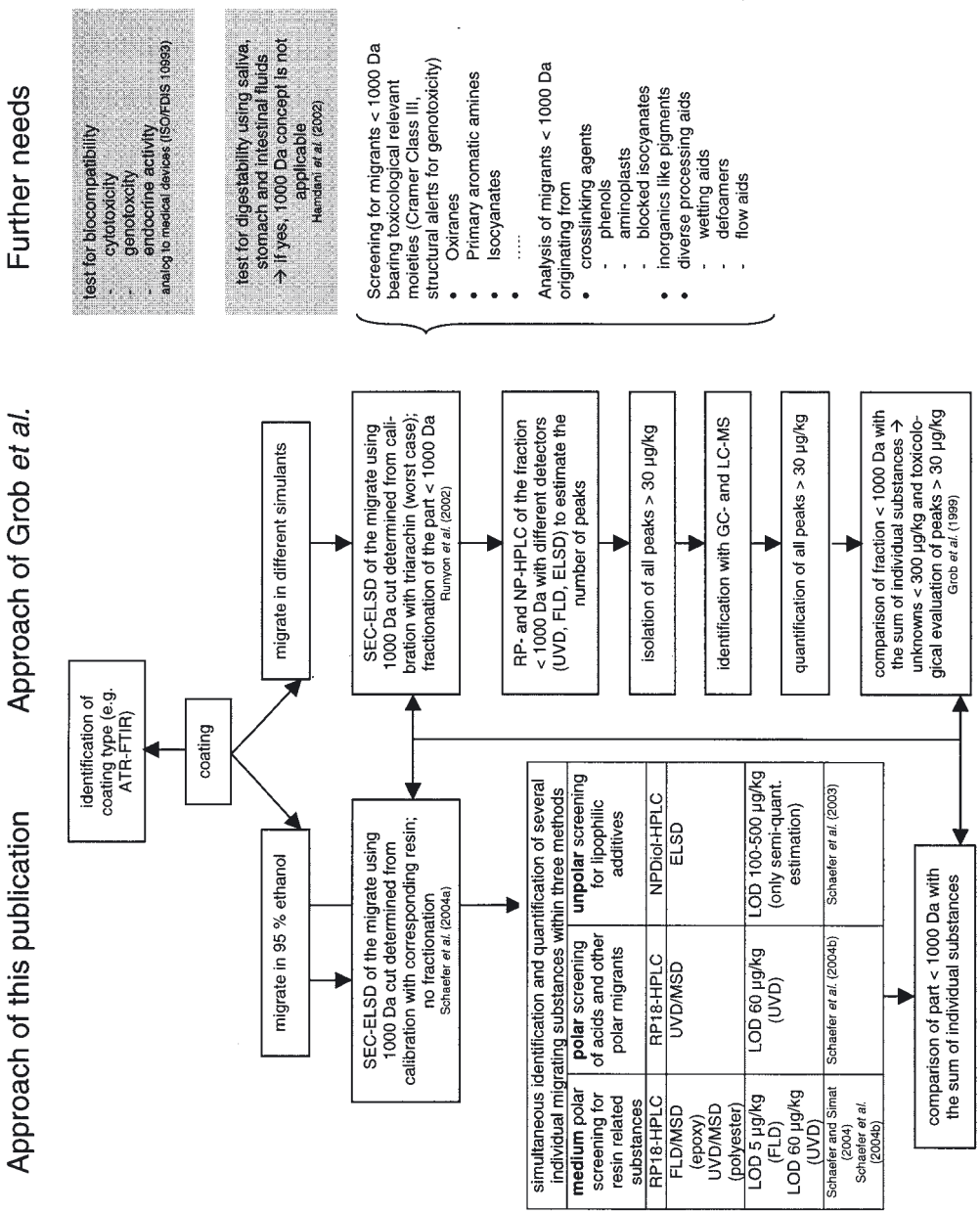


Figure 7.5. Comparison of concepts for the analytical elucidation of coating migrates and future prospects

be done to identify **crosslinking agents**. Trimellitic acid (TMA) widely used for anhydride hardened coatings was determined within the 'polar screening method'. However Noti and Grob (2004) revealed that most TMA in the coating migrates is esterified and determined 50-225 µg total TMA/sdm in commercial unfilled food cans. Besides anhydrides phenolic resins are frequently used for crosslinking purposes. To the best knowledge of the authors several methods for the determination of monomeric phenols have been published (Brauer and Funke 1992, Johnson 2001), however, nothing was published about the composition of oligomeric phenols < 1000 Da and their reaction products migrating from coatings. (Concerning amine based hardeners Fuchslueger *et al.* (1996) published a method based on the determination of derivatised (with 2,4-dinitrofluorobenzene) amines which were released from complex polyamines after hydrolysis. To the best authors' knowledge no investigations have been published about the migration of blocked isocyanates and their reactions products in coating migrates, respectively. Although additives like pigments, wetting aids, defoamer and flow aids should not make up a major part in the migrate this should be proven by adequate methods.

7.4.6 A proposal for further legal recommendations for food cans (and other food contact materials)

As already stated in the introduction the legal regulations for migrants from food can coatings are not harmonised. The only applicable directive regulates the amount of selected BADGE- and BFDGE-monomer derivatives (2002/16/EC, European Commission 2002b). Directive 2002/72/EC (European Commision, 2002a) is not applicable to can coatings, however, this may be changed within the new superdirective (European Commission 2003b).

Besides the limit for the overall migration a further limit for the sum of migrating substances below 1000 Da could be considered. It is proposed that, reliable analytical methods for substance groups of special toxicological concern (Cramer Class III, structural alerts for genotoxicity, Kroes *et al.* 2004) with a molecular weight below 1000 Da like e.g. oxirane-containing substances, primary aromatic amines and isocyanates have to be established and legal limits for these groups will be appropriate in addition

to the limits for starting materials. The directive 2002/16/EC (European Commission 2002b) follows a similar concept, limiting amongst other related substances Novolac glycidyl ethers (NOGE) below 1000 Da with at least one intact oxirane ring.

The structural identification and quantification of migrants is the first step of the analysis of coating/food-interactions. Further toxicological evaluation of these migrants is needed in order to ensure consumer safety. In case of identified migrants this can be enabled by comparison of the chemical structure with databases due to estimate structurally related toxicological effects (QSRT) or by application of the concept of structure-based thresholds of toxicological concern (TTC, Kroes *et al.* 2004)

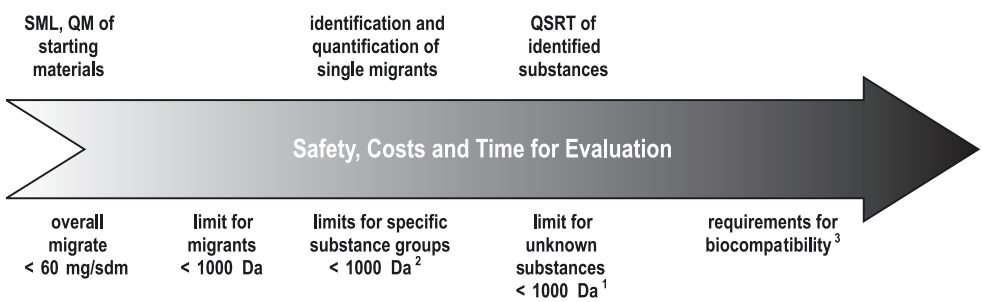
However, even the best concept for analysis of migrates will remain some substances unknown or undetectable. Therefore toxicological screening tests (for mutagenicity, cytotoxicity, etc.) as proposed for medical devices (ISO/FDIS 10993-1 2003) could be applied on the overall migrate. If positive effects were detected the causative substances would have to be elucidated and subsequently evaluated. With regard to feasibility of these expensive tests they could be applied only to one representative of a product line (e.g. one binder/hardener-system). The effects of different used additives and change of raw materials can be estimated by comparison of fingerprints (HPLC-UVD/MSD) without further biocompatibility tests. Figure 7.6 is summarising the proposed concept for hazard identification of materials in food contact.

7.5 Conclusion

This investigation has been focussed on the migrating substances from polyester and epoxy coatings, which are besides organols the most frequently used coating types. By analysis of binder resin-related substances, lubricants and organic acids in the 10 % and 95 % ethanol migrates, respectively, about 42 to 96 % of the migrate below 1000 Da could be elucidated. Thereby, more than 80 % of the resin-related substances have been structurally identified. The fact that nearly 60 % of the migrate below 1000 Da in the 95 % ethanol migrate of the polyester coating remained unknown is dissatisfying.

A reasonable uncertainty of the determined values especially due to the substance depending response of the ELS-detector (determination of the overall migrate below 1000 Da and of the lubricants) has to be considered when discussing the amount of unknowns. Moreover this analysis scheme should be enlarged by the determination of hardener components (especially for coatings cured with phenols or aminoplasts) and of other additives like pigments, catalysts, defoamers and flow aids in order to enhance the comprehensiveness. On the other hand screening methods for substances of toxicological concern below 1000 Da should be established. A different approach would apply toxicological screening tests for identification of potential hazards and subsequent elucidation of the causing agents as well as their evaluation.

Nevertheless, this publication presents one of the first approaches to identify the actual migrating substances from coatings below 1000 Da.



[1] limits of detection may be calculated from the concept of structure-based thresholds of toxicological concern (TTC) compared with an estimate of human exposure (Kroes *et al.* 2004) for certain groups like epoxides homologues and isomers (e.g. NOGE) should be calculated in sum

[2] substances of Cramer class III (e.g. epoxides, isocyanates) (Kroes *et al.* 2004), which are suspected to migrate from the food contact material

[3] broad spectrum of toxicity tests applied on the overall migrate, e.g. mutagenicity, cytotoxicity, endocrine disruption (if positive, causative agents should be identified and evaluated) (according to the concept for medicinal devices (ISO/FDIS 10993-1, 2003))

Figure 7.6. Concept for hazard identification of materials in food contact

7.6 Acknowledgements

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7.7 References

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8

Concluding statement

8.1 Research intention

Metal-based containers are normally coated internally in order to prevent metal corrosion occurring, particularly with aggressive food ingredients. However, interactions between packaging surface (coating) and packed foodstuff take place. This thesis focuses on the mass transfer (migration) from coating components into foodstuffs. Although many methods have been published previously for the identification and quantification of single migrants or the total migrate (see chapter 1.6) this thesis has been the first approach for establishing of a comprehensive analysis concept (see figure 8.1) considering legal requirements (global migration limit, specific migration limits, absorption barrier of 1000 Da and threshold of

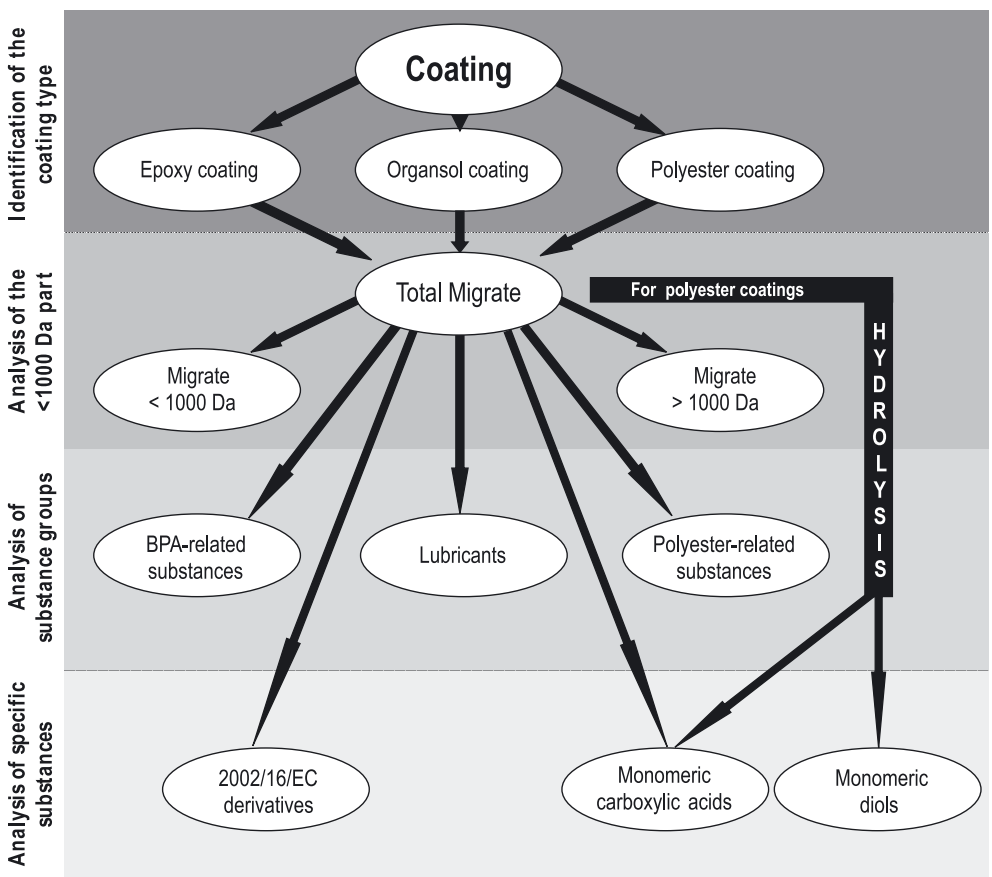

Figure 8.1. Analysis concept as concluding statement of all methods

regulation) and applicability (time, equipment, costs). The analysis concept includes the identification of the binder-hardener system, the determination of the total migrate and the part below 1000 Da as well as the elucidation of this toxicologically relevant part as components traced back to the resin, the added lubricants and hardeners.

8.2 Identification of the coating type

Since the **binder-hardener systems** of all analysed samples were known, a preceding identification of the coating type was not necessary. However, the analysis concept should be applicable to samples with unknown composition. Therefore, the analysis of the binder type by attenuated total reflection-fourier-transformation-infrared-(ATR-FT-IR)-spectroscopy can be switched prior to the determination of the migrants as successfully used by Petersen *et al.* (2002). A special atlas of spectra containing various exemplary spectra and flow-diagrams for the identification of the coating type is available (Brezinski 1991). In case of insufficient significance the presence of functional groups can be confirmed by chemical reactions (Krause *et al.* 1983).

Epoxy-containing coatings (also including epoxy-stabilised organsols) can be identified using the IR-signals at 1510 cm^{-1} and 1250 cm^{-1}, which are characteristic for an ether function, as well as the IR-signal at 830 cm^{-1}, which represents p,p-substituted aromatic rings. Polyester-based coatings can be identified using the IR-signals at 1723 cm^{-1} and 1240 cm^{-1}, which are characteristic for a carboxylic acid alkyl ester, as well as the IR-signals, which represent substituted aromatic rings, e.g. 730 cm^{-1} for esters based on isophthalic acid.

8.3 Overall migrate and part below 1000 Da

The **overall migrate** covers the total amount of non-volatile substances traced back to the mass transfer between the packaging and the foodstuff or simulants,

respectively. Due to the fact that the reference method based on gravimetry volatile substances are not included into this definition. Since only few volatile, potentially migrating substances remain in the coating at curing temperatures above 180 °C this value provides information about the total mass transfer and therefore the suitability for materials intended to come into food contact.

Considering the Scientific Committee on Food (SCF, European Commission 2001) emphasising the need to determine the identity, quantity and the toxicological profile of all potentially absorbable **substances with a molecular weight below 1000 Da** this part of the total migrate is important for the evaluation of the safety of a coating.

8.3.1 Overall migrate

The Council of Europe Coating's Resolution AP(96)5 (1996) imposes a limit of 10 mg/dm^2 (60 mg/kg) for the sum of substances migrating from food contact coatings which can be controlled by the gravimetric reference method of the European Committee for Standardisation (EN 1186, CEN 2002a). The method developed and introduced in this thesis enables the determination of the global migrate using analytical size exclusion chromatography (SEC) followed by evaporative light scattering detection (ELSD). Since the ELSD detects all non-volatile substances the amount of the total migrate can be calculated from the total area of the SEC-chromatogram. The ELSD was calibrated using a dissolved binder resin. Both major binder resins, epoxy and polyester gave the same response in the ELSD. This SEC-ELSD method was critically compared with the reference method (slightly modified and in-house validated) in consideration to accuracy, precision, detection limits as well as applicability.

The SEC-ELSD-method provides a better reproducibility (0.12 mg/dm^2, gravimetry 0.16 mg/dm^2), a lower limit of detection (0.04 mg/dm^2, gravimetry 0.35 mg/dm^2 in-house validated) and is less time consuming. However, the SEC-ELSD method applied to the total migrate of samples from five different coating types gave significantly lower values compared to the official gravimetric method (EN 1186, CEN 2002a):

the values corresponded to 65-90 % of the gravimetrically determined values. The differences between gravimetry and SEC-ELSD can be explained by a lower response of the ELS-detector for substances other than binder components or by too high values obtained by gravimetry due to inclusion of solvents (a weight increase of 8 % was observed after drying of an epoxy resin solution in THF /95 % ethanol).

Actually, the SEC-ELSD is not an equivalent substitute for the gravimetric reference method due to significantly lower results. However the new method still provides coherent results for coatings of similar chemistry and exhibits a helpful tool for coating development and optimisation.

8.3.2 Migrating substances with a molecular weight below 1000 Da

The SCF (European Commission 2001) mainly focuses on the potentially absorbable migrants with a molecular weight below 1000 Da for which neither a legal limit nor an official method has been established to date. However, a draft method exists for their determination using SEC and an appropriate detection (prCEN/TS 14577, CEN 2002b). Thus, two analytical approaches were compared: firstly the use of the SEC separation of the total migrate by preparative SEC followed by determination of the fraction below 1000 Da and secondly the use of the SEC-ELSD method as described above which enables the simultaneous determination of the total migrate and the portion below 1000 Da. Both methods were compared whereas in this case both methods are equivalent since both are not official methods. The extended gravimetric method for the portion below 1000 Da is very time consuming and results in a lower reproducibility (0.10 mg/dm^2, SEC-ELSD 0.035 mg/dm^2) and a higher limit of detection (0.35 mg/dm^2 gravimetry in-house validated, SEC-ELSD 0.04 mg/dm^2). Since the amount of migrating substances in all aqueous simulants (see chapter 2) was below 0.35 mg/dm^2 the gravimetric method is not suitable for these simulants. The values obtained by the SEC-ELSD method were significantly lower for substitutes of the simulant oil compared to those measured gravimetrically.

8.3.3 Molecular weight calibration of the SEC columns

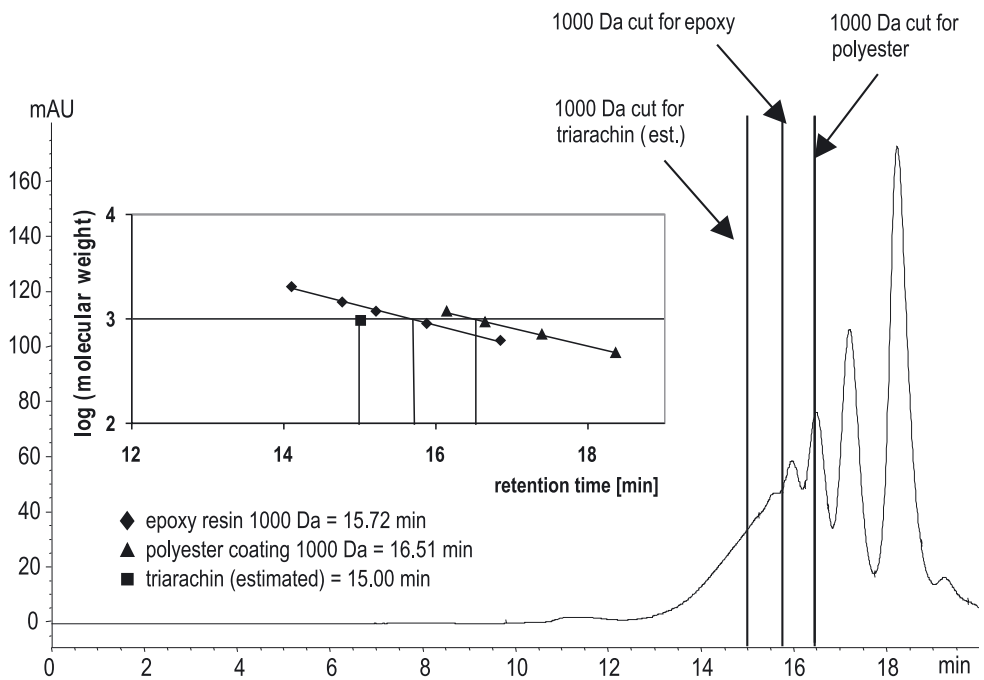

Figure 8.2. SEC-chromatogram of a polyester coating, comparison of the part below 1000 Da using different exclusion cuts

Besides the type of mass detection (gravimetry or ELSD) the determination of the exclusion cut (1000 Da) is critical for the accuracy of the methods. The SEC does not separate by the mass but by the shape of the molecule. The SEC column was calibrated using a low molecular weight epoxy resin and an acetonitrile extract of the polyester coating since a low molecular weight polyester resin containing cyclic oligomers was not available. Thus, a difference of 0.8 min of the calculated 1000 Da cut has been observed for the linear shaped bisphenol A (BPA)-related migrants from epoxy coatings and cyclic migrating oligoester from polyester coatings. Runyon *et al.* (2002) determined the SEC 1000 Da cut using triarachin, which has a molecular weight near 1000 Da and proved to exhibit the shortest retention time per unit molecular weight in SEC compared with different

oligomer types (see figure 8.2). Migrating substances with a molecular weight up to 2000 Da are incorrectly included in the below 1000 Da part, but those below 1000 Da are never excluded.

In order to gain a higher degree of accuracy and to enable a balance between the determined single components and the part below 1000 Da in this thesis the exclusion cut determined from the components of corresponding resin types was applied.

8.4 Group-specific methods

After the separation of the potentially absorbable substances from the total migrate this toxicologically relevant part should be elucidated using several methods for specific migrants and migrating substance groups. The most important group in the total migrate below 1000 Da is made up by **migrants originating from the binder resin** (epoxy- or polyester-systems).

Lipophilic substances are used for internal and external lubrication (see chapter 1.3.2) in order to facilitate the deep-drawing process and to minimise the adhesion between the packaging itself as well as to the filled food. The **lubricants** used (e.g. oil or fat) are technical mixtures and can be classified according to their polarity.

8.4.1 Epoxies

A single binder-related substance, bisphenol A-diglycidyl ether (BADGE), attracted attention in the nineties of the last century. BADGE and its hydrolysis products were foremost identified in migrates of aqueous simulants from epoxy-based coatings by Simal Gándara *et al.* (1992). Biedermann *et al.* (1996) discovered a high concentration of BADGE in the oil phase of canned fish while checking the authenticity of olive oil. Residues of epoxy adhesive components were also found in microwave susceptors (Sharman *et al.* 1995). In the past five years many methods were published for the determination of the BADGE-derivatives regulated since

2002 by Directive 2002/16/EC (European Commission 2002a) for epoxy-based food contact materials. However, likewise stated by Biedermann *et al.* (1998), migrants from epoxy coatings hardly contain any of the approved monomers but several reaction products with solvents or other additives.

The presented reverse phase-high performance liquid chromatographic (RP-HPLC)-separation was optimised for the highest resolution of all BADGE-adducts below 1000 Da in an acceptable time for analysis (max. 80 min). A gradient slope of 1 %/min provided less coelution of migrants than the rapid separation method (gradient slope 3.3 %/min) as described by Berger and Oehme (2000), who analysed BADGE-derivatives up to 2000 Da. The structure of the detected peaks was elucidated using an electrospray ionisation-mass selective detector (ESI-MSD) and their identity was confirmed by micro-syntheses of monomeric and dimeric BADGE with solvents and phenols, which provided a small data base including characteristic masses and relative retentions of 42 different BPA-related substances.

Due to its high sensitivity and selectivity fluorescence detection (FLD) was used for the quantification of the BPA-related substances as BADGE-equivalents. It had to be proven that the fluorescence activity only depends on the BPA-moiety in the molecule as previously assumed by Biedermann *et al.* (1998). Therefore, BADGE-dimer and -trimer were isolated in high amounts (55 mg dimer and 82 mg trimer) and additionally BADGE·2BuEtOH (BADGE reacted with two equivalents butoxyethanol, 48 mg) was synthesised. The difference of FLD-response between mono-, di- and trimer was less than 10 %.

However, the proportion of the fluorescence active BPA-moiety in other BADGE-adducts varies from 36 % in BADGE·2tBuPh to 86 % in BADGE·2BPA. Hence, the comparison of the calibration curves of BADGE and synthesised BADGE·2BuEtOH confirmed that the quantification of BADGE-adducts as BADGE-equivalents irrespective of the individual substance is inaccurate. Since the structural information (molecular weight and number of BPA-moieties/molecule) could be obtained by MSD, the difference of the proportion of the fluorescence-active moiety can be eliminated by using a calibration of the chromophore concentration

instead of the substance concentration. Therefore, the substance concentration was transformed by multiplication with the number of BPA-moieties in the molecule and subsequent division with the molecular weight.

The developed method using BADGE-equivalents was applied to epoxy-coatings and revealed that about 50 % (0.4 to 0.7 mg/dm^2) of the total migrate below 1000 Da consisted of BPA-related compounds which were classified according the number of epoxy groups in the molecule (SCF = substances regulated by SCF and 2002/16/EC, 2EPO = oligomers containing two epoxy groups, 1EPO = derivatives containing one oxirane ring and NEPO = derivatives without any oxirane ring). Monomeric BADGE represented less than 3 % of the BPA-related compounds, whilst 15-60 % consisted of compounds below 1000 Da with at least one oxirane ring. Consideration should be given to assess the relevance of these oxirane containing species below 1000 Da, since no toxicological information about these substances exists other than that obtained by extrapolation of the toxicological data on BADGE.

Furthermore, the sum of BPA-related substances below 1000 Da was also calculated using structural information and chromophore concentration. The difference between the results of both quantification methods is not significant due to identified migrants with a higher proportion of the fluorescence active moiety in the molecular than BADGE (e.g. cyclic-DiBADGE) and also identified migrants with a lower proportion (e.g. BADGE·BuEtOH).

Since the combined method requires an HPLC-MSD system and is time consuming as well as the difference between the results for real coatings has not been shown to be significant only the method for the determination of BPA-related substances below 1000 Da as BADGE-equivalents was fully validated. It is a sensitive (0.1 µg/dm^2 limit of detection in the FLD, estimated at a signal-to-noise ratio of 3), selective (due to the MSD) and precise (24 µg/dm^2 standard deviation) method. Due to its low detection limit the threshold of toxicological concern (see chapter 1.5.1) was considered for the first time during application of a calculation limit of 0.8 µg/dm^2 or 5 µg/kg for the individual substance.

8.4.2 Polyester

Polyester-based coatings have been commercially available for more than 30 years mostly cured with phenolic resins. Polyester-urethanes have been used commercially for internal coatings since 1987. The use of polyesters increased since they substitute epoxy-based systems, particularly during the last five years. Likewise, adhesives for lightweight containers are based on polyester-urethane systems. The object of recent research was the development of an analytical method for all representatives of the group consisting of polyester-related components below 1000 Da in a similar approach as for BPA-related substances. Since investigations concerning migrants from polyester-based coatings or adhesives have not been published before, the best references are publications describing methods for the determination of migrants from polyethylene terephthalate (PET). Begley *et al.* (1990) identified all migrants from PET as cyclic oligoesters based on terephthalic acid (TPA) and ethylene glycol (EG). Since polyester resins for can coatings or adhesives consist of different monomers (see chapter 1.3.1) cyclic oligoesters based on various acids and alcohols were expected as migrants.

The chromatographic system using the same screening gradient as described in chapter 8.4.1 separated all migrating polyester-related substances below 1000 Da. Likewise for PET, all migrants were tentatively identified as cyclic oligomers (mainly di-, tri and tetramers). Since MSD-analyses based on a single-quadrupole detection system do not provide unequivocal results for the identification of the migrants (position isomers of acids and alcohols cannot be differentiated) hydrolysis of the polyester migrate into its monomers, in accordance with the PET-hydrolysis by Castle *et al.* (1989), was developed. The resulting alcohols and acids were identified by specific methods (see chapters 8.5.2 and 8.5.3). With the knowledge of the polyester monomers it was possible to identify the main components in the migrate as cyclic oligoesters by HPLC-ESI-MSD. For further confirmation of the fragmentation pattern one cyclic oligomer, CYCLO (3IPA (isophthalic acid), 3EG) was synthesised.

The quantification of the cyclic oligoester was challenging since more than 50 substance peaks were structurally identified in only three polyester coating migrates which were traced back to 20 cyclic oligoesters and their isomers. For none of them individual standards are commercially available. A quantification method using a

calibration of a single, commercially available reference substance was required. Biedermann *et al.* (1998) and Begley *et al.* (1990) quantified all BADGE-derivatives and cyclic PET-oligomers assuming the same response factor in the FLD or ultraviolet detector (UVD) for all derivatives.

IPA and TPA, the main acids used for polyester resins for can coatings, exhibit different absorption spectra. Therefore, the wavelength (232 nm) with the identical molar absorption coefficient for both acids was determined for the quantification of different oligoesters by UVD. Considering the variety of aliphatic polyols used for polyester resins the UV-active part in the oligoester and its UVD response, respectively, depends on the molecular weight of the polyol contained in this molecule. In accordance to the quantification of the different BPA-derivatives (see chapter 8.4.1) the difference of the UV-active content can be improved by a calibration with a commercial available linear standard (e.g. bis-hydroxyethylene terephthalate) using chromophore concentration instead of substance concentration. However, it is not suitable for polyesters consisting only of aliphatic monomers, e.g. in the case of adipic acid.

The migration of the potentially absorbable oligomers from three polyester coatings into a 95 % ethanol added up to 0.1-0.8 mg/dm^2, which is comparable to the amount of BPA-related compounds below 1000 Da migrating from recent epoxy coatings into food. In consequence, the migrate below 1000 Da mainly (about 50 %) consists of components of the basic resin of the respective coating. To date nothing is known about gastro intestinal hydrolysis, absorption and physiological effects of these cyclic oligomers.

Since the UV-detection is less sensitive, the calculation limit (10 µg/dm^2) is about ten times higher than the threshold of toxicological concern (0.8 µg/dm^2, see chapter 1.5.1).

8.4.3 Lubricants

Waxes, paraffins, fats and oils as well as partial acyl glycerols or fatty acid amides are used as lubricants for internal or external applications (see chapter 1.3.2). Each lubricant consists of a technical mixture of substances with the same characteristic polar part in the molecule (e.g. triacyl glycerol backbone in fats and oils) but

different non-polar parts (e.g. different hydrocarbon chains in fats and oils). Thus, the group of lubricants was divided into twelve subgroups (lipid classes) covering all lubricant types and their by-products (paraffin, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amide).

Concerning migrating lubricants from food contact materials prior published examinations have mostly been carried out in order to determine one or two single classes using gas chromatography (GC). Paraffins were determined by Jickells *et al.* (1994) and fatty acid amides by Cooper and Tice (1995). The intention of this thesis was the development of a liquid chromatographic method for the identification and quantification of the lubricants separated into lipid classes according to their polar groups, thereby avoiding a specific differentiation into single substances forming these classes. Thus, chromatographic systems based on reverse phase columns are not suitable since these phases separate by the non-polar part in the molecule.

Elfman-Börjesson and Härröd (1997) achieved a separation of nine of these twelve classes on a normal phase (diol) column during their investigation of enzymatically synthesised lipids. Since the authors used an unusual flash chromatography with a high flow rate (3 mL/min for a column diameter of 4 mm) and several gradient steps the mobile phase was replaced. Analyses were carried out by gradient elution using isooctane and 0.1 % acetic acid in tert-butyl methyl ether. Paraffin exhibited no retention on any of the examined normal phases (silicagel, cyanopropyl, diol) irrespective of the solvent used as the mobile phase (heptane, hexane). Tert-butyl methyl ether as the polar component led to a reasonable separation of all twelve lipid classes. Although not desirable, a peak splitting of lipid classes which was observed for defined triacyl glycerols (trilaurin, tripalmitin) and two natural oils (olive oil, soybean oil) as well as for different defined fatty acid methyl ester standards (C18:0, C18:1, C18:2, C18:3) due to the different degrees of saturation and due to different chain lengths could not be completely avoided.

The detection was facilitated by the use of an ELSD. Therefore, all three parameters of the ELSD, N_2-pressure (3.5 bar), nebuliser temperature (20 °C) and vaporiser temperature (35 °C) were optimised in order to enhance the sensitivity for all lipid

representatives. The method was calibrated using a second order regression with representatives of each class in working ranges of about 5-150 mg/L, depending on the lipid class. Intra-day variances for all representatives ranged from 1.9 to 5.1 %, inter-day variances from 7.0 to 26.5 % and the limits of detection from 0.79 to 3.65 mg/L (except for two classes).

However, a comparison of the ELSD-sensitivity (calculated by slopes of the calibration curves at a concentration of 50 mg/L) for all representatives indicated that the detector response depends on the chain length and the degree of saturation. Thus, the method provides only an approximate quantitative estimation.

The sample preparation for migrants from external lubricants differs from the sample preparation for migrants from internal lubricants. Since external lubricants are located on the inner surface of the packaging, but the opposite side of the film to the metal, they migrate directly into the simulant isooctane and can be analysed after concentration. 95 % ethanol was chosen as simulant for internal lubricants in coatings. Therefore, the analytes were transferred into isooctane by using a liquid-liquid-extraction.

The developed method was applied to a commercial can coating (epoxy-anhydride) containing carnauba wax and a partial acyl glycerol as internal lubricants as well as to a laminated commercial light weight container with external (partial acyl glycerol) and internal lubrication (fatty acid amide) of the polypropylene film. The identified migrating lubricants were estimated as 0.3 mg/dm^2 for the can coating and 5.5 mg/dm^2 for the laminate excluding paraffin.

8.5 Specific methods

With regard to the present legal regulation (2002/16/EC, European Commission 2002a) some migrating **BADGE-derivatives** need a special consideration. They are already included in the method presented for the determination of all BPA-related

substances, but for the surveillance of the legal limit they have to be quantified with a more specific and valid method. Other specific methods are required for the identification of the polyester monomers. A GC-method was established for **polyols** (hydroxy-functional monomers). The polyvalent **carboxylic acids**, of which the trimellitic acid also belongs to the group of anhydride hardener monomers, were determined using a specific HPLC-method.

8.5.1 Regulated BADGE-derivatives

The migration of BADGE and its reaction products with water and hydrochloric acid has recently been limited to 1 mg/kg foodstuff by Directive 2002/16/EC (European Commission 2002a). An RP-HPLC-ESI-MSD/FLD method for the simultaneous identification and quantitation of these substances and their precursor BPA in food is presented introducing bisphenol A di-(3-hydroxypropyl) ether (BADHPE) as an internal standard.

The introduction of an internal standard leads to the elimination of losses in analyte recovery and the enhancement of accuracy of results. BADHPE does not occur in migrates and is detectable by FLD as well as by MSD. The internal standard bisphenol F diglycidyl ether used elsewhere (Summerfield *et al.* 1998) is not appropriate, because it might also occur in migrates from can coatings and it is a mixture of three isomers giving three single peaks under the chromatographic conditions used.

Concerning the chromatographic separation, numerous RP-HPLC methods including all relevant BADGE derivatives were published (Biedermann *et al.* 1999, Lintschinger and Rauter 2000). However, both systems did not include an internal standard or BPA and Lintschinger and Rauter (2000) did not achieve a separation of all BADGE derivatives by using one system. They introduced a second chromatographic separation under isocratic conditions for the resolution of BADGE·H_2O and BADGE·HCl·H_2O. The use of a ternary gradient (organic modifier: methanol/acetonitrile, 2/1, v/v, buffer: ammonium formate) enabled the separation of all eight substances within an acceptable analysis time (30 min).

Fluorescence detection (275 nm/305 nm) was used in order to achieve the lowest limit of detection and minimal interference by the matrix. The fluorescence intensity of all derivatives was shown to be equal and previous assumptions proved to be valid (Biedermann *et al.* 1999), but the synthesis and use of individual reference substances represents a significant enhancement of the validity of the developed method. Since 2000 reference standards are commercially available. The calculated detection limits (according to DIN 32645) ranged from 50 µg/kg to 120 µg/kg fatty foodstuff or from 10 to 20 µg/kg fatty foodstuff calculated by a signal-to-noise ratio of 3. However, estimated detection limits for food simulants calculated were the same as for the epoxy screening method since the same detector and sample preparation were used (0.1 µg/dm^2). Variation coefficients ranged between 2.5 % and 5 % for food simulants and between 6 % and 15 % for fatty foodstuff. Additional information in the case of suspect samples was obtained by MSD using selected ion monitoring for characteristic molecular ions and fragments.

Sample preparation was done by lipid extraction with diethyl ether as a moderately polar solvent after removal of water using kieselguhr, followed by lipid-lipoid-extraction using acetonitrile and clean-up by RP18-solid phase extraction. High and equal recovery rates (91-108 %) for all analytes over a broad range of polarity were achieved after internal standard correction. The extensive investigations led to a simple, rapid and valid method for the identification and quantification of BADGE and its derivatives in various food matrices. Thus, the enforcement of legal restrictions is possible for both producers and control authorities. The introduction of HPLC-MSD for additional identification and an internal standard for the enhancement of method precision and elimination of analyte losses make up the most important improvements compared to previously published methods.

8.5.2 Carboxylic acids

Carboxylic acids may occur in migrates from food packagings as residues from polyester resin monomers (three phthalic acid isomers and adipic acid) or from anhydride hardeners (trimellitic anhydride or acid). The Directive 2002/72/EC (European Commission 2002b) limits the residual amount of some acids in the

packaging (quantitative maximum, QM(t)) or their specific migration (specific migration limit, SML): trimellitic acid (QM(t) = 5 mg/kg), isophthalic acid (SML = 5 mg/kg) and terephthalic acid (SML = 7.5 mg/kg). However, this directive compromises only plastic food contact materials and does not regulate coatings or light weight containers. A specific HPLC-method is needed for the identification and quantification of these carboxylic acids. Since all polyvalent carboxylic acids react like a chelating agent, a separation could only be achieved on a special (free of heavy metal ions) RP-18 column with an ammonium formate buffer. In addition the UVD, MSD was used in order to detect the sparse UV-active adipic acid. Whilst this method was only used for qualitative purposes with regard to the identification of the polyester monomers, it had to be validated in order to quantify free residual trimellitic acid in migrates from epoxy-anhydrides. Due to the limit of detection of 1 µg/dm^2, this method enables the quantification of free carboxylic acid under consideration of the legal limits and threshold of toxicological concern for single migrants (0.8 µg/dm^2, see chapter 1.5.1).

8.5.3 Polyols

Polyvalent alcohols are used as monomers for polyester resins indended to come into contact with food. A specific GC-method using a flame ionisation detector (FID) was applied for the identification of these monomers in polyester resins. The polyols are mostly aliphatic (ethylene glycol, butandiols) or cyclo-aliphatic diols (cyclohexyl dimethanol) as well as triols (trimethylolpropane). Due to their significantly varying boiling points, the temperature program with a high slope on a special column (Poraplot Q HT) was used for the separation of all ten polyols in a single chromatographic run.

This method was only used for qualitative purposes with regard to the identification of the polyester monomers, therefore it was not fully validated. However, the GC-FID method was optimised in order to achieve acceptable detection limits which enabled the identification of the polyols obtained after hydrolysis.

8.6 Balance of migration

Various analytical methods have been developed for the determination of migrating binder components below 1000 Da (chapter 3, 4 and 6) hardeners residues or polyester monomers (chapter 3) and lubricants (chapter 5) for a comprehensive elucidation of migrates below 1000 Da from epoxy- and polyester-based coatings or adhesives, respectively. A **mass balance of migration** (see figure 8.3) was achieved by comparing the sum of the individual values for these migrate components with the value determined for the total migrate below 1000 Da (chapter 2).

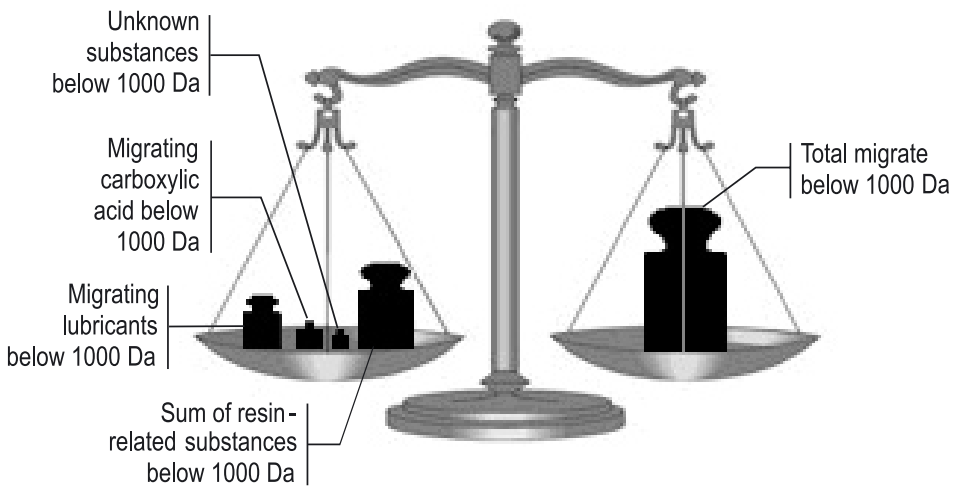

Figure 8.3. Balance of migration

A complete mass balance was calculated for two standard can coatings, an epoxy-anhydride and a polyester-urethane coating. Detailed quantitative results are presented in chapters 7.4.3 and 7.4.4 for the 10 % and 95 % ethanol migrates of these coatings. About 20 % (0.32 mg/dm²) of the total migrate below 1000 Da in 95 % ethanol and about 30 % (0.07 mg/dm^2) in the 10 % ethanol migrate are still unknown for the investigated epoxy-anhydride coating. The total migrate below 1000 Da of the polyester coating in 10 % ethanol was almost completely (96 %) elucidated whereas about 60 % (0.77 mg/dm^2) of the 95 % ethanol migrate below 1000 Da are still unknown.

Concerning the validity of the balances the variances of the applied methods have been discussed in chapter 7.4.3. Therefore, the calculated amount of unknowns for the total migrate below 1000 Da of the epoxy coating in 95 % ethanol may be within the uncertainty of the applied methods, especially because of the high coefficient of variance of the lubricant determination.

A significant unknown residue was calculated for the total migrate below 1000 Da of the epoxy coating in 10 % ethanol, but can be estimated below 0.1 mg/dm^2. Thus, in both migrates of the epoxy coating unidentified single components up to 0.05 mg/dm^2 are still anticipated.

However, one major component of polyester migrate in 95 % ethanol is still unknown that could not be explained by the uncertainties of measurements concerning quantification methods.

Moreover, the uncertainty of the exclusion cut has to be considered. Substances with a specific molecular weight elute on the SEC over a period of time (not at specific point of time) due to their different three-dimensional structure and their interaction with the stationary phase. Therefore, an exclusion cut set at 1000 Da due to the calibration of one substance group (here the binder resin) will always exclude some substances below 1000 Da and will also include some substances above 1000 Da, but in most cases the amounts of incorrectly excluded and included substances are the same. Moreover, this cut will be different to the cut for other migrating groups with a different molecular structure (see figure 8.2). Furthermore, the determination of the specific migrants below 1000 Da was done from simulant solutions containing the overall migrate without prior separation. Since all migrants are dissolved this technique enabled the quantification of all toxicologically relevant migrants. Therefore, the scale of figure 8.3 cannot be balanced in some cases.

The Swiss working group analysed migrants below 1000 Da after pre-separation by SEC (Biedermann *et al.* 1998). For the problem of quantification of all specific migrants below 1000 Da they proposed a worst case cut using an standard (triarachin) exhibiting the shortest retention time for calibrating of the 1000 Da cut (Runyon *et*

al. 2002). Thus, the total migrate below 1000 Da could be greatly overestimated, which might lead to a wrong interpretation about the toxicologically relevant part. The scale could be balanced if they would quantify all migrants in the separated fraction independent of their molecular weight.

Grob *et al.* (1999) proposed a limit for the sum of unknown migrating substances below 1000 Da of 300 µg/kg (which equates to 10 to 50 µg/dm^2 depending on the size if the packaging). For the first time five different methods were applied to elucidate the total migrate below 1000 Da. It could be shown that for aqueous simulants the sum of unknowns is below (polyester coating) or near (epoxy coating) this limit. However, at least 0.3 mg/dm^2 are still unknown for fatty food simulants migrants, this is ten times higher than the limit requested by Grob *et al.* (1999), which seems unrealistic at this point of time.

8.7 Perspectives

8.7.1 Epoxy-based coatings

The analysis concept has been almost comprehensive for the 95 % ethanol migrate of the epoxy-anhydride-system (see figure 7.2a, above 75 % elucidation). However, another RP-HPLC-UVD-screening method has been applied covering in the gradient the polar range from 10 % to 60 % acetonitrile. Some new UV-active peaks were detected whose identity and quantity has to be elucidated.

Furthermore, a ‘gap’ is the injection peak in the lubricant screening method. This peak might be traced back to higher boiling hydrocarbons (boiling point below 180 °C) which are components present in technical mixtures of solvents, since they may not be completely evaporated during the curing at about 190 to 200 °C. During the presented mass balance the amount of this peak was not considered. An application of a specific GC-MSD or GC-FID technique would lead to further elucidation.

Considering the application of this concept to epoxy systems with another crosslinking agent than anhydrides (phenol- and aminoplasts) methods for identification and quantification of migrants from these hardeners are not established to date. Since phenols are not easily ionisable in the LC-MS-system and since various phenol-group-containing derivatives below 1000 Da are possible, a derivatisation of these phenols to dimethylglycine derivatives could enable another group-specific method (Johnson 2001). Concerning amine based hardeners Fuchslueger *et al.* (1996) published a method based on the determination of derivatised (with 2,4-dinitrofluorobenzen) amines which were released from complex polyamines after hydrolysis.

8.7.2 Polyester-based coatings

The amount of elucidated substances from the 95 % ethanol migrate of the analysed polyester-urethane system is much lower (see figure 7.2c below 50 %). Here, the identification of the mentioned injection peak (see chapter 7.4.4) should be the next step. Polyester resins used for coating lacquers are commonly dissolved in mixtures of hydrocarbons, some with high boiling points, prior to the addition into liquid lacquer composition. The presence of free isocyanates from the urethane-system is not probable, but can be confirmed using a specific HPLC-FLD method after derivatisation with 1-(2-pyridyl)-piperazine as developed by Steinhart *et al.* (2003).

8.7.3 Organosol coatings

In this thesis only migrants from epoxy-based stabilisers have been investigated. Migrants from the polyvinyl chloride binder have to be determined using ELS detection and mass selective detection for the identification of chlorides due to their isotopic pattern.

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Lebensmittelchemische Gesellschaft, Regionaltagung Nord, 18th March 2002, Kiel-Kronshagen, Germany.

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