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Chemical migration from packaging materials into foods. Method development and validation

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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ ΔΙΠΛΩΜΑΤΟΣ ΕΙΔΙΚΕΥΣΗΣ

Χημική μετανάστευση από υλικά συσκευασίας σε τρόφιμα. Ανάπτυξη και επικύρωση μεθόδου

ΣΤΥΛΙΑΝΗ – ΦΑΝΗ Π. ΜΕΓΡΕΜΗ

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ABSTRACT

The use of novel food packaging materials has increased the number of occurring hazards due to the migration from packaging material to food. Commonly, polymers have been investigated for the potential migration of substances. The regulations and the directives of the EU tend to become stricter and great effort has been made to uniformly utilize food simulants and testing conditions for migration studies. Furthermore, the list of hazardous monomers, oligomers, and additives continues to grow in order to comply with HACCP requirements and assure consumers' safety.

A comprehensive overview of overall migration is presented. Special focus is made on plastic packaging materials which are mostly used as packaging materials globally. However, several substances from plastics have caused great health problems such as bisphenol-A (BPA) in baby bottles. For this reason, Regulation (EU) 321/2011 was issued regarding the restriction of Bisphenol A use in plastic infant feeding bottles. It has to be noticed that, the determination of Bisphenol-A belongs to specific migration experiments which differ from overall migration experiments.

In our study, three methods for overall migration were developed and one of these was validated. In total immersion method the plastic was immersed in a specific glass tube, in cell method the plastic was put in a specific cell for migration tests and in tenax method the plastic was put in specific dishes (petri dishes) to come into contact with the food simulant. The tested samples, were obtained by Yotis S.A company (BOPP, LDPE, PE), by a market place ("intestine"), German reference office (PA) and Fapas proficiency testing provider (Nylon film,PA) and were tested with three different food simulants. Simulant A (10% EtOH), fatty food simulant (95% EtOH) and modified polyphenylene oxide (MPPO-Tenax) were used according to the Regulation (EU) 10/2011.

The intestine showed the highest overall migration (16,6 mg/dm^2) among all the materials tested. The three packaging materials provided by Yotis S.A showed no overall migration. In this aspect, validations experiments could not be performed. Most importantly, the lab took part in two different proficiency testing procedure where we succeeded acceptable results in both of them (z' score < 2).

In conclusion, the results obtained from the present work proved that the reliability of the methods developed as the demands criteria of ISO 17025 were satisfied. Also, this work

illustrates the importance of studying overall migration from plastic packaging materials in order to protect the human health and improve the shelf-life of food.

SUBJECT AREA: Food chemistry

KEYWORDS: chemical migration, overall migration, plastics packaging materials, total immersion, cell, tenax

ΠΕΡΙΛΗΨΗ

Η χρησιμοποίηση νέων υλικών συσκευασίας τροφίμων αύξησε τον αριθμό των κινδύνων που προκύπτουν λόγω της μετανάστευσης από το υλικό συσκευασίας στα συσκευασμένα τρόφιμα. Τα πολυμερή έχουν, κυρίως, μονοπωλήσει το ενδιαφέρον των δοκιμών μετανάστευσης και του πειραματισμού. Οι κανονισμοί και οι οδηγίες της ΕΕ τείνουν να γίνονται αυστηρότεροι και δίδεται έμφαση στην επίτευξη συναίνεσης όσον αφορά τους προσομοιωτές τροφίμων και τις συνθήκες δοκιμών για μελέτες μετανάστευσης. Επιπλέον, ο κατάλογος των επικίνδυνων μονομερών, ολιγομερών και προσθέτων συνεχίζει να αυξάνεται, προκειμένου να διασφαλιστεί ότι η ασφάλεια των καταναλωτών βρίσκεται σε συμφωνία με το σύστημα HACCP.

Σε αυτή τη διπλωματική εργασία παρουσιάζεται μια γενική εικόνα της συνολικής μετανάστευσης. Ιδιαίτερη έμφαση δίνεται στα πλαστικά υλικά συσκευασίας που είναι αυτά που χρησιμοποιούνται περισσότερο σε πολλά τρόφιμα από πολλές χώρες. Ωστόσο, υπήρξαν αρκετές ουσίες από πλαστικά που προκάλεσαν μεγάλα προβλήματα υγείας όπως η δισφαινόλη-Α (BPA) σε πλαστικές φιάλες για βρέφη. Για αυτό το λόγο εκδόθηκε ο κανονισμός (ΕΕ) 321/2011 σχετικά με τον περιορισμό της χρήσης δισφαινόλης Α σε πλαστικές φιάλες για βρέφη. Ωστόσο, ο προσδιορισμός της δισφαινόλης-Α ανήκει σε πειράματα ειδικής μετανάστευσης τα οποία διαφέρουν από τα πειράματα ολικής μετανάστευσης.

Στη μελέτη αυτή αναπτύχθηκαν τρεις μέθοδοι για την ολική μετανάστευση και μία από αυτές επικυρώθηκε. Στην μέθοδο ολικής εμβάπτισης το πλαστικό βυθίστηκε σε ένα συγκεκριμένο γυάλινο σωλήνα, στην μέθοδο με τα ειδικά κελιά το πλαστικό τοποθετήθηκε σε συγκεκριμένο κελί για δοκιμές μετανάστευσης και στη μέθοδο tenax που είναι η λιγότερο χρησιμοποιούμενη στα πειράματά μας το πλαστικό τοποθετήθηκε σε συγκεκριμένα τρυβλία (τρυβλία Petri) για να έρθει σε επαφή με τον κατάλληλο προσομοιωτή. Τα δείγματα που εξετάστηκαν, προμηθεύτηκαν από την εταιρία Γιώτης (BOPP, LDPE, PE), από ένα εμπορικό κατάστημα (υλικό συσκευασίας για λουκάνικα, «έντερο»), το Γερμανικό γραφείο αναφοράς DRRR (PA) και τον εξουσιοδοτημένο φορέα διοργάνωσης διεργαστηριακών δοκιμών Fapas (Nylon film, PA) και δοκιμάστηκαν με τρεις διαφορετικούς προσομοιωτές τροφίμων. Σύμφωνα με τον κανονισμό (ΕΕ) αριθ. 10/2011, οι προσομοιωτές που χρησιμοποιήθηκαν ήταν ο προσομοιωτής Α (10% EtOH), ο προσομοιωτής λιπαρών τροφίμων (95% EtOH) και το τροποποιημένο πολυφαινυλενοξείδιο (MPPO-Tenax).

Το υλικό συσκευασίας για λουκάνικα (έντερο) ήταν το υλικό με το υψηλότερο ποσοστό ολικής μετανάστευσης (16,6 mg / dm^2) και τα υλικά από την εταιρεία Γιώτης χαρακτηρίστηκαν ως αδρανή επειδή είχαν μηδενική ολική μετανάστευση. Πραγματοποιήθηκαν δύο διεργαστηριακές δοκιμές όπου και οι δύο πραγματοποιήθηκαν με επιτυχία (z' score <2).

Συμπερασματικά, τα αποτελέσματα που προέκυψαν από την παρούσα εργασία καταδεικνύουν τη σπουδαιότητα της μελέτης ολικής μετανάστευσης από διάφορα πλαστικά υλικά συσκευασίας στα τρόφιμα για την αύξηση του χρόνου ζωής του τροφίμου αλλά και την προστασία της υγείας του καταναλωτή.

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Χημεία Τροφίμων

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: χημική μετανάστευση, ολική μετανάστευση, πλαστικά υλικά συσκευασίας, ολική εμβάπτιση, κελιά, tenax

Στην οικογένεια μου

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ΠΕΡΙΕΧΟΜΕΝΑ

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PREFACE

This master thesis was conceived and performed at the Laboratory of Analytical Chemistry (HRIC, Hellenic Research & Innovation Centre, Institute of Food Safety) in YOTIS S.A company with supervisor Dr Komaitis Efstratios in corporation with the Food Chemistry Laboratory of NKUA. I am really thankful to have the opportunity to work in a real workplace and I would like to thank all of my collaborators for providing a great working environment.

1. Migration from Food Contact Materials (FCMs)

1.1 Food Contact Materials

Food Contact Materials (FCMs) are called all type of materials and articles that come into contact with food during its production, preparation and serving, before its eventual consumption. Food contact materials are either intended to be brought into contact with food, are already in contact with food, or can reasonably be brought into contact with food or transfer their constituents to the food under normal or foreseeable use. Some examples are the containers for transporting food, the packaging materials, the kitchenware and the tableware. European legislation for food contact materials also covers materials that contact water intended for human consumption, e.g. bottles, but excludes fixed public or private water supply equipment (Panel, Materials, & Aids, 2016). They can be from variety of materials including plastics, glass, rubber, paper, ceramics, silicone, metal, etc. Often a combination of materials is used like a fruit juice carton which include layers of plastic, aluminium, paper and ink. All these food contact materials serve important functions because they protect food from microorganisms increasing the shelf -life of food(Marsh & Bugusu, 2007).

An important function of food packaging is to protect the food from external contamination by microorganisms, foreign bodies or chemical contaminants. The chemical contaminants can include the chemicals, which are generally foreign to foods, or can include chemical species present in some foods that cause taint in other foods. A typical example is cross contamination of bland biscuits from flavoring materials in citrus flavored biscuits, especially in retail displays where the two types may be in physical contact. Long-term storage in a freezer can also result in cross contamination of bland foods by highly flavored foods. In the choice of food packaging materials, the design of adequate barrier properties to prevent cross-contamination can be of great importance. Food packaging materials can themselves act as a potent source of tainting chemical species, however. This can be particularly serious in long

shelf-life foods that are stored for considerable periods of time in intimate contact with the packaging material, especially liquids - conditions which maximise the risk of migration. In addition, even when contamination of the food itself does not occur, release of odorous volatiles on opening a food pack can generate consumer complaints and should be regarded as a taint problem (*Food Taints and Off-Flavours*, 1996).

However, food safety always draws the attention both of consumers and regulatory bodies. The basic concepts for ensuring the safety of the substances migrating from FCMs into food are from the 1960–70s. Hence, control started with the substances used, as they were known and available for toxicological tests. In the early European legislations, the concept was materialised based on the evaluated substances used and the gravimetrically determined overall migration. Migration testing methods were established that enabled the control with the means available at that time, in particular simulation with solvents (to avoid analysis in food) and gravimetric measurement. Conditions were legally specified, as simulation was known to be a rather rough approximation. Lists of authorised substances were started, intended to become positive, i.e., to the exclusion of all others. Of the 17 types of FCMs listed in Regulation (European Commission (EC)) 1935/2004, plastics and regenerated cellulose were dealt with first, probably assuming that the others could be tackled soon afterwards – which was not the case. With this concept, control fitted into the procedures of the official enforcement authorities: for the FCMs of then still more simple composition, the substances used could be guessed at; the migration limits and the procedures were known. Hence, FCMs could be picked from the market and checked in the laboratory (Grob, 2017).

1.2 Legislation

The most important aspect in the use of FCMs is that they should be sufficiently inert so that their constituents neither adversely affect consumer health nor influence the quality of the food. For this reason, European Commission established laws and rules for business operators to protect the consumers. The European Union (EU) rules for food contact materials apply neither to all FCMs nor to specific materials for more specific protection of consumers.

For every food contact material there are specific substances that can be used and for their safety is responsible the European Food Safety Authority (EFSA). The safety of food contact materials must be tested by the business operators placing them on the market, and by the competent authorities of the Member States during official controls. Scientific knowledge and technical competence on testing methods is being maintained by the European Reference Laboratory for Food Contact Materials (EURL-FCM).

Regulation (EC) No 1935/2004 provides a harmonised legal EU framework. It sets out the general principles of safety and inertness for all Food Contact Materials. The principles set out in Regulation (EC) No 1935/2004 require that materials do not:

Release their constituents into food at levels harmful to human health

Change food composition, taste and odour in an unacceptable way

Moreover, the framework provides:

- for special rules on active and intelligent materials (they are by their design not inert)
- powers to enact additional European Union measures for specific materials (e.g. for plastics)
- the procedure to perform safety assessments of substances used to manufacture FCMs involving the EFSA
- rules on labelling including an indication for use (e.g. as a coffee machine, a wine bottle, or a soup spoon) or by reproducing the appropriate symbol.

Figure 2: Do not eat symbol

Figure 1: Food contact symbol

Generally, Regulation (EC) No 1935/2004 shall apply to materials and articles, including active and intelligent food contact materials and articles which in their finished state:

(a) are intended to be brought into contact with food

Or

(b) are already in contact with food and were intended for that purpose

Or

(c) can reasonably be expected to be brought into contact with food or to transfer their constituents to food under normal or foreseeable conditions of use.

Instead, shall not apply to:

(a) materials and articles which are supplied as antiques;

b) covering or coating materials, such as the materials covering cheese rinds, prepared meat products or fruits, which form part of the food and may be consumed together with this food

(c) fixed public or private water supply equipment.

The definition of intelligent food is that “Intelligent food contact materials and articles” (hereinafter referred to as intelligent materials and articles) means materials and articles which monitor the condition of packaged food or the environment surrounding the food.

According to the Regulation (EC) No 1935/2004, the materials and articles which may be covered by specific measures are:

1. Active and intelligent materials and articles
2. Adhesives
3. Ceramics
4. Cork
5. Rubbers
6. Glass
7. Ion-exchange resins
8. Metals and alloys
9. Paper and board
10. Plastics
11. Printing inks
12. Regenerated cellulose
13. Silicones
14. Textiles
15. Varnishes and coatings
16. Waxes
17. Wood

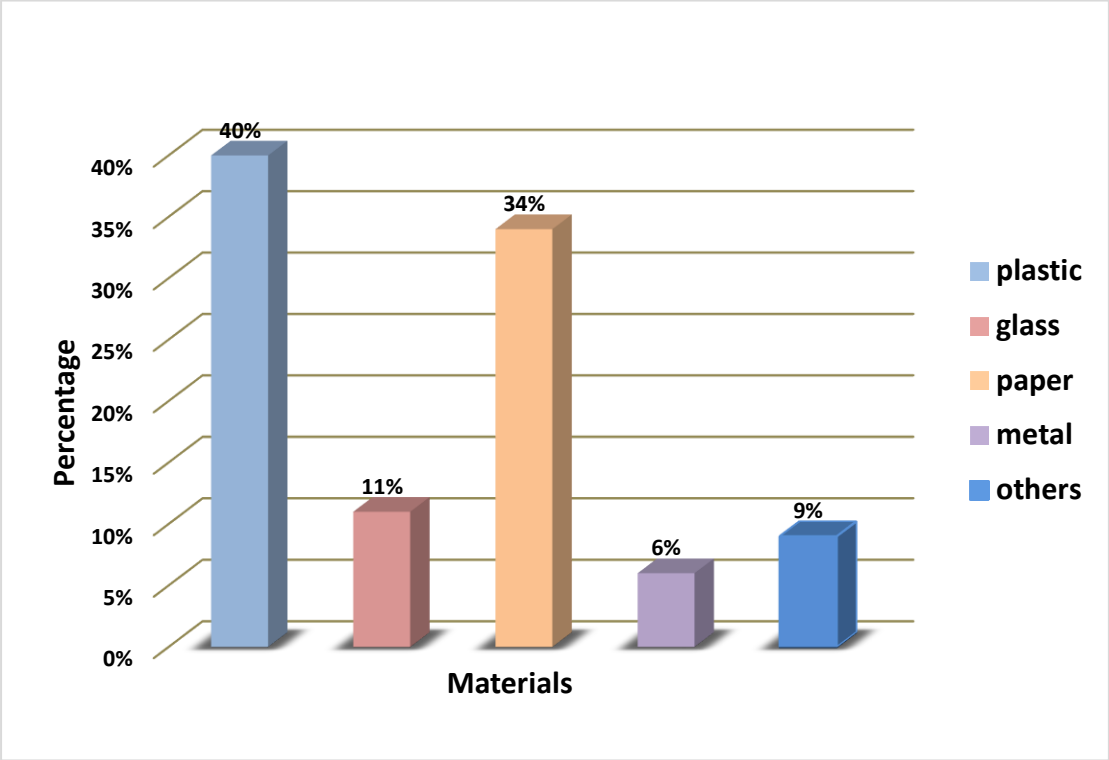

Figure 3: Food Contact Materials

1.3 Chemical Migration

Since ancient times, food was consumed where it was found, and it was the most important concern for humans to survive. For the transportation of liquid food, humans used animal skins or large plants and later baskets from stiff grasses or reeds. First time that ceramics used, was in the Middle East and making sacks of fabrics while Egyptians were using glass containers to protect the food from insects and microorganisms (Ossberger, 2015). More freedom and individualisation of the population followed the inventions of the large new varieties of packaging materials and their marketing. New functions of the packaging, including environmental compatibility and consumer convenience, have and will lead to new solutions of packaging materials such as paper and paperboard, aluminium, and collapsible, soft metal tube packaging materials (Berger, 2005). From the majority of materials, glass has the major advantages of “no migration” properties and recyclability. 'Migration', in English and many other languages, derives from the Latin verb migrate - prime meaning, to remove or depart (to another place), and the noun migration - prime meaning, removal or changing of habitation. The modern language usage appeared in the 17th century, applying to mass movements of animals, especially seasonal, and in particular of birds. Later the word was extended to cover human populations, but it was not until around the beginning of the 20th century that it entered the language of physical and chemical science to describe the movement of small particles - atoms, ions, molecules - within a relatively static matrix or across the boundary, especially under the influence of an electric field it was easy for the word to be taken over to describe the movement of contaminants into food from a package. Migration is when some chemical substances of materials which make contact with food, might be transferred into the food and to be a risk for human health (Exciton, Wells, & Blank, n.d.). The most possible way to proof conformity of the material is to measure the migration by analytical methods and compare the result with the migration limit.

The chemical migration is separated to overall migration and to specific migration. From the one hand, overall migration is the maximum permitted amount of non-volatile substances released from a material or article into food simulants. From the other hand, specific migration is the maximum permitted

amount of a given substance released from a material or article into food or food simulants. Also, the rate of migration of substances from materials into foods is called critical factor for migration. This is influenced by the substance itself, the type of material, the type of the food and the condition of contact such as the temperature and the duration. For example, migration into dry food is more limited than in aqueous food or fat food. The substances that can migrate into foods are separated to two categories, IAS (Intentionally Added Substances) and NIAS (Non-Intentionally Added Substances). In the category of IAS are known ingredients such as monomers, catalysts, additives etc. and in the category of NIAS are unknown ingredients, contamination from indirect sources such as printing inks, external coatings, adhesives, secondary packaging et all.

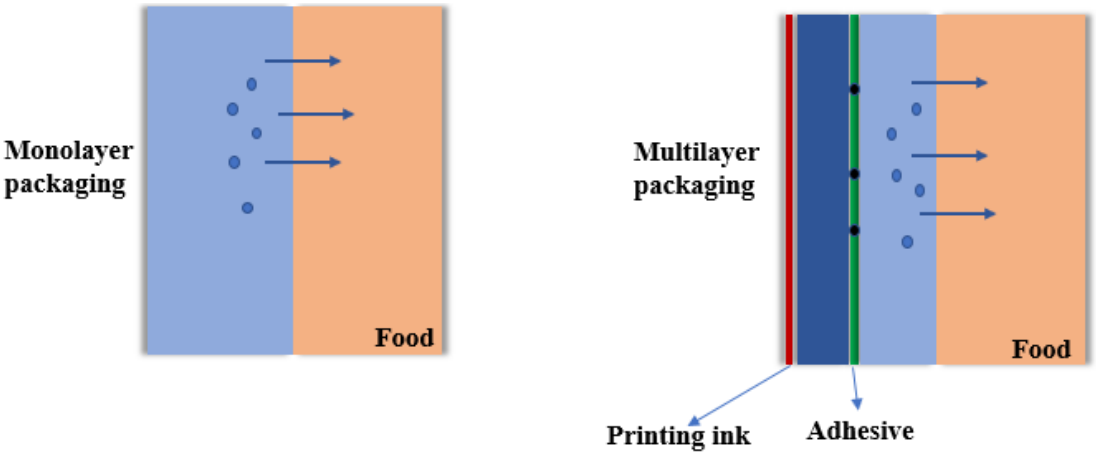

Figure 4: Migration from monolayer and multilayer packaging material

There are many Regulations about migration. The basic Community legislation that covers all food contact materials and articles is the Regulation (EC) 1935/2004 which lays down the general principles for eliminating the differences between the laws of the Member States as regards food contact materials. After that, the Regulation (EC) 2023/2006 has been established about Good Manufacturing Practice and for every material (plastics, ceramics, active and intelligent materials) or for dangerous substances like bisphenol-A, BADGE (Bisphenol A diglycidyl ether), BFDGE (Bisphenol F diglycidyl ether), NOGE (Novolac glycidyl ethers), nitrosamines and nitrosatable substances.

The basic Regulation that used for the experiments is the Regulation (EC) No 10/2011 about plastic materials and articles intended to come into contact with food. This Regulation is a specific measure within the meaning of Article 5(1) of Regulation (EC) No 1935/2004. This Regulation should establish the specific rules for plastic materials and articles to be applied for their safe use and repeal Commission Directive 2002/72/EC of 6 August 2002 on plastic materials and articles intended to come into contact with foodstuffs.

1.3.1 Temperature

As all chemical and physical processes, the migration of chemicals is accelerated by heat. So, it will occur faster if the temperature is raised. Packaging materials are increasingly used under a very wide range of temperature conditions, ranging from storage deep frozen, refrigerated and at ambient temperature, to boiling, microwaving and even baking in the pack. Clearly, a material suitable for one particular application may not necessarily be suitable for another (Figge, 1996).

1.3.2 The duration of contact

Materials suitable for short duration contact may not be suitable for longer service times. The time (duration) of contact for common packaging can vary enormously:

- minutes (e.g. take-away foods)
- hours (e.g. fresh bakery, sandwiches)
- days (e.g. fresh milk, meat, fruit and vegetables)
- weeks (e.g. butter, cheese)
- months and years (e.g. frozen foods, dry goods, canned foods, drinks).

1.3.3 Health issues

There can be absolutely no doubt that food packaging has greatly improved human health both now and through the ages by helping to provide regular and reliable supplies of safe, wholesome and nutritious foods. But chemical migration is always undesirable and if not controlled it could be hazardous to the health of consumers. The exception is for ‘active packaging’ which may be intended to release substances into the food with beneficial effects, such as antioxidants or preservatives. The main health concerns are for possible effects from chronic exposure to migrating substances. There are two specific exceptions to this, where an acute effect may arise (Tovar, Salafranca, Sánchez, & Nerín, 2005). One is migration of tin from tinplated steel into canned tomato products where high tin concentrations in food may cause short-term stomach upsets in some people but without any lasting harm. The other concerns latex allergen transfer which could have serious implications for some individuals.(Copestake, 1994) Recent research sponsored by the UK Food Standards Agency has shown that latex allergens may be present in some food packaging materials and that there is a theoretical possibility of transfer from the material to the food. The materials include cold seal adhesives based on latex and latex food-handling gloves. Further work is being done to improve methods to detect and quantify latex allergens in packaging and foods, to see if these allergens do migrate into food. To address possible long-term health concerns, the risk assessment process involves describing the toxicological hazard profile of the chemical substance, using qualitative and quantitative data, and coupling this to an estimate of exposure to a chemical migrant, to assess any risk. Consequently, the information that is required on packaging chemicals comprises data on (i) toxicity, and (ii) dietary exposure. As a general principle, the higher the exposure the more toxicological information is required.

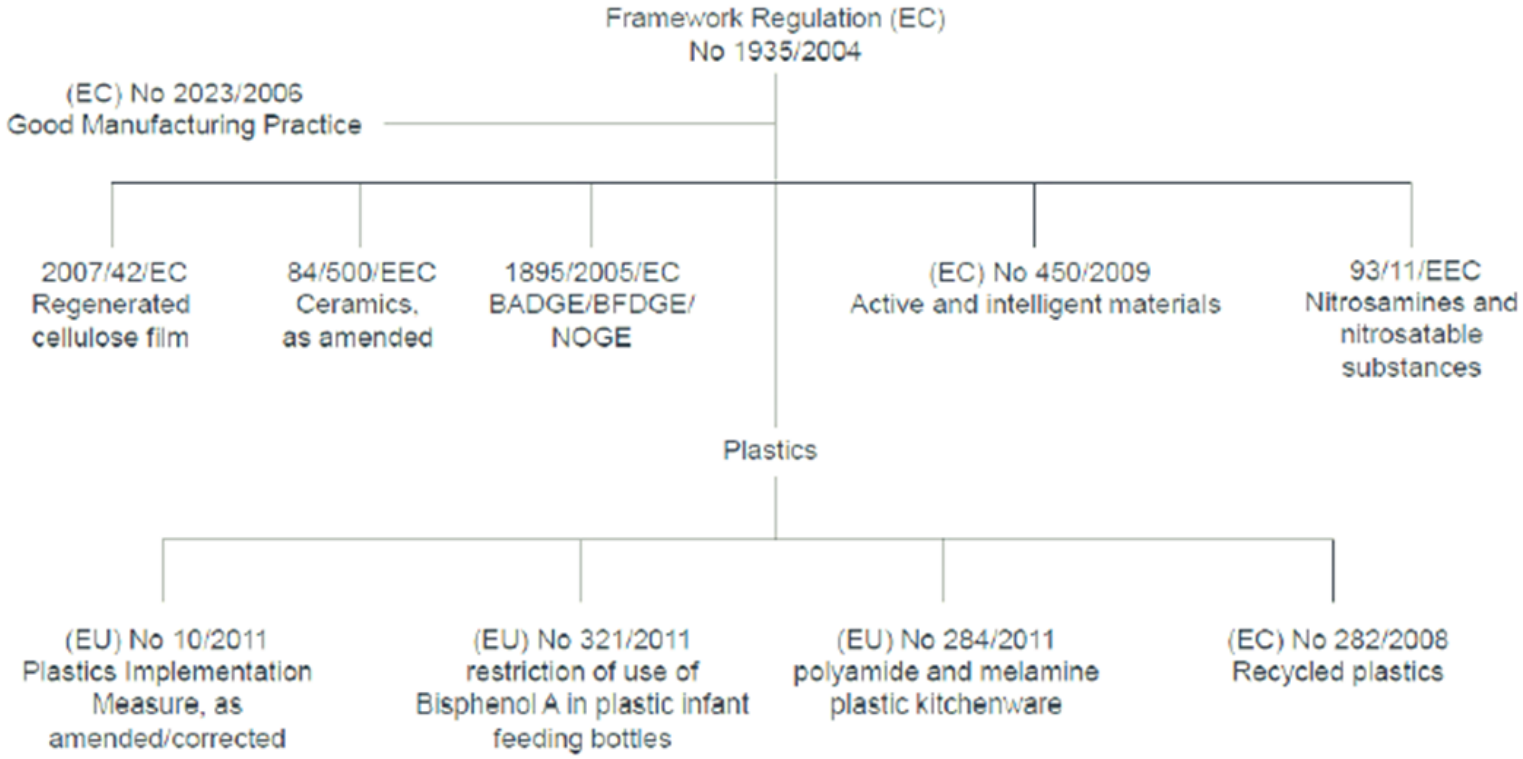

Figure 5: Regulations for FCMs

1.4 Active and intelligent packaging

The application of active packaging is certainly not new. The most likely, oldest application of active packaging is the use of wood barrels for the storage and maturation of wine, whisky and other distillates. It was discovered a long time ago that due to the release and absorption of substances from wood barrels, the sensory properties of the products were improved. Now there is a great focus on packaging materials that are deliberately developed to influence the quality, shelf life or appearance of the packaged food. Any packaging material will have that function, but conventional packaging materials act only as a barrier to influences from the outside and they do not modify the conditions of the packaged food. Generally, they are referred to as 'passive packaging'. Active packaging has an additional function by removing or adding substances from or to the packaged food. In active packaging two different types of active compounds can be assigned: one absorbs, the other releases. Some materials do both (De Kruijf, Van Beest, Sipiläinen-Malm, Paseiro Losada, & De Meulenaer, 2002). A barrier film, preventing gas transmission between packed food and its environment, is clearly passive packaging. A polymer film with built-in chemicals to react with the oxygen inside the food package is clearly defined as active packaging. But a film made of polymer blends, with selective permeability to different gases that allows the food to breath and thus influences the atmosphere inside the package (so-called equilibrium modified atmosphere packaging, EMAP) may cause doubts. In the EMAP film no specific chemicals are added and thus it should be classified as passive packaging. Intelligent packaging does not affect food. It provides information on the conditions of the packaged food. This information can be related to storage conditions, gas composition (generation of CO2, leakage detector) (Dainelli, Gontard, Spyropoulos, Zondervan-van den Beuken, & Tobback, 2008). It can also detect metabolites or chemical reaction products. Accordingly. Intelligent packaging is placed either inside or outside the primary food packaging.

Intelligent packaging provides information on packaged food. This information may relate to storage conditions and to the quality of the food. It may be readable by the consumer but in some cases the information is accessible only to manufacturers or retailers. Some examples of the information are:

Time/temperature indicators

Many time-temperature indicators (TTIs) are available today. Most of the indicators are based on the diffusion rate of one layer into a second layer of the TTI, leading to colour change. Some indicators register only the time or the temperature. A system using enzymatic reaction is commercially available. In general, all these indicators are readable by the consumer. An example of an indicator intended only for the retailer is a printed bar code label. The bar code will change in time and that package will be recognised as unsuitable for sale. All these indicators are positioned on the outside of the packaged food.

Oxygen indicators

These indicators can detect the presence of oxygen and are mainly used to detect any leakage in, for example, modified atmosphere packaging. Carbon dioxide indicators are available in the form of a label stuck to the inside of primary packaging. When the indicator changes its colour the generation of carbon dioxide is detected.

Microbial growth indicator

Indicators to detect metabolites are of great interest, but reliable systems are not yet commercially available.

Ripening indicator

Packaged fruits may be provided with an indicator that shows the ripening of fruit

The exact definition of active and intelligent materials according to the regulation (EC) No 1935/2004 is ‘Active food contact materials and articles means materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food. ‘Intelligent food contact materials and articles’ (hereinafter referred to as ‘intelligent materials and articles’) means materials and articles which monitor the condition of packaged food or the environment surrounding the food’.

1.4.1 Migration from active and intelligent packaging into foodstuffs

Active packaging and intelligent packaging are considered to be food contact materials. This means that the migration of packaging substances should be examined according to the existing EU directives or national provisions. At the EU level the framework Regulation 1935/2004 sets the general requirements of which Article 3, stating that the packaging material should not endanger human health, is the most important. EU regulatory control of active and intelligent packaging will therefore have to fit in with the well-established system of controls from the EC 10/2011.

A food contact material is brought into contact with the selected simulant under selected conditions of time and temperature. Homogeneous materials are contacted by submersion while multi-layers or thin films are often in single-sided contact with the food simulant. After the contact period the simulant is separated from the food contact material and the overall migration is determined gravimetrically while specific migration is determined using a suitable analytical method like gas or liquid chromatography with spectroscopic detection. The determination of the overall migration in olive oil is more complex and sensitive to analytical and systematic errors (López-Cervantes, Sánchez-Machado, Pastorelli, Rijk, & Paseiro-Losada, 2003). Some active and intelligent materials function as primary packaging by wrapping or holding the food, but most active and intelligent packaging has varying shapes, sizes and compositions. In many cases conventional migration tests use single-sided contact and cannot be applied for technical reasons related to, e.g., the size of the packaging. It was concluded that determination of overall migration from active and intelligent packaging using conventional methods is not applicable in many cases. There is a need for dedicated tests that simulate better the conditions of contact for some types of active and intelligent packaging (López-Cervantes et al., 2003).

1.5 Plastics and chemical migration into food

1.5.1 Plastics

Plastics are organic macromolecular compounds obtained by polymerization, polycondensation, polyaddition or any similar process from molecules with a lower molecular weight or by chemical alteration of natural molecules (EN-1186-1, 2002). They are classified as permeable materials and they offer some limited resistance to migration, but this can occur not only from the surface but also from the interior of the material. The resistance to mass transfer depends on the structure, density, crystallinity, etc., of the material. (Karen, Sinclair, & Watson, 2007) They are the most versatile and popular materials used in the manufacture of food packaging and other food contact materials (FCMs) with approximately 50% of all Europe's food packaged in plastics. Their biggest advantages are that they are robust and light in weight. However, only glass, metal and paper packaging materials have been recycled and reused in the packaging area for several decades in contrast with plastic materials which were recycled only in the non-food area. Within the last two decades, recycling processes have been developed or optimised due to their cleaning efficiency (Guart, Bono-Blay, Borrell, & Lacorte, 2011). Modern recycling and decontamination technology is, in some cases, able to reduce the post-consumer contaminants in polymers to levels below analytical detection limits.

Plastics can be placed into two main categories, thermoplastic and thermoset. Thermosets cannot be softened and remoulded on heating and have few applications in food packaging, except for the inner linings used for can coatings and many adhesives, as used, for example, in multilayer materials (J.H & Katan, 1996). A limited range of food contact materials is made from thermosets, predominantly melamine resins and unsaturated polyesters used in tableware and utensils. Thermoplastics can be softened repeatedly by heating and are more easily recyclable. Thus, they are used most often in food contact applications.

Food packaging materials are grouped into two categories in flexible and rigid. For rigid packaging materials, a significantly higher proportion of PET, (e.g. in beverage bottles) and polystyrene (e.g. expanded polystyrene food trays and

high impact polystyrene cups, and pots) is used. The category of flexible packaging materials include biaxially oriented polypropylene (BOPP), crystal polypropylene (PP), oriented polyamide (PA), polyethylene (PE), polyvinyl chloride (PVC), ethylene vinyl alcohol (EVOH) etc. (Karen et al., 2007).

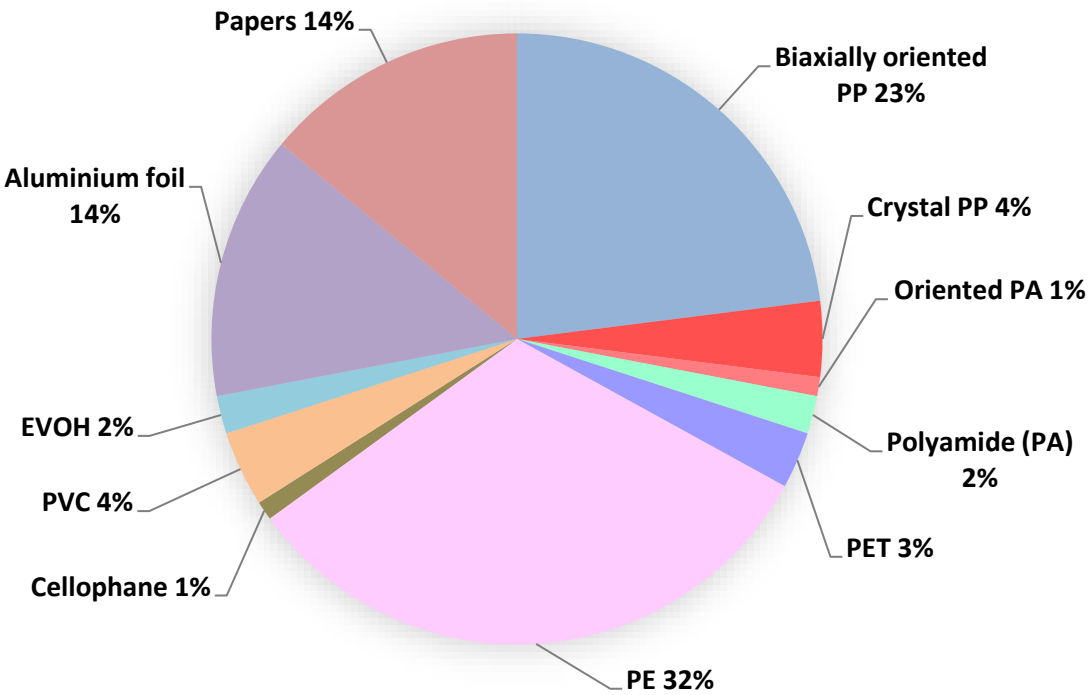

Figure 6: Main types of plastics and other materials used as substrates in flexible packaging applications (Karen et al., 2007)

Plastic materials and articles were the first materials to be covered by Community harmonization. The plastics Directive 2002/72/EC covers plastic monolayer and multilayer structures that purely consist of plastic. A monolayer structure may be a PE bag, a multilayer structure such as a plastic tray for pre-packaged food consisting of different plastic layers, e.g., ethylene vinyl alcohol copolymer/polyethylene (EVOH/PE) (Silva, García, Cooper, Franz, & Losada, 2006). Multilayers that consist of plastic and other materials such as plastic covered paper board, as in beverage cartons, do not fall under the specific

Community legislation on plastics. Plastic coatings, adhesives and epoxy resins are only covered in part by specific Community legislation on plastics by Regulation (EC) No 1895/2005.

According to the Regulation (EC) No 10/2011, plastics are made of monomers and other starting substances which are chemically reacted to a macromolecular structure, the polymer, which forms the main structural component of the plastics. Additives are also added to the polymer to achieve defined technological effects. The polymer as such is an inert high molecular weight structure. As substances with a molecular weight above 1000 Da usually cannot be absorbed in the body, potential health risk from the polymer itself is minimal. Potential health risk may occur from non- or incompletely reacted monomers or other starting substances or from low molecular weight additives which are transferred into food via migration from the plastic food contact material. Therefore monomers, other starting substances and additives should be risk assessed and authorised before their use in the manufacture of plastic materials and articles.

Table 1: Plastic recycling symbols

	PLASTICS	USES	SYMBOLS
PET	Polyethylene terephthalate	carbonated drinks bottles, peanut butter jars, plastic film and microwavable packaging	
HDPE	High-density polyethylene	detergent bottles, milk jugs and molded plastic cases	
PVC	Polyvinyl chloride	food wrap, plumbing pipes, and detergent bottles	
LDPE	Low-density polyethylene	plastic bags	
PP	Polypropylene	bottle caps, drinking straws, yogurt containers,	
PS	Polystyrene	foam peanuts, food containers, plastic tableware, disposable cups, plates, cutlery,	

- PET (Polyethylene terephthalate)

PET is made by polymerising ethylene glycol with terephthalic acid or transesterification with dimethyl terephthalate, commonly using an antimony trioxide catalyst. The use of PET plastics has increased significantly over the last ten years replacing glass and, to some extent PVC, in applications for water and soft drinks. The PET used in high-temperature applications have a higher crystallinity and is opaque compared to the transparent amorphous material used to make bottles. By-products of polymerizing or processing the components of PET are diethylene glycol and acetaldehyde, although acetaldehyde scavengers, such as anthranalamide (2- aminobenzamide), can be used to reduce the level of acetaldehyde which can cause taint/odour problems (Bradley et al., 2010a).

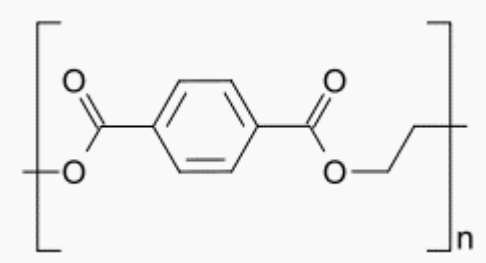

Figure 7: Structure of Poly(ethyl benzene-1,4-dicarboxylate)

- HDPE (High Density Polyethylene)

HDPE is used for blow-moulded bottles for milk and other drinks, and in food storage containers. HDPE has high usable temperature (up to about 100–120 ^{o}C) making it suitable for 'hot fill' and pasteurisation applications. HDPE is used for meat and poultry packaging because of its greater strength and puncture resistance at thinner gauges and for beverages. A typical formulation for a HDPE bottle would be: HDPE 100 parts per hundred (pph), octadecyl-3-(3,5-di-tert-butyl-4- hydroxyphenyl)propionate 0.05 pph, phosphorous acid, tris(2,4-di-tertbutylphenyl) ester 0.1 pph (Karen et al., 2007).

- PVC (Polyvinyl chloride)

Vinyl chloride can be polymerised to form polyvinyl chloride (PVC) which is fairly brittle and unsuitable for food contact applications, so it is mixed with plasticisers to soften the polymer and impart flexibility. Plasticised PVC is used to make stretch films and flexible PVC. Flexible PVC used for tubing and gaskets may contain di(2-ethylhexyl)phthalate, and stretch films will probably contain di(2-ethylhexyl)adipate and a polymeric adipate plasticiser. 'Rigid' PVC is used to make trays for fresh meat having good clarity and water bottles (Leadbitter, 2003).

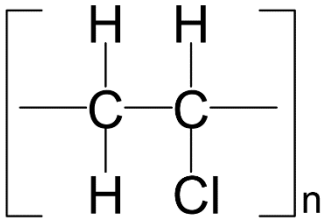

Figure 8: Structure of poly(1-chloroethylene)

- LDPE (Low Density Polyethylene)

This type of plastic is used frequently for food bags, which are produced by blowing a film. As LDPE is very flexible it is also used to make lids for food storage containers, produced by injection moulding techniques. The polymer is relatively cheap with good water vapour and moisture resistance but has poor barrier properties to gases and low molecular weight organic chemicals. LDPE is often used as a film or coating on other materials, such as paper and aluminium foils to provide flexibility and heat sealability (Karen et al., 2007).

- PP (Polypropylene)

PP is stiffer than LDPE or HDPE and has superior tensile strength, good clarity and grease resistance. PP can be converted by a range of procedures to make films, pouches, closures, containers, bottles and injection moulded containers and articles that can withstand retorting and microwave reheating. A typical formulation for a polypropylene (PP) film used for wrapping biscuits would be: PP 100 pph, pentaerythritol tetrakis [3-(3,5-di-tertbutyl-4-hydroxyphenyl) propionate] 0.1 pph, phosphorous acid, tris(2,4-di-tertbutylphenyl) ester 0.1 pph, erucamide 0.05 pph (International Life Sciences Institute, 2002).

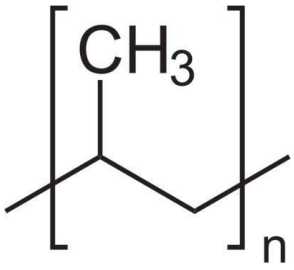

Figure 9: Structure of polypropylene

- PS (Polystyrene)

Polystyrene homopolymer, often referred to as general purpose polystyrene (GPPS) or 'crystal' polystyrene, is a hard, fairly brittle polymer with excellent transparency. GPPS is used for making disposable tableware and plastic glasses. Styrene polymers are used in a range of food contact applications including tableware, wine/beer glasses, yoghurt pots, coffee cups, and thermoformed trays for meat and fish.(Bradley et al., 2010b)

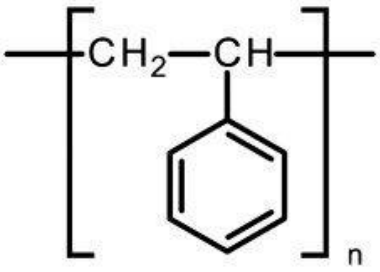

Figure 10: Structure of Poly(1-phenylethene)

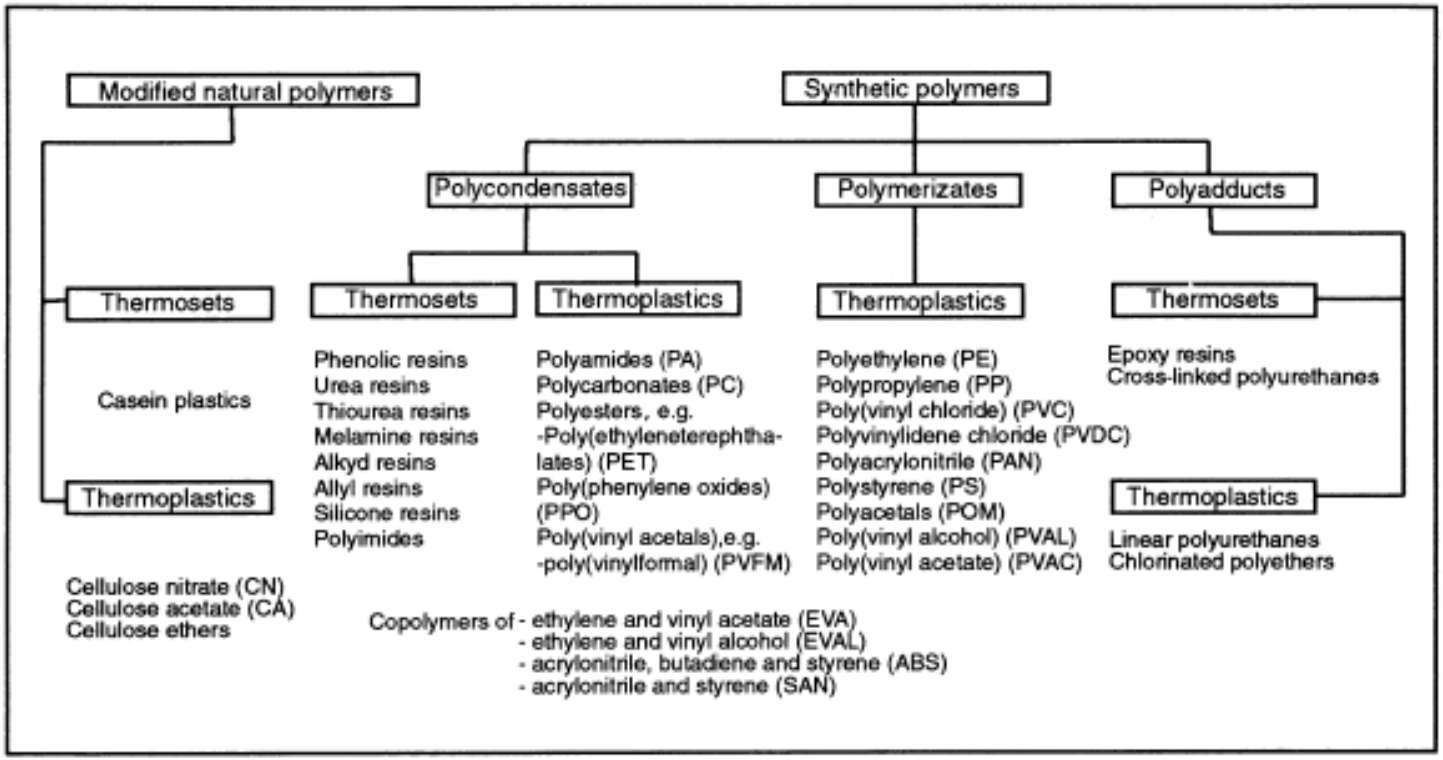

Figure 11: Basic polymers, (Figge, 1996)

1.5.2 Future trends

Future trends in food contact plastics are most likely to be directed more environmentally friendly or sustainable materials, such as those that are biodegradeable and plant derived, to reduce the negative environmental impacts created by landfill and incineration of plastics (Weber, Haugaard, Festersen, & Bertelsen, 2002). There is also an ever-increasing trend in the development and use of active and intelligent packaging with the associated benefits of increased food safety for the consumer. In particular, the availability of oxygen scavengers that can be incorporated into inner layers has been a key element in the design of new PET beer bottles.

Figure 12: PET beer bottles

In addition, the petitioner should provide evidence that the substance is not used to replace the normal hygienic measures required in handling foodstuffs. At present, nanotechnology is being applied in plastics packaging to a limited degree to improve the properties of materials and increase the efficiency of the packaging production process (Lagarón et al., 2005). Potential benefits include improved barrier properties (delivering longer shelf life or allowing material substitution) and better temperature performance using titanium, zinc, aluminium and iron oxides. Applications are also being developed in areas of active and antimicrobial food packaging.

1.5.3 Overall migration

According to the EN 1186:1-2002 “overall migration” is the mass of material transferred to the food simulant or test media. Furthermore, according to the Regulation (EC) No 10/2011 “overall migration limit” (OML) means the maximum permitted amount of non-volatile substances released from a material or article into food simulants. Overall migration is the basic analysis for migration. The migration tests are carried out with the exposure of material with a food simulant in specific conditions of temperature and for specific time.

According to good manufacturing practice it is feasible to manufacture plastic materials in such a way that they are not releasing more than 10 mg of substances per 1 dm^2 of surface area of the plastic material. In order to achieve comparable results in the verification of compliance with the overall migration limit, testing should be performed under standardised test conditions including testing time, temperature and test medium (food simulant) representing worst foreseeable conditions of use of the plastic material or article (O’Brien, Leach, & Cooper, 2000). In conclusion, plastic materials and articles shall not transfer their constituents to food simulants in quantities exceeding 10 milligrams of total constituents released per dm^2 of food contact surface (mg/dm^2) plastic materials and articles intended to be brought into contact with food intended for infants and young children, as defined by Commission Directives 2006/141/EC([1]) and 2006/125/EC([2]) shall not transfer their constituents to food simulants in quantities exceeding 60 milligrams of total of constituents released per kg of food simulant (Grob et al., 2007). That is the limit for the infants and young children because they have a higher consumption of food per kilogram bodyweight than adults and do not yet have a diversified nutrition, special provisions should be set in order to limit the intake of substances migrating from food contact materials.

1.5.4 Tests for overall migration plastics

EN 1186-1 is intended to give advice on the selection of the most appropriate type of test, test conditions and test method for a given application of a plastics article and is intended to be read in its entirety before testing. For most plastic articles, methods in EN 1186-2 to EN 1186-9 are suitable, according to the form in which the article is tested. Subsequent Parts of this standard are intended to be used in conjunction with the methods in EN 1186-2 to EN 1186-9 for more difficult samples and for other exposure temperatures. The methods are listed in Figure 13.

Reference	Title
Plastics	Materials and articles in contact with foodstuffs – Plastics -
EN 1186-1:2002	Part 1: Guide to the selection of conditions and test methods for overall migration
EN 1186-2:2002	Part 2: Test methods for overall migration into olive oil by total immersion
EN 1186-3:2002	Part 3: Test methods for overall migration into aqueous food simulants by total immersion
EN 1186-4:2002	Part 4: Test methods for overall migration into olive oil by cell
EN 1186-5:2002	Part 5: Test methods for overall migration into aqueous food simulants by cell
EN 1186-6:2002	Part 6: Test methods for overall migration into olive oil using a pouch
EN 1186-7:2002	Part 7: Test methods for overall migration into aqueous food simulants using a pouch
EN 1186-8:2002	Part 8: Test methods for overall migration into olive oil by article filling
EN 1186-9:2002	Part 9: Test methods for overall migration into aqueous food simulants by article filling
EN 1186-10:2002	Part 10: Test methods for overall migration into olive oil (modified method for use in cases where incomplete extraction of olive oil occurs)
EN 1186-11:2002	Part 11: Test methods for overall migration into mixtures of C-labelled synthetic triglycerides
EN 1186-12:2002	Part 12: Test methods for overall migration at low temperatures
EN 1186-13:2002	Part 13: Test methods for overall migration at high temperatures
EN 1186-14:2002	Part 14: Test methods for 'substitute tests' for overall migration from plastics intended to come into contact with fatty foodstuffs using test media iso-octane and 95 % ethanol
EN 1186-15:2002	Part 15: Alternative test methods to migration into fatty food simulants by rapid extraction into iso-octane and/or 95 % ethanol

Figure 13: CEN standards related to overall migration in plastics (Simoneau, 2009)

Migration tests may be performed in four ways: using a migration test cell, by preparation of a pouch, by total immersion and by article filling. According to the form and the dimensions of the material or article to be tested, one of these methods is chosen

- Testing by total immersion

According to the EN 1186-1:2002, in method for determining overall migration by total immersion, the samples are tested at a fixed ratio of surface area of test specimen to food simulant volume. In order to ensure that all parts of the test specimen are in contact with the food simulant, glass tubes of the appropriate diameter are used. However, minor adjustments to the level of the simulant in the tubes may be made by adding glass rods or glass beads sufficient to ensure complete immersion of all of the surfaces of the test specimen. Overall migration tests shall be performed in such a way that only those parts of the sample intended to come into contact with foodstuffs in actual use will be in contact with the foodstuff or simulant. In the total immersion test, both the surface which is intended to come into contact with the foodstuff and the outside surface are in contact with the food simulant. In cases where the overall migration limit is exceeded when testing by total immersion, the test shall be repeated using a method applying single sided contact. The surface to volume ratio in the total immersion test is conventionally 1 dm² of food contact area to 100 ml of food simulant.

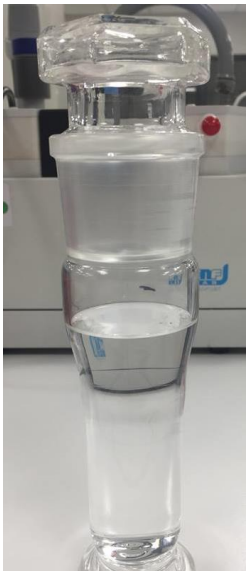

Figure 14: Glass tubes for total immersion method

- Single sided testing using a migration cell

Where single surface testing is the preferred procedure, particularly important for multi-layer articles, this may be carried out in a specific cell for migration tests. The test specimen was placed in the base of specific cell and after that the simulant was added. When the test is done with 3 % w/v aqueous acetic acid (food simulant B) the materials of the cell must not influence the final result, e.g. cells constructed from aluminium may not be suitable in contact with 3 % w/v aqueous acetic acid. The surface to volume ratio in a classic cell is conventionally 2,5 dm² of food contact area to 125 ml of food simulant.

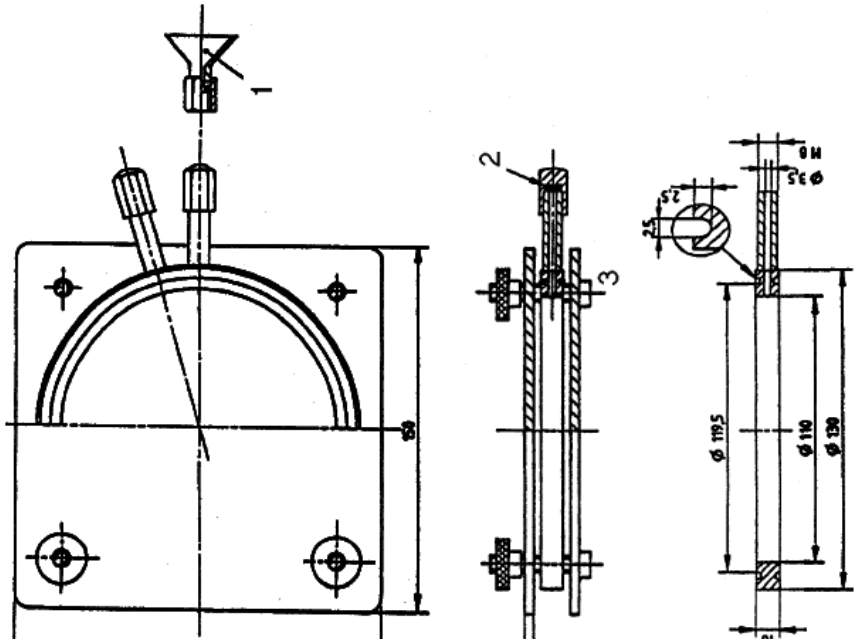

Figure 15: Specific cell for migration tests

- Single side testing using a migration pouch

For flat articles which have sufficient seal strength to form durable pouches, single surface testing in a pouch is used as this does not require specialized apparatus and allows more efficient use of oven space. The surface to volume ratio in the pouch is conventionally 2 dm² of food contact area to 100 ml of food simulant.

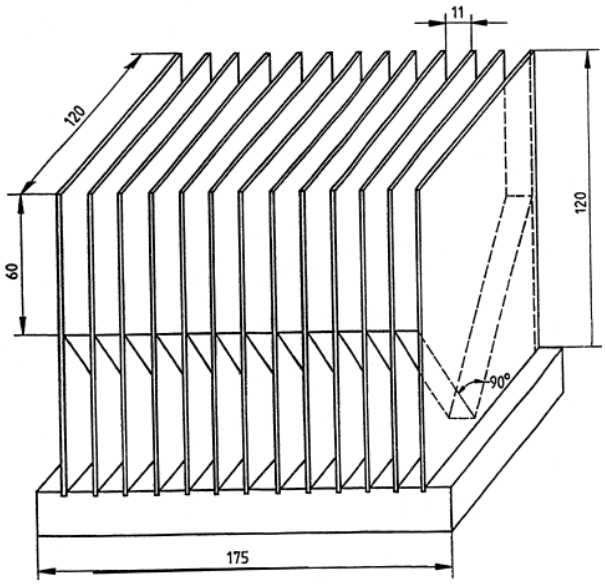

Figure 16: Pouch holder for migration tests

- Single side testing by filling

For articles in container form, e.g. bottles and trays, it is often most convenient to test them by filling with food simulant. For very large containers testing by filling may not be practicable and it may be necessary to fabricate smaller test specimens representing the article to be tested.

Figure 17: Example of cups for article filling method

- Tenax method - Adsorption by modified polyphenylene oxide (MPPO)

For flexible thin film and sheet materials it is often most convenient to test them with petri dishes by using as simulant the modified polyphenylene oxide (Tenax). Modified polyphenylene oxide is a porous polymer with a high molecular weight, 500 000 to 1 000 000, a very high temperature stability (T_{max} = 350 °C), a high surface area and a low specific mass (0,23 g/cm^3) (Aurela, Ohra-aho, & Söderhjelm, 2001). The surface of the article is covered with modified polyphenylene oxide and held at the selected time temperature test conditions where the maximum temperature applicable is 175 °C.

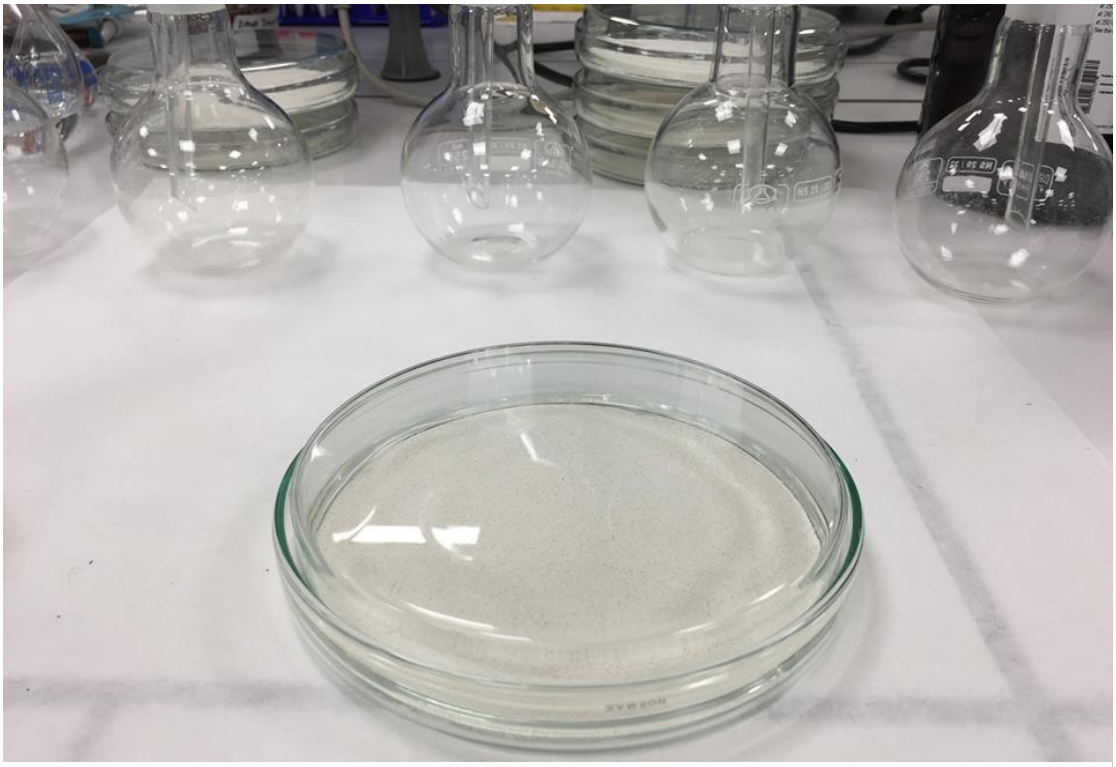

Figure 18: Petri dish for migration test with Tenax

1.5.5 Food simulants

According to the Regulation (EC) 10/2011 “food simulant” means a test medium imitating food, where the food simulant mimics migration from food contact materials. There are six food simulants which are used for migration test from plastic materials and they listed in Figure 6.

List of food simulants

Food simulant	Abbreviation
Ethanol 10 % (v/v)	Food simulant A
Acetic acid 3 % (w/v)	Food simulant B
Ethanol 20 % (v/v)	Food simulant C
Ethanol 50 % (v/v)	Food simulant D1
Vegetable oil (*)	Food simulant D2
poly(2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm	Food simulant E

(*) This may be any vegetable oil with a fatty acid distribution of

No of carbon atoms in fatty acid chain: No of unsaturation	6-12	14	16	18:0	18:1	18:2	18:3
Range of fatty acid composition expressed % (w/w) of methyl esters by Gas chromatography	< 1	< 1	1,5-20	< 7	15-85	5-70	< 1,5

Figure 19: Food simulants, EC 10/2011

General food simulants A, B and C are assigned for foods that have a hydrophilic character and are able to extract hydrophilic substances. Food simulant B shall be used for those foods which have a pH below 4.5. Food simulant C shall be used for alcoholic foods with an alcohol content of up to 20 % and those foods which contain a relevant amount of organic ingredients that render the food more lipophilic. Food simulants D1 and D2 are assigned for foods that have a lipophilic character and are able to extract lipophilic substances. Food simulant D1 shall be used for alcoholic foods with an alcohol content of above 20 % and for oil in water emulsions. Food simulant D2 shall be used for foods which contain free fats at the surface. Food simulant E is assigned for testing specific migration into dry foods.

For testing migration from materials and articles not yet in contact with food the food simulants that corresponds to a certain food category shall be chosen according to a Table in Regulation (EC) 10/2011 which depicts the food and the necessary simulant for the test (Figure 20).

(1)	(2)	(3)					
Reference number	Description of food	Food simulants					
		A	B	C	D1	D2	E
01	**Beverages**						
01.01	Non-alcoholic beverages or alcoholic beverages of an alcoholic strength lower than or equal to 6 % vol.:						
	A. Clear drinks:		X(*)	X			
	Water, ciders, clear fruit or vegetable juices of normal strength or concentrated, fruit nectars, lemonades, syrups, bitters, infusions, coffee, tea, beers, soft drinks, energy drinks and the like, flavoured water, liquid coffee extract						
	B. cloudy drinks:		X(*)		X		
	juices and nectars and soft drinks containing fruit pulp, musts containing fruit pulp, liquid chocolate						
01.02	Alcoholic beverages of an alcoholic strength of between 6 %vol and 20 %.			X			
01.03	Alcoholic beverages of an alcoholic strength above 20 % and all cream liquors				X		
01.04	Miscellaneous: undenaturated ethyl alcohol		X(*)			Substitute 95 % ethanol	
02	**Cereals, cereal products, pastry, biscuits, cakes and other bakers' wares**						
02.01	Starches						X
02.02	Cereals, unprocessed, puffed, in flakes (including popcorn, corn flakes and the like)						X
02.03	Cereal flour and meal						X

Figure 20: Food category specific assignment of food simulants, EC 10/2011

1.5.6 Testing conditions

Overall migration testing shall be performed under the standardised testing conditions. The overall migration test for materials and articles intended for the food contact conditions described in column 3 of Figure 21 shall be performed for the time specified and at the temperature specified in column 2. However, where a material or article is intended to come into repeated contact with foods, the migration test shall be carried out three times on a single sample using another sample of the food simulant on each occasion. Its compliance shall be checked on the basis of the level of the migration found in the third test. However, if there is conclusive proof that the level of the migration does not increase in the second and third tests and if the overall migration limit is not exceeded on the first test, no further test is necessary.

Standardised testing conditions

Column 1	Column 2	Column 3
Test number	Contact time in days [d] or hours [h] at Contact temperature in [°C]	Intended food contact conditions
OM1	10 d at 20 °C	Any food contact at frozen and refrigerated conditions.
OM2	10 d at 40 °C	Any long term storage at room temperature or below, including heating up to 70 °C for up to 2 hours, or heating up to 100 °C for up to 15 minutes.
OM3	2 h at 70 °C	Any contact conditions that include heating up to 70 °C for up to 2 hours, or up to 100 °C for up to 15 minutes, which are not followed by long term room or refrigerated temperature storage.
OM4	1 h at 100 °C	High temperature applications for all food simulants at temperature up to 100 °C.
OM5	2 h at 100 °C or at reflux or alternatively 1 h at 121 °C	High temperature applications up to 121 °C.
OM6	4 h at 100 °C or at reflux	Any food contact conditions with food simulants A, B or C, at temperature exceeding 40 °C.
OM7	2 h at 175 °C	High temperature applications with fatty foods exceeding the conditions of OM5.

Figure 21:Standarised testing conditions, EC 10/2011

1.5.7 Specific migration

Specific migration is the amount of a specific component that migrates from the food contact material to the food during contact. According to the Regulation (EC) 10/2011 “specific migration limit” (SML) means the maximum permitted amount of a given substance released from a material or article into food or food simulants and “total specific migration limit” (SML(T)) means the maximum permitted sum of particular substances released in food or food simulants expressed as total of moiety of the substances indicated (Alin & Hakkarainen, 2011). Also, Regulation (EC) 10/2011 contains a Union list (Figure 23) of authorised monomers, other starting substances and macromolecules which obtained from microbial fermentation, additives and polymer production aids for plastic materials. In this catalogue there is a column about specific migration limit of every substance (Column 8). The SML is expressed in mg substance per kg food and it is indicated ND if the substance shall not migrate in detectable quantities.

For the testing of specific migration of materials and articles which are already in contact with food, the material or article is stored as indicated on the packaging label or under conditions adequate for the packaged food if no instructions are given. The food is treated in accordance with the cooking instructions on the package if the food is to be cooked in the package. Parts of the food which are not intended to be eaten shall be removed and discarded. The remainder shall be homogenised and analysed for migration. The analytical results expressed on the basis of the food mass that is intended to be eaten, in contact with the food contact material.

For the materials and articles which are not yet in contact with food, migration is determined on the material or article or, if this is impractical, on a specimen taken from the material or article, or a specimen representative of this material or article (Zygoura, Paleologos, & Kontominas, 2011). For each food simulant or food type, a new test specimen is used. Only those parts of the sample which are intended to come into contact with foods in actual use shall be placed in contact with the food simulant or the food. Materials and articles intended for contact with all types of food shall be tested with food simulant A, B and D_2. The sample shall be placed in contact with the food simulant in a manner

representing the worst of the foreseeable conditions of use. If it is found that carrying out the tests under the combination of contact conditions specified causes physical or other changes in the test specimen which do not occur under worst foreseeable conditions of use of the material or article under examination, the migration tests shall be carried out under the worst foreseeable conditions of use in which these physical or other changes do not take place (COMMISSION REGULATION (EU) No 10/2011, 2011)

Contact time

Contact time in worst foreseeable use	Test time
t ≤ 5 min	5 min
5 min < t ≤ 0,5 hour	0,5 hour
0,5 hours < t ≤ 1 hour	1 hour
1 hour < t ≤ 2 hours	2 hours
2 hours < t ≤ 6 hours	6 hours
6 hours < t ≤ 24 hours	24 hours
1 day < t ≤ 3 days	3 days
3 days < t ≤ 30 days	10 days
Above 30 days	See specific conditions

Table 2

Contact temperature

Conditions of contact in worst foreseeable use	Test conditions
Contact temperature	Test temperature
T ≤ 5 °C	5 °C
5 °C < T ≤ 20 °C	20 °C
20 °C < T ≤ 40 °C	40 °C
40 °C < T ≤ 70 °C	70 °C
70 °C < T ≤ 100 °C	100 °C or reflux temperature
100 °C < T ≤ 121 °C	121 °C (*)
121 °C < T ≤ 130 °C	130 °C (*)
130 °C < T ≤ 150 °C	150 °C (*)
150 °C < T < 175 °C	175 °C (*)
T > 175 °C	Adjust the temperature to the real temperature at the interface with the food (*)

(*) This temperature shall be used only for food simulants D2 and E. For applications heated under pressure migration testing under pressure at the relevant temperature may be performed. For food simulants A, B, C or D1 the test may be replaced by a test at 100 °C or at reflux temperature for duration of four times the time selected according to the conditions in Table 1.

Figure 22: Tables of conditions for specific migration tests, EU 10/2011

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
145	10630	0000079-06-1	acrylamide	no	yes	no	ND			
146	23890 82000	0000079-09-4	propionic acid	yes	yes	no				
147	10690	0000079-10-7	acrylic acid	no	yes	no		(22)		
148	14650	0000079-38-9	chlorotrifluoroethylene	no	yes	no	ND			(1)
149	19990	0000079-39-0	methacrylamide	no	yes	no	ND			
150	20020	0000079-41-4	methacrylic acid	no	yes	no		(23)		
151	13480 13607	0000080-05-7	2,2-bis(4-hydroxyphenyl) propane	no	yes	no	0,6			
152	15610	0000080-07-9	4,4′-dichlorodiphenyl sulphone	no	yes	no	0,05			
153	15267	0000080-08-0	4,4′-diaminodiphenyl sulphone	no	yes	no	5			
154	13617 16090	0000080-09-1	4,4′-dihydroxydiphenyl sulphone	no	yes	no	0,05			
155	23470	0000080-56-8	α-pinene	no	yes	no				
156	21130	0000080-62-6	methacrylic acid, methyl ester	no	yes	no		(23)		
157	74880	0000084-74-2	phthalic acid, dibutyl ester	yes	no	no	0,3	(32)	Only to be used as: (a) plasticiser in repeated use materials and articles contacting non-fatty foods; (b) technical support agent in polyolefins in concentrations up to 0,05 % in the final product.	(7)
158	23380 76320	0000085-44-9	phthalic anhydride	yes	yes	no				

Figure 23: Page 10 of union list of authorised monomers, other starting substances, macromolecules obtained from microbial fermentation, additives and polymer production aids, EC 10/2011

The analytical technique that must be selected for the migration study is the most difficult task when the sample arrived at the laboratory. As mentioned before, a clear protocol for the compounds that must be presented in a sample and migrate to the food is available in Regulation (EC) 10/2011 but there is not a protocol about the identification of NIAS. For this reason, the analysis is performed with the same migration tests as those mentioned in EU 10/2011(COMMISSION REGULATION (EU) No 10/2011, 2011). For polymer analysis, the following techniques can be used: (a) direct thermal desorption from the polymers and GC–MS analysis (either by headspace or SPME analysis); (b) direct MS analysis such as ASAP (atmospheric solids analysis probe), DART (direct analysis in real time) (Bentayeb, Ackerman, & Begley, 2012) or DESI (desorption electrospray ionization); (c) total dissolution of the polymer and analysis either by GC–MS or LC–MS; (d) solvent extraction and final analysis by GC–MS or LC–MS. These techniques are mainly used for confirmation, as there are no separation steps and identification based only on the MS fragments is extremely difficult without having previous experience and knowledge of the compounds likely to be present. The main advantage of the two first approaches (a and b) is that they do not require sample pretreatment before the main instrumental analysis. However, in the last two approaches (c and d) a dissolution or extraction step is necessary before the analysis (Nerin, Alfaro, Aznar, & Domeño, 2013). When NIAS determination is performed in a solution after the migration test, most of the analytical procedures involve only concentration steps before the instrumental analysis. Since solutions usually contain more than one compound, hyphenated analytical techniques in which a chromatographic separation is coupled online with one or more information-rich detectors are indispensable tools for NIAS identification or confirmation. GC–MS is the most frequently used technique.

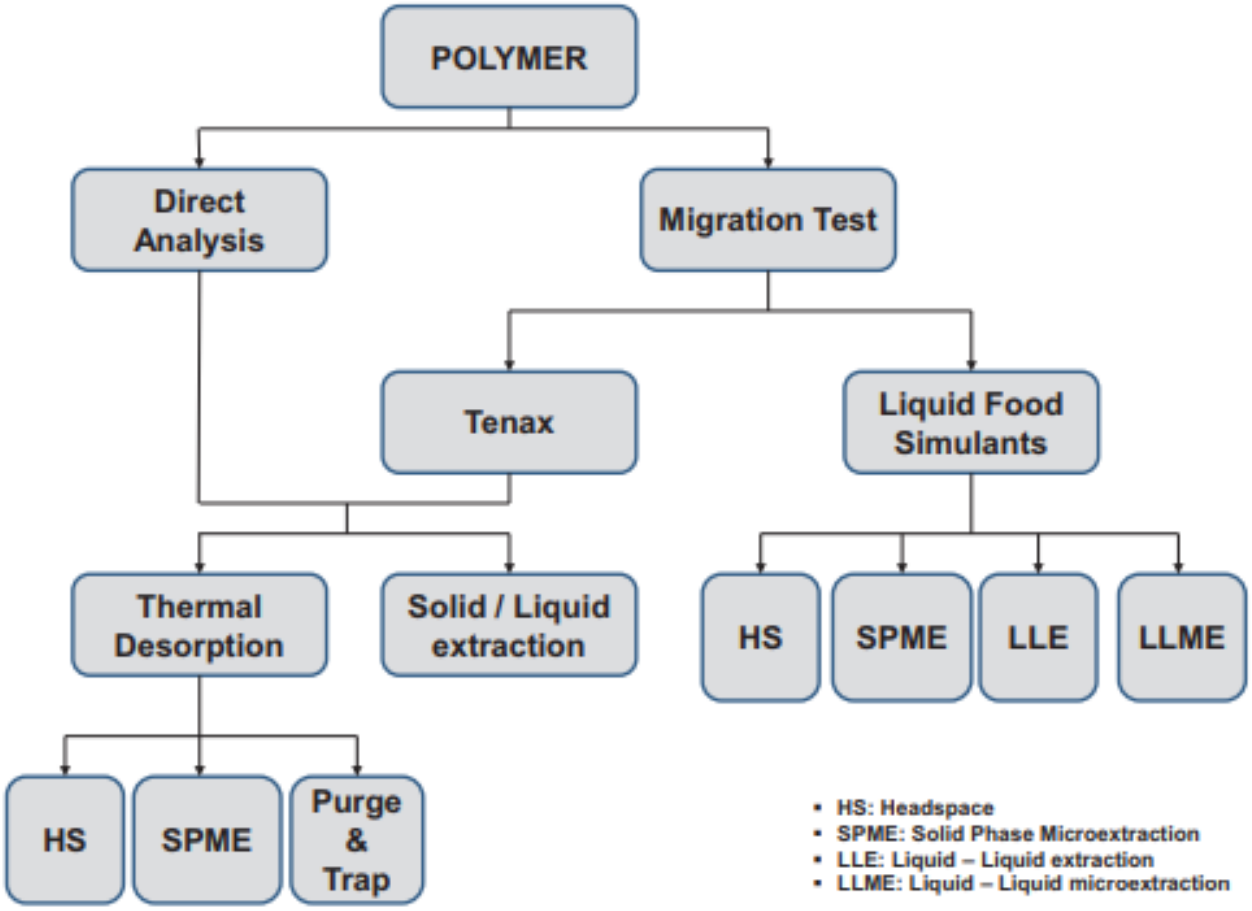

Figure 24: Sceme for sample treatment procedure for the NIAS determination, (Nerin et al., 2013)

CEN document “EN 13130-1:2004 Materials and articles in contact with foodstuffs – Plastics substances subject to limitation – Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants” is the document with general information about how to determine specific migration. Also, there are more established methods from CEN for the specific migration (Figure 25)

EN 13130-1:2004	Materials and articles in contact with foodstuffs – Plastics substances subject to limitation – Part 1: Guide to test methods for the specific migration of substances from plastics to foods and food simulants and the determination of substances in plastics and the selection of conditions of exposure to food simulants
EN 13130-2:2004	Determination of terephthalic acid in food simulants
EN 13130-3:2004	Determination of acrylonitrile in food and food simulants
EN 13130-5:2004	Determination of vinylidene chloride in food simulants
EN 13130-7:2004	Determination of monoethylene glycol and diethylene glycol in food simulants
CEN/TS 13130-9:2005	Determination of acetic acid, vinyl ester in food simulants
CEN/TS 13130-10:2005	Determination of acrylamide in food simulants
CEN/TS 13130-11:2005	Determination of 11-aminoundecanoic acid in food simulants
CEN/TS 13130-12:2005	Determination of 1,3-benzenedimethanamine in food simulants
CEN/TS 13130-13:2005	Determination of 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A) in food simulants
CEN/TS 13130-14:2005	Determination of 3,3-bis(3-methyl-4-hydroxyphenyl)-2-indoline in food simulants
CEN/TS 13130-15:2005	Determination of 1,3-butadiene in food simulants
CEN/TS 13130-16:2005	Determination of caprolactam and caprolactam salt in food simulants
CEN/TS 13130-18:2005	Determination of 1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, 4,4'-dihydroxy-benzophenone and 4,4′ dihydroxybiphenyl in food simulants
CEN/TS 13130-19:2005	Determination of dimethylaminoethanol in food simulants
CEN/TS 13130-21:2005	Determination of ethylenediamine and hexamethylene-diamine in food simulants
CEN/TS 13130-23:2005	Determination of formaldehyde and hexamethylenetetramine in food simulants
CEN/TS 13130-24:2005	Determination of maleic acid and maleic anhydride in food simulants
CEN/TS 13130-25:2005	Determination of 4-methyl-1-pentene in food simulants
CEN/TS 13130-26:2005	Determination of 1-octene and tetrahydrofuran in food simulants
CEN/TS 13130-27:2005	Determination of 2,4,6-triamino-1,3,5-triazine in food simulants
CEN/TS 13130-28:2005	Determination of 1,1,1-trimethylolpropane in food simulants

Figure 25: CEN methods for specific migration tests

Bisphenol – A (BPA) is the most common substance determined by using specific migration methods. Bisphenol A (BPA) is used as a monomer in the synthesis of polycarbonates (PC), a type of polymer utilized in the manufacture of plastic food containers, such as infant feeding bottles. Exhibiting high transparency, low weight and high heat and impact resistance, polycarbonate plastics are increasingly replacing glass in such applications. In the early 1990s, it was reported that BPA which was released from polycarbonate flasks, exhibits estrogenic activity in in-vitro assays at concentrations of 10–25nM (2–5 ng ml^{-1}) (Krishnan, Permuth, & Alto, 2018). Later studies reported that exposure to low doses of BPA causes reproductive toxic effects, such as increases in the murine prostate gland and reduced sperm efficiency (vom Saal FS1, 1998).

The presence of unreacted BPA in PC baby bottles has been reported, with the residual amount of the monomer ranging from 7 to 58 mg g^{-1} in samples purchased in Washington and from <3 to 141 mg g^{-1} in samples purchased in Singapore (Wong, Leo, & Seah, 2005). The occurrence of BPA in the plastic, the fact that baby bottles are intended to be used repeatedly and the potential adverse effects of BPA at low doses, especially regarding infants, has generated the need for a thorough investigation of BPA migration from this type of food contact material. Migration studies of BPA from infant feeding bottles have been conducted under diverse treatments and diverse conditions using water (vom Saal & Hughes, 2005). They reported that residual BPA, observed to migrate from PC bottle to 10% aqueous ethanol at 100 ^{o}C for 30 min over four cycles of use, decreased rapidly after an initial ‘‘bloom’’ and then leveled out (Gándara, Mahía, Losada, Lozano, & Abuín, 1993). In contrast, another migration study conducted on 12 PC baby bottles supports the finding that dishwashing, boiling and brushing significantly increased the migration of BPA during incubation with water at 100 ^{o}C for 1 h (Brede, Fjeldal, Skjevrak, & Herikstad, 2003).

The most common techniques used for the analysis of Bisphenol-A are gas chromatography-mass spectrometry (GC-MS), liquid chromatography with fluorescence detection (HPLC–FLD) or liquid chromatography-mass spectrometry (LC–MS). The procedure for the migration test is the same with

the methods of overall migration and before the evaporation of the simulant, the solution is taken and analysed with the aforementioned techniques.

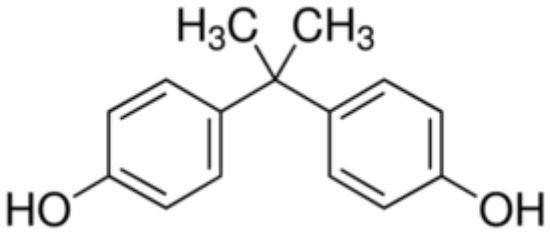

Figure 26: Structure of Bishpenol-A (BPA)

1.6 Validation

The development and validation of a method is a crucial analytical challenge. Validation is defined as the confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled (ISO, 1994). The essential parameters that analysts need to assess in order to check whether a method satisfies previously defined analytical requirements are the performance criteria or performance characteristics. In this stage all analytical parameters such as sample pretreatment, simulant preparation or evaporation have to be optimized in order to achieve the best results. The validation part, it is an important analytical issue as it demonstrates if the developed method is fit to purpose. The users of test results also require, for example, that the estimated value be repeatable under certain conditions, that the analytical method provide similar results when the experimental conditions are slightly modified and that the test method be capable of quantifying very low concentrations of the analyte (Ricard Boqué, Alicia Maroto, Jordi Riu, & Rius, 2002). It is important to notice that although nowadays the attention for the presence of basic validation data in scientific publications is surely higher than in past years, unfortunately a significant number of methods reported in literature are completely not validated. This fact raises some doubts on the reliability of the method as validation represents an essential component

of the measures that any laboratory should implement to produce reliable analytical data. If the laboratories that produce the results are compared to other similar laboratories and can show that they use good tools properly (validated methods under quality assurance conditions), that they internally control their processes and demonstrate proficiency by comparing their performance to similar laboratories, there are reasons to think that the general quality of the laboratories is translated to their individual results. In this way, the results of the precision (repeatability) and accuracy in a specific overall migration method and the validation process are presented and discussed in the following pages.

2. Scope and Objectives

European Union regulations focus on the consumers' protection since consumers daily use various materials, especially plastic, for storing their food. Plastics show numerous advantages such as accessibility, light weight and recycling capability. Consequently, it is necessary to test the suitability of these materials which are used as food contact materials with migration tests.

Taking into consideration the above mentioned points, the aim of this master thesis is to evaluate the suitability of selected plastic materials to come in contact with foodstuffs using overall migration tests. Particularly, the objectives of this study are:

- the determination of overall migration from plastic food packaging materials
- the development and validation of overall migration methods, using several simulants and many different conditions in each migration test

3. Materials and methods

3.1 Evaporation dishes calibration

3.1.1 Reagents, Apparatus

- Distilled water
- Acetone
- Evaporation dishes
- Thermostatically controlled oven
- Desiccator
- Analytical balance

3.1.2 Procedure

The calibration of evaporation dishes was performed to check their suitability for the overall migration test because it is a gravimetric method. The dishes were washed with soap and water and after that were rinsed with distilled water and acetone. Subsequently, the dishes were left to dry and then were placed in a thermostatically controlled oven at 105 ^{o}C to 110 ^{o}C for a period of 1 hour, to completely dry. Subsequently, the dishes were placed in a desiccator and allowed to reach ambient temperature for 45 minutes. At the end, the dishes were weighed, and the masses of each dish were recorded as mass 1 (m_1). The procedure was repeated three more times and the mean mass value of dishes, the weighing uncertainty (U_d), the uncertainty of difference (U) and average of absolute value were calculated. If the uncertainty of difference was lower than the average of absolute value, the dishes were suitable for use.

Figure 27: Evaporation dish

3.2 Total immersion

3.2.1 Reagents, Apparatus

- Distilled water
- Ethanol 10 % (v/v) in aqueous solution (simulant A)
- Ethanol 95 % (v/v) in aqueous solution
- Cutting slab (glass 25 x 25 cm)
- Scissors
- Rule (25 mm wide)
- Analytical balance
- Specimen supports constructed of stainless steel with cross arms attached by welding
- Glass tubes with ground neck (internal dimeter 35 mm and length 100 – 200 mm)
- Thermostatically controlled oven
- Evaporation dishes
- Steam bath
- Desiccator
- Measuring cylinder 100 ml
- Tweezers

The analytical balance and the thermostatically controlled oven have been calibrated by an authorised company. This is very important parameter on overall migration methods because are gravimetric methods and it is also prerequisite for ISO 17025.

3.2.2 Sample preparation - Procedure

At the beginning, three test specimens were required for sample for the migration test in the form of thin films. Every test specimen was laid on the cutting slab from glass and cut 1 dm^2 (100 x 100 mm), using the rule and the scissors. After that, every test specimen was cut into four equal test pieces (25 x 100 mm) and assembled onto the support of stainless steel.

For the exposure of test specimen into food simulant, five glass tubes were used, three for the test specimens and two for the blanks. 100 ml ± 2 ml of the food simulant were added into each tube and the tubes were stopped by the stoppers. The five tubes were placed in the already preheated thermostatically controlled oven at the test temperature and left until the simulant has attained the test temperature. During this procedure, the temperature of simulants was cross checked by a thermometer. When the five tubes achieve the desired test temperature, the three test specimens were placed into each of three tubes and stopped with stoppers. Each tube was marked for identification and also marked the liquid level on the outside of each tube with a suitable marker. The test specimens must be totally immersed in the simulant and if they were not then glass beads were added into the tube to raise the level of the simulant until total immersion was achieved. All of the tubes were replaced in the thermostatically controlled oven at the test temperature for the selected period of time after the temperature of the simulant has reached a temperature within the permitted tolerance for the test temperature. This procedure is very fast (only 2 hours), so it is necessary not to lose the tubes or the simulant the reached temperature. For exposure times of 24 hours or more it is acceptable to monitor the temperature of the airbath of the thermostatically controlled oven instead of the temperature of the simulant. After the end of time exposure to food simulant, the tubes were removed from the oven and the level of simulant was checked in each one. If the level of the simulant was fallen to more than 10 mm below the mark or any part of the test pieces was exposed, the test was repeated using new test specimens. If the level of simulant in a tube was less than 10 mm below the mark, the test

Figure 28: Glass tube for total immersion with specimen support

specimen removed and checked if the recovery of simulant was at least 90%. If it was not, then the test was repeated.

During the exposure of the test specimens to food simulant, five dishes were placed in another thermostatically controlled oven at 105 ^{o}C to 110 ^{o}C for a period of 1 hour, to dry. Afterwards, the dishes were placed in a desiccator and allowed to cool to ambient temperature for 45 minutes. Finally, the five dishes were weighed, and the masses of each dish were noticed as mass 1 (m_1).

Subsequently, the simulants from every tube were poured into separate dishes and then the dishes were placed to the steam bath for the evaporation of the simulants. Each of the tubes was washed with two lots of 10 ml of unused simulant and these washings were poured into the respective dishes. When the simulant had almost completely evaporated, the dishes were placed in the oven at 110 ^{o}C for a period of 1 hour, to dry. After the oven the dishes were placed in a desiccator and allowed to cool to ambient temperature for 45 minutes. After that, the five dishes were weighed, and the masses of each dish were noticed as mass 2 (m_2) and then the difference in masses m_1 and m_2 was calculated. At the end, the average of the three differences of masses was calculated (m_a). The same procedure was repeated for the 2 blanks dishes, where the final mass was m_b.

Figure 29: Evaporation dishes in steam bath

3.2.3 Calculations

The overall migration was expressed as milligrams of residue per square decimeter of the surface of the sample and were calculated using the following type:

$$M = \frac{(m_a - m_b) \times 1000}{S}$$

Where:

M: is the overall migration into the simulant in milligrams per square decimeter of surface area of sample intended to come into contact with foodstuffs

m_a: is the mass of the residue from the test specimen after evaporation of the simulant in which it had been immersed, in grams

m_b: is the mass of residue from the blank simulant, in grams

S: is the surface area of the test specimen intended to come into contact with foodstuff, in square decimeters

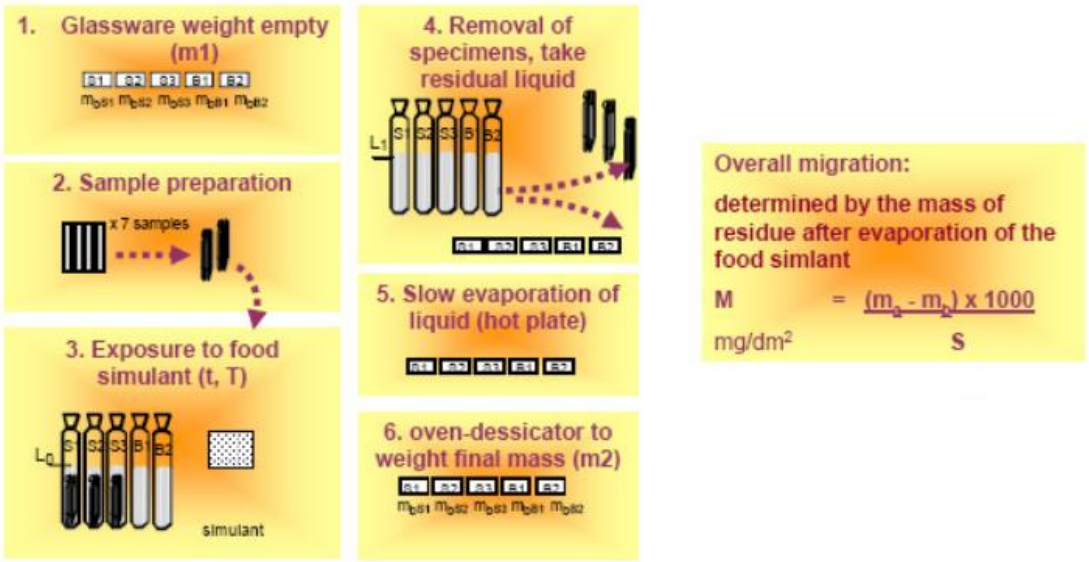

Figure 30: Principle for overall migration in aqueous simulants

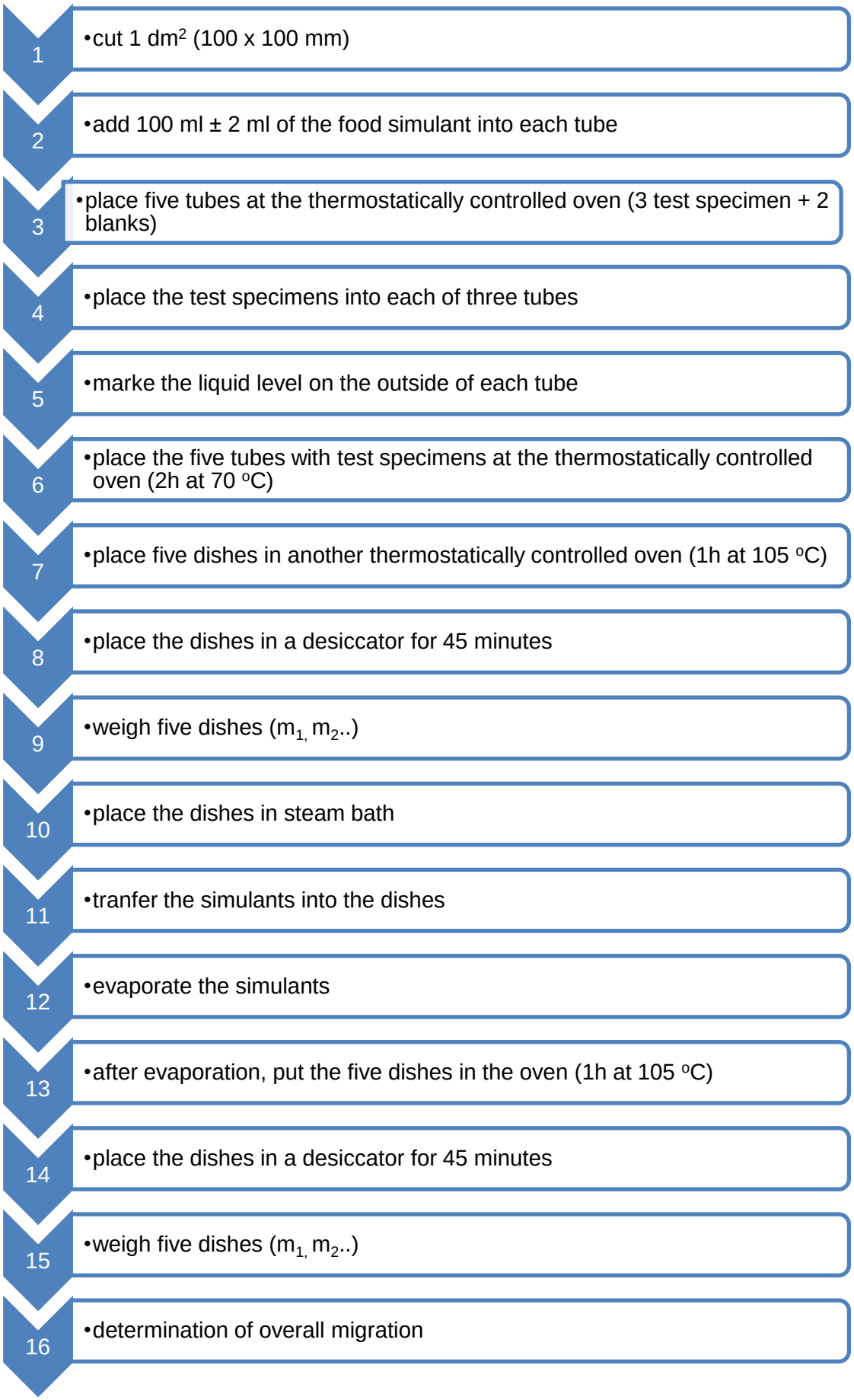

Figure 31: Flow diagram of Total immersion

3.3 Cell test

3.3.1 Reagents, Apparatus

- Distilled water
- Ethanol 10 % (v/v) in aqueous solution (simulant A)
- Ethanol 95 % (v/v) in aqueous solution
- Cutting slab (glass 25 x 25 cm)
- Scissors
- Rule (25 mm wide)
- Analytical balance
- Standard cell, type A
- Glass tubes with ground neck (internal dimeter 35 mm and length 100 – 200 mm)
- Thermostatically controlled oven
- Evaporation dishes
- Steam bath
- Desiccator
- Measuring cylinder 100 ml
- Tweezers

3.3.2 Sample preparation – Procedure

At the beginning, three test specimens were required for each sample for the migration test in the form of thin films. Every test specimen was laid on the cutting slab from glass with the surface to be in contact with the food simulant uppermost. The test specimens were cut by using the outer edge of the ring from the standard cell type A (Figure 32).

Figure 32: Cell type A with test specimen

For the exposure of test specimen into food simulant, five glass tubes were taken, three of them for the test specimens and the other two for the blanks. 100 ml ± 2 ml of the food simulant were inserted into each tube and the tubes were stopped by the stoppers. The five tubes and three standard cells were placed in the thermostatically controlled oven at the test temperature until the simulant and the cells have attained the test temperature. During this procedure the temperature of simulants was checked with a thermometer. When the cells achieved the test temperature, were removed from the oven and the test specimens were placed on the base of each cell. The cells were reassembled and then the tubes with the simulant were removed from the oven and the simulants were poured into each cell. The simulants for the blanks were remained into the two tubes. The three cells and the two tubes were replaced in the thermostatically controlled oven at the test temperature for the selected period of time after the temperature of the simulant has reached a temperature within the permitted tolerance for the test temperature. For 24 hours of exposure time or more, it is acceptable to monitor the temperature of the airbath of the thermostatically controlled oven instead of the temperature of the simulant. After the time of exposure to food simulant, the cells and the two tubes were taken for the oven.

During the exposure of the test specimens to food simulant, five dishes were placed in another thermostatically controlled oven at 105 ^{o}C to 110 ^{o}C for a period of 1 hour, to dry. Afterwards, the dishes were placed in a desiccator and allowed to cool to ambient temperature for 45 minutes. At the end the five dishes were weighed, and the masses of each dish were noticed as mass 1 (m_1).

Subsequently, the simulants from every cell and tube were poured into separate dishes and placed to the steam bath for evaporation. Each of the tubes was washed with two aliquots (10 ml each) of unused simulant and the washings were poured into the respective dishes. When the simulant had almost completely evaporated, the dishes were placed in the oven at 105 ^{o}C to 110 ^{o}C for a period of 1 hour, to dry. Afterwards, the dishes were placed in a desiccator and allowed to cool to ambient temperature for 45 minutes. Then, the five dishes were weighed, and the mass of each dish were noticed as mass 2 (m_2) and then the difference in masses m_1 and m_2 was calculated. At the end, the average of the three differences of mass (expressed as m_a) was calculated. The same procedure was carried out for the 2 blanks dishes, where the final mass was m_b.

3.3.3 Calculations

The overall migration was expressed as milligrams of residue per square decimeter of the surface of the sample and were calculated using the following type:

$$M = \frac{(m_a - m_b) \times 1000}{S}$$

Where:

M: is the overall migration into the simulant in milligrams per square decimeter of surface area of sample intended to come into contact with foodstuffs

m_a: is the mass of the residue from the test specimen after evaporation of the simulant in which it had been immersed, in grams

m_b: is the mass of residue from the blank simulant, in grams

S: is the surface area of the test specimen intended to come into contact with foodstuff, in square decimeters

Figure 33: Dish after evaporation

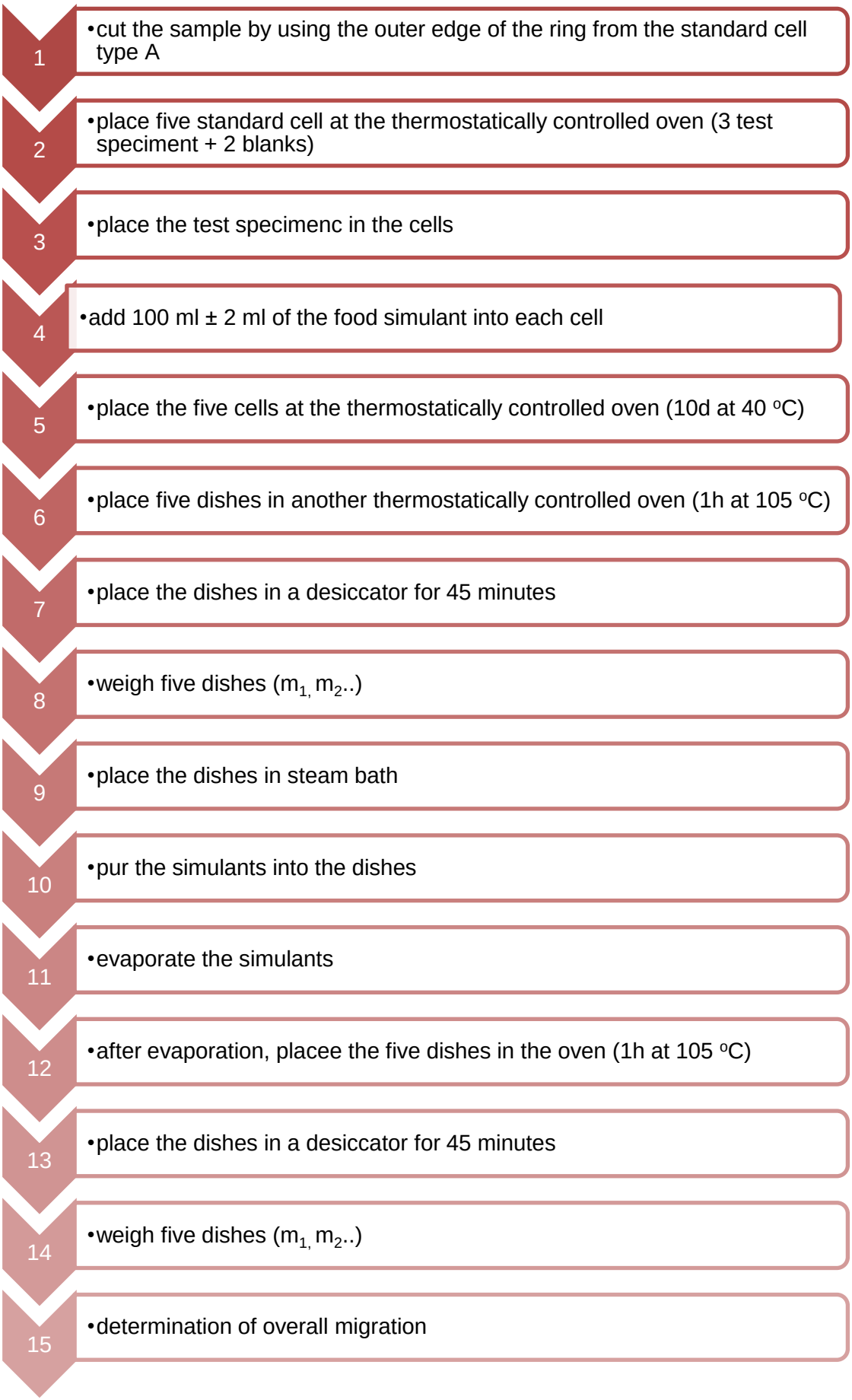

Figure 34: Flow diagram of Cell method

3.4 Adsorption by modified polyphenylene oxide (MPPO – TENAX)

3.4.1 Reagents, Apparatus

- Diethylether of 99,8% purity
- Modified polyphenylene oxide (MPPO)
- Nitrogen, purity 99,999%
- Scissors
- Rule
- Analytical balance
- Oven
- Petri dishes
- Glass rings
- Erlenmeyer flasks
- Filter funnels
- Vials, glass with capacities of 10 ml and 100 ml
- Pasteur pippetes

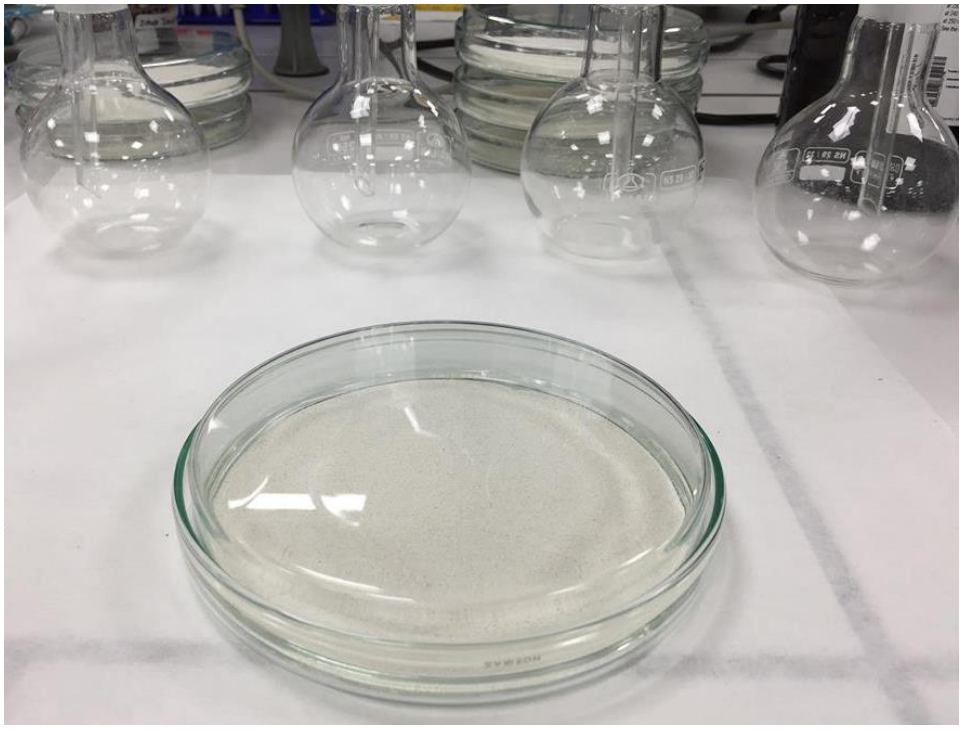

Figure 35: Petri dish with TENAX

3.4.2 Sample preparation – Procedure

At the beginning, three test specimens were required for each sample for the migration test in the form of thin films. Every test specimen was laid on the cutting slab from glass with the surface to be in contact with the food simulant uppermost. The test specimens were cut by using the glass ring.

Three test specimens were placed into three glass Petri dishes and then 5,64 g MPPO was placed on the surface of each test specimen. The same procedure was carried out for blank determination, omitting the test specimens. The oven was set at 60 ^{o}C and the test specimens were left in the oven for 10 days. After 10 days the test specimens were removed from the oven and allowed to cool to room temperature. The MPPO was transferred into the Erlenmeyer flask with the aid of a funnel and then 50 ml diethylether were poured through the funnel into the flask. Subsequently, a folded filter was placed into the funnel and the MPPO with the diethylether were poured into the funnel to another flask and after that the filter was rinsed with 30 ml diethylether. This procedure was repeated for 3 times and subsequently, the diethylether extract was evaporated to dryness with a rotary evaporator. The residue was reconstituted with 3 ml diethylether and the solution was transferred quantitatively to a pre-weighed vial (m_1). The solutions were evaporated to dryness, using a stream of nitrogen and the vials were weighed again (m_2). Finally, the average of the three differences of mass (m_a) was calculated. Similarly, the final mass (m_b) from the 2 blanks dishes was calculated.

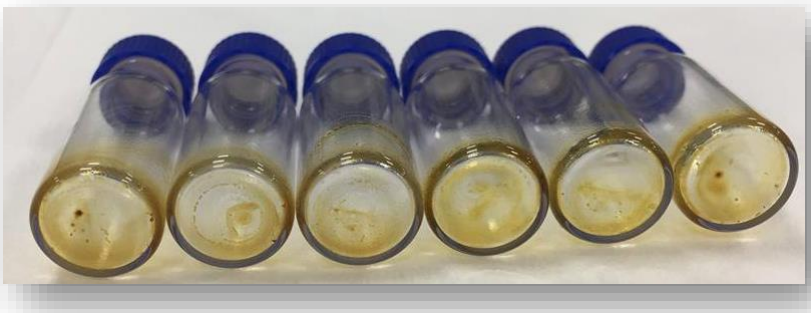

Figure 36: Vials with evaporated diethylether solution

3.4.3 Calculations

The overall migration was expressed as milligrams of residue per square decimeter of the surface of the sample and were calculated using the following type:

$$M = \frac{m_a - m_b}{s}$$

Where:

M: is the mass of migrated substances adsorbed onto MPPO from the test specimen, in milligrams per square

m_a: is the mass of the residue from the MPPO that had been in contact with the test specimen, in milligrams

m_b: is the mass of residue from the MPPO that had not been in contact with the test specimen, in milligrams

S: is the surface area of the test specimen that was in contact with the MPPO, in square decimeters

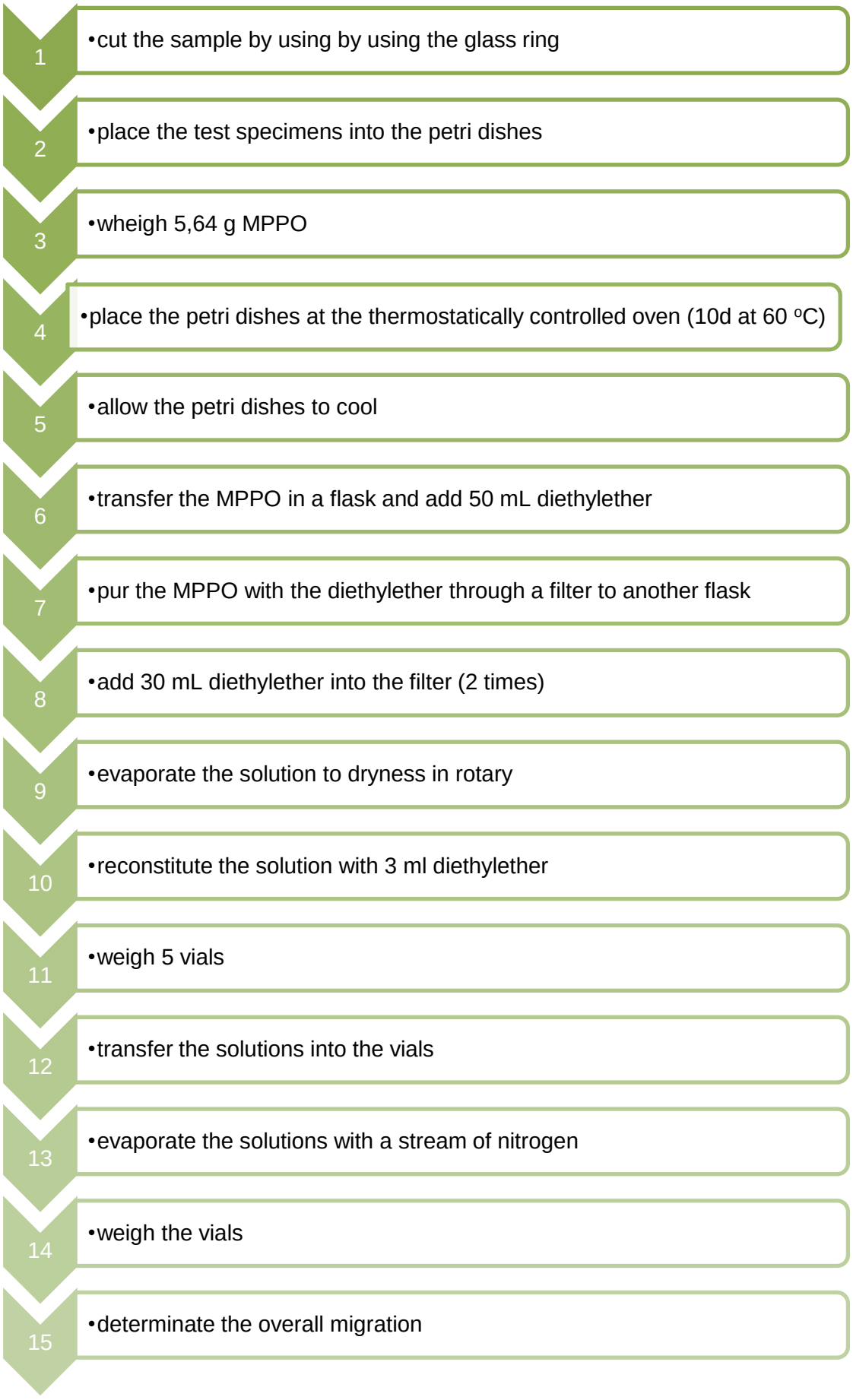

Figure 37: Flow diagram of Tenax method

3.5 Sample provision and storage

Twenty-six plastic samples were collected from Yotis company, a nylon sample for wrapping sausages “intestine” from a market place, and two samples of PA from two different proficiency tests (DRRR, Fapas). The plastics that were tested for overall migration were BOPP.T, PE, LDPE from Yotis. Details considering origin, name and number of plastics samples are presented in Table 2.

Table 2: Plastic samples

Samples	Name	Origin
PET.T	Polyethylene Tetraphthalate. Transparent	Yotis S.A.
BOPP perla	Biaxially-Oriented Polypropylene perla	
PET.MAT	Polyethylene Tetraphthalate. Mat	
PET.MET	Polyethylene Tetraphthalate. Metallic	
BOPP.T	Biaxially-Oriented Polypropylene Transparent	
BOPP white	Biaxially-Oriented Polypropylene white	
PP cast	Polypropylene cast	
OPA	Oriented Polyamide	
PE white	Polyethylene white	
PE	Polyethylene	
PE EVOH	Polyethylene Ethylene vinyl alcohol	
Paper		

PET	Polyethylene Tetraphthalate	
LDPE white	Low Density Polyethylene white	Yotis S.A.
LDPE	Low Density Polyethylene white	
LDPE thick	Low Density Polyethylene thick	
PVC	Polyvinyl Chloride	
OPP MAT	Oriented Polypropylene MAT	
OPP MET	Oriented Polypropylene Metallic	
OPP white	Oriented Polypropylene white	
OPP	Oriented Polypropylene MAT	
PA	Polyamide	German proficiency test (DRRR)
PE	Polyethylene	German proficiency test (DRRR)
Intestine		Super market
Nylon film (PA)		Fapas

4. Results and discussion

4.1 Validation of evaporation dishes

Fourteen dishes were validated to use them in the gravimetric method of overall migration. The procedure of the validation was described in chapter 3.1 and the average of the mass dishes, the weighing uncertainty (u_d), the uncertainty of difference (u) and the average of absolute value was calculated. Finally, we checked if the average (absolute value) was smaller than uncertainty of difference (u). The calculations based on the following equations

- Average: $\mathbf{m_d = \sum_{i=1}^{4} mi \,/\, 4}$
  m_d: average of masses
  $\Sigma_{i=1}$ mi: sum of mi

- u_d (weighing uncertainty): $\mathbf{u_d = f(md) \,/\, k}$
  f(m_d): function of extended uncertainty as a function of mass
  k: coverage factor for a certain confidence level (k=2 for confidence level 95%)

- u (uncertainty of difference): **$u = sqrt(2) * u_d$**

Table 3: Calculations for validation of evaporation dishes

Dishes	Mass Average (g)	u_d (weighing uncertainty)	u (uncertainty of difference)	Average (absolute value) (g)
1	59.3889	0.0011	0.0017	0.0005
2	60.0463	0.0012	0.0017	0.0005
3	62.1795	0.0012	0.0017	0.0006

4	58.7374	0.0011	0.0017	0.0007
5	63.5839	0.0012	0.0017	0.0007
6	63.5479	0.0012	0.0017	0.0007
7	61.1695	0.0012	0.0017	0.0003
8	57.9050	0.0012	0.0017	0.0011
9	61.1630	0.0012	0.0017	0.0009
10	63.5802	0.0012	0.0017	0.0006
11	59.3578	0.0012	0.0017	0.0008
12	63.5419	0.0012	0.0017	0.0009
13	59.4043	0.0012	0.0017	0.0006
14	58.7331	0.0012	0.0017	0.0004

As we observe, all the dishes were suitable for the overall migration tests.

4.2 Overall migration tests

From Table 2, the samples which were selected for overall migration test were BOPP, PE, LDPE, PA, Intestine and the sample from Fapas proficiency test (nylon film). Each one was tested in specific condition and temperature, with different simulant and with different method. At the beginning BOPP, PE and LDPE were tested with total immersion method, for 2 hours at 70 ^{o}C with simulant A (10% EtOH) but did not show any overall migration. After that BOPP was tested with simulant A and simulant B (20% EtOH) at 70 ^{o}C for 4 hours with total immersion method with no overall migration. The last test with BOPP was with cell method, simulant A and for 2h at 70 ^{o}C and did not show any migration again.

Table 4: Overall migration tests

TEST	SAMPLES	METHOD	SIMULANT	DONDITIONS	MIGRATION
1	BOPP, PE, LDPE	Total immersion	10% EtOH	2h 70 ºC	NO
2	BOPP	Total immersion	10% EtOH	4h 70 ºC	NO
3	BOPP	Total immersion	20% EtOH	4h 70 ºC	NO
4	BOPP	Cell	10% EtOH	2h 70 ºC	NO
5	PA	Cell	95% EtOH	10 d 40 ºC	YES
6	PA	Tenax	Tenax	10 d 60 ºC	YES
7	Intestine	Total immersion	10% EtOH	2h 70 ºC	YES
8	Intestine	Total immersion	95% EtOH	2h 70 ºC	YES
9	Nylon film (Fapas)	Total immersion	95% EtOH	6h 60 ºC	YES

Subsequently, a PA sample from Germany for a proficiency test was received which showed a migration to food simulant 95 % EtOH. This sample was from the one side PA and from the other side polyethylene (PE) and for this reason

the appropriate method for this multilayer material was the cell method to get into contact with food simulant only with the PA side. The sample was tested for 10 days at 40 ºC with 95 % EtOH and we achieved a successful test.

Table 5: Overall migration of PA with Cell method (Proficiency test, Germany)

	BEFORE (Evaporation) (g)	AFTER (Evaporation) (g)	DIFFERENCE (g)	OVERALL MIGRATION (mg/dm^2)
A	57.9077	57.9143	0.0066	
B	63.5820	63.5878	0.0058	
C	58.7338	58.7405	0.0067	*2.5*
BLANK	59.4050	59.4052	0.0002	
STDEV			0.0005	

Figure 38: Logo from the German reference office

After that, the same sample was tested with the Tenax method for 10 days at 60 ºC. The Tenax method was successful but the results were much lower than the results from the cell method. This could happened because the tenax that we used, was not well cleaned with Soxhlet extractor or because it had not come into good contact with the material. Generally, the biggest problem in tenax method is the contamination of the Tenax because is the only reusable simulant in contrast with A, B, C and D which are prepared from the beginning

each experimental day. The Tenax every time at the end of the test is subjected to a series of solvent purification-cleaning using Soxhlet extractor.

Table 6: Overall migration of PA with Tenax method (Proficiency test, Germany)

	BEFORE (Evaporation) (g)	**AFTER (Evaporation) (g)**	**DIFFERENCE (g)**	**OVERALL MIGRATION (mg/dm^2)**
A	2.3362	2.3374	0.0012	*0.7*
B	2.3781	2.3796	0.0015	
C	2.3371	2.3383	0.0012	
D	2.3530	2.3541	0.0011	
BLANK	2.3460	2.3463	0.0003	
STDEV			0.0002	

Afterwards, a commercial special plastic packaging material for wrapping sausages “intestine” was tested. From the “intestine” we cut 0.5 dm^2 and it was tested with the total immersion method for 2 h at 70 ^{o}C, with two simulants. The first one was 95% EtOH and the second one simulant A (10% EtOH). The intestine had the biggest overall migration with the simulant 95% EtOH as compared to the other samples and a lower migration with the simulant 10% EtOH.

Table 7: Overall migration of intestine, 95% EtOH 2h 70 ºC

	BEFORE (Evaporation) (g)	AFTER (Evaporation) (g)	DIFFERENCE (g)	OVERALL MIGRATION (mg/dm^2)
A	59.3549	59.3639	0.0090	
B	60.0366	60.0444	0.0080	
C	61.1570	61.1654	0.0080	*16.6*
BLANK	50.7302	50.7301	-0.0001	
STDEV			0.0006	

It can be observed that the results of overall migration of intestine with simulant 95% EtOH for 2h at 70 ºC exceeded the migration limit which is 10 mg/dm^2. However, this does not mean that intestine exceeded the migration into sunflower oil or olive oil which are simulants D and they are used to determine the migration in fatty foodstuff. To make a correct conclusion for fatty foodstuff we need more experiments with simulant D. The use of isooctane or 95% ethanol significantly simplifies testing procedures.

Table 8: Overall migration of intestine, 10% EtOH 2H 70 ºC

	BEFORE (Evaporation) (g)	AFTER (Evaporation) (g)	DIFFERENCE (g)	OVERALL MIGRATION (mg/dm^2)
A	59.3867	59.3894	0.0027	
B	57.8047	57.8083	0.0036	
C	58.7365	58.7389	0.0024	*5.4*
BLANK	61.2902	61.2904	0.0002	
STDEV			0.0006	

At the end, we participated in one more proficiency test Fapas. In this test we cut 1 dm^2 from Nylon film (PA), add 95% EtOH simulant for 6 h at 60 ^oC with total immersion method. From the results we concluded that the sample had a migration 2.7 mg/dm^2, the assigned value was 2.6 mg/dm^2 and our z' score was 0.1.

Table 9: Overall migration of nylon film (PA), 95% EtOH 6h 60 ^oC

	BEFORE (Evaporation) (g)	**AFTER (Evaporation) (g)**	**DIFFERENCE (g)**	**OVERALL MIGRATION (mg/dm^2)**
A	59.3544	59.3572	0.0028	
B	59.3804	59.3833	0.0029	
C	58.7295	58.7324	0.0029	*2.7*
BLANK	61.1558	61.1560	0.0002	
STDEV			0.0006	

Figure 39: Logo of the fapas

We studied the literature and compared our results in order to reveal similarities and differences (Table 10 and 11). Although all plastics are not the same with other studies. Czerniawski & Pogorzelska (1997) tested three different plastics (HDPE, PA, PS) with three different methods but in the same conditions (10d 40 ^{o}C). The similar plastic with our experiments was PA but it was tested with total immersion and pouch method in the same conditions. However, according to Directive 85/572/EEC2 their work should be performed using the recommended aqueous food simulants, i.e. distilled water, 3% (w/v) acetic acid in aqueous solution and they used also isooctane for non-polar plastics, 95% ethanol for polar plastics. According to their results, they found low migration limits for every plastic and especially for PA which is the similar plastic, the overall migration in pouch method was 0.9 mg/dm^2 with 95% EtOH and 1.5 mg/dm^2 with isooctane as a food simulant with total immersion method.

Goulas et al. (2002) tested monolayer commercial samples (HDPE, BOPP) of plastic packaging materials. They used as food simulants distilled water and 3% aqueous acetic. Iso-octane was also used as an alternative fatty food simulant. The migration was tested by the total immersion method for 10 d at 40 ^{o}C and for 2 d at 20 ^{o}C. The overall migration values into distilled water and isooctane was significantly lower than the upper limit (10 mg/dm^2) set by the EU in all cases. However, in the present study, BOPP material was tested using the same method with 10% EtOH as a simulant but in different conditions (duration, temperature) and the overall migration was found 0 mg/dm^2. On the contrary, in their study BOPP had an overall migration with isooctane as a simulant 2.4 mg/dm^2.

In another study (Antonios E. Goulas, 2001), overall migration was determined in multilayer films (PA/tie/LDPE/LDPE/LDPE) where rectangular strips of each film sample (surface area 100 cm^2) were placed in two side contact (total contact surface area 200 cm^2) with 100 ml of food simulant (distilled water, 3% aqueous acetic acid, or iso-octane) in glass beakers. The conditions were 10 d at 40 ^{o}C and for 2 d at 20 ^{o}C and the higher overall migration was with isooctane as a food simulant (2.9 mg/dm^2). However, we didn't test overall migration in multilayer films, but we had tested the outer films of their multilayer films like PA and LDPE.

Galotto & Guarda (2004) investigated the suitability of alternative fatty food simulants on overall migration determination under thermal treatment and microwave heating in different plastic materials, which were used for packs subjected to thermal treatment or microwave heating. PA/PE, PA and PP were tested with the cell method and were in contact for 10 d at 40 ^{o}C with10% EtOH, isooctane and 95% EtOH. PP presented a high inert behavior during microwave heating, although contact time facilitates diffusion of migrants. PA/PE presented high values of overall migration (7.5 mg/dm^2 and 4.8 mg/dm^2) but it was under the overall migration limit. For the PA which is the same plastic with our study, the overall migration is exactly the same with our result (2.4 mg/dm^2) with the same method, in the same conditions and with the same simulant (95% EtOH).

Material	Method	Conditions (duration, temperature)	10% EtOH (Simulant A)/Migration (mg/dm^2)	3% Acetic acid (Simulant C)/Migration (mg/dm^2)	Isooctane/ Migration (mg/dm^2)	95% EtOH/Migration (mg/dm^2)	References
HDPE	CELL	10d 40 °C	0.3	0.2		0.4	(Czerniawski & Pogorzelska, 1997)
	TI	10d 40 °C			0.9		
PA	PM	10d 40 °C	0.4	0.3		0.9	
	TI	10d 40 °C			1.5		
PS	TI	10d 40 °C	1.5	0.8	6.1	1.3	
HDPE	TI	10d 40 °C	0.9	2.1			(A E Goulas, Riganakos, Badeka, & Kontominas, 2002)
BOPP	TI	10d 40 °C	0.3	0.7			
HDPE	TI	2d 20 °C			4.2		
BOPP	TI	2d 20 °C			2.4		
PA/tie/LDPE/LDPE/LDPE	TI	10d 40 °C	0.4	0.5			(Antonios E. Goulas, 2001)

Material	Method	Conditions	10% EtOH (Simulant A)/Migration (mg/dm^2)	3% Acetic acid (Simulant C)/Migration (mg/dm^2)	Isooctane/ Migration (mg/dm^2)	95% EtOH/Migration (mg/dm^2)	References
PE/tie/PA/tie/PE	TI	10d 40 °C	0.7	0.8			(Antonios E. Goulas, 2001)
PE/tie/PA/tie/PE	TI	2d 20 °C			2.9		
PA/PE	CELL	10d 40 °C			7.5	4.8	(Galotto & Guarda, 2004)
PA	CELL	10d 40 °C	1.5			2.4	
PP	CELL	10d 40 °C	0.8			0.2	
BOPP	TI	2h 70 °C	0				OURS
PE	TI	2h 70 °C	0				
PA	CELL	10d 40 °C				2.4	
	Tenax	10d 60 °C				0.7	
Intestine	TI	2h 70 °C	5.4			16.6	
Nylon film (PA)	TI	6h 60 °C				2.7	

Table 10: Overall migration (mg/dm^2) of plastics samples from different studies and our experiments

4.3 Validation of overall migration method by cell

As mentioned in the chapter 4.1 the laboratory participated in a proficiency test from the German reference office for proficiency testing and reference materials, (Detaches Referenzbüro für Ringversuche und Referenzmaterialien GmbH). A polyamide sample was tested, using the overall migration method by cell, with 95% EtOH for 10 days at 40 ^{o}C. The value of overall migration was 2.5 mg/dm^2. After that the sample was used as reference material to conduct the validation of the method. The sample was tested three times with four replicates each time and one blank sample. The best estimate for the true value was 5.58 ± 1.01 mg/dm^2 (uncertainty 95.5%).

4.3.1 Precision in terms of Repeatability and Reproducibility

In ISO 5725, accuracy of a measurement result or a measurement method or a measurement system is a general term which involves trueness and precision. Trueness, the closeness of agreement between the average value obtained from a large series of measurement results and an accepted reference value, is usually expressed in terms of bias which is the difference between the expectation of the measurement results and the accepted reference value. Precision, the closeness of agreement between independent measurement results obtained under stipulated conditions, is usually expressed in terms of standard deviation of the measurement results.

Repeatability is the closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement (Jcgm, 2008). The measurements are taken by a single person or instrument on the same item, under the same conditions, and in a short period of time. Reproducibility is measurement results under reproducibility conditions where measurement results are obtained with the same method on identical test items in different laboratories with different

operators using different equipment. An “internal reproducibility” limit, R, can be calculated from the run-different intermediate standard deviation.

The results showed RSD_r % = 6.65 which shows the good repeatability of the developed method and reproducibility RSD_R % = 8.59.

Table 11: Repeatability of migration method with cell and 95% EtOH, Day 1

	BEFORE (Evaporation) (g)	**AFTER (Evaporation) (g)**	**DIFFERENCE (g)**	**OVERALL MIGRATION (mg/dm²)**	**RSD_r%**
A	62.1812	62.1866	0.0054	*2.5*	*6.65*
B	58.7344	58.7401	0.0057		
C	59.4063	59.4125	0.0062		
D	63.5818	63.5872	0.0054		
BLANK	59.4050	59.4052	0.0002		
STDEV			0.0004		

Table 12: Reproducibility of migration method with cell and 95% EtOH

	DAY 1	**DAY 2**	**DAY 3**	**DAY 1-3**
AVERAGE (g)	0.0064	0.0057	0.0056	0.0058
STDEV	0.0005	0.0004	0.0004	0.0005
RSD_R %				8.59

4.3.2 Trueness

Trueness is usually estimated using certified reference materials (CRM), but in cases where this is not feasible, measurements through recovery of additions of known amounts of the analytes to a sample (blank matrix) can be utilized. Trueness can also be assessed when the laboratory takes part in a proficiency testing scheme. In this case, the reference value corresponds to the consensus value obtained by the participating laboratories (Ricard Boqué et al., 2002). In our case, the trueness was assessed when our laboratory was taken part in the RVEP 180556 overall migration (fatty foodstuffs) proficiency test and we participated with great success with z'-score: 1.92 < 2.

4.3.3 Accuracy

Accuracy is one of the key parameters to be assessed for method validation and involves common systematic errors (bias). Accuracy of the method is calculated as accuracy = (repeatability) + (trueness). Therefore, since our method is repeatable and trueness, then it is also accurate.

5. Conclusions

Packaging materials protect foods from spoilage, but they may also transfer chemicals substances which have a negative impact on the quality and safety of food. At the same time, efforts are being made to create inert materials and study various migration cases. To this end, three methods were optimized, and one was validated for the overall migration. Noticeably, the developed methods were tested based on the British Standard methods (1186:2002). All the quality characteristics of the validated method including precision (repeatability, reproducibility), trueness and accuracy were acceptable. Consequently, the developed validated method was capable for the determination of overall migration with 95 % EtOH as the food simulant.

Additionally, this master thesis evaluated the overall migration of various plastic food packaging materials. The overall migration of determined materials ranged from 0 mg/dm^2 to 16,6 mg/dm^2. The plastic material with the higher overall migration was the “intestine” while plastics materials from Yotis S.A did not show any migration potency.

Positively, the materials from Yotis did not show any migration to the aqueous simulants (10% and 20% EtOH) tested. Furthermore, even if we increased the contact time with the simulants, the plastics remained inert. The polyamide from German reference office was the first material which presented migration into the food simulant (95% EtOH). However, the same material was tested with the Tenax method and the results were lower than the results from the cell method applied.

The intestine was the material with the higher proportion of overall migration (16,6 mg/dm^2) and it was tested both with aqueous and fatty simulants. The proportion of overall migration with 95% EtOH, as the stimulant, was three times higher than the migration with 10% EtOH and it exceeded the overall migration limit (10 mg/dm^2). Additionally, during the experiments we realized that the material had to be cut only with special tongs, otherwise the method would resulted in negative errors.

Overall migration was significantly higher when using the fatty simulants (isooctane and 95% EtOH) compared to aqueous simulants (10% EtOH and

3% Acetic acid), which came in accordance to other migration studies. Consequently, packaging materials intended to be used with fatty matrices have to be carefully tested.

We also noticed that the PA was the most studied plastic material using the total immersion for 10 days at 40 ^{o}C.

Moreover, the validation experiments were satisfactory, and the 2 proficiency tests were successful for both methods tested (z' score = 1,92, z' score = 0,1).

Finally, through the daily experiments we succeeded higher repeatability, accuracy and reliability of these gravimetric methods and the results illustrate the importance of studying overall migration from plastics packaging materials. However, after validating all overall migration methods we should proceed in specific migration determinations (e.g. Bisphenol-A by HPLC-DAD).

ABBREVIATIONS AND ACRONYMS

FCM	Food Contact Material
EC	European Commission
EFSA	European Food Safety Authority
EURL-FCM	European Reference Laboratory for Food Contact Material
EU	European Union
IAS	Intentionally Added Substances
NIAS	Non-Intentionally Added Substances
BADGE	Bisphenol A diglycidyl ether
BFDGE	Bisphenol F diglycidyl ether
NOGE	Novolac glycidyl ethers
PET	Polyethylene terephthalate
BOPP	Biaxially oriented polypropylene
PP	Polypropylene
PA	Polyamide
PE	Polyethylene
PVC	Polyvinyl Chloride
EVOH	Ethylene vinyl alcohol
OML	Overall Migration Limit

SML	Specific Migration Limit
MPPO	Modified Polyphenylene Oxide
OPA	Oriented Polyamide
LDPE	Low Density Polyethylene
HDPE	High Density Polyethylene
OPP	Oriented Polypropylene
CRM	Certified reference material
EMAP	Equilibrium modified atmosphere packaging
BPA	Bisphenol – A

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