DETERMINATION OF PARTITION AND DIFFUSION COEFFICIENTS OF COMPONENTS OF TWO RUBBER ADHESIVES IN DIFFERENT MULTILAYER MATERIALS

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Abstract

Rubber adhesives both natural and synthetic are widely used in the manufacture of multilayer laminates, commonly used as food packaging. For this reason, it is very important to identify the compounds that could migrate from the laminate into the food. Twenty nine compounds were identified in two different rubber adhesives, some of them with high toxicity levels according to the theoretical model of Cramer, such as benzene 4–cyanocyclohexene and benzene isothiocyanate. Partition of these compounds between the adhesive and different substrates and diffusion in both media were experimentally determined obtaining great variability of values depending on the properties of the compounds and the substrates used. Finally, only three compounds were found to migrate, benzene 4-cyanocyclohexene, butylated hydroxytoluene and 2-cyclopentyl-1.3.5-trimethylbenzene, with values of migration of 1.79, 22.9 µg/dm^2 and 4.57 µg/dm^2 respectively. These values were below the specific migration limits (SML) enforced by the legislation of the European Union (EU) and the values recommended by Cramer.

Keywords: adhesives, natural and synthetic rubber, diffusion and partition coefficients, migration.

1. Introduction

Natural and synthetic rubber adhesives are elastomeric resins. Both usually have excellent peel strength but low shear strength properties [1].They are generally supplied as solvent or water solutions and as pressure sensitive tapes.

Natural rubber has been used in adhesives for more than a hundred years and is highly suitable for the purpose because of its excellent flexibility and good tack retention properties. These properties make it ideal for pressure sensitive adhesive applications. Natural rubber solutions are usually employed for bonding paper, felt and textiles in the manufacture of stationery, carpets and for packaging to attach metal foil to paper or wood. In general, chemical catalysts or accelerators may be used at ambient temperature, or heat curing can be used to vulcanize the natural rubber to improve the strength and temperature resistance. Natural rubber has good resistance to water, but poor resistance to oils, organic solvents, and chemical oxidizing agents. Natural rubber adhesives set usually by solvent or water evaporation [1]. One natural rubber adhesive has been studied in this work.

Rubber adhesives can also be synthetic. Synthetic rubber adhesives can be based on different substances such as butyl, polyisobutylene, nitrile or styrene butadiene. In this work a styrene butadiene rubber (SBR) was studied. SBR copolymers are often used as the primary components in elastomeric adhesives, and they are also used as a major additive to adjust properties of other resin-based adhesives. They are available in a variety of formulations. Water dispersions are very common, as solvent solutions or as hot melt formulations. Often tackifiers, plasticizers, antioxidants and other additives are added to SBR adhesives formulations. SBR adhesives are used as pressure sensitive adhesives and laminating adhesives for paper, textile, leather, plastic films and metal foils [1].

As can be seen a wide variety of substances are added to these adhesives to improve their properties. When these adhesives are used for the manufacture of food packaging, these substances can diffuse trough the packaging and migrate to food what represents a potential risk for the health of the consumers. For this reason it is very important to determine the potential of migration from the adhesives. As it was described in previous works [2, 3], two main mechanisms affect the migration processes from packaging to food, partition and diffusion. In the case of multilayers manufactured with an adhesive bonding two substrates it is necessary to know the diffusion coefficients in each layer of the laminate as well as the partition coefficient at each interface of the laminate-food system. Among these coefficients, the partition between the adhesive and its substrate, K_{AS}, plays an important role in determining the level of migration from the laminate into the food. Unfortunately little is known yet about the K_{AS} coefficients for rubber adhesives.

Because of that one of the main aims of this work was to determine them for natural and synthetic rubbers and different substrate materials. These diffusion and partition coefficients will be later used to implement the mathematical models for predicting migration values.

In practice, laminates with adhesives are manufactured not only using plastic films as substrates but often adhesives are used to stick plastic/paper or plastic/cardboard as well as to stick paper/cardboard to paper/cardboard. Little is known about the diffusion of substances which are contained in the rubber adhesives in cardboard or paper. Therefore in this work some results are reported on the diffusion of several compounds coming from rubber adhesives through different substrates.

2. Materials and methods

2.1. Reagents and solutions

The standards 4-vynil-1-cyclohexane, 1,2,4-trimethylcyclohexane, ethylbenzene, 4-tert-butyltoluene, phenylcyclohexane, 4-cyanocyclohexene, 1-phenyl-1-cyclohexene, benzene isothiocyanate, styrene, 1,3-diethylbenzene, 1-methylethylbenzene, butylated hydroxytoluene, 1-methyl-4-(1-methylpropyl)-benzene, toluene, 4-tert-butyltoluene, alkanes standard solution C_8-C_{20} and *p*-tertbutylphenol were purchased from Sigma-Aldrich Química S.A (Madrid, Spain). All of them were of analytical quality. Dichloromethane, methanol and hexane were purchased from Scharlau Chemie S.A (Sentmenat, Spain). All of them were HPLC grade. Purified water was obtained from a Milli-Q 185 Plus system from Millipore (Bedford, MA, USA).

Solutions of *p*-tertbutylphenol of 190 µg/g in methanol (solution A), 190 µg/g in hexane (solution B), 10 µg/g in methanol (solution C) and 3 µg/g in hexane (solution D) were used as internal standard solutions.

2.2. Samples and laminates

Two rubber adhesives with very different composition were studied, a water based synthetic rubber adhesive (SR) and a solvent-based natural rubber adhesive (NR). Samples were provided by 2 adhesive companies. SR was an aqueous dispersion of carboxylated styrene butadiene copolymer (SBR) with a 53% solid content and with an aqueous dispersion of acrylate and butadiene copolymer as thickening agent. NR was a solution of natural rubber in an organic solvent. No more precise information about formulation can be supplied due to confidentiality reasons.

Laminates with the structure [substrate-adhesive-substrate] were manufactured in the laboratory. The laminates structures were as follows:

- Laminate 1: [paper – SR (9.6 ± 1.1 g/m^2) – paper]
- Laminate 2: [PVC – NR (22.8 ± 0.0 g/m^2)– PVC]

In laminate 1, paper used as substrate was a glossy paper, treated with a white kaolin for having a smooth surface that makes the paper more suitable for the printing inks, grammage of the paper was 66.7 g/m^2, thickness was 52 µm and density was 1.284 g/cm^3. The adhesive, layer had a thickness of 10 µm and a density of 0.932 g/cm^3.

In laminate 2, PVC used as substrate was a PVC-H film with a grammage of 44 g/m^2, a thickness of 30 µm and a density of 1,458 g/cm^3. The adhesive layer had a thickness of 20 µm and a density of 0.95 g/cm^3

For the application of the adhesive on the substrate at laboratory scale different Meyer rods, with different notchs, were used. After having prepared the laminates, they were passed through a laminator at 90°C. Finally, the laminates were cut into 10 cm by 10 cm cut-outs and wrapped in aluminum foil, leaving them for 1 week to reach the equilibrium. The grammage of adhesive expressed as g/m^2 was obtained by weight over the dried sample.

2.3. Gas chromatography – mass spectrometry

The equipment used was a CTC Analytics CombiPal autosampler coupled to a HP 6890N gas chromatograph with a mass spectrometer MS 5975B detector, all of them from Agilent Technologies (Madrid, Spain). The capillary column used was a HP-5MS (30 m x 0,25 µm x 250 µm) from Agilent Technologies (Madrid, Spain). The oven program was as follows: 40ºC for 2 min, with rate of 10°C/min up to 250 º C, maintained for 3 min. The mass detector was set at SCAN mode (in the range m/z 45-350) for the identification of the compounds and in SIM mode for quantification purposes. Quantification ions are shown in table 1.

2.4. Optimization of HS-SPME conditions

The first step was to select the most appropriate solid phase microextraction (SPME) fiber for the HS-SPME-GC-MS analysis. The fiber selection was based on obtaining the maximum number of peaks and a high signal intensity for the detected peaks. Five different fibers were tested 100 µm polydimethylsiloxane (PDMS), 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 50/30 divinylbenzene/Carboxen /Polydimethylsiloxane (DVB/Carboxen/PDMS), 100 µm polyacrylate and 70 µm carbowax/divinylbenzene (CW/DVB). All of them were from Sigma-Aldrich Química S.A (Madrid, Spain).

The second step was the optimization of the SPME extraction conditions. Three different parameters were optimized: extraction temperature (T_{ext}) from 40 to 80ºC, extraction time (t_{ext}) from 10 to 30 min, and desorption time (T_{des}) from 1 to 5 min. The optimization procedure was carried out using an experimental design by means of Modde software. For this purpose, a response surface design method (RSM) was used**.** Final HS-SPME extraction conditions were: T_{ext} 80ºC, t_{ext} 30 min and T_{des} 1 min. The fiber selected for both adhesives was the PDMS fiber.

2.5. Identification of the compounds present in the rubber adhesives and toxicity classification

For the identification of the compounds present in SR and NR adhesives, 1 gram of pure adhesive was placed in a 20 ml vial and analyzed by HS-SPME-GC-MS.

The identification of the compounds was based on the comparison of the retention time and the mass spectrum with the pure standards. The identified compounds were then classified according to the toxicity Cramer rules with the software Toxtree v1.51 (Ideaconsult Ltd.).

2.6. Determination of the initial concentration profile (CP_0) of rubber adhesives

For the determination of the CP_0 in the SR adhesive the samples were water diluted (1/200 w/w) prior to the analysis. Three aliquots of 5g of the water diluted sample were spiked with 10 µL of solution A and analyzed by HS-SPME-GC-MS.

Due to the difficulty of finding all the necessary standards, some of the identified compounds were quantified with standards that had a similar chemical structure.

The calibration curve was prepared by diluting the standards in water at different concentration levels. Three replicates of each point of the calibration curve were prepared and analyzed.

The NR adhesive was not available for its extraction. Due to this, it was necessary to work with the laminates for the CP_0 determination of this adhesive. A liquid extraction of laminate 2 was carried out. For this purpose laminate 2 was cut into small cut-outs of approximately 0.5 cm x 0.2 cm. The first step was the selection of the most appropriate extraction solvent. Three different polarity solvents were used, hexane, methanol and dichloromethane. For each solvent, 1 g of laminate 2 was extracted with 6g of solvent during 24 hours at 40ºC; 3 consecutive extractions were made on each sample and afterwards the 3 extracts were joined in a vial and concentrated using a N_2 current up to 1g [3]. Final extraction protocol was as follows: 1 gram of laminate 2 cut-outs was three consecutive times extracted with 6g of hexane during 24 hours at 40ºC, the 3 extracts were joined, spiked with 20 µL of solution B and concentrated using a N_2 current up to 1g solution. The PVC substrates were also extracted following the same procedure as with the laminate.

The calibration curve was prepared in hexane at different concentration levels. Three replicates of each point were prepared and analyzed.

2.7. Determination of the partition and diffusion coefficients

Partition and diffusion experiments were carried out in laminates 1 and 2.

The partition coefficient of a compound C between the adhesive and the substrate, $K_{A,S}$, can be calculated according to next equation.

$$K_{\mathrm{A,S}} = \frac{[C_{eq}]_A}{[C_{eq}]_S} \quad (1)$$

Where $[C_{eq}]_A$ and $[C_{eq}]_S$ are the concentrations of compound C – units mg/cm³ - in the adhesive and respectively in the substrate at equilibrium.

As it was impossible to separate the substrates from the adhesive at equilibrium since they were glued, the methodology used by Canellas et al [2] and Vera et al [3] for calculating $K_{A,S}$ was used. In this methodology the laminate was sandwiched between two substrates, identical to those used to manufacture the laminate:

- Paper-[paper – SR– paper]-paper
- PVC-[PVC – NR– PVC]-PVC

These sandwiches were placed in a migration cell that consists of two aluminum plates of 1 dm by 1 dm of surface which can be tightened together with a controlled torque of 0.8 Nm. The cells were kept closed in a constant temperature oven at 40 for 30 days in order to assure that the compounds reached the equilibrium concentration in each layer of these sandwiches. All the experiments were carried out in duplicate. After this period, cutouts from the central part of the two sandwiched substrates (5 cm by 5 cm) were spiked with 10 µL of solution C (for paper samples) or solution D (for PVC samples), left equilibrate for 24 hours at room temperature and analyzed by HS-SPME-GC-MS. Afterwards, the concentration of the compound at equilibrium in the sandwiched substrate was determined $[C_{eq}]_S$..

The $[C_{eq}]_A$ was calculated with a simple balance equation proposed by Canellas et al [2] and straightforward the $K_{A,S}$ coefficients were determinate with equation 1.

To determine diffusion coefficients of the compounds in the adhesives and substrates, the laminates 1 and 2 were sandwiched between 10 sheets of virgin substrates placed at each side of the laminate, and these sets were kept in the migration cells used previously for the partition experiments at 40 and 60 ºC during 24 hours. After this period, cut-outs of 5 cm by 5 cm from the central parts of the added 2x10 substrates were produced and they were analyzed by HS-SPME-GC-MS. The results obtained were mean concentrations of migrants in each of the 2 x10 added substrates at time t, $[C_t]_S$. The same procedure was followed with other migration cells after 48 h respectively.

Calibration curves for quantifying paper and PVC samples were built by spiking 5 cm by 5 cm cut-outs of paper and PVC virgin samples with 10 µL of the compounds, in methanol and hexane respectively, at different concentration levels. Afterwards the samples were spiked with 10 µL of solution C (for paper samples) or solution D (for PVC samples), left equilibrated for 24 hours at room temperature and analyzed by HS-SPME-GC-MS.
Knowing, for a sandwich made of the laminate and virgin substrates the CP_0 and the $K_{A/S}$ calculated previously, the concentration profile $[C_t]_S$ of a compound diffusing from the adhesive into the stack of virgin substrates can be calculated with an appropriate numerical solution of the diffusion equation [3-6]. The fitting parameters in these calculations are the diffusion coefficients D_A and D_S of the compound in the adhesive and respectively the substrate material. The iteration method used to derive D_A and D_S from the experimental concentration profiles $[C_t]_S$ was presented previously [3-6].

2.8. Determination of migration results.

Migration tests from these two laminates were carried out using Tenax as food simulant due to these kinds of laminates are commonly used for dry food packaging and Tenax is recommended for the migration test.

For migration tests the methodology proposed by Canellas and Vera et al.[2, 3] was used. Cut-outs of each laminate, size 1 cm by 8.5 cm, were covered with 0.34 grams of Tenax forming an uniform layer (UNE-EN 14338)[4]. This system was placed inside a Petri dish and kept in the oven at 40 ºC for 10 days. Then Tenax was extracted 2 consecutive times with 3.4 mL each time of acetone, solutions were put together, added with 10 µL of internal standard solution, concentrated under a stream of N_2 to 200 mL and finally analyzed by GC-MS.

To quantify the migration data different solutions of the compounds at different concentration levels were prepared in acetone and analyzed by GC-MS. Three replicates of each concentration level were analyzed.

The migration was calculated by mg of compound that migrated to Tenax per dm^2 of laminate in contact with it.

3. Results and Discussion

SR adhesive is commonly used for manufacturing metallic complexes on porous media such as the adhesion of foil or metallized plastic film and paper (cardboard boxes or trays, food flexible packaging, candies wrapper).

The main function of NR adhesive was the sealing of cardboard boxes through its application in the form of tape under pressure. It was used primarily in the secondary packaging, although in many cases the cardboard boxes are the primary packaging (candy, sweets, etc.). The materials that NR usually joins are PVC and paper.

The first step was to identify the substances present in the adhesives and likely acting as migrants when in contact with food.

3.1. Identification of the compounds present in the rubber adhesives and toxicity classification

Five different SPME fibers with different polarities were used during the optimization of the rubber adhesive analysis. For both adhesives the best results were obtained with the PDMS fiber, which provided the best sensitivity. The compounds detected were identified and classify according to Cramer toxicity rules. These rules are not based on toxicity studies but on the capacity of a compound for being metabolized by the organism depending on its chemical structure. Three structural classes have been established and a maximum daily intake (mg/ person/ day) has been proposed as follows:

- Class I (low toxicity): 1.8 mg/ person/ day
- Class II (moderate toxicity): 0.54 mg/ person/ day
- Class III (high toxicity): 0.09 mg / person / day

This classification was selected in order to compare the theoretical toxicity between the compounds identified.

SR adhesives are obtained from processing hydrocarbons. One of the most commonly used SR is the adhesive used in this work, a copolymer of styrene and 1,3-butadiene (SBR). Figure 1 shows the chromatogram obtained in the HS-SPME-GC-MS analysis of the SR adhesive with a PDMS fiber. Fourteen different compounds and the internal standard (*p*-tertbutylphenol) were detected, 11 were identified and 3 were unknown compounds. Most of the identified compounds were aromatic compounds. Table 1 shows the toxicity of the compounds according to theoretical model of Cramer. One compound was classified as class III toxicity, 4-cyanocyclohexene, a reagent used as hardening in adhesives and coatings. Six compounds with class II toxicity were obtained, such as 4-phenylcyclohexene and the five isomers of cyclohexane,1-phenyl-3,4-divinyl and the rest of the compounds were classified as class I toxicity. Among the class I compounds, a monomer used in the formation of SBR polymer, styrene, and some solvents such as ethyl benzene and 1-methylethylbenzene were detected.

NR is solid polyisoprene which is extracted from a certain type of plants after it is tapped. Latex comes out of this tree after tapping and then solid rubber is separated from it. Figure 2 shows the chromatogram obtained in the HS-SPME-GC-MS analysis of the NR adhesive with a PDMS fiber. A total of 13 compounds were detected and 8 of them were identified. A class III toxicity compound (table 2), benzene isothiocyanate and 2 class II toxicity compounds were found, a solvent (toluene) and a common antioxidant (BHT).

A high partition coefficient (K_{AS}) together with low diffusion coefficients (D_A and D_S) of the compounds may reduce the level of migration below the limits of detection. For this reason, it is very important to evaluate the physicochemical properties of these compounds and especially to determine these coefficients in the different materials used in the laminates. Once K_{AS}, D_A and D_S are known for a given laminate one can apply mathematical modelling to predict migration into a food or a food simulant.

3.2. Determination of the initial concentration profile (CP_0) of rubber adhesives

Once the compounds were identified, a quantification method was developed in order to find out the amount of the compounds in the cured adhesives.

Table 1 shows the analytical parameters and the concentration of the compounds found in the SR adhesive. Samples were analyzed by HS-SPME and analytical parameters were satisfactory in terms of linearity, limits of detection and reproducibility. For some compounds like phenylcyclohexane the limits of detection found were very low as 2.01 picogram per gram of hexane (pg/g). Moreover, concentration of the compounds ranged from 0.04 to 27.60 µg/g. The highest concentration was found for 4-cyanocyclohexene (27.6 µg/g). This compound was classified in the Cramer class III, the most toxic according to this classification, so the study of the behavior of this compound in the laminate is very relevant. Nevertheless, the lowest concentrations were found for the isomers of cyclohexane, 1-phenyl-3,4-divinyl classified as medium toxicity according to Cramer rules.

Table 2 shows the analytical parameters and concentration of the compounds found in the NR adhesive. Analytical parameters were satisfactory in terms of linearity, limits of detection and reproducibility. For most of the compounds the limits of detection found were below 0.02 µg/g. Moreover, the concentration of the compounds ranged from 7.40 to 165 µg/g. Then, in general the concentration of compounds in NR was higher than those analyzed in SR. The highest concentration was found for BHT (165.5 µg/g). This compound was classified in the medium toxicity class by Cramer so the study of the behavior of this compound in the laminate was very important.

3.3. Determination of the partition and diffusion coefficients.

Once the concentration of the compounds in both SR and NR was obtained it was necessary to analyze their amount in the substrates (paper and PVC) at equilibrium in order to calculate partition and diffusion coefficients. As a result, an analytical method using SPME was designed. Table 3 shows the analytical parameters for this method applied to the analysis of the compounds in paper and table 4 shows the analytical parameters for this method applied to the analysis of the compounds in PVC. As can be seen, very good limits of detection were obtained for both. In the case of paper analysis very low limits of detection (LOD) were reached since phenylcyclohexane had only 0.02

ng/g. In PVC analysis even lower LOD were reached, as two compounds (toluene and 4-tert-butyltoluene) had a LOD of 0.01 μg/g.

3.3.1.. Determination of the partition coefficients.

Partition coefficients between two polymers are mainly influenced by different factors like polarity, hydrogen bonds formation, temperature, chemical structure and size of the compound [5].

Table 5 and table 6 show the partition coefficients of some of the compounds identified in the adhesives under study and some of their properties, like their octanol-water partitioning coefficient (logP_{OW}), molecular weight (M_w), and number of hydrogen acceptors and donors.

As it can be seen, only one compound, cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_1 had a higher tendency to remain in the paper rather than in the adhesive as the $K_{adhesive/paper}$ was below 1. Moreover, 4-cyanocyclohexene showed the higher tendency to remain in the adhesive with a $K_{adhesive/paper} = 200.7$. This compound had the lowest log P_{OW} and therefore is the most polar of the substances studied. According to the composition given in paragraph 2.2, SR has a polar character so the polar compounds such as 4-cyanocyclohexene had a preference to stay in it. This is a relevant data since this substance was classified in the class III, the most toxic class according the Cramer classification. As a result 4-cyanocyclohexene will have a low tendency to migrate to the food in contact with the laminate while cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_1 will have a higher tendency to reach the paper in contact with food. Moreover it can be seen that not only polarity but also molecular structure influenced the partition coefficients as this value was different in all the different cyclohexane, 1-phenyl-3,4-divinyl isomers studied.

Table 6 shows the partition coefficients of some of the compounds identified in the natural rubber adhesive between the adhesive and PVC. The adhesive studied is commonly used to bond PVC in laminates for food contact so it was the reason to study the partition coefficients $K_{adhesive/PVC}$. Table 6 shows that one of the compounds, butylated hydroxytoluene had a high tendency to remain in the adhesive as its partition coefficient $K_{adhesive/PVC}$ was 779.9. This compound is the only one that had a hydrogen donor and therefore, it could form hydrogen bonds with some compounds that have hydrogen donors in the structure of this adhesive as butylated hydroxytoluene.

3.3.2. Determination of diffusion coefficients.

The diffusion coefficients for the SR adhesive and the paper substrate used to manufacture the laminate 1 are shown in table 5. It can be observed that the magnitude of these coefficients depends mainly on the molecular weight of the migrants, the temperature and the matrix where the diffusion occurs (adhesive or paper)

The literature has shown that the diffusion of a molecule in a polymer is related to some characteristics of the polymer (matrix) such as its molecular weight, degree of crystallinity, glass transition temperature, the temperature of the environment where the diffusion occurs as well as the size, the shape, the chemical nature and the polarity of the diffusing molecule [6, 7, 8]. In general the diffusion coefficients are higher for small molecules and for polymers with a low crystallinity degree [6, 9].

Table 5 shows that, as expected, diffusion coefficients values in adhesive SR at 40ºC for the compounds with the lowest molecular weight such as 4-cyanocyclohexene and 4-phenylcyclohexene were higher than those of the highest molecules. The similar trend was found for the diffusion coefficients of the compounds in the paper substrate.

As shows table 5, the temperature is an important factor affecting to the diffusion in the adhesive and the substrate. The diffusion in rubbery polymers is a thermally active process which depends on the temperature according to the Arrhenius-equation [8], where the diffusion of the migrants at high temperatures is facilitated by the expansion related with the increase of free volume in the polymer produced at high temperatures [10, 11]. In this case, it was shown that diffusion coefficients were significantly higher at 60 ºC than at 40ºC ($p<0.01$).

Only a few publications dealing with the diffusion processes in paper were found. The diffusion in this medium depends on properties such as its porosity, tortuosity and permeability [12-14]. Thus, in fact, the D_S data listed in table 5 for paper should be regarded as macroscopic “apparent” diffusion coefficients. The diffusion of all compounds in paper (D_S) were lower than in the adhesive (D_A) at both temperatures.

Table 6 shows the diffusion of butylated hydroxytoluene in natural rubber and PVC substrate used to make the laminate 2. For the rest of compounds, the diffusion in both media could not be calculated as the compounds did not diffuse under the test conditions listed above.

Butylated hydroxytoluene was also found in a laminate manufactured with a hotmelt adhesive and cardboard as substrate studied by Vera et al. [3]. Its diffusion in this adhesive was lower than in the NR adhesive at two temperatures. In contrast, its diffusion in the cardboard substrate was higher than that in the PVC substrate also at both temperatures.

Pure PVC is a brittle crystalline polymer with a glass transition temperature (T_g) of about 70°C. Diffusion coefficients in rigid PVC are very low [15]. When PVC resin is mixed with a plasticizer the polymer becomes more flexible, reduces its T_g and at the same time diffusion coefficients may increase several orders of magnitude [16]. From 20 to 60°C the PVC substrates used in this work were highly flexible. This indicates that they were plasticized samples (about 35 to 45% w/w) and implies that their glass transition temperature T_g layed obviously below ambient temperature. Such PVC samples usually exhibit diffusion coefficients in the same range low density polyethylene (LDPE). In fact the D_S coefficients reported in Table 6 for butylated hydroxytoluene are in the same range as its diffusion coefficient in LDPE [17].

3.4. Determination of migration results.

Table 7 shows the migrant compounds found for the laminate 1 and 2 using Tenax as food simulant. Migration results were expressed as micrograms of migrant compound per dm^2 of laminate in contact with the simulant. The analytical parameters of GC-MS for standard solution in acetone used to quantify the different migrant compounds were also shown.

Only three compounds migrated as 4-cyanocyclohexene, butylated hydroxytoluene and 2-cyclopentyl-1.3.5-trimethylbenzene with values of migration corresponded to 1.79, 22.9 µg/dm^2 and 4.57 µg/dm^2 respectively.

In order to study the possible risks of these migrant compounds, migration values were compared with the specific migration limits (SML) stipulated by the EU legislation. Only butylated hydroxytoluene compound has a SML of 3 mg/Kg [18]. For NR the migration result was 22.9 mg/dm^2, corresponding to 0.14 mg/Kg of food (assuming a cube with a surface area of 6 dm^2 in contact with 1 Kg of food [19]), and therefore this value was bellow of its SML.

For the rest of the compounds the estimated daily intake (EDI) was calculated according to the U.S. Food & Drug Administration (FDA) equation:

$$EDI\left(\frac{mg}{person\ x\ day}\right) = migration\ value\ \left(\frac{mg}{kg}\right) x3Kg(total\ food\ intake\ per\ person\ per\ day)xCF$$

Where CF is the consumption factor, what means the fraction of the daily diet expected to be in contact with a specific packaging material (for adhesives CF is 0.14).

As the table 7 shows none of the migration values of EDI exceeded the recommended Cramer exposure values for compounds of class I, II and III.

4. Conclusion

Two rubber adhesives (synthetic and natural) commonly used in food packaging materials have been studied. Twenty seven compounds were identified, most of them had a similar chemical structure. Only two of the compounds determined showed a high toxicity according to the theoretical model of Cramer, benzene isothiocyanate and 4-cyanocyclohexene nevertheless only 4-cyanocyclohexene migrated, with a migration value bellow the recommended Cramer exposure values as well as the rest of migrant compounds. Partition coefficients between adhesive and substrate used to manufacture of the laminate showed a wide variation of values mainly depending on the nature of the migrating compound and the substrates used in the laminates was found.

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Table 1 Identified compounds in the synthetic rubber adhesive, toxicity according to Cramer (Tox), quantification ions (QI), quantification standards, analytical parameters of the quantification method and initial concentration profile (CP_0)

Compounds	**Tox**	**QI**	**Quantification standard**	**Calibration range (ng/g)**	**Linearity (R^2)**	***LOD* (pg/g)**	***RSD* (%)**	***CP_0* *(µg/g)***
4-Vinylcyclohexene	I	79.1	4-Vinylcyclohexene	0.697-50.0	0.999	209	6.7	3.17 ± 0.31
Ethylbenzene	I	91.0	Ethylbenzene	0.613-50.0	0.999	18.4	4.7	5.33 ± 0.40
Styrene	I	104.1	Styrene	0.276-50.0	0.997	82.7	5.4	5.48 ± 0.43
1-Methylethylbenzene	I	105.1	1-Methylethylbenzene	0.081-50.0	0.999	24.2	7.0	3.61 ± 0.42
4-Cyanocyclohexene	III	79.1	4-Cyanocyclohexene	62.30-250	0.997	18700	10.1	27.6 ± 1.50
4-Phenylcyclohexene	II	104.1	1-Phenyl-1-cyclohexene	0.012-49.8	0.996	3.81	3.4	9.40 ± 0.32
Cyclohexane,1-phenyl-3,4-divinyl_1	II	104.0	Phenylcyclohexane	0.007-50.4	0.999	2.01	3.8	0.04± 0.00
Cyclohexane,1-phenyl-3,4-divinyl_2	II	104.0	Phenylcyclohexane	0.007-50.4	0.999	2.01	3.8	0.69 ± 0.04
Cyclohexane,1-phenyl-3,4-divinyl_3	II	104.0	Phenylcyclohexane	0.007-50.4	0.999	2.01	3.8	0.93 ± 0.05
Cyclohexane,1-phenyl-3,4-divinyl_4	II	104.0	Phenylcyclohexane	0.007-50.4	0.999	2.01	3.8	0.25 ± 0.02
Cyclohexane,1-phenyl-3,4-divinyl_5	II	104.0	Phenylcyclohexane	0.007-50.4	0.999	2.01	3.8	*<LOQ*

Table 2. Compounds identified in the natural rubber adhesive, toxicity according to Cramer (Tox), quantification ions (QI), quantification standards, analytical parameters of the quantification method and initial profile concentration profile (CP_0)

Compounds	**Tox**	**QI**	**Quantification standard**	**Calibration range (µg/g)**	**Linearity (R^2)**	***LOD* (µg/g)**	***RSD* (%)**	***CP_0* *(µg/g)***
Toluene	I	91.0	Toluene	0.08-10.0	0.995	0.01	10.3	154.6 ± 60.3
1, 1, 2-Trimethylcyclohexane	I	69.0	1, 2, 4-Trimethylcyclohexane	0.13-10.8	0.998	0.04	9.3	10.3 ± 2.4
2-Tert-butyltoluene	I	133.0	4-Tercbutyltoluene	0.06-10.3	0.999	0.02	7.8	13.6 ± 2.0
1, 3-Diethy-5-methyllbenzene	I	133.0	1, 3-Diethyl-5-methylbenzene	0.06-10.2	0.999	0.02	7.3	7.40 ± 1.2
1-Methyl-4-(1-methylpropyl)-benzene	I	119.0	1-Methyl-4-(1-methylpropyl)-benzene	0.05-10.0	0.999	0.01	8.2	18.5 ± 3.1
Benzene isothiocyanate	III	135.0	Benzene isothiocyanate	0.07-10.3	0.995	0.02	2.2	79.2 ± 11.3
Butylated hydroxytoluene	II	205.0	Butylated hidroxytoluene	0.05-10.3	0.999	0.01	7.0	165.5 ± 24.7
2-Cyclopentyl-1, 3, 5-trimethylbenzene	II	159.0	Phenylcyclohexane	0.02-10.2	0.994	0.01	1.7	41.7 ± 6.4

Table 3. Analytic parameters for the HS-SPME-GC-MS method applied to the analysis of paper.

Compound	**Linear range (ng/g)**	**Linearity (R^2)**	**LOD (ng/g)**	**LOQ (ng/g)**	**RSD (%)**
4-Vinylcyclohexene	22.4-142	0.996	6.71	22.4	26.7
Ethylbenzene	11.8-2360	0.998	3.53	11.8	23.5
Styrene	2.20-2410	0.992	0.60	2.20	18.1
1-Methylethylbenzene	3.80-2360	0.990	1.2	3.8	19.7
4-Cyanocyclohexene	872-2380	0.990	262	872	8.3
1-Phenyl-1-cyclohexene	4.50-2350	0.999	1.30	4.50	6.7
Phenylcyclohexane	0.07-2360	0.999	0.02	0.07	4.4

Table 4. Analytic parameters of the HS-SPME-GC-MS method applied to the analysis of PVC.

Compound	**Linear range (µg/g)**	**Linearity (R^2)**	***LOD* (µg/g)**	***LOQ* (µg/g)**	***RSD* (%)**
Toluene	0.02-6.10	0.982	0.01	0.02	31.1
1, 2, 4-Trimethylcyclohexane	0.81-4.37	0.992	0.24	0.81	34.1
4-Tert-butyltoluene	0.04-6.09	0.993	0.01	0.04	28.9
1,3-Diethylbenzene	0.30-6.18	0.994	0.09	0.30	25.9
1-Methyl-4-(1-methylpropyl)-benzene	0.25-6.26	0.991	0.07	0.25	21.3
Benzene isothiocyanate	0.57-36.2	0.998	0.17	0.57	7.0
Phenylcyclohexane	0.18-6.26	0.999	0.06	0.18	8.7
Butylated hydroxytoluene	0.28-6.20	0.996	0.08	0.28	8.5

Table 5: Molecular weight (Mw), LogP_{OW}, partition coefficient adhesive/paper (K_{AS}) and diffusion coefficients in paper (D_S) and in adhesive (D_A) at 2 different temperatures (40 and 60 ºC) of the identified compounds in the synthetic rubber (SR) adhesive.

Compound	**Mw (g/mol)**	**Log P_{OW}**	**$K_{AS40\ ºC}$**	**D_A 40ºC (cm^2/s)**	**D_A 60ºC (cm^2/s)**	**D_S 40ºC (cm^2/s)**	**D_S 60ºC (cm^2/s)**
4-Cyanocyclohexene	107,1	1,70±0,27	200,7 ± 4,1	1,65E-07	1,13E-06	3,18E-08	1,87E-07
4-Phenylcyclohexene	158,2	4,04±0,41	27,0±0,0	1,69E-07	6,04E-07	1,32E-08	5,25E-08
Cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_1	212,2	5,15±0,47	0,1±0,0	6,74E-08	5,13E-07	1,12E-08	3,90E-08
Cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_2	212,2	5,15±0,47	4,1±0,0	8,67E-08	4,66E-07	1,16E-08	2,67E-08
Cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_3	212,2	5,15±0,47	8,9±0,0	5,33E-08	6,46E-07	4,90E-09	2,36E-08
Cyclohexane,1-phenyl-3,4-divinyl, (1R,3cis,4cis)_1	212,2	5,15±0,47	2,8±0,0	8,54E-08	5,39E-07	7,33E-09	3,13E-08

Table 6. Molecular weight, LogP_{OW}, number of hydrogen acceptors and donors, partition coefficients adhesive/PVC (K_{AS}) and diffusion coefficients in PVC (D_S) and in the adhesive (D_A) at 2 different temperatures of the identified compounds in the natural rubber (NR) adhesive.

Compound	**Mw (g/mol)**	**Log P_{OW}**	**Nº hydrogen acceptors**	**Nº hydrogen donors**	**D_A (cm^2/s, 40 ºC)**	**D_A (cm^2/s, 60 ºC)**	**$K_{AS40^{o}C}$**	**D_S (cm^2/s, 40^0 C)**	**D_S (cm^2/s, 60^0 C)**
1-Methyl-4-(1-methylpropyl)-benzene	148,2	4,28±0,39	0	0	ND	ND	44.9±8.0	ND	ND
Benzene isothiocyanate	135,2	3,18±0,25	1	0	ND	ND	2.1±0.2	ND	ND
2-Cyclopentyl-1.3.5-trimethylbenzene	184,1	5,06±0,6	0	0	ND	ND	43.2±7.2	ND	ND
Butylated hydroxytoluene	220,3	5,06±0,37	1	1	2,675E-08	1,945E-07	779.9±24.6	5.53E-09	4.73E-09

ND: Non-detected compound in diffusion experiments

Table 7: Migrant compounds in the synthetic and natural rubber, quantification ions (QI), quantifications standards, analytical parameters of the quantifications method, values of migration (µg/dm^2), the estimated daily intake (EDI) expressed as mg/(person x day) and the recommended values Cramer exposure values.

Compound	**QI**	**Quantification Standard**	**Calibration range (µg/g)**	**Linearity (R^2)**	**RSD (%)**	**LOD (µg/dm^2)**	**Migration (µg/dm^2)**	**EDI (*mg/(person x day)*)**	**Cramer (*mg/(person x day)*)**
4-Cyanocyclohexene	79.1	4-Cyanocyclohexene	4.46-9.56	0.996	6.73	1.34	1.79	0.0045	0.09
4-Phenylcyclohexene	104.1	1-Phenyl-1-cyclohexene	2.96-60.4	0.998	4.25	0.89	<LOD		
Cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_1	104.0	Phenylcyclohexane	2.56-10.3	0.999	3.74	0.77	<LOD		
Cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_2	104.0	Phenylcyclohexane	2.56-10.3	0.999	3.74	0.77	<LOD		
Cyclohexane,1-phenyl-3,4-divinyl, 1R,3trans,4trans)_3	104.0	Phenylcyclohexane	2.56-10.3	0.999	3.74	0.77	<LOD		
Cyclohexane,1-phenyl-3,4-divinyl, (1R,3cis,4cis)_1	104.0	Phenylcyclohexane	2.56-10.3	0.999	3.74	0.77	<LOD		

1-Methyl-4-(1-methylpropyl)-benzene	119.0	1-Methyl-4-(1-methylpropyl)-benzene	0.13-11.5	0.997	2.41	0.04	<LOD		
Benzene isothiocyanate	135.0	Benzene isothiocyanate	0.17-12.3	0.996	6.81	0.05	<LOD		
Butylated hydroxytoluene	205.0	Butylated hydroxytoluene	0.27-104	1	7.24	0.08	22.9	0.06	0.54
2-Cyclopentyl-1.3.5-trimethylbenzene	159.0	Phenylcyclohexane	2.56-10.3	0.999	3.74	0.12	4.57	0.11	0.54

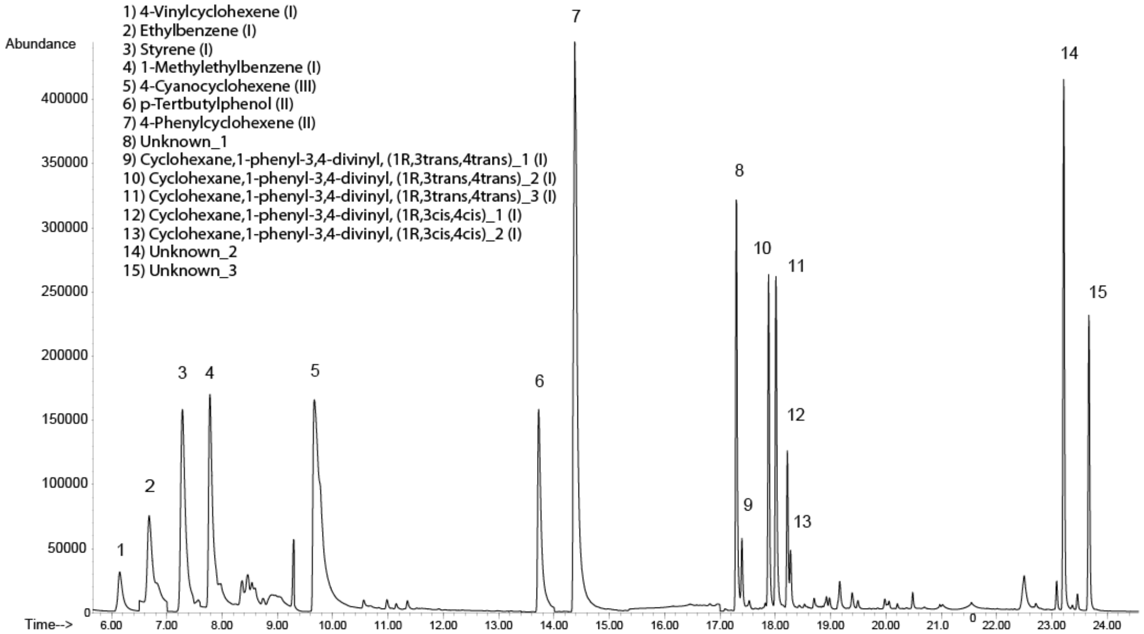

Figure 1. Chromatogram of the synthetic rubber adhesive sample analyzed by HS-SPME-GC-MS using a PDMS fiber, compounds identification and index toxicity(I: low toxicity, II: medium toxicity, III: high toxicity)

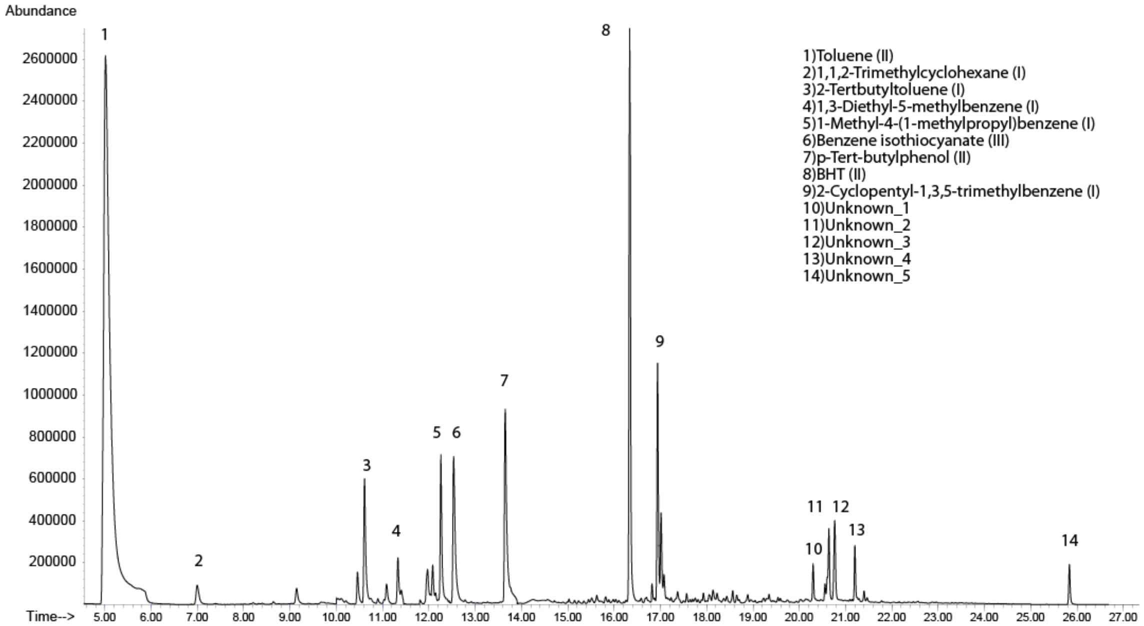

Figure 2 Chromatogram of the natural rubber adhesive sample analyzed by HS-SPME-GC-MS using a PDMS fiber, compounds identification and toxicity index (I: low toxicity, II: medium toxicity, III: high toxicity)