UNIVERSITÉ DU QUÉBEC À MONTRÉAL

DEVELOPMENT OF A METHOD FOR QUANTIFICATION OF TOLUENE DIISOCYANATES AND METHYLENEDIPHENYL DIISOCYANATE MIGRATION FROM POLYURETHANE FOAM SAMPLE SURFACE BY HPLC-UV-MS

MÉMOIRE
PRÉSENTÉ
COMME EXIGENCE PARTIELLE
À LA MAÎTRISE EN CHIMIE

BY
ALEKSANDRA DONCHENKO

AUGUST 2020

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DÉVELOPPEMENT D'UNE MÉTHODE DE QUANTIFICATION DE LA MIGRATION DE TOLUÈNE DIISOCYANATES ET DE MÉTHYLÈNEDIPHÉNYL DIISOCYANATE À PARTIR D'UNE SURFACE D'ÉCHANTILLON DE MOUSSE DE POLYURÉTHANE PAR CLHP-UV-SM

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LIST OF ABBREVIATIONS AND ACRONYMS

CV	Coefficient of variation
DBA	Di-*n*-butylamine
DMSO	Dimethylsulfoxyde
EPA	Environmental Protection Agency
FL	Fluorescence
GF	Glass fibre
HPLC	High-performance liquid chromatography
III	International Isocyanate Institute Inc
IRSST	Institut de recherche Robert-Sauvé en santé et en sécurité du travail
LOD	Limit of detection
LOQ	Limit of quantification
MAMA	9-(*N*-methylaminomethyl)anthracene
MDA	4,4'-methylenedianiline
MDI	4,4'-methylenediphenyldiisocyante
1,2-MP	1(2-Methoxyphenyl)piperazine
MS	Mass spectrometry
NIOSH	National Institut for Occupational Safety and Healt
OSHA	Occupational Safety and Healt Administration
OEL	Occupational Exposure Limits
PDA	Photodiode array detector
PFA	Polyureathane Foam Association
PP	1-(2-pyridyl)piperazine
PU	Polyurethane
REL	Reference Exposure Levels
TDA	Toluenediamine
TDI	Toluene diisocyanate
TRYP	Triptamine, 3-(2-aminoethyl)indole
UV	Ultraviolet
UQÀM	Université du Québec à Montréal

LIST OF SYMBOLS AND UNITS

°C	degree Celsius
cm	centimeter
cm^2	square centimeter
g	gram
h	hour
kV	kilovolt
L	Litre
mEq	milliequivalent
mg	milligram
min	minute
mL	millilitre
mm	millimeter
ng	nanogram
ppm	parts per million
V	volt
µg	microgram
µL	microliter
µm	micrometer

RÉSUMÉ

Les diisocyanates sont des composés organiques avec deux fonctions isocyanates très réactives. Ils sont principalement utilisés dans la production de polyuréthane formée par polymérisation avec des polyols. Ces polymères sont largement utilisés dans notre environnement: meubles, matériaux de construction, colle et autres. En général, le produit final ne contient pas de diisocyanate résiduels. Des études antérieures ont été effectuées pour déterminer la quantité d'isocyanate libre qui peut éventuellement rester dans un produit final. Ce diisocyanate a été extrait de mousse de polyuréthane flexible en utilisant différents solvants organiques. Les résultats obtenus ont montré que le diisocyanate extrait ne peut pas être considéré comme un diisocyanate libre et une méthode différente de détermination de la migration d'un diisocyanate à partir d'une surface était nécessaire.

Une méthode de quantification de la migration du toluène diisocyanate (TDI) et du méthylènediphényl diisocyanate (MDI) à partir de la surface d'un échantillon en mousse de polyuréthane (PU) a été mise au point conformément à l'une des recommandations du protocole d'essai de l'US EPA. Pour cette méthode, la migration du TDI et du MDI d'une surface a été évaluée en utilisant des filtres en fibre de verre imprégnés de 1-(2-méthoxyphényl) pipérazine (1,2-MP) en tant qu'agent de dérivation mouillé avec la solution de sueur synthétique. Suivant les recommandations de l'EPA, six solutions de sueur synthétique ont été préparées et utilisées pour l'évaluation de la performance de récupération d'isocyanate. Les dérivés 1,2-MP ont été analysés par HPLC équipée d'un détecteur UV pour la quantification et d'un détecteur MS pour qualifier les pics. La méthode validée a été utilisée pour quantifier la migration des diisocyanates à partir d'une surface d'échantillons de mousse de polyuréthane souple fournie par l'association de mousses de polyuréthane (PFA). Selon les tests de validation, les limites de détection de cette méthode sont de 0,002 µg / mL pour le 2,6-TDI, de 0,011 µg / mL pour le 2,4-TDI et de 0,003 µg / mL pour le MDI. Les limites de quantification étaient respectivement de 0,006 µg / mL, 0,037 µg / mL et 0,010 µg / mL. Les résultats, obtenus à partir de cinq échantillons de mousse flexible les plus couramment utilisés dans la production de produits commerciaux et présentant le potentiel le plus élevé en diisocyanate résiduel, ont montré que les diisocyanates ne sont pas détectés à la surface.

Mots clés: isocyanate, toluène diisocyanate, méthylènediphényl diisocyanate, polyuréthane, migration, 1-(2-méthoxyphényl)pipérazine, LCUP-UV.

ABSTRACT

Diisocyanates are organic compounds with two isocyanate functional groups that are very reactive. They are predominatly used in a polyurethane production formed by polymerization with polyols. These polymers are largely used in our environment: furniture, building materials, glue and others. In general, the final product does not have any uncured diisocyanate. Previous studies have been done to determine the quantity of free isocyanate that can possibly stay in a final product. The free diisocyanate was extracted using different organic solvents from flexible polyurethane foam. Howevere, it was shown that extracted diisocyanate can not be considered as a free diisocyanate and a different method of determination diisocyanate migration from a surface was needed.

A method for quantification of toluene diisocyanate (TDI) and methylenediphenyl diisocyanate (MDI) migration from polyurethane (PU) foam sample surface was developed according to one of the US EPA testing protocol recommendations. For this method, migration of the TDI and MDI from a surface was evaluated using glass fibre filters impregnated with 1-(2-methoxyphenyl)piperazine (1,2-MP) as a derivative agent wetted with the synthetic sweat solution. Following the EPA recommendations six synthetic sweat solutions were prepared and used for the evaluation of isocyanate recovery performance. 1,2-MP-derivatives were analyzed using HPLC equipped with a UV detector for quantification and a MS detector to identify peaks. The validated method was used to quantify the migration of the diisocyanates from a surface of polyurethane flexible foam samples supplied by polyurethane foam association (PFA). According to validation tests the limits of detection for this method are 0.002µg/mL for 2,6-TDI, 0.011µg/mL for 2,4-TDI, and 0.003µg/mL for MDI. The limits of quantification were 0.006µg/mL, 0.037µg/mL, and 0.010µg/mL, respectively. Results, from five flexible foam samples most commonly used in customer products production and with highest potential of residual diisocyanate, showed that diisocyanates are not detected on the surface.

Keywords: isocyanate, toluene diisocyanate, methylenediphenyl diisocyanate, polyurethane, migration, 1-(2-methoxyphenyl) piperazine, HPLC-UV.

CHAPTER I-THEORETICAL FRAME

INTRODUCTION

Polyurethane's history starts in 1937 when it was produced for the first time by Otto Bayer and his coworkers (Szycher, 1999; Six et Richter, 2000). Since 1950s it was commercialized and used in almost all areas of industry and our daily lives. This exceptional polymer is obtained by the chemical reaction between an isocyanate and a polyol. A great variety of products of amazing quality and properties can be synthetized thanks to a huge selection of raw materials: isocyanates and polyols. Isocyanates are chemical compounds that were first synthesized by Wurtz in 1848 (Allport *et al.*, 2003; Six et Richter, 2000).

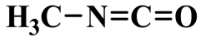
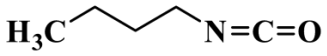
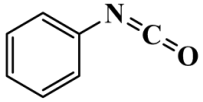
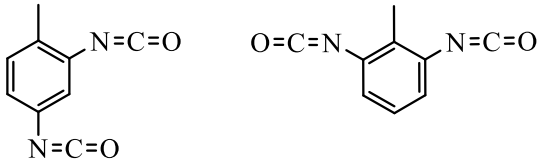
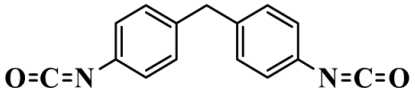
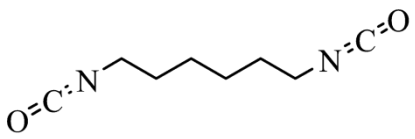
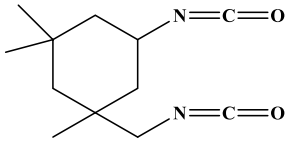
1.1 Isocyanates general information

1.1.1 Nomenclature of isocyanates and their uses

Isocyanates are molecules that have one or more functional isocyanate (NCO) groups attached to an organic molecule. The number of NCO groups attached to a molecule divides them to monoisocyanates, diisocyanates and polyisocyanates. The functional group can be attached to aliphatic, alicyclic or aromatic molecule giving a large diversity of existing isocyanates (Allport *et al.*, 2003; Guglya, 2000; Six et Richter, 2000). The most common isocyanates from monoisocyanates are methyl isocyanate, butyl isocyanate, phenyl isocyanate (Table 1.1). They are typically used for the production of herbicides, antidiabetic drugs and carbamic acid derivatives. Typical diisocyanates (with two functional groups NCO) are methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI), isophorone

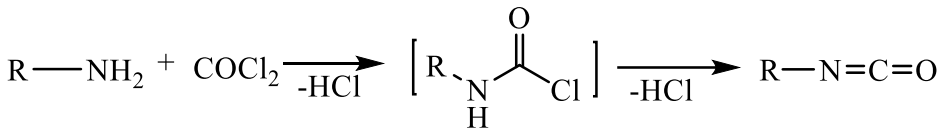
diisocyanate (IPDI) (Table 1.1). They are predominantly used in polyurethane production.

Table 1.1 Most common Isocyanates, structures and their basic applications (Allport *et al.*, 2003).

	Name (abbreviation)	Structure	Applications
Monoisocyanates	Methyl isocyanate (MIC)		Insecticides and fungicides production
	n-Butyl isocyanate		Antidiabetic drugs production; insecticides
	Phenyl isocyanate (phI)		Insecticides and fungicides production
Diisocyanates	Toluene diisocyanates (TDI)	 **2,4-TDI** **2,6-TDI**	Polyurethane production (Furniture)
	4,4'-Methylene diphenyl diisocyanate (MDI)		Polyurethane production (insulation, footwear, furniture)
	1,6-Hexamethylene diisocyanate (HDI)		Polyurethane production (light-stable polyurethane coatings)
	Isophorone diisocyanate (IPDI)		Polyurethane production (light-stable polyurethane coatings)

1.1.2 Isocyanate production

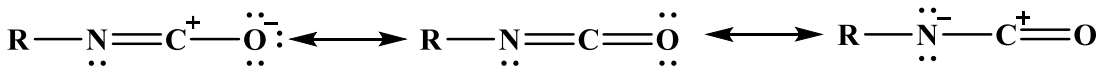
Isocyanate was first obtained by phosgenation reaction of an amine. This process is most efficient for large-scale isocyanates production and it can be carried out in liquid or gas phase (Allport *et al.*, 2003; Lowe, 1970; Six et Richter, 2000). The solvents used for this purpose are toluene, xylene, decahydronaphthalene, chlorobenzene, and ortho-dichlorobenzene.



Other methods of isocyanate synthesis were developed because of the toxicity of phosgene used as a reagent, and also of the corrosive hydrogen chloride formed during the reactions (Knölker *et al.*, 1995). Unfortunatly, these non-phosgene methods are not commercially profitable and are not used for industrial production of isocyanates.

1.1.3 Reactivity of isocyanates and their typical reactions

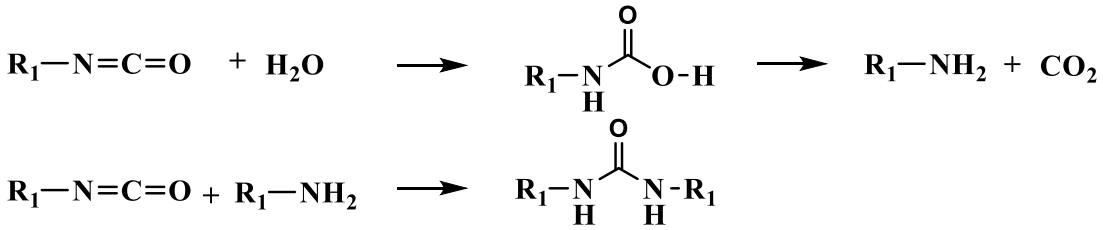
Isocyanates are highly reactive molecules due to NCO group. According to electronic resonance structures they react with nucleophiles due to the positive charge on the carbon atom of the successive double bond:



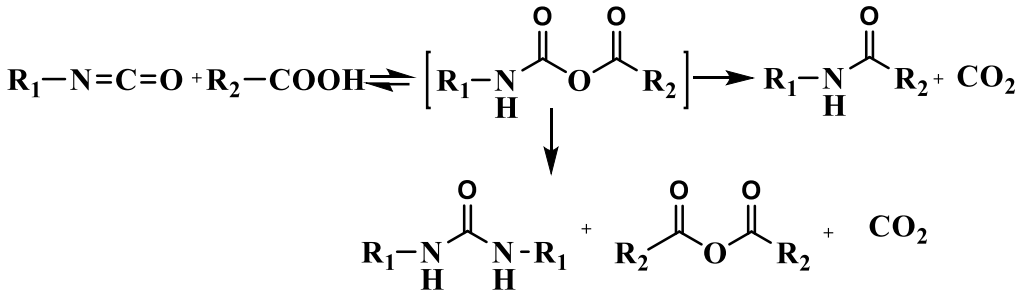
They will react with chemical substances containing mobile hydrogen in there molecules. The typical reactions for isocyanates are reactions with water, alcohols (OH group), amines (NH group) and carboxylic acid (Allport *et al.*, 2003; Six et Richter, 2000).

The reaction with water gives the polymeric urea in two steps. At first, the reaction of isocyanate with water gives a carbamic acid which is unstable and breaks down into

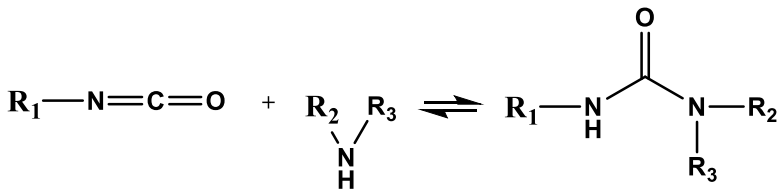
carbon dioxide and amine. The last then reacts with isocyanate still available in the system to form a substituted urea (Allport *et al.*, 2003; Guglya, 2000).



Isocyanates reacting with carboxylic acid form anhydrides which are usually unstable and will then decompose into an amide or carboxylic anhydride depending on the starting materials composition, and a substituted urea (Allport *et al.*, 2003):

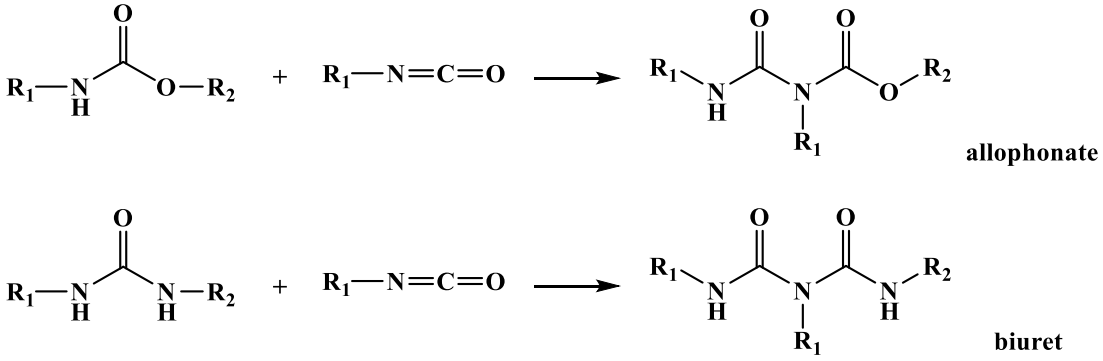


Primary and secondary amines also react with isocyanate forming substituted ureas:

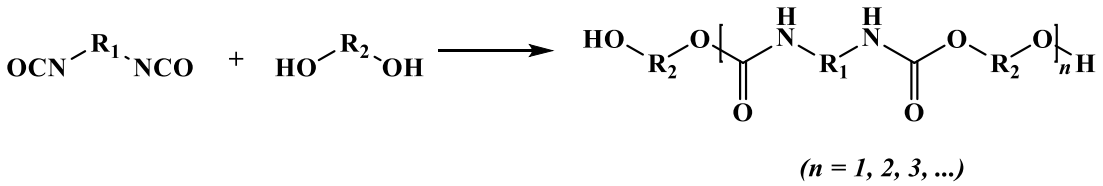


Diamines are used as reagents with TDI and MDI to form polyureas (Allport *et al.*, 2003). This type of reaction allows to extend chains or to do crosslinking and is used to modify the physical characteristics of formed polyurethane.

An increase of the temperature causes further reactions of isocyanates with NH groups of carbamates and ureas that lead to allophanates and biurets formation (Allport *et al.*, 2003; Delebecq *et al.*, 2013):



The exothermic reaction of isocyanates with alcohols is commercially the most important reaction. It leads to an urethane formation. A variety of chemical substances can catalyze this reaction (Delebecq *et al.*, 2013). The similar reaction between diisocyanate and diol or poliol will lead to polyurethane (PU) formation with possible branching and crosslinking in case of polymer formed with any or both reactants with functionality beyond two (Allport *et al.*, 2003; Delebecq *et al.*, 2013; Six et Richter, 2000):



1.2 Polyurethane (PU)

The developement of a new universal material for the replacement of rubber, plastics and metal began in the 1930s in the USA and Germany. At that time, several studies were carried out in the United States on the synthesis of polyamides by W.H.

Carothers (Szycher, 1999). He synthesized an artificial rubber and later nylon which were patented. At the same time (1937) methods for the synthesis of elastomers were developed in Germany by Otto Bayer and his coworkers. Based on compositions of diisocyanates and polyols, they were able to synthesize elastic and solid polyurethane elastomers for the first time (Szycher, 1999). These new materials have similar properties to rubber and nylon patented by the Americans.

The beginning of World War II delayed the development of the PU industry for several years. It was used for the coating of the planes during the War (in small quantities). Soon after the War, PU was commercialized due to its exceptional physical properties, huge number of possible applications, and new types of polymers with different properties for various purposes were created (Szycher, 1999).

Currently used in industries, polyurethanes include an extensive class of polymers containing urethane (–NHCOO–) groups which can be very different in chemical nature, chain structure and properties (Allport *et al.*, 2003). Elastic, semi-rigid and rigid materials can be generated based on PU. Almost all existing technological methods such as extrusion, pressing, casting, pouring can be used for the processing of PUs. Types of materials and products that can be obtained from these processes are: filled, reinforced, foamed, laminated, in the form of plates, sheets, blocks, profiles, fibers, and films (Delebecq *et al.*, 2013).

1.2.1 Types of polyurethane

Depending on the type of isocyanate, alcohol and additives used for the polymerization reaction, a huge variety of a final products can be obtained (Delebecq *et al.*, 2013). The types of PU and their most common uses are shown in the table below (Allport *et al.*, 2003):

Table 1.2 Polyurethane types and uses

PU Types	Uses
Flexible foam	Household furniture, bedding, automotive seating, textile laminates, cushioning
Rigid foam	Thermal insulation, pipelines, storage tanks, refrigerators, freeze equipment
Semi-rigid integral skin foam	Furniture elements, steering wheels, headrests, automotive interior components, furniture elements, sport goods (skis, surf boards)
Others	Fibres, wheels (rollers, skateboards, etc.), vehicle body panels, shoe soles, construction and automotive industries sealants, conveyors

PUs are mostly produced as thermosets which cannot be heat shaped without degradation. PUs are also produced as thermoplastics basically from pure MDI or modified MDI. These types of polymers can be formed thermally by injection molding or extrusion at high temperature and used for high performance footwear (ski boots), automotive parts, hoses and electrical cabling. Modified MDI are prepolymers or blends that can be obtained from pure MDI to give products with different chemical and handling properties (Allport *et al.*, 2003; Delebecq *et al.*, 2013).

1.2.2 Polymerization reaction and possible risks

As indicated before, PU is obtained by the reaction between diisocyanate and polyol with heat liberation (Allport *et al.*, 2003; Delebecq *et al.*, 2013). To obtain a good commercial product large number of different additives are used: catalysts for the speed regulation, surfactants to control the interaction between nonhomogeneous components, chain extenders and cross-linkers to modify the polymer's structure properties, fire retardants, fillers and colorants to enhance the final product properties. Different densities and thicknesses are obtained using blowing agents that can be reactive or non-reactive. One of the reactive blowing agents used in PU production is water. Water reacts with a diisocyanate generating the carbon dioxide gas in the mixture which reacts as a blowing agent. Concentration of the chemicals in a mixture and the type of blowing agent give the possibility to control the density and the thickness of the formed polyurethane.

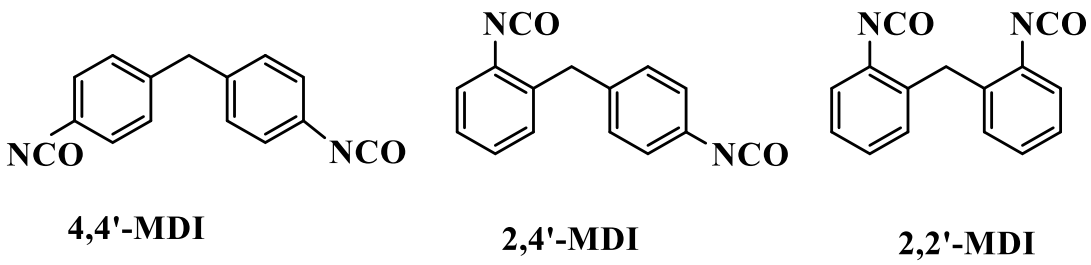
As the polymerization reaction is exothermic, heat liberated during this process has to be constantly dessipated from the reaction. Indeed, high temperature can promote the reverse reaction and the monomeric diisocyanate used will be released in this case. The other possible source of unreacted diisocyanate in a final PU product can be due to its excess than the stoichiometric quantities added during polymerization. In these two cases residual diisocyanate trapped into a PU can hypothetically migrate to the surface shortly after production (Allport *et al.*, 2003).

1.3 MDI and TDI

The two most important diisocyanates used in PU production are toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI). A large variety of flexible and rigid polyurethane products are also prepared from these chemicals.

1.3.1 MDI

Pure monomeric MDI is a white solid at 25°C predominantly at 98% 4,4'-MDI isomer that contains some percentage of 2,4'-MDI and 2,2'-MDI isomers:



4,4'-MDI **2,4'-MDI** **2,2'-MDI**

A mixture containing monomeric isomers of MDI and higher molecular weight polyisocyanates (pMDI) is obtained from a mixture of methylenediphenyl diamine (MDA) and higher oligomers (pMDA) by phosgenation in monochlorobenzene. The mixture of MDA is obtained from condensation reaction of aniline with formaldehyde. Pure MDI can be isolated from this mixture by distillation or crystallization. Hydrogen chloride and phosgene are recovered at the end of the process and recycled (Allport *et al.*, 2003).

MDI is almost insoluble in water and can be dissolved in aprotic organic solvents such as toluene, acetonitrile, DMSO and others.

1.3.2 TDI

Mostly monomeric TDI is manufactured as a mixture of isomers containing 20% of 2,6-TDI isomer and 80% of 2,4-TDI isomer (Table 1.1). These two isomers (both colorless liquids at 25^{o}C) are also produced as pure chemicals for special applications and laboratory uses. Similarly to MDI, TDI can be produced by phosgenation of toluene diamine (TDA). The solvents and by-products (hydrogen chloride) are also recycled at the end of the reaction (Allport *et al.*, 2003; Six et Richter, 2000). The

TDA raw material is manufactured starting from toluene, which is dinitrated with mixed acids and subsequently catalytically reduced under hydrogen pressure.

Similarly to MDI, TDI is hydrophobic and can be dissolved in organic solvents (toluene, acetonitrile, DMSO, acetone, benzene). Having two isocyanate groups, TDI and MDI are very reactive. These chemicals easily react with nucleophiles, as described before. The most important reaction is with diols (and polyols) that lead to a PU formation. As it discussed before, the polymerization reaction can leave some unreacted diisocyanate trapped into a final product.

1.3.3 MDI and TDI health problems

Over exposure to TDI and MDI is known to cause skin and respiratory tract irritations. It can also cause contact dermatitis and sensitization among the sensible person (Banks *et al.*, 1986; Baur *et al.*, 1994; Verschoor et Verschoor, 2014). They are also known to cause a work-related asthma (Mapp *et al.*, 1999; Redlich et Karol, 2002). 2,4-TDI and 2,6-TDI are considered a potential occupational carcinogen according to the *International Agency for research on cancer* (IARC, 1986). Airborne isocyanates represent the principal route for exposure to these chemicals and a principal concern. However, some animal studies investigated the skin exposure in order to evaluate the health effects due to dermal exposure (Bello *et al.*, 2006; Vanoirbeek *et al.*, 2004). These studies demonstrated that skin exposure can lead to an isocyanate sensitization or even induced this sensitization.

As isocyanates are reactive chemical substances, they readily react with nucleophiles like amines, alcohols, thiols, carboxylic acids and water in nucleophilic addition reactions to form urethanes, thiourethanes, amines or to give urea bonds (Allport *et al.*, 2003; Delebecq *et al.*, 2013; Lowe, 1970). Since these nucleophilic functional groups are present in biological molecules: the external layer of the epidermis of human skin stratum corneum is rich in OH groups (i.e. found in ceramides,

cholesterol, and carboxylic acids), NH groups (contained in ceramides, amino acids, proteins), and also SH groups included in cysteine residues of keratin. All these groups can spontaneously react with migrated isocyanates even without any catalyst (Bello *et al.*, 2006). The mechanism of isocyanate reaction after dermal exposure is still not well studied especially in humans due to ethical aspects. However, the aggravated respiratory effects were associated with previous dermal exposure in some studies (Petsonk *et al.*, 2000; Vanoirbeek *et al.*, 2004).

After the development of occupational asthma due to exposure to isocyanate, even concentrations lower than the regulated limits can trigger the asthmatic reaction (Baur *et al.*, 1994).

1.3.4 TDI and MDI regulations

Due to their toxicity TDI and MDI are included in a list of dangerous chemicals. North-American governmental agencies have established Occupational Exposure Limits (OEL) and Reference Exposure Levels (REL) for these chemicals; the limits are intended for use to determine airborne concentrations in the workplace (American Conference of Governmental Industrial Hygienists., 2017) or in general population (Office of Environmental Health Hazard Assessment (OEHHA), 2016). Absorbed diisocyanates mainly by inhalation or dermal contact are metabolized and eliminated in urine. To analyze diisocyanate, corresponding diamine extracted from urine can be quantified (Budnik *et al.*, 2011; Lepine *et al.*, 2019; Rosenberg et Savolainen, 1986; Sennbro *et al.*, 2006). In these cases there are limits for the corresponding diamines in creatinine. There are no exposure limits for isocyanates on surfaces or on skin so it is impossible to evaluate the concentrations of these substances being transferred *via* skin contact and their subsequent health effect. Occupational exposure limits fixed at 5 ppb (American Conference of Governmental Industrial Hygienists., 2017) were used for analytical development of the method for this study project.

1.3.5 Previous analysis methods for TDI and MDI

There are different methods that exist to analyze TDI and MDI. The sampling procedures will depend on the type of particles to be analyzed, and analysis methods will depend on the type of instrument chosen for analyses. Some of the methods used in the past for isocyanates analysis are being replaced by more selective, robust and sensitive methods developed more recently.

The airborne TDI and MDI can be monitored using direct-reading methods and indirect methods. In direct-reading methods sampling, collection, stabilization of the analyte and analysis are performed simultaneously *in situ* by using adequate instruments. These methods can give the instantaneous airborne concentration of analyte, its variation over a time period and also an average concentration over a sampling period. One of the direct-reading methods for TDI and MDI monitoring is a ‘‘*Paper Tape’’* methods (Gas Sensing., 2017; Honeywell Analytics., 2008). In these methods an analyte is collected on a filter paper tape that is chemically impregnated with a chromophore agent. An airborne isocyanate thus reacts and forms a derivative with a specific color. The color stain intensity is proportional to its concentration. This simple method has its limitations. The results can be affected by the relative humidity, aerosols and chemical interferences. There is also a color indication tube for direct-reading method. It consists of a glass tube containing silica gel as the inert substrate, coated with a chromophore reagent. It is possible to use more than one chromophoric reagent. The analyte is drawn in with the air by the pump and reacts with the reagent in the tube to produce a color stain (a1cbiss, 2019).

Indirect methods of analysis are more complicated and require delayed analysis response. The analyte has to be sampled on site and sent to the laboratory for analyses. Such analysis use chromatographic techniques to separate mixture of TDI and MDI derivatives before analysing them. Among the chromatographic separation techniques used, the high performance liquid chromatography (HPLC) is routine. To be able to separate a mixture of diisocyanates, they have to be dissolved in a solvent

and their –NCO functional groups have to be stabilized. As said before, TDI and MDI are hydrophobic and aprotic organic solvents are usually used (toluene, acetone, DMSO, acetonitrile, etc.). One of the first methods used ethanol to dissolve and stabilize TDI and MDI. The urethane derivatives then could be analyzed using an appropriate detector. This method has its limitations: the amines present in the atmosphere could be collected with isocyanates. The amine, having a faster rate of reaction with isocyanates will then compete with that of ethanol (Allport *et al.*, 2003). Another reagent that can be used to stabilize isocyanate group is the *p*-nitrobenzyl-*N*-*n*-propyl-amine (nitro reagent). This method was incorporated by OSHA as Method 18 for impinger sampling and NIOSH Method 2535 for a solid sorbent tube sampling for TDI. The biggest drawback with the ‘‘nitro reagent’’ is its sensitivity to day and fluorescent light exposure, it degrades into interfering side products.

A more light stable isocyanate stabilization agent is 1-(2-pyridyl)piperazine (1,2-PP). The derivatives formed with 1,2-PP can be analyzed using UV or fluorescence detectors. OSHA used 1,2-PP for the filter coating to analyze MDI and TDI (OSHA Methods 42 and 47). There are several precautions that have to be taken when using these methods: 1,2-PP evaporates at temperatures greater than 40ºC and filters can’t be stored for more than two weeks at 4ºC. Tryptamine (3-(2-aminoethyl)indole (TRYP) has been used for isocyanate stabilization in one of NIOSH methods (Method 5522). The rates of the isocyanates reactions with this amine are about 10^7 faster than with water, so the presence of moisture in a collection system does not affect the results.

The secondary amine 1-(2-methoxyphenyl)pyperazine (1,2-MP) is also used for isocyanate derivatization. This amine is more stable to the direct light and less volatile than 1,2-PP. It was regulated in the UK Health and Safety Executive method (White, 2006), in NIOSH method 5521 and it is an ISO recommended standard method for airborne isocyanates. It can be used as impinger medium or applied to filters impregnation. The latter have to be field-desorbed right after sampling.

It is also possible to form isocyanate derivatives that can be analyzed by UV or fluorescence detectors. One of the derivatizing agents used for this purpose is 9-(*N*-methylaminomethyl)anthracene (MAMA). This reagent is used in Iso-Chek method adopted by the ASTM as a standard method for TDI in air (Brown, 2001).

A relatively recent study reported the relative rates of the reaction determined for some secondary amine derivatizing agents (DBA, MAMA, MAP, MP) and diisocyanates (Tremblay *et al.*, 2003). It was shown that in general, the relative rates of the reaction for the following amines are DBA>MAP>MP>MAMA. Although the rate of the reaction could depend on the solvent used, the order is the same. It also stated that the reaction between diisocyanate and an amine is usually completed in less than 2 minutes.

There are also methods to analyze isocyanates in water, soil and in solid media (non airborne) (Brede *et al.*, 2003). In water TDI and MDI can be quantified by derivatization with dibutylamine followed by quantification of TDA and MDA (Allport *et al.*, 2003). TDI and MDI can also be evaluated on a surface (skin or on a surface such as flexible polyurethane foam) by wiping. If the wiping material contain colorimetric agent, the change of the color gives a direct response in about 3 minutes (Colormetric Laboratories). However, these methods are only qualitative and can't determine the concentration of an isocyanate.

TDI and MDI can also be extracted with a solvent from a flexible polyurethane foam sample and the concentration of the TDI and MDI derivatives can be determined using HPLC method. These studies were carried out by several authors (Gagne *et al.*, 2003; Hugo *et al.*, 2000; Mutsuga *et al.*, 2014). It was shown that the quantity of TDI and MDI extracted from the same sample can depend upon the solvent used (Vangronsveld *et al.*, 2013a). Therefore, TDI and MDI extracted from a foam cannot be considered as free isocyanate contained in a sample.

Even though there is no method for quantification of diisocyanate migration from the flexible foam surface, all methods mentioned above can be helpful in developing such a method.

1.3.6 EPA protocol

The Environmental Protection Agency is an independent agency of the United States federal government for environmental protection. It was established on December 2, 1970 and began to operate in order to protect human health by creating programs designed to reduce pollution and ensure environmental protection of the air, water and land (United States Environmental Protection Agency., 2018).

The EPA Office of Pollution has generated testing protocols providing the information, description and analytical procedures in order to evaluate the indoor exposure to chemicals from consumer products and building materials. These chemicals can migrate to air and dust media prior to the exposure. The protocols do not specify the chemicals that can represent risk for the exposure, but give the general information about potential exposure routes to human receptors. Among ten protocols, there are protocols to evaluate the migration to dust, migration to saliva (or oral exposure), migration from solid material to water and also migration to skin (or dermal exposure). For this research the dermal exposure protocol was investigated and one of the approaches was applied in order to evaluate the concentration of diisocyanates load on a surface (United States Environmental Protection Agency., 2017).

Dermal exposure protocol describe a procedure to evaluate a chemical loading on the skin surface being in direct contact with an article, dust, soil or vapor in order to quantify the availability for the dermal exposure. According to this protocol the chemical loading on a skin surface can come from the contact through application of liquid or semi-solid products, from transfer of chemicals from vapor-phase in the air

to the skin, from contact with dust or soil and migration into simulated sweat or skin lipids (oil) and from contact with surface of article or building material and migration into simulated sweat or skin lipids (oil). The PU flexible foam is a product of interest for this study. As it is used in furniture and mattresses production, the migration to a simulated sweat solution after a direct contact was identified as a possible route for dermal exposure.

A small or a large scale experiment can be used to evaluate migration to a simulated sweat solution from a direct contact with an article. Several parameters can be varied based on a chemical of interest: amount of synthetic sweat solution applied, size and thickness of the article, time and amount of pressure applied, size of skin surrogate and its material, additional barriers between article and skin. For this study project a small scale experiment was used and the amount of sweat solution applied to a filter was selected based on the size of the skin surrogate (glass fiber filter). The filter had to be well moisturized in order to facilitate the dissolution of the chemicals at the surface of the filter, but not overwetted, to avoid spreading of substances on the surface with subsequent losses. The amount of pressure applied was selected to simulate the process of compression of the foam under weight while sleeping or sitting on the product that is made of PU flexible foam. The pressure by compression of 25% of height of foam sample was applied overnight also to simulate the real life conditions. Different formulations of the simulated sweat solutions containing typical components such as water, urea, lactate, sodium, potassium, calcium, and magnesium at different pH proposed by EPA, were used as an extract solution. A small scale experiment protocol was used for this project and the surface of 37 mm in diameter was studied in order to quantify the concentration of diisocyanate migrated to a filter. For these tests the experimental cell was constructed from the parts as shown in Figure 3.5. Each filter was desorbed and evaluated separately for diisocyanates of interest. The results of the experiment are shown and discussed in chapter II.

1.4 Method development steps

For the development of a robust and reliable method, it is necessary that regulated steps be followed. These steps can be found in (OSHA, 2005) evaluation guidelines for air sampling methods utilizing spectroscopic analysis protocol. The key steps are: sampling, storage & preservation, sample preparation, sample analysis and method validation. Preliminary tests are also performed to collect all the practical information about physical and chemical properties of the studied substance such as: solubility in different solvents, possible interferences, stability of the analyte at the different temperature, toxicity, sampling and preexisting analytical procedures. To determine the analyte concentration or the dynamic range, the existing exposure limits for this substance can be used (OEL's, REL's etc.). Moreover, the preliminary tests are used to determine adequate sampling medium and analytical conditions for the method.

1.4.1 Sampling

Sample collection is an important factor that will contribute to reliable analytical results. A good sampling procedure allows to obtain the accurate results using sensitive and robust method of analysis.

Airborne isocyanates can be sampled using paper tape systems, impingers with appropriate derivatizing agent in a solvent, or a coated filter. A sampler for isocyanate can also be a system with impinger and filter together, or two filters combined in a cassette (ISO-CHEK) (Lesage *et al.*, 1992), or a denuder and a filter (ASSET EZ4-NCO with di-*n*-butylamine(DBA)) (Marand *et al.*, 2005). Impinger system gives the possibility to collect and stabilize an isocyanate in a solvent immediately during the sampling. However, organic solvents, in particular toluene, may evaporate or accidentally be spilled during the sampling procedure which leads to adversed results. This device can thus be unsafe for a worker to wear. To avoid these problems, other

solvent free sampling systems can be used. One of them is a cassette with two filters (ISO-CHEK). One filter is a Teflon filter, 5µm pore size, placed to collect aerosols. This filter has to be transferred to a desorption solution containing a derivatizing agent instantly after sampling. Under the Teflon filter glass fiber (GF) filter coated with 9-(*N*-methylaminomethyl)anthracene (MAMA) is placed to collect and stabilize the vapors immediately during the sampling. The recommended time for sampling with this device is 15 min due to limited loading capacity of the filters. ASSET EZ4-NCO is another sampling device used without solvent. It consists of a denuder and a filter that are coated with di-*n*-butylamine (DBA) to react and transformed into a derivative any isocyanate collected. The formed DBA-derivative is stable and there is no need for any field desorption right after sampling. Once extracted, the derivative can be analyzed using HPLC-MS/MS at very low detection limits. The capacity of this system allows the sampling up to 12 hours. For this study project 37-mm glass fiber filters coated with derivatizing agent were chosen for the sampling.

1.4.2 Stabilisation by amine derivatisation and storage.

Isocyanates are highly reactive molecules, they have to be stabilized during the sampling or right after it. As already mentioned above, several samplers used the following amine derivatisation MAMA, DBA. Another reactive secondary amine usually used is 1-(2-methoxyphenyl)pyperazine (1,2-MP) which was reported to form stable derivatives with diisocyanates. The rate of this reaction is fast enough, so the derivative is formed immediately at room temperature (Puscasu *et al.*, 2014). Some of these derivatives (DBA derivatives) are shown to be stable even at room temperature for 3 weeks (Spanne *et al.*, 1996) or can be stored for 14 days (Henriks-Eckerman *et al.*, 2000). For this study project 1,2-MP was used for derivatization and formed derivatives were analyzed using UV detector.

1.4.3 Analysis

The high performance liquid chromatography (HPLC) is commonly used to separate the derivatives. Depending on an agent chosen for isocyanates stabilisation, the corresponding derivatives can be analyzed using either a UV-PDa, EC, FL or MS detector (Gagne *et al.*, 2003; Hugo et *al.*, 2000; Marand *et al.*, 2005; Puscasu *et al.*, 2014; White, J., 2006; Wu et *al*, 1990). Although methods using a UV detector are low cost and more simple, the mass spectrometric (MS) detector is very sensitive, and it allows the quantification at very low concentration levels. Another advantage of a MS method is also in its selectivity, it gives the actual masses of each derivatives in the mixture of analytes.

To develop a reliable and robust method all the above steps have to be optimized.

1.4.4 Method validation

A robust and reliable method has to be validated. To this end, several analytical parameters of a method have to be evaluated (Institut de recherche Robert-Sauvé en santé et sécurité du travail, 2013). These parameters are: limit of detection (LOD), limit of quantification (LOQ), dynamic range of application of the method, method precision, recovery and accuracy. The LOD will determine the lowest concentration of the studied analyte that can be detected using the method. The LOQ determines the minimum quantifiable concentration of the analyte for the method. The precision is determined by two parameters: replicability and repeatability. Replicability is calculated by the difference between samples at different concentrations prepared by the same person the same day on the same instrument. Repeatability is also calculated by the difference between samples at different concentrations with a change of one of the elements either person, either instrument or a day. The difference for both of these parameters has to be less than 10% for good method replicability and repeatability. The accuracy of the method can be calculated by the difference between the expected

value and the obtained one. Sensitivity is calculated by the ratio of the analyte signal to its concentration. To determine the recovery of the method the recovery of the analyte has to be calculated from the difference with or without media collector (i.e. empreignated filter).

1.5 Objectives

There are analytical methods to evaluate concentration of diisocyanates present in air or soil. Some methods allow biological monitoring by measuring the corresponding isocyanate-derived diamine in urine sample (Budnik *et al.*, 2011; Mirmohammadi *et al.*, 2013). However, these methods do not provide the relationship between the concentration of an isocyanate in the air and its concentration that can be accumulated on the surface of the product available for dermal exposure. Other methods are needed to evaluate a diisocyanate migration from a surface.

The main objective of this study was to develop an analytical method that will allow the quantification of diisocyanate migration from a surface following the recommendations of one of the protocols proposed by US EPA. Two diisocyanates most commonly used in flexible polyurethane foam production were used for this purpose.

The secondary objectives of this project are:

- Adapt a method for quantification of MDI-1,2MP and TDI-1,2MP derivatives on a new instrument that is Aquity Arc from Waters with PDa and QDa detectors;
- Evaluate the recovery obtained using six synthetic sweat solutions proposed by EPA as co-solvents;
- Validate the developed method according to a DR-12-VMC method validation and IG-020 protocol of IRSST using one of the six synthetic sweat solutions with the best recovery percentage;

- Apply the validated method to evaluate the possible migration of the TDI and MDI from the surface of samples provided by the Polyurethane Foam manufactures.

CHAPTER II – ARTICLE

DEVELOPMENT OF A METHOD FOR QUANTIFICATION OF TOLUENE DIISOCYANATE AND METHYLENEDIPHENYL DIISOCYANATE MIGRATION FROM POLYURETHANE FOAM SAMPLE SURFACE TO ARTIFICIAL SWEAT BY HPLC-UV-MS

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This chapter discusses objectives related to the development of an analytical method for quantification of diisocyanate migration from a surface following the recommendations of US EPA. The article was submitted for publication in the Journal of Chromatography B. Existing methods allow the quantification of isocyanates present in the air, soil, water or even biological monitoring. However, there is no method for quantification of isocyanates migrated from a polyurethane flexible foam surface.

The following method was developed: desorption method, chromatographic parameters and analysis parameters were optimized to obtaine the best results. Six synthetic sweat solutions were prepared according to an EPA protocol. Recovery tests conducted with all six sweat solutions indicated a good recovery percentage (approximately 80%) for five of them. The recovery for the sixth sweat solution is low, approximately 30%. Then, the method was validated according to an approach used by the Quebec Center of Expertise in Environmental Analysis. The robustness of the method was obtained from the evaluation of its analytical performances. The dynamic range of application choosen for this method is from 0.025 µg/mL to 0.49 µg/mL for TDI isomers and from 0.04 µg/mL to 0.8 µg/mL for MDI. The average desorption recoveries are 89.5±4.4% for 2,6-TDI-1,2MP derivative, 92.7±11.0% for 2,4-TDI-1,2MP derivative and 80.5±3.3% for MDI-1,2MP derivative. The detection limits of the method were 0.002 µg/mL for 2,6-TDI-1,2MP, 0.011µg/mL for 2,4-TDI-1,2MP, and 0.003 µg/mL for MDI-1,2MP. Quantification limits were 0.006 µg/mL, 0.037 µg/mL, and 0.010 µg/mL respectively. TDI and MDI migration was not observed when testing was conducted on foam samples.

2.1 Abstract

The US Environmental protection agency (EPA) has published guidance that includes test procedures for evaluating indoor exposure to chemicals from products. One of the test procedures represents the migration test for evaluating potential dermal exposure from home furniture. Such an evaluation involves the chemical measurement of the sweat which is currently unavailable in the literature.The objective of this project was to develop and validate an analytical method for quantification of migration of 4,4'-methylenediphenyl diisocyanate (MDI), 2,6-toluene diisocyanate (2,6-TDI) and 2,4-toluene diisocyanate (2,4-TDI) from a polyurethane (PU) flexible foam to artificial sweatthat meets the recommendations of the EPA test protocol. Following the EPA protocol, six synthetic sweat solutions were prepared and used in evaluation of isocyanate recovery performance. The migration tests were conducted using five foam types that were chosen and supplied by PU foam manufacturers to represent the types most commonly found in commercial products, and with formulations anticipated to have the highest potential residual TDI or MDI. Migration tests were conducted using glass fiber filters (GFF) coated with 1-(2-methoxyphenyl)piperazine (1,2-MP) and analyzed using HPLC equipped with a UV detector for quantification and a MS detector to qualify peaks. The detection limits of the method were 0.002µg/mL for 2,6-TDI, 0.011 µg/mL for 2,4-TDI, and 0.003 µg/mL for MDI. Quantification limits were 0.006 µg/mL, 0.037 µg/mL, and 0.010 µg/mL, respectively. The recovery tests on a Teflon surface for 5 of the 6 EPA-recommended synthetic sweat solutions indicate the recovery percentage was approximately 80% for diisocyanates. Recovery for the sixth sweat solution was low, approximately 30%. TDI and MDI migration was not observed when testing was conducted on foam samples.

Keywords: isocyanate, polyurethane, artificial sweat, migration, 1-(2-methoxyphenyl) piperazine, HPLC-UV.

2.2 Introduction

Toluene diisocyanate (TDI) and methylenediphenyl diisocyanate (MDI) are organic compounds that have two isocyanate (NCO) groups in their molecules. These two diisocyanates are largely used in polyurethane (PU) production. The polymer is formed by chemical reaction between an isocyanate with two or more NCO groups and a diol or polyol in the presence of a catalyst, a chain extender and other additives [1]. The different nature of isocyanates and polyols used for polymerization create a wide variety of materials, from high performance elastomers to tough thermoplastics and foams that can be used in applications such as construction (for thermal insulation), furniture (bedding, mattresses, chairs, sofas), and automobiles (molded rigid foams), among others. Increasing the temperature during polymerization can cause a reverse reaction that leaves some residual isocyanate attached to the formed product [2]. The concentration of free isocyanate in freshly prepared foam will decrease within a few days after production [1]. The method of quantification of diisocyanate migration studied in this project can confirm or determine at what point the diisocyanates are no longer available to migrate to a sweat solution. Therefore, this can be a useful tool in the estimation of the potential risk for dermal exposure. However, isocyanates analysis has not yet been reported from sweat samples and needs to be addressed.

TDI and MDI are known to cause respiratory tract, eye and skin irritation [3-6]. Over-exposure can lead to sensitization and asthma [7-9]. Possible induction of sensitization to isocyanates due to dermal exposure has been studied in animals [10-12]. It can also cause contact dermatitis on a sensible person [6, 13, 14]. Furthermore, if MDI or TDI are able to migrate, they will eventually hydrolyse to the corresponding MDA or TDA side products [15]. The latter has been classified category 2B, as a potential cancer suspect agent for human by the *International*

Agency for research on cancer [16]. It is also known that the MDA can be absorbed via skin contact [17]. Because of the frequent and prolonged contact with PU foam based furniture's in every day life, it is of the outmost importance to develop an efficient method to determine the migration of potential residual diisocyanates from such foams.

There are exposure limits established by the governmental agencies for these chemicals that are intended for use to determine airborne concentrations [18-20]. Unfortunately, there are no exposure limits for diisocyanates on surfaces or on skin so it is impossible to evaluate the concentrations of these substances being transferred *via* skin contact and their subsequent health effect.

To be able to analyze diisocyanates it is necessary to stabilize the NCO groups by a derivatization reaction. Secondary amines used in this reaction can include 1-(9-anthracenylmethyl)piperazine (MAP), N-(4-nitrobenzyl)-N-propylamine (OSHA method 18), 1-(2-Pyridyl)piperazine (1,2-PP) (OSHA 42 and 47 methods), 1-(2-methoxyphenyl)piperazine (1,2-MP) and are routinely used for derivatization of isocyanates. These stable derivatives can be further analyzed in laboratory using reversed phase chromatography with UV, diode-array or Mass Spectrometry (MS) detection [21, 22].

It's been demonstrated in some studies that residual TDI can be extracted from foam using organic solvents, treated with a derivatizing agent and then analyzed [23-25]. The concentrations of TDI extracted from foam are very low and usually decrease with foam aging. It was also reported that the concentration of TDI found depends on the solvent used for extraction [24]. Assuming that solvent extraction may lead to detection of unreacted TDI, those concentrations should not be considered 'free TDI' which is available for migration to surface of the foam, leading to skin contact from articles made with PU foams. For this reason, another approach was needed for

evaluation of diisocyanate that may migrate from polyurethane products and potentially result in dermal exposure.

US EPA's Office of Pollution Prevention and Toxics (OPPT) has published ten testing protocols providing general information and descriptions of sampling and analytical procedures used to evaluate indoor exposure to chemicals in consumer products and articles [26]. The latter include building materials used in the indoor environment. One of the testing procedures described in the protocol is for migration to simulated sweat to determine the quantity of a chemical accumulated on skin surface from direct contact with an object. Five simulated sweat solutions containing typical components of sweat (i.e., water, lactate, urea, sodium, potassium, calcium, magnesium cations) at different pH (5.4; 4.5; 2.8) were proposed by EPA (Table 2.1).

Table 2.1. Synthetic sweat solutions №1-№5 components

	NaCl	$CaCl_2$	$MgSO_4$	KH_2PO_4	Lactic acid	Urea
Sweat №1 pH5.4	0.1706 g/L	0.0092 g/L	0.0147 g/L	0.1388 g/L		
Sweat №2 pH 4.5	0.3208 g/L	0.1842 g/L	0.0294 g/L	0.1851 g/L		
Sweat №3 pH4.5	0.3208 g/L	0.3685 g/L	0.0294 g/L	0.1851 g/L		
Sweat №4 pH4.5	0.3208 g/L	0.7369 g/L	0.0294 g/L	0.1851 g/L		
Sweat №5 pH2.8	5 g/L				1 g/L	1 g/L

A sixth artificial sweat solution (Table 2.2) was added to the protocol later, and is more likely to represent the composition of a biological fluid [27-30]. All six simulated sweat solutions were used in this study to evaluate TDI and MDI migration from a polyurethane flexible foam sample. Even if these sweat mixtures are available from EPA, there is no example reported on how the samples should be prepared for

analytical measurements. The development of an efficient method is therefore needed to extract isocyanates from the sweat.

Table 2.2. Synthetic sweat №6 solution components (pH adjust to 5.3)

Sweat Ingredients	**Measured Qty,**
Electrolytes and Ionic Constituents	(g/L)
Sodium Sulfate	5.83×10^{-2}
Copper Chloride anhydrous	1.60×10^{-4}
Ammonium Hydroxide	1.82×10^{-1}
Iron sulfate Heptahydrate	2.72×10^{-3}
Sulfur	7.37×10^{-2}
Lead- Reference Solution 1000 ppm	2.49×10^{-5}
Manganese- Reference Solution 1000 ppm	1.38×10^{-4}
Nickel- Reference Solution 1000 ppm	2.46×10^{-5}
Zinc - Reference Solution 1000 ppm	8.5×10^{-4}
Sodium Bicarbonate	2.52×10^{-1}
Potassium chloride	4.55×10^{-1}
Magnesium Chloride Hexahydrate	1.67×10^{-2}
Sodium Phosphate Anhydrous Monobasic	4.84×10^{-2}
Calcium Chloride Dihydrate	7.65×10^{-1}
Sodium chloride	5.84×10^{-2}
Organic Acids and Carbohydrates	
Acetic Acid	7.81×10^{-3}
Butyric Acid	2.11×10^{-4}
D(+) –Glucose	3.06×10^{-2}
Lactic Acid	1.57
Essential Amino Acid Mix	2.5 mM each : 17 AA
Nitrogenous Substances	
Ammonium Chloride	9.92×10^{-3}
Urea	6.01×10^{-1}
Creatinine	9.50×10^{-3}
Sebum Ingredients	
Squalene	0.5151
Palmityl Palmitate (saturated)	0.9718
Triolein (Unsaturated)	0.5345
Cholesteryl Oleate	0.0972

The objective of this study was to develop and validate a method for the quantification of diisocyanate from synthetic sweat samples according to the EPA published protocol described above in order to assess the migration potential from foam furniture. Glass fiber (GF) filters coated with 1,2-MP were used to collect isocyanates, simultaneously transforming them into stable derivatives that were analyzed by HPLC coupled with both UV-and mass detectors. Five polyurethane foam samples, representing the types most commonly found in commercial products and with formulations anticipated to have the highest potential residual TDI or MDI were supplied by manufacturers and were analyzed using the developed method. Synthetic sweat solutions (6 proposed by EPA) were used to evaluate each of the five foams for determining optimum recovery results. The use of a mass detector allowed simultaneous confirmation of the identity of the peaks detected by UV.

2.3 Methods

2.3.1 Chemicals

All chemicals were used without any further purification. 2,4-TDI (96% purity), 2,6-TDI (97% purity), 4,4'-MDI (98% purity), 1-(2-Methoxyphenyl)piperazine (1,2-MP; 98% purity), Acetic anhydride (AcA; 98% purity), Amino acid mix, Squalene, Glyceryl trioleate, Cetylpalmitate, N-Butyric acid were obtained from Sigma-Aldrich (Milwaukee, USA). Sodium sulfate (99% purity), Lead reference 1000 ppm, Manganese-reference 1000 ppm, Nickel-reference 1000 ppm, Zinc-reference 1000 ppm, Calcium chloride, Copper chloride, Ammonium hydroxide (trace metal grade), Creatinine were obtained from Fisher Scientific (Markham, ON, Canada). Cholesteryl oleate, Sulfur (99%) and Lactic acid were obtained from Alfa Aesar (Tewksbury, MA, USA). Ammonium chloride, Sodium chloride and Iron sulfate (>99% purity) were obtained from VWR (Ville Mont-Royal, QC, Canada). Sodium phosphate and Sodium bicarbonate were obtained from Bioshop (Burlington, ON, Canada). Urea

was obtained from Bio Basic (Markham, ON, Canada). Acetonitrile (ACN) and water (H_2O), both HPLC grade were obtained from Fisher Scientific (Canada). Glacial acetic acid was obtained from J.T. Baker. Potassium chloride (99 % purity) and D-Glucose were obtained from EMD Millipore Corp. (Billerica, MA, USA). Ammonium acetate (99% purity) was obtained from Fluka Chemical Corp. (Ronkonkoma, NY).

2.3.2 Instruments and analytical conditions

The HPLC-UV-QDa system was an Acquity Arc from Waters (Beverly, Massachusetts, USA). The analyses were carried out using a Kinetex core shell C_{18} column of 2.6 µm (2.1 mm × 100 mm) from Phenomenex. A SecurityGuard™ Ultra Cartridge System (C18 4.6mm) was also used to protect the analytical column from an accumulation of any possible precipitants.

The elution was effectuated using an isocratic method with 50% of aqueous phase (2 mM ammonium acetate buffer at pH 6 adjusted using acetic acid) and 50% of organic phase (acetonitrile) for 4 minutes. The column temperature was maintained at 35 °C and a constant eluent flow rate of 0.5 mL/min was used. The injection volume was 10 µL and the sample temperature was maintained at 18 °C. The UV spectrum was obtained using a photodiode array detector operating between 200 and 400 nm. The quantification was done at 242 nm for 2,6-TDI, 2,4-TDI and 250 nm for MDI. Areas for TDI and MDI quantification were determined using the automatic integration feature and manual adjustments were done only where auto-integrations did not cover the entire peak. The calibration curve regression equation was determined using least-squares linear regression fitted of the peak area versus concentration data, with a concentration weighting factor of 1/x.

The QDa was operated in positive mode. Cone voltage was adjusted to 15 V, capillary voltage was set at 0.8 kV and the probe temperature was set at 600 °C. The detector was scanning the ion masses between 100-700 Da. The peaks of TDI-1,2-MP

were confirmed by the ion mass 559 Da and the peak of MDI-1,2-MP had the ion mass 635 Da.

2.3.3 Synthetic sweat solutions

Artificial sweat solutions were prepared for use as sample collection solvents in migration tests with foam samples and on a Teflon surface for transfer efficiency.
Five sweat solutions were prepared in water using sodium, potassium, calcium, magnesium, lactate and urea in different concentrations as shown in Table 2.1. The sixth sweat solution was prepared partially in water and partially in a mixture of organic solvents (3 parts chloroform and 1 part methanol). These two parts were mixed together in a 1:1 proportion and mixed on a Vortex mixer prior to the experiment (Table 2.2).

2.3.4 Standard curve preparation

The stock solution of isocyanates mixture (200 µg/mL of MDI and 124.4 µg/mL for the 2,6-TDI, 2,4-TDI isomers) used to make the calibration standards was prepared in ACN. For the stock solution, about 20 mg of MDI was weighted accurately on a microbalance and dissolved in 80 mL of ACN; 10 µL of each isomer of TDI were added to the volumetric flask and was placed in a sonicating bath for 1 minute; then ACN was added to a flask up to 100 mL. Several dilutions of the stock solution used for the calibration curve were also done in ACN. The preparation of the calibration curve samples was as follows: known aliquots from diluted stock solutions were spiked into 0.1 mg/mL 1,2-MP/ACN followed by addition of 0.5% AcA. One blank and 5 calibration solutions containing TDI-1,2-MP derivatives of concentrations ranging from 0.025 µg/mL to 0.490 µg/mL (0 µg/mL, 0.025 µg/mL, 0.050 µg/mL, 0.125 µg/mL, 0.250 µg/mL, 0.490 µg/mL); and from 0.04 µg/mL to 0.8 µg/mL (0 µg/mL, 0.040 µg/mL, 0.080 µg/mL, 0.200 µg/mL, 0.400 µg/mL, 0.800 µg/mL) for MDI-1,2-MP derivative were made. All the calibration samples were filtered on a

syringe cartridge containing a 0.22 µm pore size filter. Final sample concentration solutions were obtained by adding 500 µL of the above solutions and 500 µL of water as mobile phase solvents.

The calibration concentration range of the method is equivalent to 0.009 µg/cm^2 to 0.178 µg/cm^2 of TDI at the filter surface and 0.015 µg/cm^2 to 0.291 µg/cm^2 of MDI at the filter surface. To convert units from µg/mL to µg/cm^2, the concentrations were multiplied by 4/11. Given that each 37 mm filter (11cm^2 in surface) was desorbed in 2 ml of desorption solution and then diluted 1:2 and analyzed, 4 mL of sample represents the amount removed from 11 cm^2 of the surface.

2.3.5 GF/1,2-MP coated filters preparation

The 1,2-MP solution for the coating was prepared by dissolving approximately 750 mg of 1,2-MP in 100 mL of acetonitrile. GF filters were placed in a beaker and soaked with the coating solution for ~ 30 minutes. The filters were removed from the solution, placed on a clean aluminum foil surface and into a drying box; they were allowed to dry under nitrogen atmosphere overnight. Dried filters were transferred to a clean closed container and stored in a dark refrigerated (2-8ºC or cooler) place for no more than 3 months.

2.3.6 Desorption efficiency tests

Teflon squares used for the tests were cut from a sheet, wiped with methanol and kept in a sealed plastic bag.

Three procedures were attempted to evaluate the desorption efficiency of the spiked isocyanates from the Teflon filter as well as the possibility of hydrolysis to occur:

Procedure 1 Wiping of the Teflon surface with a 37mm GF/1,2-MP impregnated filter:

The desorption efficiency was determined by spiking 40µL of acetonitrile solution

containing 19.36µg/mL of each isomer of TDI and 37.77µg/mL of MDI on Teflon and immediately wiping with a 37mm GF/1,2-MP filter. The filter was transferred to a glass jar containing 2 mL of desorption solution (0.5% of acetic anhydride in acetonitrile). The solution in the jar was placed on a shaker for thirty minutes to facilitate desorption and then filtered using a filter syringe with a 0.22µm pore size. 500µL of filtered solution was combined with 500µL of water, mixed on a Vortex mixer, and analyzed.

***Procedure 2 and 3 Wiping of the Teflon surface with a sweat solution moistened 37mm GF/1,2-MP impregnated filter VS a sweat solution moistened 37mm filter containing no derivatizing agent**:*

A similar procedure using a GF/1,2-MP filter wetted with 0.5 mL of a synthetic sweat solution (one out of six) and a GF non-impregnated filter wetted with a synthetic sweat solution (one out of five) was used to evaluate the desorption efficiency. Sweat 6 was not evaluated with non-impregnated filters due to low recovery performance with impregnated filters. In this case the filters with diisocyanate migrated were placed in a jar containing 1,2-MP solution (0.1 mg/mL) in acetonitrile for derivatization. In both cases, each filter was transferred to a separate jar after 5 min and 60 min to evaluate possible hydrolysis.

2.3.7 Analytical performance evaluation

The analytical performance evaluation was completed in accordance with DR-12-VMC method validation [30]. The limit of detection (LOD) and limit of quantification (LOQ) were measured with ten sample replicates (0.025 µg/mL of TDI isomers; 0.040µg/mL of MDI). The concentrations of replicates were distributed over the calibration range. The standard deviation of the sample peak areas was determined. The LOD and LOQ were calculated as three and ten times the standard deviation, respectively.

The influence of the "matrix effect" of the media solution (GF/1,2-MP filter wetted with 0.5 mL of a synthetic sweat solution) was investigated for each isocyanate (2,6-TDI, 2,4-TDI and MDI). This was done by comparing measured concentrations of isocyanates-1,2-MP derivatives, obtained from five replicate samples where a mixture of the three isocyanates was spiked (at five different concentrations) to the media solution, and was compared to those obtained by spiking the same isocyanates mixture to 1,2-MP solution without any GF/1,2-MP filter and synthetic sweat solution.

Intra-day precision was assessed from six replicates of four different concentrations injected the same day. Inter-day precision was also determined using four concentrations and repeated on six different days using the same instrument and analyst. The accuracy of the method was calculated against a different TDI and MDI stock solution that was carried through the entire extraction procedure and injected during each day of the validation period. The concentration of the solutions that were used for the accuracy was 0.250 µg/mL for TDI isomers and 0.400 µg/mL for MDI. The analytical uncertainty (u) was obtained according to the formula of method DR-12-VMC and incorporated a bias and overall precision. This formula uses the total relative standard deviation (TRSD). The extended uncertainty (U) is also calculated by applying a coverage factor k = 1.96 to u [31].

2.3.8 Polyurethane foam samples migration tests

The foam used for these experiments was received from the Polyurethane Foam Association (PFA). The Polyurethane Foam Association represents manufacturers and suppliers to the flexible polyurethane foam industry, providing support and education on industry topics, and guidance to members on regulatory compliance. All samples were prepared and packaged for shipping according to the procedures outlined in the CertiPUR program [32] and analyzed in the range of 7-23 days post-

production.

The foam samples received were cut in cylinders of 37 mm in diameter (Ø) and 6 cm in height one day before the test, and were kept in a sealed plastic bag. The experimental cell was assembled and a 0.25 mm thick Teflon square, large enough to cover the metal plate at the bottom, was utilized. Two GF/1,2-MP filters wetted with 0.5 mL of synthetic sweat solution (each) were placed on the Teflon square. The sample was then placed on these two filters with two more GF/1,2-MP sweat-wetted filters then placed on top of the sample and covered with another Teflon square. The top metal part of the cell was then installed and the foam was compressed by 25% (1.5 cm) of its height using wing nuts (Figure 2.1). The cell was then left overnight at ambient laboratory conditions. The cell was disassembled and each single filter was transferred to a separate glass jar containing 2 mL of desorption solution (0.5% of acetic anhydride in acetonitrile). The solution in the jar was placed on a mechanical shaker for thirty minutes to facilitate desorption. Lastly, the solution was filtered through a pore size 0.22 µm filter syringe with 500 µL of the filtered solution added to 500 µL of water, mixed by Vortex and analyzed.

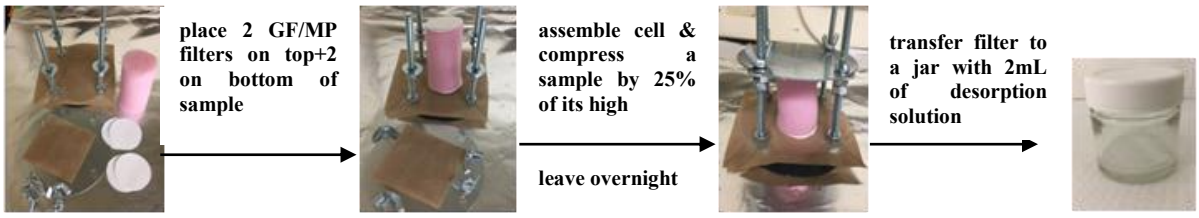


Figure 2.1. Migration cell apparatus

2.4 Results and discussion

2.4.1 Method strategy

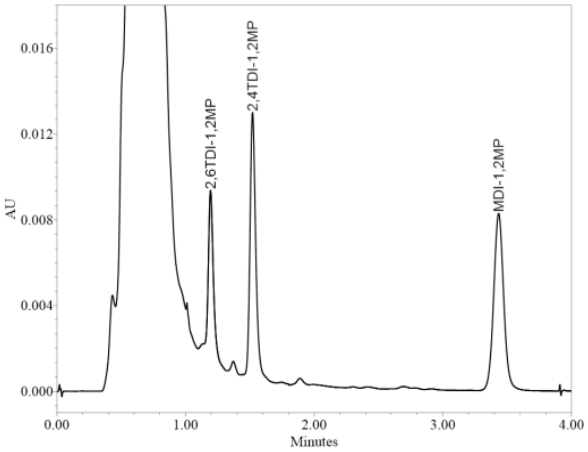
The analysis of isocyanates chemical from sweat samples is novel and it is of high importance in the current scheme where dermal exposure must be evaluated. A typical sweat is a matrix containing several components such as salts, electrolytes and inorganic constituents. It is therefore important to ensure that these constituents won't interfere with the derivatization needed with diisocyanates analysis.

Even though it has been demonstrated that residual TDI and MDI can be extracted from foam samples using organic solvents [23-25], the test procedures outlined in the EPA protocol and used in this study represent a more realistic evaluation of the potential for skin contact. For the derivatization of the isocyanates, 1,2-MP-impregnated glass fiber filters were used due to their efficiency in stabilizing the isocyanate group through formation of a stable derivative [21, 23] that can be easily analyzed using UV detector [22].

The synthetic sweat solutions were used to moisten the surface of the filter to facilitate the migration of the chemicals from the surface in a fashion that is representative of the skin surface environment. The desorption solution was prepared in acetonitrile instead of commonly used toluene [23] or methanol [21], to allow injection directly into the column, avoiding a long evaporation step in the sample work up procedure.

The use of a Waters Acquity Arc with diode-array and QDa detectors with subsequent UV spectrum allowed quantification without the use of an internal standard, which can add some complication due to the matrix effect. Figure 2.2 (a) shows the UV chromatogram of the 2,6-TDI-1,2-MP, 2,4-TDI-1,2-MP and 4,4'-MDI-1, 2-MP.

a) PDA Timed Wavelength (0.00min, 242.0nm) (1.80min, 250.0nm) standard chromatogram



b) MS standard chromatogram with Extract Ion Chromatograms (XIC) for 2,6-TDI-1,2-MP (at 1.186 min), 2,4-TDI-1,2-MP (at 1.506 min) and MDI-1,2-MP (at 3.392 min)

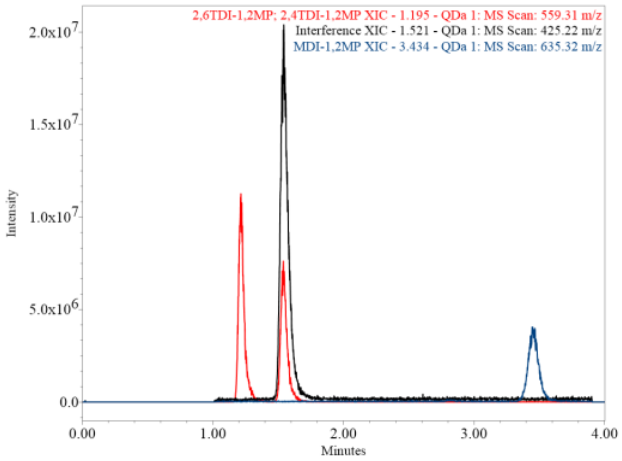


Figure 2.2 UV (a) and MS (b) chromatograms of 2,6-TDI-1,2-MP, 2,4-TDI-1,2-MP, MDI-1,2-MP

The QDa detector allows for validation of peak identities using ion masses, and also allows for the identification of any possible interference in cases where peaks are coeluting or overlapping as shown in Figure 2.2 (b). Using the Extract Ion Chromatograms (XIC) for the peaks of interest, two peaks with different masses coeluting together can be seen at the place of 2,4-TDI derivative (at 1.5 min Fig. 2.2 (b)). The peak of unknown interference has a mass of 425 Da.

2.4.2 Dynamic range, detection limits, precision and accuracy

For method validation, analytical performances were determined and are summarized in Table 2.4. The dynamic range was established to cover the range from 0.025 µg/mL to 0.490 µg/mL for TDI-1,2MP isomers (0.009-0.178 µg/cm^2 of foam surface) and from 0.040 µg/mL to 0.800 µg/mL for MDI-1,2-MP (0.015-0.291 µg/cm^2 of foam surface) with $R^2 \geq 0.990$. The minimum reported value was chosen as the concentration of the standard with minimal concentration. The detection limits of the method, that correspond to an analytical performance of the instrument, were 0.002 µg/mL for 2,6-TDI, 0.011 µg/mL for 2,4-TDI, and 0.003 µg/mL for MDI. Quantification limits were 0.006 µg/mL, 0.037 µg/mL, and 0.010 µg/mL, respectively. The average intra-day precision is less than 4% for all of the diisocyanates derivatives and average inter-day precision is less than 10% as shown in Table 2.4. This indicates that the method is robust and can produce quantitative data. The accuracy is >81% at a target level around 0.250 µg/mL (for TDI isomers) and 0.400 µg/mL (for MDI). Validated parameters assured that the method can be used for real sample quantification.

2.4.3 Recovery and matrix effect

The recovery and the matrix effect were investigated by comparing the derivatives concentrations (obtained by spiking the isocyanate standards in the 1,2-MP solution) without any filter and sweat solution to the one spiked on the GF/1,2-MP wet filter.

The results are summarized in Table 2.4. The recovery performance is possibly due to insufficient desorption or incomplete derivatization on the filter surface, or the spiking technique.

2.4.4 Desorption efficiency tests results

To evaluate the recovery percentage of TDI and MDI in all assays, spiking the Teflon surface with a standard solution containing TDI and MDI was performed. Stabilization of the spiked diisocyanates was done both using GF/1,2-MP without any synthetic sweat solution and using all six sweat solutions. In the case of GF filters not coated with 1,2-MP and moisturized with sweat solution, the stabilization with 1,2-MP was done subsequently. According to the results summarized in Table 2.3, the derivatizing agent needs to be applied directly on the filter surface for good recovery of isocyanates derivatives (entry 2-6). The data (entry 7-10) also indicate that four of the six synthetic sweat solutions used for these tests give an acceptable recovery (> 80% for TDI isomers and >70% for MDI) and any one of them can be used for the validation of the method and for migration test of real samples. The fifth synthetic sweat solution gives a recovery that is slightly lower (entry 11) than the other four (entry 7-10), probably due to the low pH. The sixth synthetic sweat solution did not provide a good recovery (entry 12). Although the formulation used in the sixth synthetic sweat solution best mimics biological fluid, the chloroform used as a solvent to solubilize the sebum ingredients cause interferences in the chromatogram when applied to the filter. In addition, it doesn't give a homogeneous solution when mixed with water and it dries very quickly. The recovery percentages were very similar for the filters transferred after 5 or 60 minutes to the desorption solution, thus confirming that there is no interference of isocyanate-1,2-MP derivatization by the sweat solution.

Table 2.3. Results of recovery tests on Teflon surface (time 5/60 min)

Entry	**Glass Filter (n=№ of replicates)**	**Synthetic Sweat soln.№**	**2,6-TDI %**	**2,4-TDI %**	**MDI %**
1	GF/MP no sweat (n=8)	-	107	105	86
2	GF + sweat	1	0	0	8/0
3	(n = 4)	2	0	0	9/4
4		3	0	0	9 /0
5		4	0	0	10/2
6		5	0	0	7/0
7	GF/MP +sweat	1	81/85	100/97	77/79
8	(n = 2)	2	80/85	86/97	76/81
9		3	82/89	88/99	77/86
10		4	88/91	103/109	74/77
11		5	81/82	68/82	65/71
12		6	58/52	32/17	31/20

Table 2.4. Method performance parameters

Parameters	**2,6-TDI-1,2-MP**	**2,4-TDI-1,2-MP**	**MDI-1,2-MP**
LOD (µg/mL) n=10 (µg/cm^2 of foam surface)	0.002 0.0007	0.009 0.0033	0.003 0.0011
LOQ (µg/mL) n=10 (µg/cm^2 of foam surface)	0.006 0.0022	0.030 0.0109	0.010 0.0036
Dynamic range (µg/mL) (µg/cm^2 of foam surface)	0.025-0.49 0.009-0.178	0.025-0.49 0.009-0.178	0.040-0.80 0.015-0.291
Intra-day precision (n=24)	1.40	3.58	1.03
Inter-day precision (n=24)	8.55	9.16	4.25
Recovery (%)	89.5±4.4	92.7±11.0	80.5±3.3
Accuracy (%) (n = 10)	83.8	87.8	81.1
Analytical uncertainty (%)	2.0	8.7	1.9
Extended uncertainty (%)	4.0	17.1	3.8

2.4.5 Analytical performance

The samples used for evaluating analytical performance were prepared by spiking the isocyanate standards diluted in acetonitrile on the 1,2-MP-coated GF filter wetted with the synthetic sweat solution No. 3 followed by the desorption solution.

2.4.6 Specificity and selectivity

The chromatographic retention time and the precise wavelength (242 nm for TDI isomers and 250 nm for MDI) were used for the method specificity and selectivity. A possible interference was investigated by comparing the chromatograms of derivatives obtained by spiking the standards in 1,2-MP solution or on a coated filter

wetted with the No. 3 sweat solution. One peak of interference was observed while stabilizing the isocyanates with GF/1,2-MP filter. An unknown substance was coeluting with the peak of 2,4-TDI-1,2-MP (Figure 2.2(b)). The QDa data allows for easily distinguishing between the peaks of interference due to different ion mass. This peak is also observed in the chromatogram of the media blank of GF/1,2-MP filter wetted with the sweat solution. The average concentration of three media blank filters was subtracted from the concentration of the standards during the method validation tests.

2.4.7 Polyurethane foam samples migration tests results

The validated analytical method was used to evaluate the migration of isocyanates from the surface of the flexible PU foam supplied by the PFA. All the foam samples were analyzed within 7 to 23 days post-production. Results obtained are summarized in Table 2.5. As can be seen in these assays, none of the analytes (2,4- and 2,6-TDI and MDI) were detected in the migration samples. The results of this study suggest that unreacted diisocyanates (MDI, TDI) would not be expected to be present on the surface of, or migrate to skin in contact with, consumer foam products such as those tested here.

Table 2.5. Results from PU foam samples provided by PFA

Foam type/name	**№days produced-analyzed**	**Sweat solution №**	**Isocyanate found**
Super soft grade	7	1-6	Not detected*
Conventional	13	1-6	Not detected
Viscoelastic	13	1-6	Not detected
HR grade	23	1-6	Not detected
250 gel Viscoelastic	13	1-6	Not detected

* Detection limits: 2,6-TDI: 0.0007 µg/cm^2; 2,4-TDI: 0.0033 µg/cm^2; MDI: 0.0011 µg/cm^2

2.5 Conclusion

A method to quantify diisocyanates from sweat samples according to an EPA protocol was developed and validated in order to establish the potential of migration from foam materials. The method was shown to be robust, reliable and accurate. It is therefore possible to analyze diisocyanates such as TDI and MDI from sweat samples. Among six synthetic sweat solutions proposed by EPA, five resulted in good recovery and can be used as a co-solvent during migration tests. This method can be used to quantify any of the three diisocyanates commonly used in flexible PU production. Testing of five “worst-case” foam samples provided by foam manufacturers showed that free diisocyanates were not detected.

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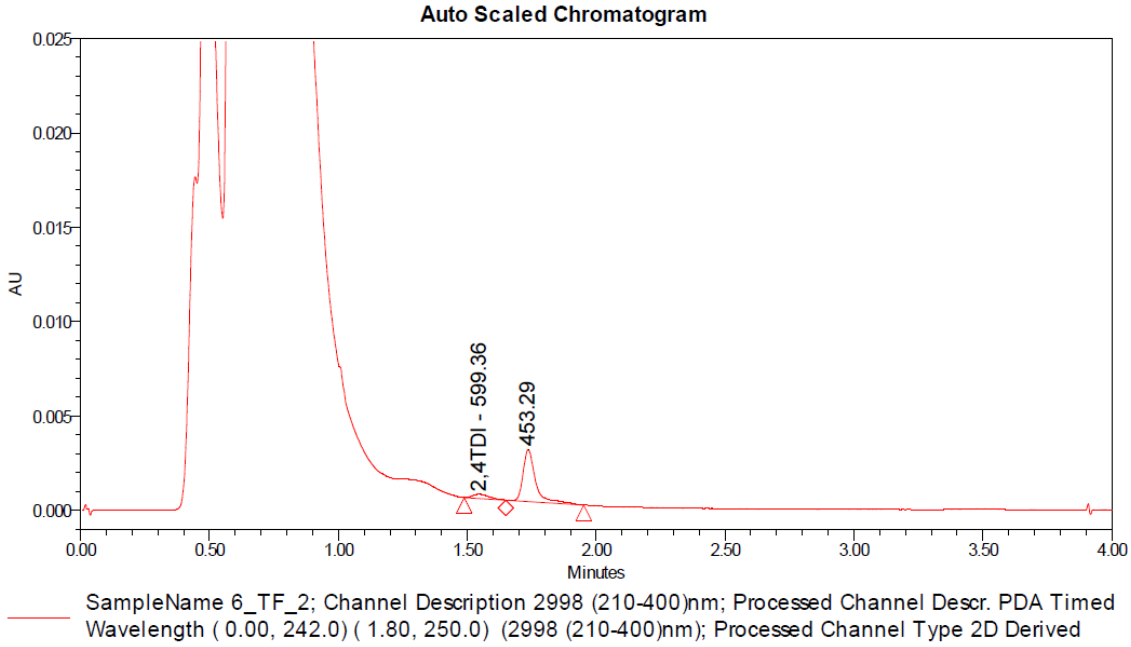
CHAPTER III
GENERAL DISCUSSION

3.1 Sweat solutions

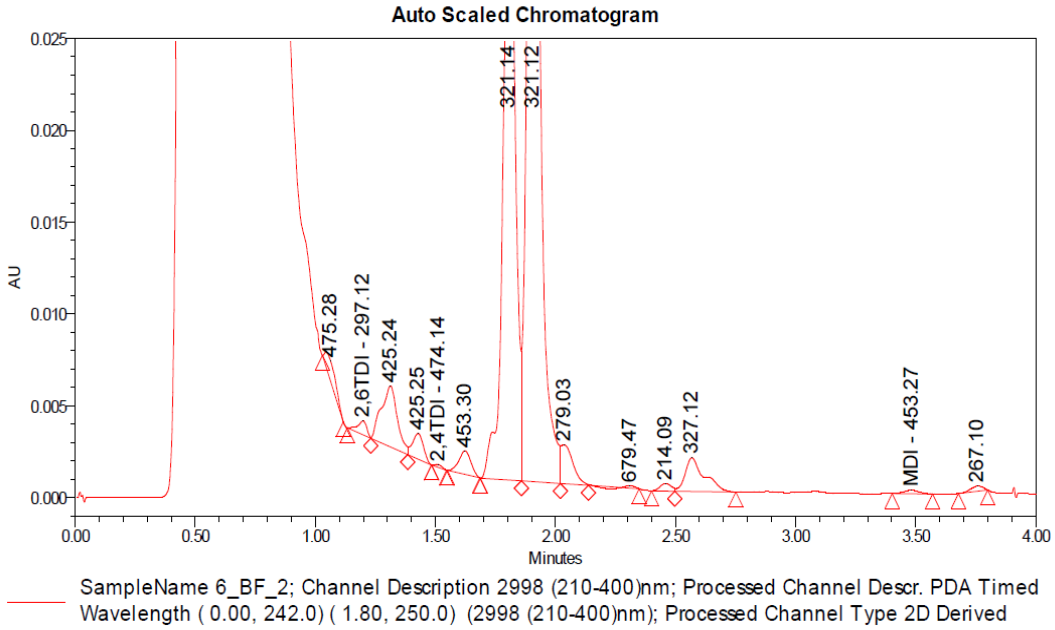
All synthetic sweat solutions proposed by EPA for determination of chemicals migration via skin contact were prepared and evaluated in transfer efficiency tests in order to verify their potential matrix effect. The results summarized in chapter II (Table 2.3) have demonstrated that low pH (2.8) doesn't affect the recovery in spite of the possible hydrolysis of TDI- and MDI-1,2-MP derivatives. It can also be seen that the recovery of the diisocyanate-1,2-MP derivative does not depend on pH of the sweat solution. Five of six sweat solutions give a similar recovery percentage and could be used in a sampling of diisocyanates. Given that all these five sweat solutions behave in a same manner, only synthetic sweat solution 3 was used in the method validation; pH (4.5) of this sweat solution is similar to sweat solutions 2 and 4. Sweat solution 5 has the lowest pH among all the solutions and was not chosen for the method validation. As for the sweat solution 6, it's shown in a table (Table 2.3) that the recovery is very low compared to the other sweat solutions. This sweat solution has more complex formulation and is partitioned between water and organic solvents mixture (chloroform:methanol 3:1). For preliminary tests, two parts of this sweat solution were mixed in two different manners:

Procedure 1 of sweat №6 solution preparation: 0.25 mL of sebum part is applied, then 0.25 mL of aqueous part of sweat solution is applied.

Procedure 2 of sweat №6 solution preparation: Two parts of the sweat solution are mixed together in a proportion 50%:50% and vortexed. Then 0.5 mL of this mixture was applied to a filter. According to the chromatograms, none of these procedures gave the appropriate homogeneous mixture. The chromatograms of the different filters from the same sample differ too much from each other, revealing the peaks of unknown substances (Figure 3.1 (a) and (b)). The filter wetted with this sweat solution dries very fast. For the rest of the tests, procedure 2 was used in order to obtain mixture that is more homogeneous. However, the results of the migration tests showed that this sweat solution should not be used.



a) top filter using synthetic sweat №6 solution as co-solvent



b) bottom filter using synthetic sweat №6 solution as co-solvent

Fig.3.1 PDA Timed Wavelength (0.00min, 242.0nm) (1.80min, 250.0nm) standard chromatogram of foam sample; (a) top and (b) bottom filter wetted with synthetic sweat №6 solution

3.2 Method validation

The developed method was validated according to an IRSST protocol IG-020 (Institut de recherche Robert-Sauvé en santé et sécurité du travail, 2013). The analytical data obtained are summarized in chapter II Table 2.4. The analytical detection limits were verified by preparing the standards at concentrations corresponding to the calculated limits. In the following Tables, are shown detailed analytical data obtained from the

intra-day, inter-day precision and desorption tests for 2,6-, 2,4-TDI-1,2-MP, and MDI-1,2-MP derivatives.

Table 3.1 Analytical data of desorption test for the 2,6-TDI-1,2-MP derivative

2,6-TDI	Concentration (µg/mL)	0.025 (n = 6)	0.049 (n = 6)	0.125 (n = 6)	0.250 (n = 6)	0.490 (n = 6)
With media (sweat solution +filter)	Mean (µg/mL)	0.014	0.040	0.118	0.235	0.473
	Standard deviation (µg/mL)	0.0003	0.0003	0.0036	0.0060	0.0024
	CV%	1.99	0.72	3.08	2.58	0.50
Without media	Mean (µg/mL)	0.015	0.044	0.127	0.268	0.565
	Standard deviation (µg/mL)	0.0018	0.0010	0.0081	0.0045	0.0146
	*CV%	11.67	2.35	6.41	1.67	2.59
	desorption coefficient %	92.86	90.4	93.08	87.52	83.82

**CV is coefficient of variation*

The desorption percentage found was around 90% for the 2,6-TDI derivative. This percentage decreased when spiking the standards with higher concentration of diisocyanate. That can be explained by a decreased capacity of the impregnated filter to derive spiked diisocyanate, probably due either to the limited quantity of the 1,2-MP on the filter or to an insufficient filter surface.

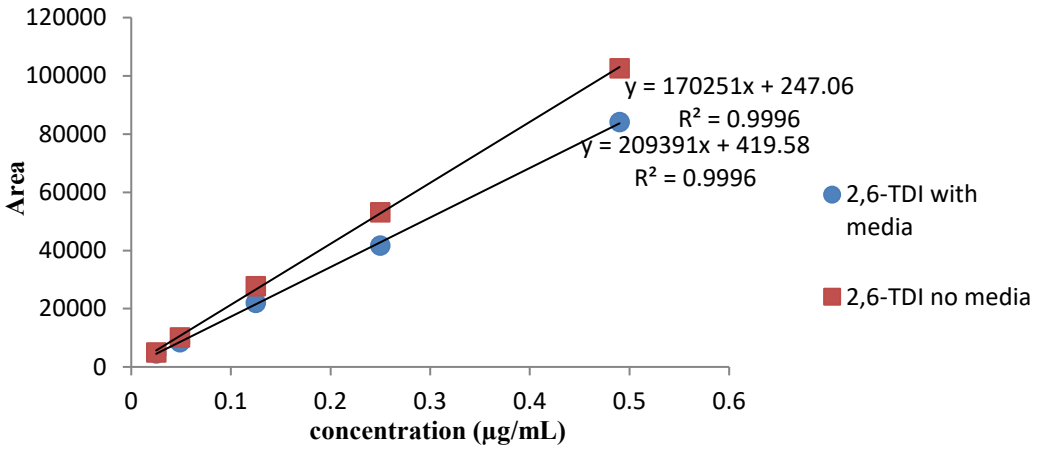


Figure 3.2 Calibration curve for the 2,6-TDI-1,2MP standards prepared by spiking the wetted impregnated filter (with media) or into a 1,2-MP solution (no media).

The calibration curves for standards prepared by derivatization using the coated filter wetted with sweat solution (with media) with those prepared by derivatization in the 1,2-MP solution (no media) are shown in Figures 3.2 to 3.4. As it can be seen, the curve prepared without any media solution (without filter and sweat solution) is more sensitive, having a higher slope. A similar tendency was noted with the other two diisocyanate derivatives. This can be explained by the fact that the sampling media interfered by decreasing the instrument signal.

Table 3.2 Analytical data of desorption test for the 2,4-TDI 1,2-MP derivative

2,4-TDI	Concentration (µg/mL)	0.025 (n = 6)	0.049 (n = 6)	0.125 (n = 6)	0.250 (n = 6)	0.490 (n = 6)
With media (sweat solution +filter)	Mean (µg/mL)	0.028	0.051	0.111	0.205	0.395
	Standard deviation (µg/mL)	0.0038	0.0080	0.0048	0.0101	0.0055
	CV%	*13.98*	*15.51*	*4.35*	*4.94*	*1.39*
Without media	Mean (µg/mL)	0.026	0.050	0.128	0.243	0.469
	Standard deviation (µg/mL)	0.0004	0.0009	0.0020	0.0019	0.0031
	*CV %	*1.33*	*1.79*	*1.61*	*0.79*	*0.66*
	desorption coefficient %	104.27	103.42	87.29	84.27	84.30

**CV is coefficient of variation*

As seen in the table above, the recovery concentration for the 2,4-TDI-1,2MP standard derivative prepared with a media solution is slightly higher than without any media solution at lower concentration. This unexpected result is explained by the presence of an interfering peak that is coeluting with 2,4-TDI-1,2MP. This peak is also present in the chromatogram of the blank filter.

The corrected concentration of the standards were obtained, by subtracting from each result, the average concentration of the 2,4-TDI-1,2MP obtained from the blank filters. The interfering peak affects mostly the results at low standard concentration. However, it does not affect significantly the recovery percentage.

In the same manner observed with 2,6-TDI-1,2MP derivative, the percentage of desorption coefficient decreased with a higher concentration of the spiking standard.

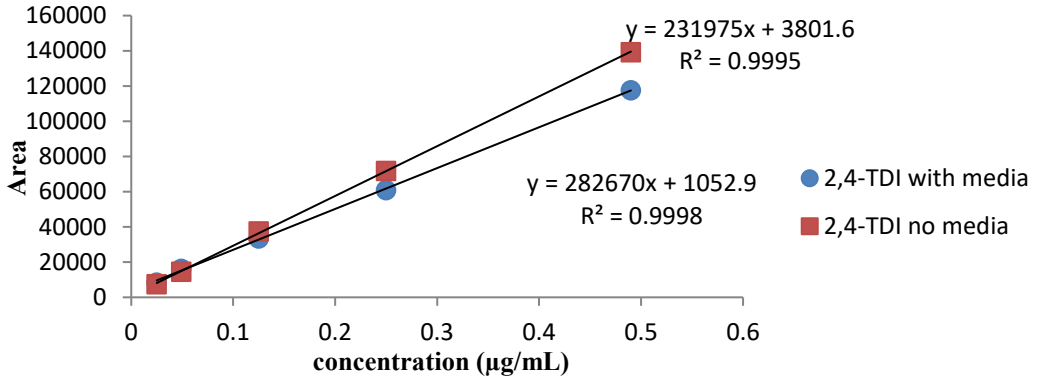


Figure 3.3 Calibration curve for the 2,4-TDI-1,2MP standards prepared by spiking the wetted impregnated filter (with media) or into a 1,2-MP solution (no media).

Similar tendency was observed for the MDI-1,2MP derivative and are shown below.

Table 3.3 Analytical data of desorption test for the MDI-1,2MP derivative.

MDI	Concentration (µg/mL)	0.039 (n = 6)	0.079 (n = 6)	0.197 (n = 6)	0.394 (n = 6)	0.788 (n = 6)
With media (sweat solution +filter)	Mean (µg/mL)	0.024	0.061	0.172	0.352	0.718
	Standard deviation (µg/mL)	0.0007	0.0006	0.0040	0.0079	0.0035
	CV%	*2.68*	*0.97*	*2.30*	*2.24*	*0.48*
Without media	Mean (µg/mL)	0.030	0.078	0.204	0.440	0.922
	Standard deviation (µg/mL)	0.0006	0.0006	0.0118	0.0040	0.0107
	*CV%	*2.08*	*0.74*	*5.78*	*0.91*	*1.16*
	Desorption coefficient %	82.21	78.29	84.18	79.98	77.87

**CV is coefficient of variation*

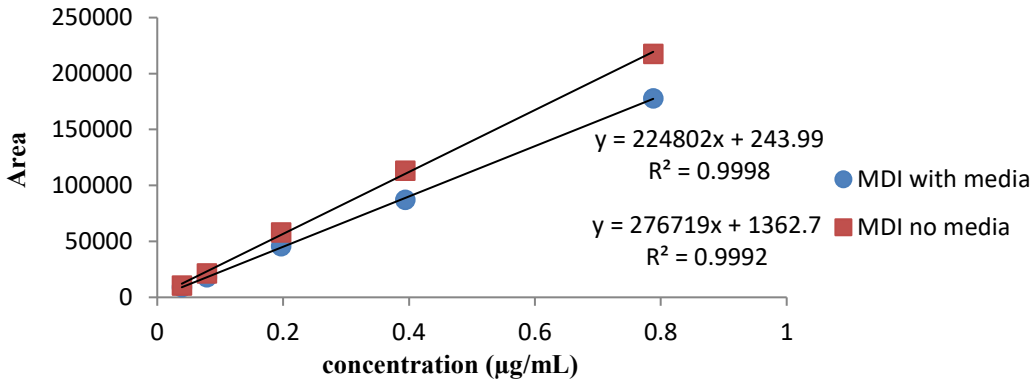


Figure 3.4 Calibration curve for the MDI-1,2MP standards prepared by spiking the wetted impregnated filter (with media) or into a 1,2-MP solution (no media).

The precision data obtained for the three derivatives are shown in the tables below.

Table 3.4 Precision data for the 2,6-TDI-1,2MP derivative.

2,6-TDI	Intra-day precision				Inter-day precision			
Concentration (µg/mL)	0.049 (n = 6)	0.125 (n = 6)	0.250 (n = 6)	0.490 (n = 6)	0.049 (n = 6)	0.125 (n = 6)	0.250 (n = 6)	0.490 (n = 6)
Mean (µg/mL)	0.036	0.095	0.195	0.396	0.043	0.102	0.206	0.419
Standard deviation (µg/mL)	0.0010	0.0006	0.0010	0.0062	0.0056	0.0072	0.0150	0.0282
**CV%*	2.88	0.65	0.51	1.56	13.10	7.06	7.31	6.74

**CV is coefficient of variation*

The percentage of coefficient of variation for precision for intra-day is lower than the one for inter-day. The analytical data vary more from one day to another when several parameters are changed (for example fresh solutions prepared and variation in instrument signal), adding more error to results. Nevertheless, the averaged

fluctuations were less than 10%. For all three diisocyanates the percentage of coefficient of variation is greater at lower concentration of standards.

Table 3.5 Precision data for the 2,4-TDI-1,2MP derivative.

2,4-TDI	Intra-day precision				Inter-day precision			
Concentration (µg/mL)	0.049 (n = 6)	0.125 (n = 6)	0.250 (n = 6)	0.490 (n = 6)	0.049 (n = 6)	0.125 (n = 6)	0.250 (n = 6)	0.490 (n = 6)
Mean (µg/mL)	0.032	0.097	0.205	0.418	0.041	0.099	0.202	0.401
Standard deviation (µg/mL)	0.0024	0.0031	0.0013	0.0124	0.0072	0.0097	0.0089	0.0198
**CV%*	7.52	3.24	0.61	2.97	17.45	9.84	4.42	4.95

**CV is coefficient of variation*

Table 3.6 Precision data for the MDI-1,2MP derivative.

MDI	Intra-day precision				Inter-day precision			
Concentration (µg/mL)	0.079 (n = 6)	0.197 (n = 6)	0.394 (n = 6)	0.788 (n = 6)	0.079 (n = 6)	0.197 (n = 6)	0.394 (n = 6)	0.788 (n = 6)
Mean (µg/mL)	0.059	0.154	0.317	0.626	0.067	0.160	0.322	0.646
Standard deviation (µg/mL)	0.0007	0.0010	0.0018	0.0104	0.0052	0.0058	0.0101	0.0157
**CV%*	1.24	0.65	0.58	1.64	7.82	3.61	3.15	2.44

**CV is coefficient of variation*

3.3 Sampling

The migration test cell was built using two filter supports, four threaded rods, eight hexagonal nuts and four wing nuts to fit the threaded rod used. The components and the assembled test cell are shown in the Figures 3.5 (a,b).

a) test cell components

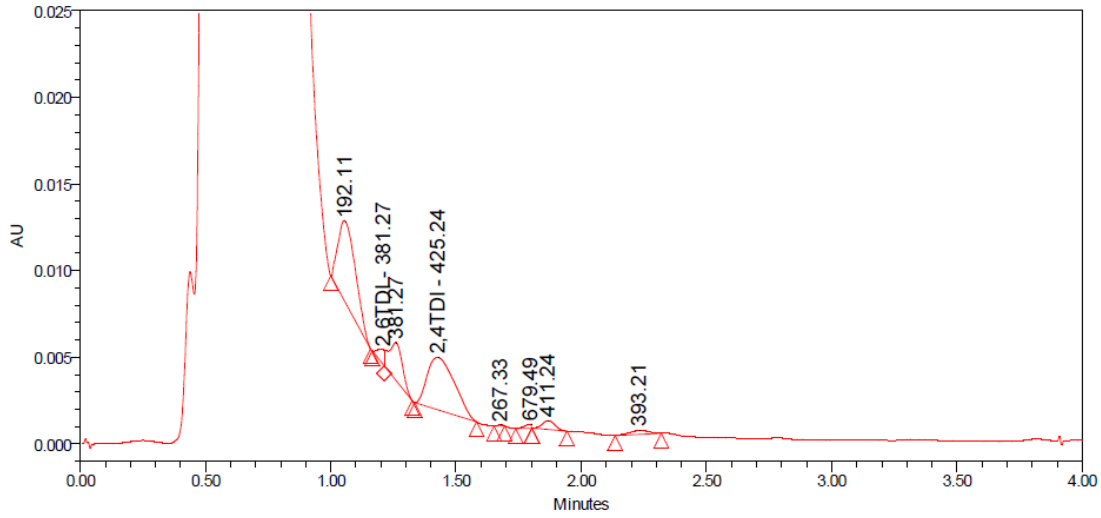
b) assembled cell

Figure 3.5 (a) Test cell components and (b) assembled test

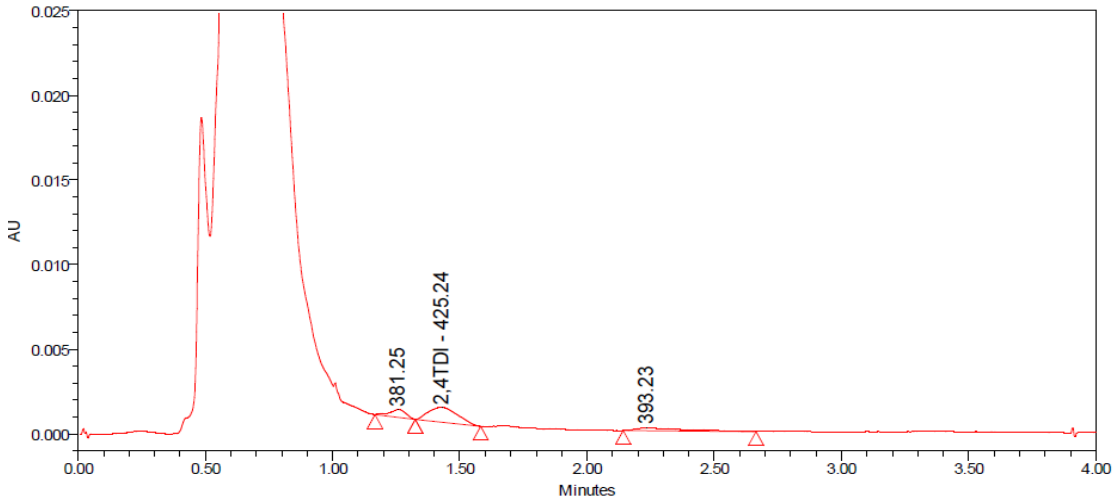
For the analysis of foam samples, all six sweat solutions were used in order to compare the migration test results. Based on the previous desorption tests results (Tables 3.1 to 3.3), two impregnated filters from each side of the sample were used to ensure proper derivatization at high concentrations of diisocyanates. It is believed that

the compression of the sample by 25% is enough to release a possible diisocyanate present in a sample, and that it will be able to migrate to one or both surfaces.

The chromatograms of the filters showed that the blank filter has many unknown interfering peaks that are increasing with air exposure time. Presumably, these unknown substances are side products generated from air oxidation of 1,2-MP. These interfering peaks were absent or are much smaller in the chromatograms of the samples (Figure 3.6 (a; b)).



a) filter blank wetted with synthetic sweat solution after 12 hrs exposed to ambient air.



b) filter wetted with synthetic sweat solution after 12 hrs contact with the foam sample

Fig. 3.6 PDA Timed Wavelength (0.00min, 242.0nm) (1.80min, 250.0nm) standard chromatogram of foam sample; (a) filter blank wetted with synthetic sweat solution after 12 hrs exposed to ambient air. and (b) filter wetted with synthetic sweat solution and after 12 hrs contact with the foam sample.

The obtained analytical data from validation tests reassure that any of three diisocyanate present at the surface of the PU foam at the LOD of the method can be detected. Fortunately, none of these diisocyanates was detected on the PU foam surface during the real samples evaluation.

CHAPTER IV

GENERAL CONCLUSION

Today, the large number of polyurethane applications make this material indispensable in particular as flexible foam, which is largely used in the production of upholstered furniture and mattresses. The fact that raw material for polymer production, MDI and TDI, can be trapped in the final product, might make it unsafe for customer uses. Indeed, low quantity of these diisocyanates can be extracted from a flexible foam sample. However, it was shown that the method of diisocyanate extraction from a foam does not provide the actual concentration of residual diisocyanate available for transfer at the surface. A different analysis method was needed for this purpose.

The objective of this study was to develop a method for quantification of MDI and TDI migration from a surface of flexible polyureathane foam sample according to EPA recommendation.

The approach used for this method was to simulate a situation in which an isocyanate, located on a foam surface, is able to migrate to the skin upon contact with such a product. Six different simulated sweat solutions were prepared and used to generate environmental conditions that were analogous to the real ones. Five of these synthetic sweat solution gave a recovery of approximately 80%. The sixth synthetic sweat solution gave a low level of recovery, around 30% and was thus discarded.

The migrated isocyanate was collected and stabilized using a GF filter coated with 1-(2-methoxyphenyl)piperazine and wetted with sweat solution, desorbed in 0.5% acetic anhydride in acetonitrile and analyzed with UV detector for quantification and MS detector for confirmation. The method was validated according to a standard validation protocol. Dynamic range of application of the method is from 0.025 µg/mL to 0.49 µg/mL (0.009 µg/cm^2-0.178 µg/cm^2 of foam surface) for TDI-1,2MP isomers derivatives and from 0.04 µg/mL to 0.80 µg/mL (0.015 µg/cm^2-0.291 µg/cm^2 of foam surface) for MDI-1,2MP derivative. The average desorption recoveries are 89.5±4.4% for 2,6-TDI-1,2MP derivative, 92.7±11.0% for 2,4-TDI-1,2MP derivative and 80.5±3.3% for MDI-1,2MP derivative. Limites of detection are 0.002 µg/mL (2,6-TDI-1,2MP), 0.009 µg/mL (2,4-TDI-1,2MP), 0.003 µg/mL (MDI-1,2MP), and limites of quantification are 0.006 µg/mL, 0.030 µg/mL and 0.010 µg/mL, respectfully. Intra-day precision is less than 4% for all three diisocyanate derivatives and inter-day precision is less than 10%.

Five flexible PU foam samples commonly used in consumer products, and with formulations anticipated to have the highest potential for residual TDI or MDI, were supplied by Polyurethane Foam Association and analyzed using the validated method. Diisocyanates were not detected on both surfaces of these matured PFA foam samples.

The developed method was proved to be robust and reliable. Unlike existing extraction methods from polyurethane with different solvents, this method represents a model that is more likely to reflect real life conditions. It makes it possible to calculate the concentration of residual diisocyanate available for surface transfer, representing a potential toxicity.

CHAPTER V

PERSPECTIVES

The method developed was showed to be reliable and robust during validation tests. However, the lack of detected disocyanates in the tested samples is due to concentration below the LOD. To be able to detect possibly present unreacted diisocyanates on a surface of a flexible foam sample, the analytical method can be used just at the end of the polyurethane production line before these blocks are taken away to be stored for a maturation time. The use of this method immediately after the polyurethane production will allow to evaluate the possibility of leakage of isocyanates from PUs samples right after their production. This procedure can be included to a list of tests that should be performed in order to evaluate the quality and safety of a product.

The method can also be used for any other chemical of interest. For example, the sample can be taken from a cutting a piece of foam from a couch. This sample with a layer of fabric can be examined in a similar manner for the substances that can migrate from a fabric. For such needs, the method has to be optimized for that specific chemical. For example, the method can be used to evaluate the possible migration of MDA from the polyurethane foam sample made from MDI. In this case filters can be coated with acid to be able to recover the MDA and the desorption solution has to be also modified.

Although for this study objective the cell was compressed by 25% of it's height, other modes of compression (such as rubbing or placing the filter inside the sample) can be tried to capture the possible release of unreacted diisocyanate. Furthermore, it would be interesting to expose the cell with a foam sample to UV light or to high temperature and to evaluate if there is any release of diisocyanate due to foam degradation.

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