Universitat de Girona

DEVELOPMENT OF NEW SOLVENT-FREE MICROEXTRACTION TECHNIQUES FOR THE ANALYSIS OF VOLATILE ORGANIC COMPOUNDS: APPLICATION TO THE USE OF BREATH ANALYSIS AS A TOXICOLOGICAL TOOL FOR EXPOSURE ANALYSIS

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DOCTORAL THESIS

Development of new solvent-free microextraction techniques for the analysis of volatile organic compounds: application to the use of breath analysis as a toxicological tool for exposure analysis

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El Doctor Juan Manuel Sánchez Navarro, professor titular del Departament de Química de la Universitat de Girona,

CERTIFICO:

Que aquest treball, titulat **Development of new solvent-free microextraction techniques for the analysis of volatile organic compounds: application to the use of breath analysis as a toxicological tool for exposure analysis,** que presenta la doctorand Mònica Alonso Roura, ha estat realitzat sota la meva direcció i que compleix els requisits per poder optar a Menció Internacional.

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1. Alonso, M.; Castellanos, M.; Martín, J.; Sánchez, J.M. **"Capillary thermal desorption unit for near real-time analysis of VOCS at sub-trace levels. Application to the analysis of environmental air contamination and breath samples"**. *Journal of Chromatography B*, 877 (**2009**), 1472-1478.

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2. Alonso, M.; Castellanos, M.; Sánchez, J.M. **"Evaluation of potential breath biomarkers for active smoking: assessment of smoking habits"**. *Analytical and Bioanalytical Chemistry*, 396 (**2010**), 2987-2995.

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3. Alonso, M.; Godayol, A.; Anticó, E.; Sánchez, J.M. **"Assessment of environmental tobacco smoke contamination in public premises: significance of 2,5-dimethylfuran as an effective marker"**. *Environmental Science and Technology*, 44 (**2010**), 8289-8294.

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4. Alonso, M.; Godayol, A.; Anticó, E.; Sánchez, J.M. **"Needle microextration trap for on-site analysis of airborne volatile compounds at ultra-trace levels in gaseous samples"**. *Journal of Separation Science*, 34 (**2011**), 2705-2711.

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   Field, rank: Chemistry, Analytical; position 25 of 73 (2nd quartile)
   Times cited: 2 (29/09/2012)

5. Alonso, M.; Cerdán, L.; Godayol, A.; Anticó, E.; Sánchez, J.M. **"Headspace neddle-trap analysis of priority volatile organic compounds from aqueous samples: application to the analysis of natural and waste waters"**. *Journal of Chromatography A*, 1218 (**2011**), 8131-8139.

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   Times cited: 1 (29/09/2012)

6. Alonso, M.; Castellanos, M.; Besalú, E.; Sánchez, J.M. **"A headspace needle-trap method for the analysis of volatile organic compounds in whole blood"**. *Journal of Chromatography A*, 1252 (2012), 23-30.

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7. Alonso, M.; Castellanos, M.; Sánchez, J.M. **"Evaluation and comparison of solvent-free concentration techniques for the analysis of volatile organic compounds in whole blood at sub-trace levels"**. *Analyst*, submitted

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LIST OF ACRONYMS

A/D: analogical/digital

AOAC: association of official analytical chemists

API: atmospheric pressure ionization

BEL: biological exposure limits

BTEX: benzene, toluene, ethylbenzene, and xylene isomers

CHS-NTD: cycling headspace-needle trap device

CMS: carbon molecular sieve

CW: carbowax

CW/DVB: carbowax/divinylbenzene

DVB/CAR/PDMS: divinylbenzene/Carboxen/polydimethylsiloxane

EBC: exhaled breath condensate

EBV: exhaled breath vapour

EPA: environmental protection agency

ETS: environmental tobacco smoke

GC: gas chromatography

GC/MS: gas chromatography/ mass spectrometry

GC-FID: gas chromatography-flame ionization detector

GCXGC: comprehensive two dimensional gas chromatography

HS: headspace

HS-NT: headspace-needle trap

HS-NTD: headspace- needle trap device

I/O: inner/outer ratio

ID: internal diameter

IMS: ion mobility spectrometry

IS: internal standard

ITEX: in tube extraction

LCD: lowest concentration detected

LOD: limit of detection

LOQ: limit of quantification

MESI: membrane extraction with sorbent interface

MS: mass spectrometry

NIOSH: national institute for occupational safety and health

NT: needle trap

NTD: needle trap device

OD: outer diameter

PA: polyacrylate

PDMS: polydimethylsiloxane

PDMS/DVB: polydimethylsiloxane/divinylbenzene

pHS-NTD: passive head space- needle trap device

PT: purge and trap

PT-GC: purge and trap-gas chromatography

PT-NTD: purge and trap- needle trap device

PTR: proton transfer reaction

RH: relative humidity

RSD: relative standard deviation

SD: standard deviation

SIFT: selected ion flow tube

SPDE: solid phase direct extraction

SPME: solid phase microextraction

TD: thermal desorption

THM: trihalomethane

TLV: threshold limit values

TOF-MS: time-of-flight- mass spectrometry

VOC: volatile organic compound

WHO: world health organization

WWTP: waste water treatment plant

INDEX

I. Summary/ resum/ resumen

I. SUMMARY

The main objective of this thesis is the development of different analytical methodologies based on the use of micro-traps for the determination of volatile organic compounds (VOCs) at $ng \cdot m^{-3}$ levels. Firstly, it has been developed a micro-trap coupled to a GC/MS as a substitute of the original injection port of the GC. This system allows the analysis of VOCs in gaseous samples in a fast and easy way. Using this methodology, collection of the samples in specific gas sampling devices is required: Tedlar sampling bags were used in the studies performed. The sample is passed through the trap to retain target compounds with the sorbent materials. The retained compounds are thereafter desorbed using a fast heating pulse and introduced into the chromatographic column by a helium flow. This methodology allows the analysis of large sample volumes but it has the drawback of not being field portable, which leads to the possible formation of artifacts due to sampling contamination or loss of compounds for volatilization, diffusion, adsorption, etc. in the storage containers. In order to obtain a field portable system for on-site analysis, different needle trap devices (NTDs) have been developed. These NTDs allow on-site sampling and preconcentration of the compounds of interest in the trap in a single step, which reduces significantly the possibility of sampling contamination and loss of compounds.

Both developed methodologies have been applied in various studies devoted to assess exposure contamination to VOCs, which have been mainly focused in environmental tobacco smoke (ETS) contamination. The first system developed has been used for the analysis of breath samples from smokers and non-smokers to find a biomarker related to tobacco consumption. After an exhaustive study over more than 200 volunteers, it has been demonstrated that 2,5-dimethylfuran is a selective biomarker for the smoking habit, allowing to identify a smoking individual after more than 48 hours without smoking. A second study focused on ETS contamination in public premises was developed. 2,5-dimethylfuran has been detected in the air of public premises where smoking was allowed. For the first time, it has been demonstrated that a tobacco smoke biomarker can be detected in the breath of passive smokers working in premises where smoking was allowed, only after few hours of exposure, which confirms that contamination of passive smokers takes place fast. The development of NTDs have demonstrated that this methodology is also able to analyze 2,5-dimethylfuran at the

levels required to achieve the results indicated previously. Atmospheric samples from indoor and outdoor environments have also been analyzed. It has been demonstrated that the needle traps have a high sensitivity, which significantly reduces the sample volume required to reach detections limits in the $ng \cdot m^{-3}$ range.

It is necessary to demonstrate that the variations observed in breath samples are correlated with the presence of the compounds in conventional biological fluids (such blood and urine) before confirming that breath analysis can be used in exposure or toxicological studies. For this reason, the behavior of needle traps for the analysis of liquid samples has been evaluated, mainly for blood samples. For the analysis of liquid samples is necessary to couple the needle trap with the head-space technique (HS-NTD). The liquid sample is placed into the sealed vial and the head-space is analyzed using the needle trap to concentrate the analytes before the chromatographic analysis. Four different methodologies were evaluated for head-space analysis:

a. Static HS, a volume of the gaseous phase is collected with the needle trap (HS-NTD). After 50 min of equilibration at 50ºC, 4 mL of the headspace are collected at 2 $mL \cdot min^{-1}$.
b. Static HS with passive diffusion of the gaseous phase through the needle trap (pHS-NTD). Needle trap is placed in the vial during all the equilibration time. Under these conditions there is a passive diffusion of the volatile compounds through the trap due the over-pressure generated inside the vial for the increase of the temperature.
c. Active HS with the needle trap (PT-NTD). The needle trap is placed in the vial during equilibration and a purge of nitrogen of 6 $mL \cdot min^{-1}$ is used to remove volatile compounds from the aqueous to the gaseous phase.
d. Dynamic HS, using several sampling cycles (cHS-NTD). The needle trap is placed in the vial and a fixed volume of the gas phase is sampled using several sampling cycles. In each cycle 1 mL of the gaseous phase passes trough the trap and it is returned again to the vial to maintain the same pressure conditions inside the vial.

The best results considering sensitivity are obtained with the pHS-NTD methodology. However, it presents an important drawback as it requires a complex instrumentation.

Considering both, simplicity and sensitivity, best results are obtained with the HS-NTD and cHS-NTD sampling methodologies.

In a preliminary study, the simplest methodology has been used to assess the applicability of the technique for the analysis of natural and waste waters. The results obtained show that, despite the exhaustive nature of the needle traps, it is possible to achieve detection limits in the $ng \cdot L^{-1}$ range using small volumes of sample.

In the analysis of blood samples, the results obtained were compared with those obtained analyzing the same samples using the conventional SPME technique. It has been observed that needle traps and SPME are equivalent in sensitivity, but needle traps are more robust and appropriate for on-site analysis. Using small sample volumes (0.5 mL of blood) is possible to determine the presence of volatile organic compounds in the levels that appear in a non-exposed person, which suggests that this methodology is appropriate for the evaluation of contaminants in exposure analysis.

II RESUM

L'objectiu principal d'aquesta tesi ha estat el desenvolupament de diferents metodologies d'anàlisi basades en l'ús de micro-trampes d'adsorció per a la determinació de compostos orgànics volàtils a nivells de ng.m^{-3}. Inicialment es va dissenyar una microtrampa acoblada a un GC/MS en substitució de l'injector convencional del GC. Aquest sistema permet analitzar compostos volàtils en mostres gasoses de forma ràpida i senzilla. Amb aquesta metodologia, cal recollir les mostres en un recipient per a gasos, en aquest s'utilitzen bosses de mostreig de gasos Tedlar. Posteriorment, es fa passar la mostra per la microtrampa per retenir els compostos d'interès al llit d'adsorbents. Finalment, es desorbeixen els analits amb un pols ràpid de temperatura i són introduïts cap a la columna cromatogràfica amb l'ajut d'un flux d'Heli. Aquesta metodologia presenta l'avantatge que permet analitzar volums grans de mostra, però té l'inconvenient que no es portàtil i es requereix un recipient de mostreig addicional per recollir la mostra, el que pot donar lloc a la possible formació d'artefactes per problemes de contaminació o pèrdua de components de la mostra per volatilització, difusió, adsorció, etc. Amb la finalitat d'obtenir un sistema portàtil que permeti mostrejar directament *on-site* i no requereixi de recipients de mostreig addicionals, s'ha treballat en el disseny de microtrampes d'agulla. Aquestes trampes permeten mostrejar i preconcentrar a la mateixa etapa els compostos volàtils d'interès, amb el que es minimitza l'aparició d'artefactes per possibles contaminacions o pèrdues.

Els dos sistemes desenvolupats s'han aplicat a l'estudi de contaminació per exposició a compostos orgànics volàtils, focalitzant-se principalment en la contaminació pel fum ambiental del tabac. S'ha utilitzat el primer sistema per a l'anàlisi de mostres d'alè de fumadors i no fumadors amb la finalitat de trobar un biomarcador del consum de tabac. Després d'un estudi exhaustiu en una població superior a 200 persones s'ha determinat que el 2,5-dimetilfuran és un biomarcador selectiu de l'hàbit fumador d'una persona, permetent diferenciar l'estatus fumador d'una persona inclús després de més de 48 hores d'haver fumat una cigarreta. Un segon estudi s'ha adreçat a l'avaluació de la contaminació de locals pel fum ambiental del tabac. S'ha comprovat que gràcies a l'elevada sensibilitat de les microtrampes es pot detectar el 2,5-dimetilfuran en l'aire de locals on es permet fumar fins i tot quan el consum de tabac ha estat escàs. Per primera vegada s'ha pogut demostrar que un biomarcador del fum del tabac apareix en l'alè de

fumadors passius presents en espais tancats on es fuma després de poques hores de contacte, confirmant que la contaminació dels fumadors passius té lloc de forma ràpida. Estudis portats a terme amb les trampes d'agulla han permès corroborar que aquesta metodologia també permet analitzar el 2,5-dimetilfuran als nivells que es requereixen per assolir els resultats indicats anteriorment. S'han analitzat satisfactòriament mostres atmosfèriques de diferents ambients interiors i exteriors amb l'avantatge que l'elevada sensibilitat de les trampes d'agulla redueix de forma significativa el volum de mostra que es requereix per assolir límits de detecció en el rang dels $ng \cdot m^{-3}$.

Per poder corroborar la utilitat de l'anàlisi d'alè com a tècnica adequada per a mesures d'exposició a contaminants es necessari demostrar que les variacions detectades a l'alè es correlacionen amb la presència d'aquests compostos en fluids biològics convencionals en estudis d'exposició o toxicològics (com son la sang o l'orina). S'ha estudiat el comportament de les trampes d'agulla per a la determinació de compostos orgànics volàtils en mostres aquoses, focalitzant-se majoritàriament en mostres de sang. Per analitzar mostres aquoses cal combinar l'ús de les trampes amb la tècnica de l'espai de cap (HS-NTD). La mostra líquida es situa a l'interior d'un vial segellat i s'analitza l'espai de cap amb la trampa d'agulla per tal de preconcentrar els analits abans de la seva anàlisi cromatogràfica. S'han avaluat quatre metodologies diferents per al mostreig de l'espai de cap:

a. HS estàtic, recollint un volum de la fase gas amb la trampa d'agulla (HS-NTD). Després de 50 min d'equilibració a 50ºC es mostregen 4 mL de l'espai de cap a 2 $mL \cdot min^{-1}$.
b. HS estàtic, amb difusió passiva de la fase gas per la trampa d'agulla (pHS-NTD). Es deixa la trampa d'agulla connectada al vial durant tot el temps d'equilibració, de manera que té lloc una difusió passiva dels compostos volatilitzats per la trampa degut a la sobrepressió que es va generant dins el vial per l'increment de la temperatura.
c. HS actiu amb la trampa d'agulla (PT-NTD). Es deixa la trampa d'agulla dins el vial i es fa passar una purga de nitrogen a 6 $mL \cdot min^{-1}$ per afavorir el transport dels compostos de la fase líquida a la fase gas.
d. HS dinàmic, utilitzant varis cicles de mostreig (cHS-NTD). Es col·loca la trampa d'agulla al vial i es mostreja un volum fix aplicant varis cicles de mostreig. A cada

cicle es fa passar per la trampa 1 mL de la fase gas i es retorna posteriorment el volum de gas extret cap al vial per mantenir les condicions de pressió.

Els millors resultats des del punt de vista de sensibilitat s'obtenen amb la metodologia pHS-NTD, però presenta el desavantatge que es requereix una instrumentació complexa i és més difícil de portar a terme. Tenint en compte la combinació entre simplicitat instrumental i sensibilitat, els millors resultats s'obtenen amb les tècniques de HS-NTD i cHS-NTD.

Inicialment s'ha utilitzat la metodologia més simple (HS-NTD) per avaluar la utilitat de la tècnica mitjançant l'anàlisi d'aigües de depuradora i naturals. Els resultats obtinguts mostren que, tot i el comportament exhaustiu de les trampes d'adsorbents, es poden assolir límits de detecció en el rang dels $ng \cdot L^{-1}$ utilitzant volums petits de mostra amb aquesta metodologia.

En el cas de mostres de sang, els resultats han estat contrastats analitzant les mateixes mostres amb una tècnica convencional, com és la SPME. S'ha comprovat que les trampes d'agulla ofereixen la mateixa sensibilitat que la tècnica de SPME, però son més robustes i més adequades quan el mostreig s'ha de fer *on-site*. Utilitzant volums petits de sang (0.5 mL) ha estat possible determinar la presència de compostos volàtils als nivells en que apareixen en persones no exposades en aquests contaminants, el que fa possible la seva determinació en processos de exposició a contaminants per poder estudiar el seu comportament.

III RESUMEN

El objetivo principal de esta tesis es el desarrollo de diferentes metodologías de análisis basadas en el uso de micro-trampas de adsorción para la determinación de compuestos orgánicos volátiles a niveles de $ng.m^{-3}$. Inicialmente se ha diseñado una microtrampa acoplada a un GC/MS en sustitución del inyector convencional del GC. Este sistema permite analizar compuestos volátiles en muestras gaseosas de forma rápida y sencilla. Con esta metodología es necesario recoger inicialmente las muestras en un recipiente para gases, en este caso se utilizan bolsas de muestreo de gases Tedlar. Posteriormente se hace pasar la muestra por la microtrampa para retener los compuestos de interés en el lecho de adsorbentes. Finalmente, se desorben los analitos retenidos con un pulso rápido de temperatura y son introducidos en la columna cromatográfica con la ayuda de un flujo de helio. Esta metodología permite analizar volúmenes grandes de muestra pero presenta el inconveniente de no ser portátil, por lo que se requiere el uso de recipientes de muestreo adicionales para recoger la muestra, lo que da lugar a la posible aparición de artefactos debidos a problemas de contaminación o pérdida de componentes de la muestra por volatilización, difusión, adsorción, etc. Con la finalidad de obtener un sistema portátil que permita el muestreo directo *on-site* y sin recipientes de muestreo adicionales, se ha trabajado en el diseño de microtrampas de aguja. Estas trampas permiten muestrear y preconcentar en la misma etapa los compuestos volátiles de interés, con lo que se minimizan la aparición de artefactos por posibles contaminaciones o pérdidas.

Los dos sistemas desarrollados se han aplicado al estudio de contaminación por exposición a compuestos orgánicos volátiles, focalizándose principalmente en la contaminación por el humo ambiental del tabaco. Se ha utilizado el primer sistema para el análisis de muestras de aliento de fumadores y no fumadores, con la finalidad de encontrar un biomarcador del consumo de tabaco. Después de un estudio exhaustivo en una población superior a 200 personas se ha determinado que el 2,5-dimetilfurano es un biomarcador selectivo para el hábito fumador de una persona, permitiendo diferenciar el estatus fumador de una persona incluso después de más de 48 horas sin fumar un cigarrillo. Se ha llevado a cabo un segundo estudio para evaluar la contaminación de locales públicos por el humo ambiental del tabaco. Se ha comprobado que gracias a la elevada sensibilidad de las microtrampas se puede detectar 2,5-dimetilfurano en el aire

de locales donde está permitido fumar aún cuando el consumo de tabaco ha sido escaso. Por primera vez se ha podido demostrar "in-situ" que un biomarcador del humo del tabaco aparece en el aliento de fumadores pasivos presentes en espacios cerrados donde se permite fumar después de pocas horas de contacto, confirmando que la contaminación de los fumadores pasivos tiene lugar de forma rápida. Estudios llevados a cabo con las microtrampas de aguja han permitido corroborar que esta nueva metodología también permite analizar el 2,5-dimetilfurano a los niveles que se requieren para alcanzar los resultados obtenidos con el sistema inicial. Se han analizado satisfactoriamente muestras ambientales de diferentes ambientes interiores y exteriores con la ventaja que la elevada sensibilidad de las trampas de aguja reduce de forma significativa el volumen de muestra que se requiere para alcanzar límites de detección en el rango de los $ng \cdot m^{-3}$.

Para poder corroborar la utilidad del análisis del aliento como técnica adecuada para medir los niveles de exposición a contaminantes es necesario demostrar que las variaciones detectadas en el aliento se correlacionan con la presencia de estos compuestos en fluidos biológicos convencionales en estudios de exposición o toxicológicos (como son la sangre y la orina). Se ha estudiado el comportamiento de las trampas de aguja para la determinación de compuestos orgánicos volátiles en muestras acuosas, focalizándose principalmente en muestras de sangre. Para el análisis de muestras acuosas es necesario combinar el uso de las trampas con la técnica de espacio de cabeza (HS-NTD). La muestra líquida se sitúa en el interior de un vial cerrado y se analiza el espacio de cabeza con la trampa de aguja para concentrar los analitos antes de su análisis cromatográfica. Se han evaluado cuatro metodologías diferentes para el muestreo del espacio de cabeza:

a. HS estático, recogiendo un volumen de la fase gas con la trampa de aguja (HS-NTD). Después de 50 min de equilibración a 50ºC se muestrean 4 mL del espacio de cabeza a un flujo de 2 $mL \cdot min^{-1}$.
b. HS estático, con difusión pasiva de la fase gas a través de la trampa (pHS-NTD). Se deja la trampa de aguja conectada al vial durante todo el tiempo de equilibración, de este modo tiene lugar una difusión pasiva de los compuestos volátiles a través de la trampa debido a la sobrepresión que se va generando en el interior del vial por el aumento de la temperatura.

c. HS activo con la trampa de aguja (PT-NTD). Se deja la trampa de aguja dentro del vial y se hace pasar una purga de nitrógeno a 6 $mL \cdot min^{-1}$ para favorecer el transporte de los compuestos de la fase líquida a la fase gas.
d. HS dinámico, utilizando varios ciclos de muestreo (cHS-NTD). Se coloca la trampa de aguja en el vial y se muestrea un volumen fijo aplicando varios ciclos de muestreo. En cada ciclo se hace pasar por la trampa 1 mL de la fase gas y se retorna posteriormente el volumen de gas extraído hacia el vial para mantener las condiciones de presión.

Los mejores resultados, des de el punto de vista de sensibilidad, se obtienen con la metodología pHS-NTD, pero presenta el inconveniente que requiere una instrumentación compleja. Considerando la combinación entre simplicidad instrumental y sensibilidad, los resultados más satisfactorios se obtienen con las técnicas de HS-NTD y cHS-NTD.

En un primer estudio aplicado, se ha utilizado la metodología más simple (HS-NTD) para evaluar la utilidad de la técnica mediante el análisis de aguas de depuradora y naturales. Los resultados obtenidos muestran que, a pesar del carácter exhaustivo de las trampas de adsorbentes, se pueden conseguir límites de detección en el rango de los $ng \cdot L^{-1}$ utilizando volúmenes de muestra pequeños.

En el análisis de muestras de sangre, los resultados han sido contrastados analizando las mismas muestras con una técnica convencional (SPME). Se ha comprobado que las trampas de aguja ofrecen la misma sensibilidad que la técnica de SPME, pero son más robustas y adecuadas cuando el muestreo tiene que ser *on-site*. Utilizando volúmenes pequeños de sangre (0.5 mL) ha sido posible determinar la presencia de compuestos volátiles a los niveles que aparecen en personas no expuestas a contaminantes, lo que hace posible su determinación en procesos de exposición para poder estudiar su comportamiento.

1. General Introduction

In the ancient Greece physicians already knew that the specific odor of exhaled breath could be associated with certain diseases. For example, diabetes was identified as having a fruity smell, which we now know to be due to the presence of high levels of acetone; liver dysfunction could be identified as having a fishy and damp smell; urine-like odor was related to kidney problems; and a putrefactive smell was associated with lung infection and inflammation. In the eighteenth century, Lavoisier was the first to analyze breath and demonstrate that it contains carbon dioxide. In the mid-19th century, the introduction of colorimetric analysis helped to detect a limited number of volatile compounds in the breath (e.g., high levels of acetone in patients suffering from diabetes mellitus, and an ethanol breath test to demonstrate that consumed alcohol is largely metabolized by the body) [1]. However, despite this knowledge, breath analysis has had few practical applications to date. The main problem has been that the breath tests described were only able to detect either a previously consumed volatile organic compound (VOC), such as ethanol, or the metabolites of a precursor, such as acetone, at relatively large concentrations.

Interest in the analysis of VOCs in breath has increased significantly since the early 1970s, when Pauling et al. [2] reported a gas chromatography method for the analysis of breath samples and manage to detect more than 200 VOCs in the breath of healthy volunteers. However, the lack of resolution power of the chromatography instrumentation at that time made it very difficult to separate and identify the large amount of compounds present in exhaled breath. Improvements in sampling and instrumental techniques over the last decades are helping to overcome these problems. Since the 1990s researchers have begun to take a more systematic and analytical approach to this problem [3].

Breath analysis has the great advantage of being a non-invasive technique when monitoring the physiology of a person or exposure to toxic substances or environmental pollutants. Compared to blood or urine analysis, breath analysis is easier to perform and repeat, sampling is less likely to be perceived as unpleasant, and result interpretation is simpler as breath is a less complex matrix. Moreover, biomarkers present in breath can be detected faster than using blood and urine analysis, potentially permitting a quicker reaction against a specific problem.

The potential of breath analysis is based on the fact that gaseous compounds can exchange rapidly between the alveolar air and the blood stream in the blood-gas interface of the alveolar-capillary membranes. This rapid exchange indicates that an appropriate determination of VOCs in breath samples can be used in two important fields: (i) clinical diagnosis to analyze volatile compounds generated in the organism and eliminated through exhaled breath (endogenous compounds) and (ii) exposure analysis in order to have a fast and accurate knowledge of the levels of inhaled VOCs that can reach the blood stream and may produce harmful effects (exogenous compounds). Clinical diagnosis has received the greatest interest due to its potential to detect a disease state in a simple and non-invasive manner. This application has already been extensively reviewed [4-15] and is beyond the scope of this review.

Exposure assessment is of great interest in the determination of toxic substances in indoor environments as people spend more than 70-80% of their time indoors in western populations [16,17] and can be exposed to a range of indoor pollutants that may have adverse effects on health. The exposure to VOCs at large ($mg \cdot m^{-3}$) and medium (hundreds of $\mu g \cdot m^{-3}$) levels can result in both acute and chronic health effects [18]. Although there is no evidence of a health risk at the low levels (units of $\mu g \cdot m^{-3}$) normally detected in homes, some VOCs (e.g. benzene) are well established carcinogens or genotoxins, for which safe levels cannot be defined, or may be allergenics, due to poorly understood mechanisms, and so may have adverse effects on human health [19]. Exhaled breath has been analyzed to determine personal exposure to solvents and other VOCs [11,20-41]. A significant correlation has been found between the levels of certain VOCs and exposure to these substances [33,34,35].

Different biological exposure limits (BEL) have been proposed as the maximum recommended exposure levels for specific VOCs before they may be toxic for human health [29,42,43]. However, most information on VOC toxicity is based on exposure in industrial environments that typically have high levels of pollutants or has been established from animal and controlled studies with high concentrations. Levels in most indoor environments are well below the exposure limits required to demonstrate measurable health impacts [18,44]. If we also take into account the fact that there have been few epidemiological studies in these conditions, there is insufficient data to elucidate the possible relationship between VOC exposure in non-industrial

environments and their effect on human health [45,46], even when contact is repeated and prolonged [29].

The concept of contamination studies changed at the end of the twentieth century when "receptor-oriented" approaches began to substitute conventional "source-oriented" ones (i.e., analysis of contaminants in some obvious and generally highly contaminated sources) [30,47]. Receptor-oriented contamination studies require the appropriate measurement of pollutant concentrations at the contact boundary with the person. This approach also requires the evaluation of the portion of those substances that may affect human health. Although some important evidence of association with health problems has been found, most studies devoted to air contamination in non-industrial environments present limitations [48,49]: (i) there is a lack of detailed and systematic exposure measurement resulting in poor exposure data and (ii) many studies have been observational.

One of the main problems associated with the analysis of exposure in non-industrial environments is the low concentration of contaminants. Problems in quantifying indoor exposure also arise because many advanced technologies developed for measuring outdoor pollution are not suitable for indoor use due to cost, size and the amount of air they displace [50]. Analytical methodologies that can reach detection limits < 1 $\mu g \cdot m^{-3}$ are required as concentrations of most VOCs studied in these conditions are < 5 $\mu g \cdot m^{-3}$ [18,30,44,51]. In the case of breath samples, detection limits are normally in the range of $ng \cdot m^{-3}$. Moreover, the volume of breath samples is more limited than air samples. More sensitive methods are therefore needed to achieve appropriate detection limits, which allow target compounds to be detected at the levels at which they are expected to be found. Breath analysis can be a powerful tool in exposure analysis once appropriate procedures have been developed. Epidemiological studies are also required to confirm the correlation between VOC exposure and the entrance of these contaminants into the blood stream.

1.1. Composition of exhaled breath

It is necessary to distinguish between two different types of samples when we refer to exhaled breath: (i) exhaled breath vapor (EBV) and (ii) exhaled breath condensate

(EBC). EBV is only formed by volatile compounds. The main fraction (> 99%) is composed of a mixture of nitrogen, oxygen, carbon dioxide, water vapor, and inert gases. The remaining fraction (< 100 $mg.m^{-3}$) is formed by a mixture of hundreds of VOCs in a wide range of concentrations (ranging from few $mg \cdot m^{-3}$ to $ng \cdot m^{-3}$) [11,13].

EBC is more complex as it is a mixture of the breath expired from the lungs and the aerosolized droplets emerging with the breath. EBC contains both volatiles and non-volatiles and these must be recognized as separate entities with different properties [52]. However, it has been found that conventional EBC collection methods (e.g., Rtube at -80ºC) yield significantly lower sensitivity in the analysis of VOCs than specific EBV concentration methodologies, such as solid-phase microextraction [53]. All non-volatile compounds originating in the airway lining fluid or reaction products of metabolic processes in the gas phase are condensed in the EBC [54]. This non-volatile fraction contains inorganic compounds such as NO, insoluble substances, products of metabolic processes such as proteins, and condensed water vapor, which represents more than 99% of the collected fluid. When VOCs are the analytes of interest, EBV sampling is preferred.

Qualitative composition of EBV varies considerably from person to person and depends on the metabolism of each individual (endogenous compounds) and also on the environment around each person (exogenous compounds). The main VOCs present in a healthy person's breath are acetone, isoprene, methanol, and ethanol, which are produced in core metabolic processes [3]. All other VOCs are present at very low levels, from a few $\mu g \cdot m^{-3}$ to $ng \cdot m^{-3}$. Analysis of VOCs with the current analytical techniques is complicated by the complexity of breath samples themselves: the low concentration at which target VOCs are expected, the large variability in the concentrations of those compounds [21], and the limited volume of the samples.

1.2. Sampling procedures for EBV

As indicated in previous sections, one of the main problems when dealing with breath analysis is the limited volume of sample that can be obtained. Moreover, breath needs to be collected under careful conditions that include monitoring of the breathing [55]. The

average total lung capacity of an adult human male is about 6 liters of air, but only a small amount of this capacity is used during normal breathing. In each expiration, almost 500 mL of breath is expired [11,25]. The first portion (about one third of the total volume of exhaled air [25,56]) is "dead space air", which comes from the mouth, trachea and bronchi and so does not involve a gaseous exchange between air and blood. The remaining fraction is "alveolar air", which comes from the lungs and so does include a gaseous exchange between air and blood. Exhaled breath is a mixture of both dead space and alveolar air (Figure 1).

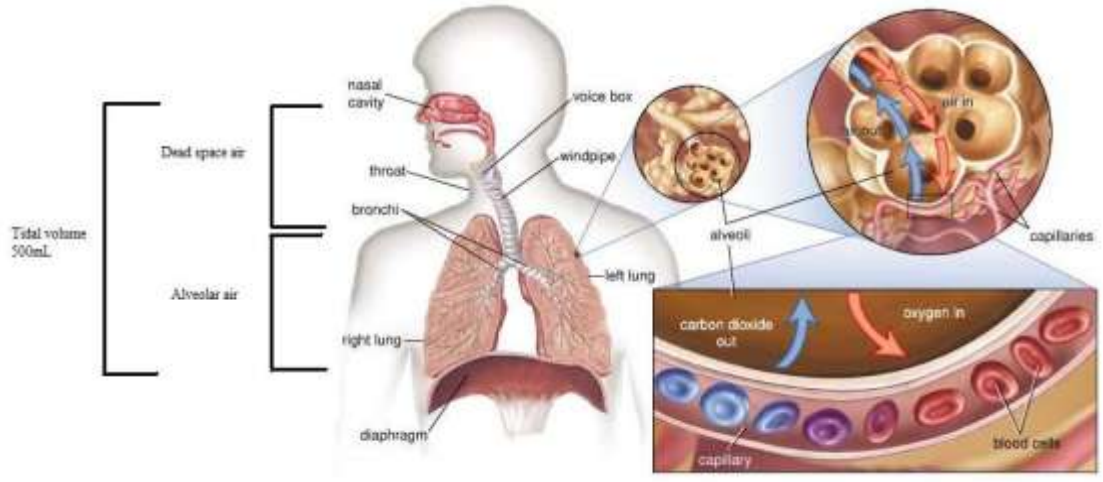

Figure 1. Scheme of the respiratory system. VOCs can also be exchanged in the alveoli together with oxygen and carbon dioxide molecules.

Preliminary EPA-sponsored studies in the 1980s relied upon a spirometer and the collection of a 40 L volume of breath [57]. This method allowed the detection of low levels of VOCs but was cumbersome and presented many drawbacks. More recent EPA studies only collect 1 L breath samples. The volume of breath sample that is usually collected currently ranges from a few milliliters when VOCs are directly retained into a sorbent device [35,58-61] to one liter when breath is collected in a gas sampling container [24,25,28,32-24,37,39,41,62-65]. To collect more than half a liter of sample it is necessary either to use forced-expiratory sampling (e.g., as the sampling methodology used with breathalyzers to estimate blood alcohol content and in spirometry tests) or to collect samples from tidal breathing over several expirations. Forced-expiratory sampling is a common sampling methodology used in different studies [63,64,66]. This procedure is very simple to perform and does not require complex instrumentation. Generally, a person is asked to take a deep breath, hold it for some seconds and then exhale into the sampling container until reaching the desired volume. This methodology

has been proposed to obtain steady and representative alveolar air but it has many drawbacks: (i) it is highly dependent on the volunteer's cooperation and effort, (ii) breath-holding with the lung full or partially emptied gives different results, and (iii) there is no control of the volunteer's breathing. Despite its widespread use in non-clinical studies, this should not be recommended as a sampling procedure for quantitative analysis.

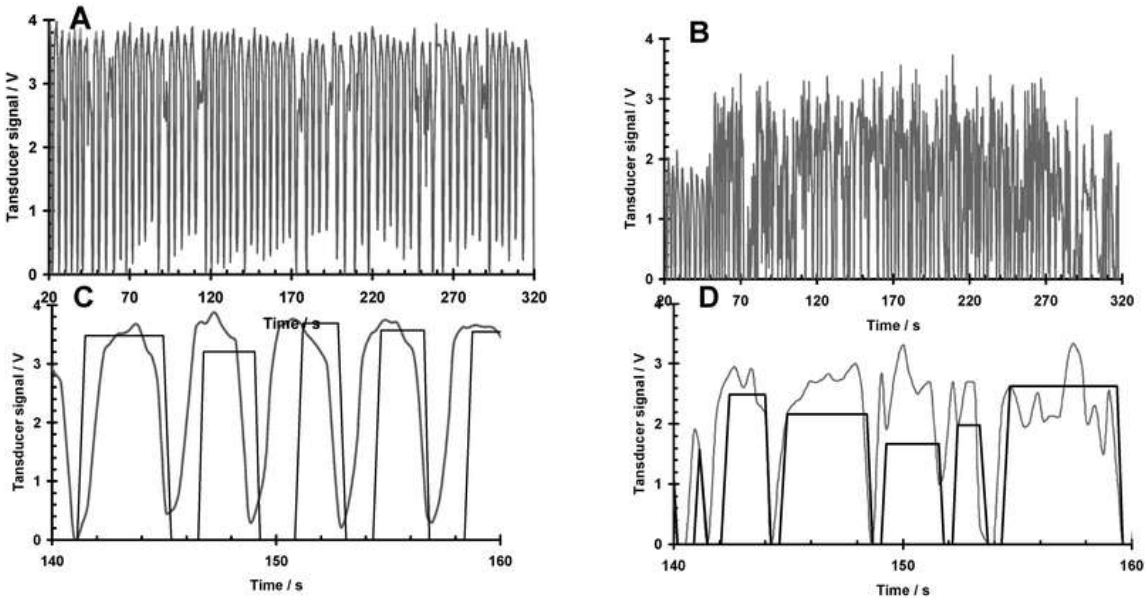

Figure 2. Example traces of breathing profiles from two persons. Trace A is a healthy subject, trace B is a subject with a chronic obstructive pulmonary disease. Traces C and and D are a portion of the profiles from traces A and B respectively. These traces illustrate the problems inherent in relying on a single breath sample, which is still more significant in a person with an impaired lung fuction [67].

Sampling by collecting different exhalations during tidal breathing would seem to be the most reliable methodology. Notwithstanding, breathing patterns are irregular and random fluctuations in breathing frequency and intensity are always present, normally associated with swallowing, yawning and taking occasional deeper breaths [67]. This variation is even more marked when people have impaired lung function (Figure 2). It is therefore necessary to collect breath samples from a series of cycles in order to obtain a representative sample. However, the collection of breath during tidal breathing also presents some problems as people tend to hyperventilate when they are asked to breathe normally [55] changing the distribution of molecules across the alveolar-capillary junction over time. It has been demonstrated that the concentration of compounds may vary considerably depending on the type of ventilation at the moment of sampling

(hypoventilation, hyperventilation and normal ventilation), leading to results that are difficult to interpret [68]. Samples should be obtained during conditions of normal ventilation, which requires introducing the volunteer to the procedure and encouraging the adoption of a relaxed natural and regular breathing profile.

In tidal breathing, different types of samples can be collected depending on the aim of the study [6,69]: (i) mixed expiratory or total breath sampling, (ii) time-controlled sampling (i.e. sampling over a predetermined time after the beginning of the expiration) and (ii) alveolar or end-tidal sampling.

In mixed expiratory sampling there is no need to identify each fraction and the whole expired air is directly collected into an appropriate device. In clinical work, this sampling procedure should not be used as it is subject to dilution and contamination by exogenous substances from the "dead space air". However, the analysis of this fraction is required when substance concentrations in the airways are of interest [69], as is the case in exposure analysis.

Time-controlled sampling presents less dilution and contamination from airways than mixed expiratory sampling, but large variations are found when repeated samples from the same individual are evaluated [69]. For this reason, this sampling methodology should also be avoided in clinical practice.

Alveolar sampling is more complex instrumentally and the difficulties in consistently capturing alveolar air samples have been known for a long time [56]. This technique has the advantage for clinical purposes that there is no contamination from the dead-space volume. Moreover, the concentration of endogenous substances is one to three times higher than in mixed expiratory samples [29,69]. As a result, it is easy to identify and quantify blood-borne substances. This is the most recommended sampling procedure for clinical applications.

The large amount of variables indicated and the fact that many different sampling methodologies are used for exhaled air makes it difficult to compare results [29]. It is therefore desirable to find a standardized system to allow comparison [55,67]. Moreover, a standardized and reproducible breath sample is required for quantitative

analysis to avoid the proportion of alveolar to tidal air varying from sample to sample [56]. The only way to obtain reliable and comparable results is to normalize samples at alveolar concentration levels [6,55,69]. Different methods have been evaluated to determine when the alveolar plateau is reached during a single expiration (e.g., monitoring CO_2, O_2 or breath temperature) [56]. The best results are obtained by the simultaneous determination of carbon dioxide in expired breath as a corrective factor [55]. A CO_2 controller is commonly used as CO_2 concentrations are higher and practically constant in the alveolar phase (Figure 3) [56,69,70]. When CO_2 levels increase and plateau out, portions of breath can be obtained using a syringe (single breath sample) [69] or by connecting a collection device to the system (several breaths) [69,70]. Miekisch et al. [71] have built an automatic CO_2 controlled device for breath sampling.

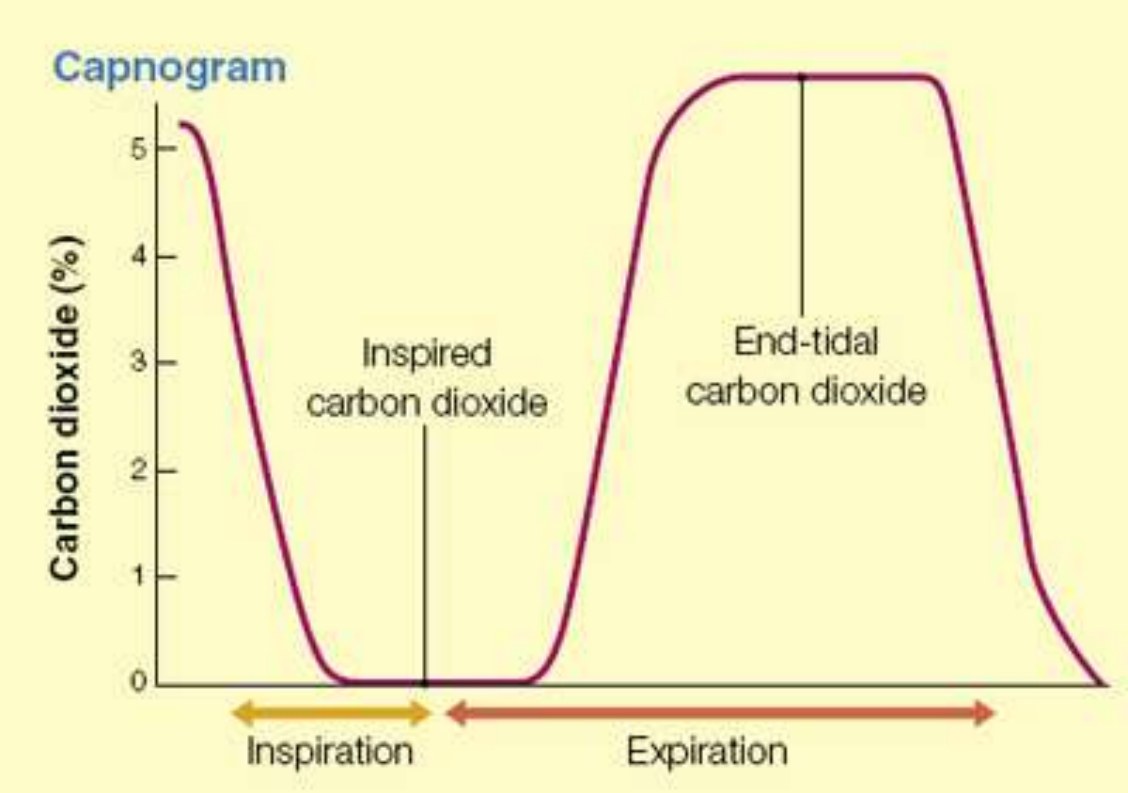

Figure 3. Illustration of a normal capnogram of the respiratory process.

1.3. EBV collection devices

Since the end of the 20th century different methods for the direct reading (real time analysis) of breath samples, such as laser spectrometry, selected ion flow tube (SIFT), atmospheric pressure ionization (API), proton transfer reaction (PTR), ion mobility spectrometry (IMS), and sensors have appeared with promising results [9,27,28,72-77]. Unfortunately, these methodologies require complex, non-portable and expensive instrumentation, which limits its applicability in exposure analysis.

Indirect methods involving collection devices to obtain and transport the samples are less expensive and, at the moment, seem to be the most appropriate methodology for obtaining on-site breath samples. Therefore, the sampling, transport and storage of exhaled breath are critical steps in the whole analytical process. The preservation of the original sample composition is a challenge for gas compounds as losses (e.g., by diffusion), adsorption (e.g., in the surface of the containers) and reactions can occur leading to artifact formation. Thus, the selection of the most appropriate container is essential in EBV sampling. Samples can be collected using different devices such as canisters, sampling bags and sorbent materials [29,78]. Containers can be grouped in two types: (i) those that collect the whole breath sample (stainless steel canisters, polymer bags and glass bulbs), and (ii) those that only collect the volatile compounds of interest (adsorbing tubes and membranes).

Canisters are used for collecting breath samples [23-25,28,33,34,36,62,79]. Stainless steel canisters are recommended devices for EPA methods TO-14A [80] and TO-15 [81] to collect atmospheric air samples for the analysis of toxic organic compounds (Figure 4). They have the disadvantage of being expensive, needing to be evacuated before sampling and requiring sophisticated equipment for cleaning [80-84]. Some authors have suggested that passivated stainless steel canisters are extremely durable for breath storage and many VOCs remain stable within these canisters for periods of 30 days or longer without any significant degradation [62]. On the other hand, Batterman et al. [85] evaluated the stability of some aldehydes and terpenes in electropolished canisters and found that recoveries for all terpenes and most aldehydes evaluated dropped substantially within the first hour, followed by a more gradual decrease later. Despite their widespread use in the collection of atmospheric samples, their use for

collecting breath samples has some important limitations. One recommended solution is to maintain under-atmospheric pressure during storage by not completely filling the canister, so preventing water condensation. [23,79].

Figure 4. Illustration of a stainless steel canister for breath analysis.

Glass bulbs can also be used for breath sampling (Figure 5) [27,35,40,65,86]. However, they are fragile (EPA methods for atmospheric sampling do not recommend the use of glass bulbs), require silanization to deactivate the interior glass surface [65,87], and must be evacuated before sampling. Moreover, loses of volatile compounds have also been observed when glass bulbs are used as containers, although at lower rates than with polymer bags [65].

Figure 5. Illustration of a commercial glass bulb.

In some cases, polymeric chambers made of polyethylene [88], Teflon® [89] and Aerochamber® [58,59] have been used to collect breath samples. Unfortunately, no information about losses and stability has been recorded. Some losses of volatile compounds are to be expected due to the polymeric structure of the chamber walls, as is also the case with polymer bags (as described in the next paragraphs).

The most common methodology for breath collection is to use polymer sampling bags due to the ease with which they can be manipulated, their reduced cost and the

possibility for them to be reused. These bags must be made of inert materials to avoid both diffusion and reactions between the compounds and the bag. The most common material used is Tedlar® (PVF: polyvinyl fluoride) [22,32,37-39,41,63,78,90-94] but other materials such as Teflon® (PTFE: polytetrafluoro ethylene) [20,73,94,95], FlexFoil® (specifically designed for storage of low molecular weight compounds, which permeate easily through the walls of Teflon® and Tedlar® bags) [94], and Nalophan® (PET: polyethylene terphthalate) [94] are also used. Prior to being used for the first time or being reused, bags must be thoroughly cleaned by flushing with pure inert gas to remove adsorbed compounds. This step plays a crucial role in the storage of gas samples [93,94]. Unfortunately, all commercial polymers suffer from diffusion and adsorption of volatile compounds [41,90,91,93,94,96-100], and temperature and storage time have a significant effect on the integrity of the gas samples [92]. Beauchamp et al. [93] evaluated the storage of different VOCs (alcohols, nitriles, aldehydes, ketones, terpenes and aromatic compounds) in Tedlar® bags and found that losses were ~35% for acetonitrile and hexanal and up to 20% for the other target compounds after 10 h of storage; and that these increased to a maximum of 50% after 70 h storage. Alonso et al. [41] found that when 2,5-dimethylfuran is a target compound, samples can only be stored in Tedlar® bags for periods <3 hours. Mochalski et al. [94] studied the behavior of six highly volatile sulfur compounds in different types of bag and found that all polymers could be used for storage periods of up to 6 h, with losses not exceeding 10%. Adsorption rates were higher for Teflon® and Nalophan® bags after this time. Given these results, it is recommended that breath samples should be analyzed as soon as possible after sampling.

Although Tedlar® bags are the most common choice for breath analysis, they also present the most significant background contamination. When Tedlar®, Teflon®, FlexFoil® and Nalophan® polymers were compared [94], only Tedlar® polymer emitted contaminants in blank tests. The main contaminants detected in Tedlar® bags are N,N-dimethylacetamide and phenol, which are both solvents that are used in the production of the film [92,93,100]. Other contaminants that have been detected in these bags are carbonyl sulfide and carbon disulfide [94].

A common commercial device for breath sampling is Bio-VOC (Markes Int. Ltd., Llantrisant, UK) [31,101-106] (Figure 6). This device is based on the collection of the

last 100-150 mL of an expired sample. Immediately after finishing sampling, a valve is opened and the collected breath is transported through an appropriate sorbent material in order to retain the VOCs. The gas sample only remains in the container for a few seconds with this device and no losses are expected. Although the manufactures state that only alveolar air is collected, there is no control of the subjects breathing and CO_2 levels, so making it difficult to compare quantitative results from different studies.

Figure 6. Illustration of the BioVOC sampling device.

As can be seen, all conventional collection devices present some specific problems related to the stability of the compounds in the container. Careful evaluation of the compounds of interest (e.g. volatility of target compounds and type or interferences) and storage time should be taken into account before choosing the most appropriate collection device. Different direct sampling methodologies have recently been developed to integrate sampling and pre-concentration into one single step, which can avoid the problems related to storage in containers. These methodologies are based on the direct collection of target VOCs on a sorbent material, which presents better stability and permits longer storage times. A modified holder connected to a solid-phase microextraction fiber (SPME) has been developed [87,89]. The use of hydrophobic membranes to eliminate water vapor and impurities followed by pre-concentration in a sorbent trap has also been proposed [58,59]. The use of a conventional R-tube for EBC sampling with a modification to introduce an SPME fiber has been suggested [53]. The use of a device called SnifProbe (Aviv Analytical Ltd., Hod Hasharon, Israel), which is based on a small length of capillary or porous-layer open tubular column for sample

collection, has been proposed [107]. An adaptive breath sampler to collect breath directly in a sorbent tube is another option [67]. Most recently, needle trap devices (NTDs) have been described [60,61,69,108]. These different methodologies have the advantages of not requiring a preliminary collection of the breath sample in a container (or the sample only stays in the container for few minutes) and of only collecting target volatile compounds on the surface of a sorbent material.

1.4. Sample enrichment

The low concentrations of VOCs in breath samples make it necessary to employ a pre-concentration technique before GC-MS of GC-FID analysis (conventional instrumental analytical techniques used for indirect EBV analysis). There are two main methodologies for this purpose: solid-phase microextraction (SPME) and concentration on solid sorbents.

1.4.1. Solid-phase microextraction (SPME)

Different procedures are followed in SPME (Table 1) [7,29,53,65,78,86,87,89-91,109,110]. Sometimes the fiber is inserted into the bag or glass bulb containing the total volume of breath collected for a predetermined period of time [65,86,90,91] and at others a fixed and small volume (a few milliliters) of the sample is transferred inside a sealed vacuum headspace vial before inserting the SPME fiber into the vial [109,110]. A modified holder for directly sampling breath from the mouth has also been proposed [87,89].

The selection of an appropriate coating is essential in the SPME method. Grote and Pawliszyn [87] evaluated the extraction efficiency of four different coatings (polydimethylsiloxane/divinylbenzene –PDMS/DVB-, polydimethylsiloxane –PDMS-, polyacrylate –PA-, and Carbowax/divinylbenzene –CW/DVB-) for the three volatile compounds usually found at the highest concentrations in breath samples (ethanol, acetone and isoprene). They found that the PDMS/DVB, PDMS and CW/PDMS fibers reach equilibrium in less than 60 seconds, with the PDMS/DVB coating being the best in terms of sensitivity (especially for the non-polar compounds). Amorim et al. [89]

compared the previous coatings with a divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coating for benzene extraction and found the DVB/CAR/PDMS coating to be the more sensitive.

Table 1. Summary of the principal studies using SPME as preconcentration technique and devoted to exposure analysis.

Coatings	Target VOCs	LOD	Sampling collection device	Ref.
CAR/PDMS	Isoprene	6 ppbv (SPME) 0.4 ppbv (sorption)	8 L Tedlar bag SPME inside bag, 10 min at 40ºC	90
PDMS/DVB	Acetone	0.05 ppbv	3 L Tedlar bag (max. storage 6 h) SPME inside bag, 4 min at 40ºC	91
PDMS	Tetrachoroethylene	0.3 $mg \cdot m^{-3}$	125 mL glass bulb (exposed 1 min)	86
PA PDMS CW/PDMS PDMS/DVB	Ethanol Acetone Isoprene	6 $nmol \cdot L^{-1}$ 2 $nmol \cdot L^{-1}$ 0.3 $nmol \cdot L^{-1}$ (PDMS/DVB coating)	Fiber directly to mouth (10 s)	87
PDMS CW/DVB PDMS/DVB DVB/CAR/PDMS	benzene	2 ppbv	Fiber directly to mouth (30 s)	89
CW/PEG	2-aminoacetophenone	50 $pmol \cdot mol^{-1}$	1 L glass bulb (24 h fiber)	65
CAR/PDMS	Acetone Acetonitrile Benzene n-butane Dimethylsulfide Furan 2-methylfuran Isoprene Limonene Toluene	2 ppbv 15 ppbv 0.05 ppbv 5 ppbv 4 ppbv 2 ppbv 2 ppbv 0.2 ppbv 2 ppbv 0.1 ppbv	3 L Tedlar (20 mL vials, 10 min 37ºC)	109
CAR/PDMS	43 VOCs	0.7-17 ppbv	3 L Tedlar (20 mL vials, 10 min 37ºC)	110

The small volume of the stationary phase is an advantage of SPME when sample sizes are not large [111]. The amount of analyte extracted by an SPME coating becomes practically constant once the sample volume is significantly larger than the product of the distribution constant and the volume of the coating [112]. Therefore, the sensitivity of SPME is not as dependent on the volume of the sample as conventional concentration on solid sorbents. LODs are commonly in the low $\mu g.m^{-3}$ range when SPME is applied to breath samples (Table 1). Unfortunately, this limits the applicability of SPME when target compounds have to be detected at lower levels. For example, 2,5-dimethylfuran, a promising breath biomarker for determining smoking status or continuous contact to environmental tobacco smoke, has to be detected at the low $ng \cdot m^{-3}$ range in breath samples in order for detection to be possible some hours after contact with tobacco smoke [32,41,108]. Analyses performed in our laboratory showed that SPME failed to detect this compound just a few minutes after smoking a cigarette, whereas the

compound was detected for more than 24 h after smoking using a multi-bed sorbent capillary trap.

An important parameter to take into account with breath analysis is the high water content in these samples. It has been found that the water content of a sample has a significant effect on the SPME sorption process when direct analysis of breath is performed [87]. For those coatings where absorption is the dominant process (PDMS), extraction efficiency is not affected by the water content of the sample. However, there is a significant change in the extraction efficiency in the case of adsorption mechanism based coatings (DVB, CAR, CW) due to the competition with water molecules for the active sites of the sorbent material. Calibration standards should be prepared at the same relative humidity (RH) as samples to avoid quantification mistakes [87,90]. However, many studies have not employed this procedure due to the complexity of the standard preparations.

1.4.2. Membrane extraction with sorbent interface (MESI)

This methodology is based on the use of silicone membranes (made of PDMS [58,113] or silicone polycarbonate [59]). They are similar in nature to nonpolar lipid bilayer cell membranes of the alveoli and preferentially transport nonpolar volatile compounds [58]. Membrane extraction minimizes analyte loss by interfacing the membrane extraction module directly to a gas chromatograph. As the membrane by itself does not provide an adequate concentration factor, the extracted analytes must be passed through a cooled sorbent trap before GC analysis is performed.

One of the advantages of the MESI methodology is the hydrophobic nature of the silicone membrane, which blocks the diffusion of water vapor through its surface. It has been found that the extraction efficiency for acetone, benzene, toluene, and ethanol does not show significant changes when the RH is increased in the 10-90% range [58]. Slight decreases (up to 12%) were observed for some terpenes when the RH increased in the 24-72% range [113].

1.4.3. Concentration on solid sorbents

Pre-concentration on solid sorbents followed by thermal desorption is the most frequent method for the analysis of VOCs in breath samples [20,22-25,28,31-40,62-64,67,76,79,95,107,114-116], including EPA [117] and recommended NIOSH methods [118]. Sorbent traps present the advantages of being less expensive and easier to manipulate [82,84], they can be prepared on a micro-scale and coupled on-line with a GC system allowing near real-time measurements [119-123], and the sorbent configuration can be easily changed to adapt to different compounds.

LODs obtained by sorbent trap techniques strongly correlate with the volume of sample analyzed. Thus, LODs obtained applying this technique decrease significantly compared to SPME limits when a large volume of a breath sample can be collected. LODs obtained with the EPA-TO-14 method in the analysis of 8 L breath samples were one order of magnitude lower than those obtained with SPME [90]. The use of a micro-trap allows LODs in the low $ng.m^{-3}$ range with samples volumes of up to 1 L to be reached [32,39].

The most common and simple sorption trap is based on a single adsorbent. In the case of exposure analysis, the most common sorbent used is Tenax [20,22,31,35,40,95]. However, the wide range of VOCs present in breath has the result that no single sorbent is capable of adsorbing all the compounds present in breath samples, and a multicomponent sorbent is necessary to complete the screening and determination of VOCs [37,63,82,115,116,119,122,123].

An important source of error when sorbent traps are used is the formation of artifacts caused by degradation reactions of both adsorbed analytes and the adsorbent itself during storage of adsorbent tubes [124-126]. This effect is more important when very low concentrations of target compounds are expected. The use of on-line traps reduces the error resulting from the degradation reactions that occur during storage [125,126].

The sorption and desorption behavior of VOCs in carbon-based sorbents is important as they determine the injection plug width and the ability to perform quantitative studies. A problem in the use of the sorbent trap technique is the high temperatures needed for

the quantitative desorption of the trapped compounds that can lead to the thermal decomposition of some compounds [127-130]. The thermal degradation of terpenes yields other different terpenes and aromatic compounds [128-132] and if one of these degradation products is also a target analyte there is the possibility that false positives and quantification errors will occur (Figure 7).

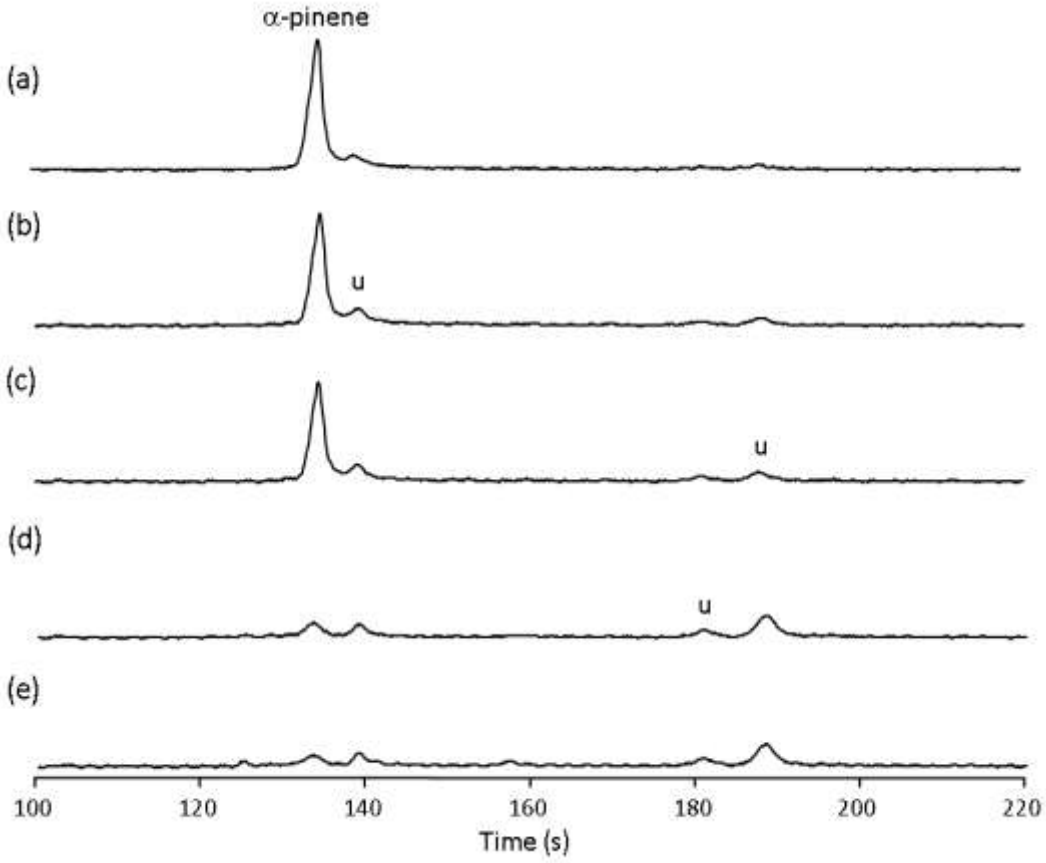

Figure 7. Chromatograms showing the thermal degradation of α-pinene when the temperature applied to the sorbent trap for desorption is increased. The sorbent trap was heated to 200ºC (a), 250ºC (b), 300ºC (c), 350ºC (d), and 380ºC (e). (u: unknown).

Sanchez and Sacks [130] evaluated the behavior of different families of volatile compounds during the thermal desorption process and found that the decomposition of analytes in carbon-based sorbents depends on both the desorption temperature applied and the time during which the compounds are in contact with the hot surface of the sorbents. Even low desorption temperatures result in thermal oxidation of the most labile compounds when they are in contact with the hot surface of the sorbent for a few seconds (Figure 8).

Alkanes and alkyl benzenes did not show thermal degradation. Other compounds that can be oxidized showed a degree of decomposition that depended on their level of oxidation (terpenes>aldehydes>ketones). These authors recommend using the lowest feasible desorption temperature and removing the desorbed compounds from the hot surface of the sorbent as fast as possible. The degradation problem is more important with conventional desorption equipment as the strict requirement for a narrow injection plug for GC analysis makes a second pre-concentration stage necessary to refocus the solutes in the analytical column. This is frequently done by cryogenic trapping, which can also result in analyte loss and the formation of artifacts [133].

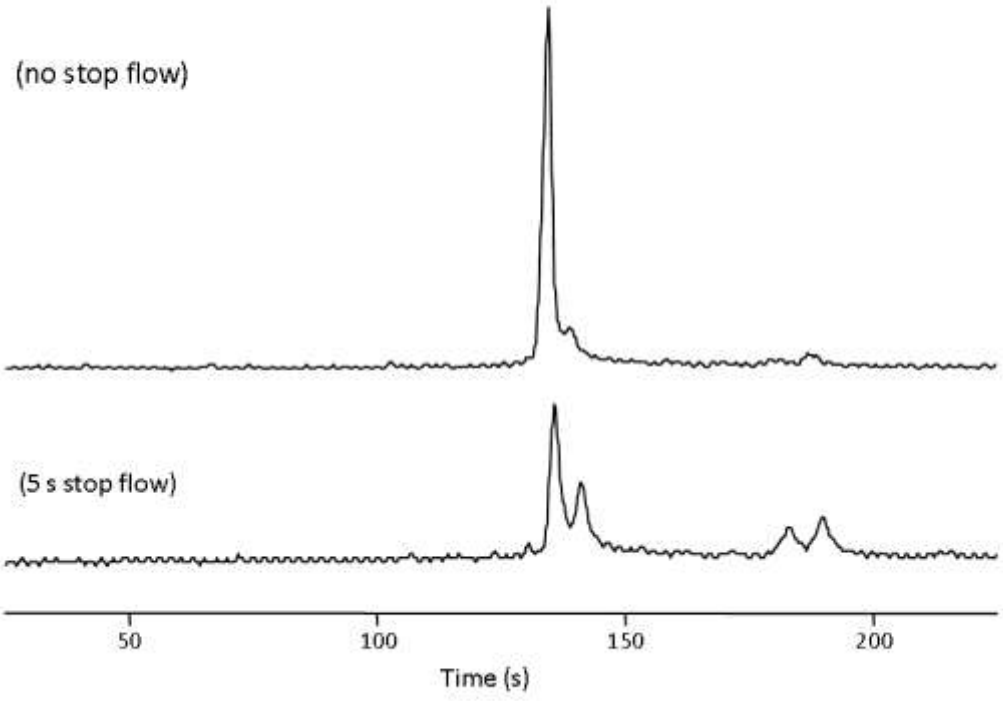

Figure 8. Chromatograms for the analysis of α-pinene when the trap is heated to 200ºC and the carrier gas is stopped for some seconds during the desorption step, allowing the compound to stay at high temperature. (u: unknown).

The sorbent material itself can generate artifacts by degradation [82]. The high sensitivity of the GCxGC/TOF-MS system has demonstrated that different VOCs can be released from the surface of carbon-based sorbents at desorption temperatures >300ºC [37]. Series of alkenes were detected at desorption temperatures >400ºC. Common

target compounds, such as benzene, toluene and styrene, can be detected at levels equivalent to conventional levels in breath samples when the desorption temperature applied is >300ºC (Figure 9). To avoid false positives, desorption temperatures of up to 300ºC are recommended for this methodology.

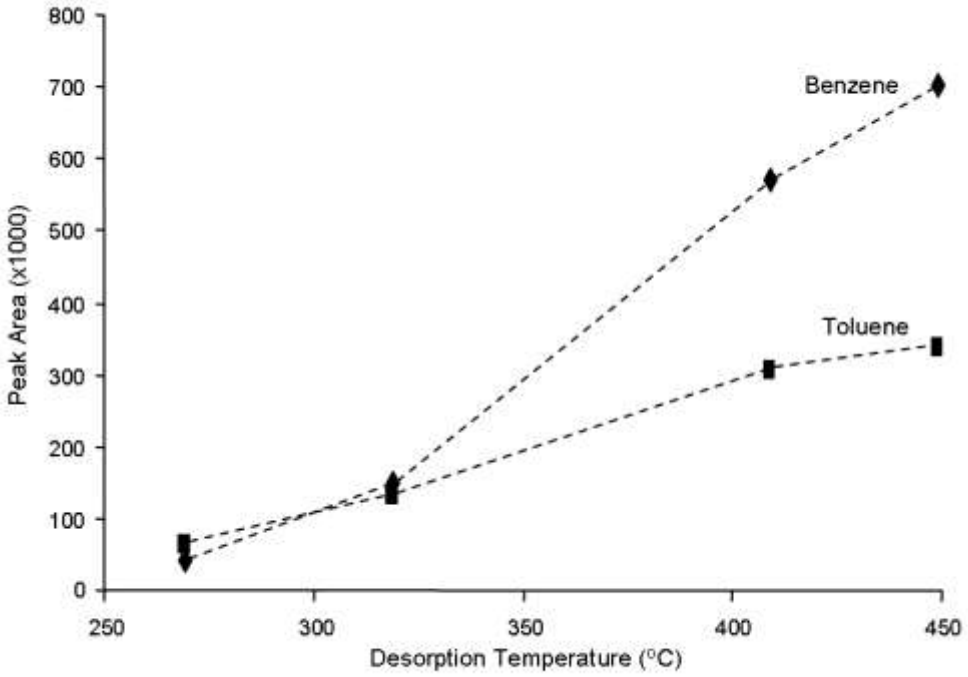

Figure 9. Increase in the peak area detected for benzene and toluene in blank analyses of a three-bed trap containing Carboxen 1000, Carbopack X and Carbopack B as sorbent materials. The blank measurements were performed at different temperatures between 270ºC and 450ºC. In order to obtain sufficient sensitivity, the analyses were performed with a GCxGC instrument (Pegasus 4D, Leco Corp., St. Joseph, MI, USA) [37].

In order to simplify the desorption process and to solve decomposition problems, different in-house capillary traps have been developed. These micro-traps eliminate the need for a second cryofocusing stage, reduce the time that the analytes stay in contact with the hot surface of the sorbent during desorption, and allow near real-time measurements [37,39,63,64,119-123]. The configuration of the micro-traps allows much greater concentration factors than those obtained with conventional thermal desorption

instruments, which also results in a smaller amount of sample being required to reach LODs in the ng·m^{-3} range [37,39,63,64].

NTDs represent a further improvement in capillary traps for breath analysis [60,61,108,134]. These devices allow direct thermal desorption inside a GC injector (equivalent to SPME) without the need for a second focusing cryotrap and yield large enrichment factors, resulting in significantly smaller LODs than those obtained with conventional desorption methods when the same sample volumes are compared. The small i.d. of the NTDs results in high linear flows through the sorbents that limit the maximum flow rate that can be used during sampling collection (≤15 mL·min^{-1}) [108]. Given this, limited volumes of sample can be collected in an appropriate time when NTDs are used as the sampling methodology [60,69]. This may lead to those target compounds that are present at very low concentrations not being detected [108].

For the sorption process, it is important to take into account the water intake of the sorbents as this can affect the quantitative analysis of VOCs. Graphitized carbon blacks and porous organic polymer adsorbents allow a high percentage of water vapor in the sample to pass through the traps during sampling without significant loss of the target compounds [135-139]. Unfortunately, if highly volatile compounds are on the target list, strong adsorbents (carbon molecular sieves) are required in order to retain them and large amounts of water are retained at the same time.

The simultaneous trapping of water vapor can cause various problems: (i) the accumulation as ice during cryogenic pre-concentration, (ii) a reduction in the adsorption efficiency during sampling on solid adsorbents, (iii) the possible loss and transformation of organic trace gases in the water/ice matrix, (iv) freeze out of water on the trap or in the GC column during cryogenic oven cooling can plug the trap or the column and interrupt the carrier gas flow, and (v) a large water background can also cause shifts in the retention times and pose problems during detection, especially in the case of an MS detector [135].

Different options have been proposed to limit the water vapor problem [135]. One option is to pass the sample flow through a trap containing a drying agent (e.g., K_2CO_3 and Na_2SO_4). The use of a membrane (Nafion) before passing the sample through the

sorbent trap for water removal is recommended in the EPA-TO-14A method [80]. This membrane allows water to permeate through it but also permits other light polar volatile compounds to pass through, resulting in losses of highly volatile compounds [82]. Another simple option is to dry the sample with a dry inert gas. This is performed after the sample concentration is completed in the sorbent trap by forward purging with clean, dry inert gas [81,135,140]. In this case, there are also limitations due to the possible loss of VOCs or the introduction of contaminants [82,135,137]. Another alternative is to heat the adsorbent during sampling [82,135,141], but this results in most volatile compounds not being quantitatively retained by the sorbent [63] (Figure 10).

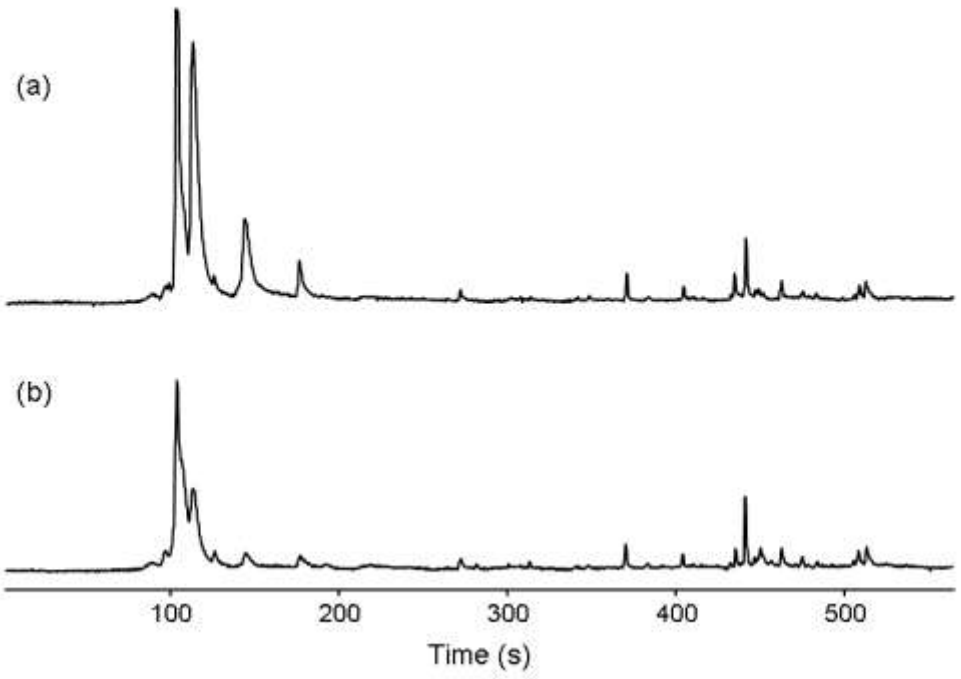

Figure 10. Effect of the temperature applied during the sorption process in the analysis of a breath sample (750 mL exhaled breath). Three bed trap containing Carboxen 1000, Carbopack X and Carbopack B as sorbent materials. As can be seen, there is a significant decrease in the peak heights for the most volatile compounds (compounds appearing at retention times <200 s) when the trap was heated at 40ºC during the sampling process (b). Sampling at 22ºC (a) yields better sensitivity for the most volatile compounds. Less volatile compounds (r.t. > 200 s) are not significantly affected by the change in the trap temperature during the sorption process.

The simplest way to reduce the water problem consists in the reduction of the volume of sample so as to reduce the amount of water vapor in the sample to below the thresholds for the proper use of the analytical instrumentation [81,135]. This option is only available, however, in those cases where small amounts of breath samples are collected (e.g., capillary traps and NTDs).

When the volume of the sample cannot be reduced satisfactorily, one possibility is to avoid the use of carbon molecular sieves (CMSs) in the design of the multi-bed sorbent traps as these materials adsorb large amounts of water, corresponding to the volume of the micropores [135,136,137]. Unfortunately, however, it is still not possible to find sorbents which are as effective as CMSs that are resistant to water adsorption [141], and CMSs are needed to adsorb highly volatile compounds. Carbotrap X, a graphitized carbon designed to adsorb highly volatile compounds, fails to intercept quantitatively any hydrocarbon that is more volatile than pentane and low-molecular weight compounds of high polarity [126].

The water vapor is rapidly and exponentially reduced to atmospheric humidity when samples at 100% RH are collected in Tedlar sampling bags (<9 h, 50% reduction in ~1h), which has been attributed to diffusion through the walls of the bags [93]. This contributes to reduce the problem of the water vapor when these sampling bags are used, but also results in a significant reduction of the most polar compounds that may be lost together with the water molecules [63].

1.5. Applications in exposure analysis

Studies found in the literature can be grouped in five categories: (i) simulations in controlled chambers [33,103], (ii) swimming and domestic water activities [24,36,88,105,142], (iii) petrol services and mechanics [34,35,40,89,143,144], (iv) solvents and volatile compounds in the workplace [31,106,145,146], and (v) active and passive smoking [28,32,37,39,41,70,108,147]. Most of the studies are focused on finding reliable exposure biomarkers.

1.5.1. Simulations in controlled chambers

These studies try to simulate conventional exposure situations in order to investigate whether breath measurements can be used as a surrogate for blood measurements. The main drawback is that controlled chambers are designed to assess exposure at levels that are equivalent to the threshold limit values (TLV) or BEL, and results cannot be extrapolated to non-exposed people.

Exposure to trichloroethene levels was evaluated from controlled inhalations at high levels for 24 hours: participants were exposed to 100 $mg \cdot m^{-3}$ trichloroethene for the first 4 hours and to purified air for the remaining 20 [33]. A model was used to predict blood levels from breath elimination curves and blood/breath partition coefficients. The results obtained gave a mean ratio of blood level calculated:measured of 0.98 and a 12% RSD. Levels in breath and blood were correlated and the study concluded that about 78% of thrichloroethene entering the body during inhalation exposure was metabolized, stored or excreted through routes other than exhalation.

Exposure to trimethylbenzene was performed in a controlled volunteer study where participants were exposed for 4 hours to this compound at 25 $mg \cdot m^{-3}$ in a laboratory controlled atmosphere facility [103]. A rapid absorption of trimethylbenzene into the blood stream was observed (steady state was reached within 1-2 hours of exposure), which is largely produced by inhalation. Elimination was determined through the analysis of exhaled breath and a metabolite in urine, and it was found that some trimethylbenzene was not eliminated via breath or urine. Breath results were significantly correlated with venous blood and urine samples, confirming the utility of breath analysis as an indicator of exposure.

1.5.2. Swimming and domestic water activities

Trihalomethanes (THMs) are important contaminants in indoor and outdoor swimming pools and also in domestic water activities. They are formed as a result of the combination of residual organic matter and chlorine-based disinfection products used in water supply systems. Exposure to these compounds was evaluated both in swimmers who were training competitively and sporadic swimmers. Lindstrom et al. [24] collected

breath samples before, during and after a 2 hour training period. They suggest that the dermal route of exposure was even more important than the inhalation route. The elimination of chloroform after exposure fitted to a three compartment model, and bromodichloromethane elimination fitted to a two compartment model. Other studies [105,142] have also found that dermal uptake for these compounds is significant. In a study specifically designed to confirm the dermal uptake of chloroform and two haloketones during bathing [88], it was found that haloketones are less permeable through skin than chloroform.

A significant increase in the breath levels of THMs was observed in some participants during bathing and showering [36]. However, other domestic water use activities, such as washing clothes or dishes, did not result in a significant increase in the breath levels even though these activities led to a significant increase in the indoor air levels.

1.5.3. Petrol services and mechanics

Benzene, toluene, ethylbenzene, and xylene isomers (BTEXs) are common compounds in petrol products. These compounds evaporate easily from the liquid and can be inhaled by people. Benzene levels in the exhaled breath of people exposed to petrol vapors are always higher than in volunteers who are not exposed [34,35,40,89,144]. These studies also found large variability in breath benzene levels for all groups evaluated, but this variability was significantly higher in the case of exposed participants. Exhaled toluene and xylenes also showed significant correlations with concentrations found by personal monitoring devices [143]. Therefore, exhaled breath levels of benzene, toluene and xylenes have been proposed as suitable for use as biological exposure indices for petrol station workers.

It has been found that naphthalene elimination from the body takes place faster than in the case of benzene [40]. An elimination rate constant for naphthalene in breath of 1.93 h^{-1} was determined (corresponding to a half life time of 21.6 min), whereas the elimination of benzene in the same samples gave a half life time of 41.8 min.

1.5.4. Solvents and volatile compounds in the workplace

Occupational exposure to benzene was evaluated in workers of a benzene production plant during their entire work shift [145]. Alveolar breath levels are significantly

correlated with ambient air and urinary and blood levels. Significant differences for alveolar and blood benzene levels were obtained between exposed and non-exposed workers. Benzene alveolar retention of around 55% was suggested.

Workers from different occupations (house painters, varnishing workers, car painters and petrol station workers) have also been evaluated [106]. Higher concentrations were detected after work shifts. Scheepers et al. [31] analyzed alveolar breath and personal exposure to BTEXs of primary school children from two different zones. They found that industrial activity made a relatively small contribution to exhaled BTEXs. Other factors, such as smoking habits, petrol services and traffic, and the use of consumer products, seem to have a greater influence on exposure to benzene and toluene.

Thrall et al. [146] developed a field-portable breath analysis system to measure selected solvents in exhaled air. Benzene and toluene were evaluated in workers from an incinerator, and trimethylbenzene, hexane and methylene chloride were determined from employees in a waste repackaging facility. The system developed has great potential for exposure analysis.

1.5.5. Active and passive smoking

The last category evaluated is focused on studies devoted to tobacco smoking, exposure to environmental tobacco smoke (ETS) and passive smoking. Buszewski et al. [70] analyzed 56 VOCs in the alveolar breath of 20 non-smokers, 14 active smokers and 4 passive smokers. Acetonitrile, furan, 3-methylfuran, 2,5-dimethylfuran, 2-butanone, octane and decane were only found in smokers and passive smokers. Berkel et al. [147] analyzed alveolar breath from 11 smokers and 11 non-smokers. They identified four VOCs as biomarkers of recent exposure to cigarette smoke: 2,5-dimethylhexane, dodecane, 2,5-dimethylfuran, and 2-methylfuran. Gordon et al. [28] evaluated the breath profiles of benzene, 1,3-butadiene and 2,5-dimethylfuran from smokers and passive smokers after smoking cigarettes in an small unventilated room. All three target VOCs were identified in the breath of non-smokers after exposure, so demonstrating their contamination by ETS.

Capillary thermal desorption units for near real-time analysis of VOCs at sub-trace levels have been developed [37,39,64] and 2,5-dimethylfuran has also been found to be a biomarker of smoking status. This compound was recently confirmed as a specific biomarker independently of the smoking status [32]. The evaluation of ETS contamination on public premises also confirmed this compound as a robust biomarker of ETS contamination [41]. The compound was also detected in the breath of non-smoking employees working on smoking premises after a few hours of the beginning of their work shift. A field-portable needle microextraction device for on-site analysis of airborne VOCs has been developed [108]. This device has been used for breath analysis of smokers and non-smokers to identify 2,5-dimethylfuran.

1.6. New instrumental techniques.

GC-MS techniques have been widely used for breath analysis during last decades. The main disadvantage of GC-MS is that it requires preconcentration and cannot be performed in real-time [9,148]. Nowadays there are some promising emerging techniques that provide reliable real-time results: SIFT-MS, PTR-MS, IMS, and laser spectroscopy [9,77,148].

Selected ion flow tube-mass spectrometry (SIFT-MS) combines the fast flow technique and mass spectroscopy. It is a real time technique for several trace gas quantification in air or breath samples [148]. An ion source generates positive precursor ions, such as H_3O^+, NO^+ or O_2^+, using chemical ionization. A quadruple mass filter selects ions and introduces them into an inert carrier gas, such as helium. Ions travel along a flow tube into which the sample is injected. Product ions formed by this reaction are then analyzed quantitatively by MS [9]. Polar substances are the principal targets, but also unsaturated carbons can be analyzed. Aliphatic hydrocarbons are not suitable for SIFT-MS. This technique is able to perform direct and immediate analysis of breath samples from single exhalation of patients, and can monitor changes of breath profiles in healthy volunteers, after ingestion, after smoking, to assess bacterial infections, and others [148]. Although SIFT-MS is less sensitive than PTR-MS, it is able to identify substances with the same molecular mass using different precursor ions [9].

Proton-transfer reaction-mass spectrometry (PTR-MS) is an innovative technique for measuring and monitoring VOCs at low concentrations in gaseous samples. It can be operated in real-time and can determine single compounds [9]. This technique is based on a chemical ionization generated by proton-transfer reactions with H_3O^+ as the primary reactant ion. The ions are mixed with a continuous flow of air sample and proton transfer takes place as the gas sample travels through the drift tube. Molecules with proton affinity greater than water will accept a proton. Most VOCs will accept a proton. This technique can be used for breath profiling, monitoring of anesthetic agents, smoking, and more [9,148]. PTR-MS is more sensitive than SIFT-MS, but cannot identify substances or differentiate between substances at the same molecular mass. Compared with GC-MS, it can yield more reliable quantitative results.

Ion mobility spectrometry (IMS) is a fast and sensitive analytical method for the detection of gas-phase analytes. A gas phase analyte is ionized by a β-radiation source. Under the influence of an external electrical field, the ions move towards a detector. During the drift to the detector, the ions collide with the drift gas molecules moving in the opposite direction. The ions are decelerated depending on their size and shape and are totally separated in the ideal case. To obtain additional information and to avoid negative effects of humidity, a multi capillary column is used for rapid pre-concentration when complex mixtures are analyzed [77]. The main advantage of IMS devices is that no vacuum systems are required and ambient air can be used as a carrier gas [9].

Laser spectroscopy is a high resolution technique that can detect specific molecular species at low concentrations. It can operate in real-time mode without the need for sample treatment or preparation, and enable determination of single compounds. This technique allows the detection of various compounds with characteristic fingerprint spectra in the mid-IR down to the ng/m^3 range [9]. The principle of this technique consists in evaluate the gas sample of interest in the gas cell with a laser beam. The laser beam can be absorbed by the molecular species of interest and the detector measures the absorbed amount of the laser beam in the gas cell, which is quantified in proportion to its concentration [148]. Laser spectroscopy is not yet been used in clinical purpose as it is still a diverse and complex technique.

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2. Objectives

The main objective of this work is **to develop new, simple and field portable techniques based on thermal desorption for the analysis of volatile organic compounds that can be applied for exposure analysis**.

Current methodologies based on thermal desorption require large sample volumes, present high limits of detection, and size of intrumentation is not appropriate for on-site analysis. Taking into account these considerations the main objective described above can be divided in more specific and detailed premises:

1. Development of new preconcentration techniques that require small sample volumes, reach low detection limits, and are easy-to-use. First approximation is the design of an in-house concentrator/injector based on a multibed sorption trap. Further experiments are based on the development of needle microextraction traps to obtain a field portable device for on-site sampling and preconcentration.

2. Application of the developed techniques to the analysis of air samples in non-contaminated environments to assess their applicability for analyzing toxic substances at the sub-$\mu g.m^{-3}$ level.

3. Application of the developed techniques to the analysis of breath samples from non-exposed people in order to confirm that the contaminants found in the surrounding air enter in contact with the human body.

4. Application of the developed techniques to the analysis of a conventional body fluid (blood) to confirm that the inhaled compounds detected in breath samples can reach human fluids and may produce some adverse health effects.

3. Publications

3. 1. **"Capillary thermal desorption unit for near real-time analysis of VOCS at sub-trace levels. Application to the analysis of environmental air contamination and breath samples"**.

Alonso, M.; Castellanos, M.; Martín, J.; Sánchez, J.M.

Journal of Chromatography B, 877 (**2009**), 1472-1478.

Alonso, M., Castellanos, M., Martín, J., Sánchez, J.M. “Capillary thermal desorption unit for near real-time analysis of VOCs at sub-trace levels. Application to the analysis of environmental air contamination and breath samples”. Journal of Chromatography B. Vol. 877, (15 May 2009) : p. 1472–1478

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Abstract

A capillary microtrap thermal desorption module is developed for near real-time analysis of volatile organic compounds (VOCs) at sub-ppbv levels in air samples. The device allows the direct injection of the thermally desorbed VOCs into a chromatographic column. It does not use a second cryotrap to focalize the adsorbed compounds before entering the separation column so reducing the formation of artifacts. The connection of the microtrap to a GC–MS allows the quantitative determination of VOCs in less than 40 min with detection limits of between 5 and 10 pptv (25 °C and 760 mmHg), which correspond to 19–43 ng m−3, using sampling volumes of 775 cm3. The microtrap is applied to the analysis of environmental air contamination in different laboratories of our faculty. The results obtained indicate that most volatile compounds are easily diffused through the air and that they also may contaminate the surrounding areas when the habitual safety precautions (e.g., working under fume hoods) are used during the manipulation of solvents. The application of the microtrap to the analysis of VOCs in breath samples suggest that 2,5-dimethylfuran may be a strong indicator of a person's smoking status.

Keywords

- Thermal desorption
- Breath
- Smoking
- Biomarker

3. 2. **"Evaluation of potential breath biomarkers for active smoking: assessment of smoking habits"**

Alonso, M.; Castellanos, M.; Sánchez, J.M..

Analytical and Bioanalytical Chemistry, 396 (**2010**), 2987-2995.

Alonso, M., Castellanos, M., Martín, J., Sánchez, J.M. “Evaluation of potential breath biomarkers for active smoking: assessment of smoking habits”. Analytical and Bioanalytical Chemistry. Vol. 396, issue 8 (April 2010) : p. 2987-2995

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Abstract

Different compounds have been reported as biomarkers of a smoking habit, but, to date, there is no appropriate biomarker for tobacco-related exposure because the proposed chemicals seem to be nonspecific or they are only appropriate for short-term exposure. Moreover, conventional sampling methodologies require an invasive method because blood or urine samples are required. The use of a microtrap system coupled to gas chromatography–mass spectrometry analysis has been found to be very effective for the noninvasive analysis of volatile organic compounds in breath samples. The levels of benzene, 2,5-dimethylfuran, toluene, o-xylene, and m- p-xylene have been analyzed in breath samples obtained from 204 volunteers (100 smokers, 104 nonsmokers; 147 females, 57 males; ages 16 to 53 years). 2,5-Dimethylfuran was always below the limit of detection (0.005 ppbv) in the nonsmoker population and always detected in smokers independently of the smoking habits. Benzene was only an effective biomarker for medium and heavy smokers, and its level was affected by smoking habits. Regarding the levels of xylenes and toluene, they were only different in heavy smokers and after short-term exposure. The results obtained suggest that 2,5-dimethylfuran is a specific breath biomarker of smoking status independently of the smoking habits (e.g., short- and long-term exposure, light and heavy consumption), and so this compound might be useful as a biomarker of smoking exposure.

Keywords

- Breath biomarkers
- Smoking
- 2,5-Dimethylfuran
- Benzene

3. 3. **"Assessment of environmental tobacco smoke contamination in public premises: significance of 2,5-dimethylfuran as an effective marker"**

Alonso, M.; Godayol, A.; Anticó, E.; Sánchez, J.M

Environmental Science and Technology, 44 (**2010**), 8289-8294.

Alonso, M., Godayol, A., Anticó, E., Sánchez, J.M. “Assessment of Environmental Tobacco Smoke Contamination in Public Premises: Significance of 2,5-Dimethylfuran as an Effective Marker”. Environmental Science & Technology. Vol. 44, issue 21 (2010) : p. 8289-8294

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Abstract

Contamination by environmental tobacco smoke (ETS) on premises where smoking is permitted is evaluated. Although all target VOCs evaluated show significant differences between smoking and nonsmoking indoors, the results obtained indicate that 2,5-dimethylfuran is the most appropriate and effective marker of ETS contamination given that this compound is only detected in environments where people have smoked and so the detection of this compound cannot be attributed to other contamination sources such as traffic. Moreover, the air levels of this compound due to coffee aroma are below the detection limits for this methodology. A preliminary study is performed to evaluate whether 2,5-dimethylfuran, a smoking breath biomarker, can be detected in passive smokers working in smoking environments. The compound was continuously detected in the breath of nonsmoking employees after being in direct contact with ETS for just a few hours. The Tedlar gas sampling bags had 5% loss of 2,5-dimethylfuran after 3 h of storage, which we took as the maximum recommended period for air sample storage.

Keywords

- Environmental tobacco smoke
- Smoking
- 2,5-Dimethylfuran

3. 4. **"Needle microextration trap for on-site analysis of airborne volatile compounds at ultra-trace levels in gaseous samples"**

Alonso, M.; Godayol, A.; Anticó, E.; Sánchez, J.M

Journal of Separation Science, 34 (**2011**), 2705-2711.

Alonso, M., Godayol, A., Anticó, E., Sánchez, J.M. “Needle microextraction trap for on-site analysis of airborne volatile compounds at ultra-trace levels in gaseous samples”. Journal of Separation Science. Vol. 34, issue 19 (October 2011) : p. 2705-2711

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Abstract

Different capillary needle trap (NT) configurations are studied and compared to evaluate the suitability of this methodology for screening in the analysis of volatile organic compounds (VOCs) in air samples at ultra-trace levels. Totally, 22 gauge needles with side holes give the best performance and results, resulting in good sampling flow reproducibility as well as fast and complete NT conditioning and cleaning. Two different types of sorbent are evaluated: a graphitized carbon (Carbopack X) and a polymeric sorbent (Tenax TA). Optimized experimental conditions were desorption in the GC injector at 300°C, no make-up gas to help the transport of the desorbed compounds to the GC column, 1 min splitless time for injection/desorption, and leaving the NT in the hot injector for about 20 min. Cross-contamination is avoided when samples containing high VOC levels (above likely breakthrough values) are evaluated. Neither carryover nor contamination is detected for storage times up to 48 h at 4°C. The method developed is applied for the analysis of indoor air, outdoor air and breath samples. The results obtained are equivalent to those obtained with other thermal desorption devices but have the advantage of using small sample volumes, being simpler, more economical and more robust than conventional methodologies used for VOC analysis in air samples.

Keywords

- Needle trap
- Screening analysis
- Thermal desorption
- VOC

3. 5. **"Headspace needle-trap analysis of priority volatile organic compounds from aqueous samples: application to the analysis of natural and waste waters"**

Alonso, M.; Cerdán, L.; Godayol, A.; Anticó, E.; Sánchez, J.M

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Alonso, M., Cerdan, L., Godayol, A., Anticó, E., Sánchez, J.M. “Headspace needle-trap analysis of priority volatile organic compounds from aqueous samples: Application to the analysis of natural and waste waters”. Journal of Chromatography A. Vol. 1218, issue 45 (11 November 2011) : p. 8131-8139

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Abstract

Combining headspace (HS) sampling with a needle-trap device (NTD) to determine priority volatile organic compounds (VOCs) in water samples results in improved sensitivity and efficiency when compared to conventional static HS sampling. A 22 gauge stainless steel, 51-mm needle packed with Tenax TA and Carboxen 1000 particles is used as the NTD. Three different HS-NTD sampling methodologies are evaluated and all give limits of detection for the target VOCs in the ng L−1 range. Active (purge-and-trap) HS-NTD sampling is found to give the best sensitivity but requires exhaustive control of the sampling conditions. The use of the NTD to collect the headspace gas sample results in a combined adsorption/desorption mechanism. The testing of different temperatures for the HS thermostating reveals a greater desorption effect when the sample is allowed to diffuse, whether passively or actively, through the sorbent particles. The limits of detection obtained in the simplest sampling methodology, static HS-NTD (5 mL aqueous sample in 20 mL HS vials, thermostating at 50 °C for 30 min with agitation), are sufficiently low as to permit its application to the analysis of 18 priority VOCs in natural and waste waters. In all cases compounds were detected below regulated levels.

Keywords

- Needle-trap
- Headspace
- Wastewaters
- Adsorption
- VOC

3. 6. **"A headspace needle-trap method for the analysis of volatile organic compounds in whole blood"**

Alonso, M.; Castellanos, M.; Besalú, E.; Sánchez, J.M.

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Alonso, M., Castellanos, M., Besalú, E., Sánchez, J.M. “A headspace needle-trap method for the analysis of volatile organic compounds in whole blood”. Journal of Chromatography A. Vol. 1252, (24 August 2012) : p. 23-30

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Abstract

Needle trap devices (NTDs) are a relatively new and promising tool for headspace (HS) analysis. In this study, a dynamic HS sampling procedure is evaluated for the determination of volatile organic compounds (VOCs) in whole blood samples. A full factorial design was used to evaluate the influence of the number of cycles and incubation time and it is demonstrated that the controlling factor in the process is the number of cycles. A mathematical model can be used to determine the most appropriate number of cycles required to adsorb a prefixed amount of VOCs present in the HS phase whenever quantitative adsorption is reached in each cycle. Matrix effect is of great importance when complex biological samples, such as blood, are analyzed. The evaluation of the salting out effect showed a significant improvement in the volatilization of VOCs to the HS in this type of matrices. Moreover, a 1:4 (blood:water) dilution is required to obtain quantitative recoveries of the target analytes when external calibration is used. The method developed gives detection limits in the 0.020–0.080 μg L−1 range (0.1–0.4 μg L−1 range for undiluted blood samples) with appropriate repeatability values (RSD < 15% at high level and <23% at LOQ level). Figure of merits of the method can be improved by using a smaller phase ratio (i.e., an increase in the blood volume and a decrease in the HS volume), which lead to lower detection limits, better repeatability values and greater sensibility. Twenty-eight blood samples have been evaluated with the proposed method and the results agree with those indicated in other studies. Benzene was the only target compound that gave significant differences between blood levels detected in volunteer non-smokers and smokers.

Keywords

- Needle trap;
- Headspace analysis
- Blood
- Volatile organic compounds
- Matrix effect

3. 7. **"Evaluation and comparison of solvent-free concentration techniques for the analysis of volatile organic compounds in whole blood at sub-trace levels"**

Alonso, M.; Castellanos, M.; Sánchez, J.M.

Analyst, submitted

Alonso, M., Castellanos, M., Sánchez, J.M. “Evaluation and comparison of solvent-free concentration techniques for the analysis of volatile organic compounds in whole blood at sub-trace levels”. Analyst

Submited

Abstract

Solid-phase microextraction (SPME) and needle trap devices (NTs) are two solvent-free concentration techniques that potentially have many applications in the field of clinical medicine for the determination of volatile substances from biological fluids. In this study, an SPME sampling procedure is evaluated for the determination of volatile organic compounds (VOCs) in whole blood samples. Comparison of the method developed with an equivalent NT method to assess the relative sensitivity, efficiency and robustness of the two methods reveals that both have significant and similar matrix effects when blood samples are analyzed. Dilution of the biological sample with water is sufficient for quantitative recoveries of the target analytes to be obtained. Although greater analytical sensitivity is expected with SPME when small volumes of samples are evaluated due to its non-exhaustive nature, no differences between the two methods are observed (detection limits are in the tens of $ng \cdot L^{-1}$ in both cases). These results show the SPME method to have greater calibration sensitivity (determined from the slopes in the calibration curves) and, therefore, to have the advantage of being able to distinguish smaller changes in analyte concentrations. However, the analysis of blank samples shows the SPME method to have more problems. Moreover, the NT methodology has larger dynamic ranges than SPME and is not found to have significant day-to-day sensitivity changes. The new NT methodology seems to perform as well or better than the conventional SPME methodology.

Keywords

- Solid-phase microextraction (SPME)
- Needle trap (NT)
- Headspaceanalysis
- Blood
- Matrix effect

4. Global discussion

This chapter includes an overall discussion of the results summarized in the reports presented in the section 3. The results obtained can be divided into two main groups. The first is focused on the development of methodologies for the analysis of volatile organic compounds (VOCs) in the range of concentrations that can be inhaled by non-exposed people. The second is devoted to the application of the developed methodologies in different matrices, such as breath, indoor and outdoor air, and liquid samples.

4.1. Development of different micro-trap methodologies for the analysis of volatile organic compounds at ng·m^{-3} levels

The current analytical methodologies for gaseous samples in the field of atmospheric contamination require collecting large volume of samples to be able to reach appropriate detections limits for determining VOCs at the legislated levels. This is not a significant problem for air analysis but becomes problematic in those matrices where only small volumes of sample can be used (e.g., breath). Therefore, the development of new methodologies for gaseous samples that permit to reach very low detection limits with small volumes of sample is of great importance.

In a first stage, an in-house capillary thermal desorption unit coupled directly to a GC/MS for the analysis of breath and atmospheric air samples at low ng·m^{-3} levels was developed. This in-house concentrator/injector device was specifically designed for quantitatively retaining target VOCs and to achieve fast and quantitative desorption of the analytes directly to the GC column without the need for a second cryofocusing device. The micro-trap allows desorbed compounds being injected into the GC column as a narrow injection band, preventing the formation of broad peaks with tails in the chromatograms. The development of this device is described in detail in publication 1. It mainly consists in a three bed sorption trap, which allows the collection of gas samples and the preconcentration of the volatiles on the sorbents. During the analysis process a vacuum pump transport the sample from a sampling bag to the trap, while a flow of carrier gas, helium, is maintained in the GC/MS system. After sampling, desorption of target compounds is carried out using a fast current pulse that allows reaching temperatures around 300ºC in less than a second. Once the compounds are

desorbed, a flow of helium is allowed to pass through the trap to introduce the analytes inside the GC column as a narrow plug.

The system was evaluated for five target VOCs, which were supposed to be probable smoking biomarkers. The developed device has the great advantage of reaching very low detection limits by analyzing a small volume of sample (LODs in the $ng \cdot m^{-3}$, or pptv, with one liter samples). Once the methodology was validated, it was used to analyze different types of samples: breath from smoking and non-smoking individuals (publications 1 and 2), environmental air (publication 1), and indoor air from public premises to evaluate environmental tobacco smoke (ETS) contamination (publication 3). All the results obtained over these applications are summarized and discussed in the following section.

Despite the applicability of the proposed methodology and the good results obtained, it presents some drawbacks that should be considered. Firstly, it is not field portable, which results in a problem when samples have to be obtained far from the laboratory. Samples have to be collected in sampling bags and analyzed as soon as possible. Under these conditions the risk of losing compounds through the walls of the bag or sample contamination due to the diffusion of some compounds into the bags has to be minimized. Storage capability of the Tedlar bags used was evaluated (publication 3) and it was found that the time that a sample can be stored in the Tedlar bag depends directly on the compounds to be analyzed. For benzene, toluene, ethylbenzene and xylene isomers, there were no losses or losses under 5% during 24 h of storage and under 10% for a period of 48 h. 2,5-dimethylfuran showed losses of 5% during the first 3 h, and around 15% after 48 h. According with the results obtained, samples should be analyzed in less than 3 h if 2,5-dimethylfuran is a target compound. Another point to consider is that the in-house injector works fixed to a GC instrument, which means that the device is not simple to modify and change as it is necessary the use of specific connections.

Considering these drawbacks, the following research was focused in developing a most simple and portable microtrap that could be used in different chromatographic instruments in a simple and fast mode. A needle trap device (NTD) for on-site analysis of volatile compounds in gaseous samples was developed (publication 4). It consists on a stainless steel needle filled with different adsorbents depending on the target analytes.

The needles used are commercially available in different sizes and configurations. A previous study carried out by our research group compared results using 20 (20G) and 22 (22G) gauge needles. It was found that 22G needles gave better results due to their smaller inner diameter, which allows a better heating of the sorbent bed and the volatile compounds are introduced as a narrower band in the GC column. As reported in publication 4, two different needle configurations were evaluated, point style 2 needles, with an end hole, and point style 5 needles, with a side hole. According to other needle traps related publications, our first experiments were performed using point style 2 needles with epoxy resin in the tip to fix the adsorbents. The main drawback detected was the impossibility to control the amount of resin used to fix the sorbent particles inside the needle, which leads to a variable and non-reproducible flow from needle-to-needle, ranging from total blockage of the needle to a maximum flow of 40 $mL \cdot min^{-1}$. A further difficulty with the use of epoxy resin is the amount of impurities that are introduced in the GC system. After 24 h of conditioning at 300ºC some impurities appear in the chromatograms, which can interfere in the detection of compounds with large retention times. To improve these problems some experiments were developed using point style 5 needles. The use of this type of needles avoids the use of epoxy resin to fix the adsorbents. A problem observed was that a particle of adsorbent can be placed just in the hole and can partially obstruct the needle. This problem was avoided by placing a second spiral plug in the tip of the needle. In these conditions a more reproducible flow was obtained, ranging from 30 to 50 $mL \cdot min^{-1}$. A conditioning time of 2-3 h was enough and impurities were not detected in blank chromatograms.

One of the main advantages of NTDs is that the thermal desorption mechanism is equivalent to SPME. After sampling, the NTD is placed in the injection port of a GC at temperatures around 300ºC. Desorbed compounds are introduced into the column by the desorptive flow produced by the internal air expansion at the hot desorption temperatures of the GC injector. It is important to achieve high linear flows to remove the desorbed compounds from the injector and introduce them into the column as a narrow band. Two different liners dimensions, with 1 and 3 mm i.d., were evaluated. It was found that improvement peak resolution is obtained as the liner inner diameter decrease. Differences between the outer diameter of the needle and the inner diameter of the liner are excessive for the 3 mm i.d. liners, which results in a dispersion of the desorbed analytes before entering to the column, giving large injection bands and poor

resolution. Using the 1 mm i.d. liners, differences are reduced and no significant dispersion of the analytes is observed, so narrow bandwidths are obtained with appropriate peak resolution.

Sampling time is correlated with the amount of sample required for reaching required LODs. Therefore, high flows will permit the use of shorter sampling times. The evaluation of the method precision at different sampling rates showed that sampling flow rates ≤15mL/min are required to obtain reproducibility values <15%. The small diameter of the needles results in high linear flows inside the trap and an incomplete adsorption of the analytes in the adsorbent material is produced at large sampling flows. The storage stability of the compounds in the NTD was also evaluated. Recoveries ranging from 91-110% were obtained for all compounds for storage times up to 48 h, which confirms the field applicability of the NTDs.

LODs for gaseous samples ranged from 4 to 10 $ng \cdot m^{-3}$. It is important to note than LOD for benzene is three orders of magnitude lower than the value regulated in the European Union for human health, which means that 10 mL of sample are enough to analyze benzene below this regulated level in ambient air samples.

The applicability of breath analysis for monitoring exposure to toxic substances requires the analysis of some body fluid to confirm that these toxic substances can affect human health. Thus, a method for determining the compounds evaluated in gas samples is needed for their assessment in a body fluid, in this case blood. When VOCs in liquid samples are analyzed the headspace technique is the most common alternative, which can be used in combination with the previously developed NTDs. Four different head-space (HS) methodologies coupled with the NTD were evaluated and compared with a conventional HS method (publications 5 and 6): (i) static HS sampling using the NTD to collect a fix volume of the head-space (HS-NTD), (ii) static HS with passive diffusion through the NTD (pHS-NTD), (iii) active HS sampling with the NTD (PT-NTD), and (iv) dynamic sampling using various sorption cycles. The use of sampling temperatures as low as possible is recommended with NTDs as competitive desorption of the most volatile compounds can take place at increased temperatures when large sampling times are used. Best results, in terms of analytical sensitivity, were obtained for the PT-NTD method and the dynamic sampling using various sorption cycles. The

pHS-NTD sampling is, however, not recommended for routine analysis as it requires a complex instrumentation to obtain reproducible results. HS-NTD and dynamic sampling using various sorption cycles were evaluated for routine analysis of liquid samples, with LODs in the ng·L^{-1} level.

In order to demonstrate the utility of the proposed NTD methodology, a conventional SPME procedure was evaluated and compared with the proposed NTD method. (publication7). The results confirm that both, SPME and NTD, are solvent-free techniques with a great potential in clinical analysis to determine volatile compounds. Although better results could be expected for SPME due to its non-exhaustive nature and the fact that low volumes of blood samples can be used, the comparison of both methods did not show significant differences. A significant matrix effect is observed in both techniques due to the complexity of the biological matrices. A simple procedure based on sample dilution solves the matrix effect and permits to obtain quantitative recoveries of the target analytes for both methods. The dilution of samples leads to an increase of the limits of detection but this problem can be overcome by decreasing the phase ratio during the HS process and, in the case of NTD, with an increase of the number of cycles used.

Both methods present equivalent analytical sensitivity for small sample volumes, with LODs in the tens of ng·L^{-1}. SPME presents better calibration sensitivity and it is able to distinguish small different concentrations. However, the better calibration sensitivity of SPME leads to a significant problem when blanks are analyzed: benzene and toluene are always detected in water blanks. The NTD methodology presents some important advantages, such as this technique does not present problems with blank analysis, achieves larger dynamic ranges, and does not show significant sensitivity changes with day-to-day. It can be concluded that NTD seems a more robust technique than SPME.

4.2 Applications of the developed micro-trap devices

Gas samples

First approximation with real samples was using the in-house thermal desorption unit. Two studies were developed with both breath and ambient samples. Breath study was

developed with the objective of finding a breath biomarker of the smoking status (publication 2). Breath samples of 204 healthy adult volunteers were collected and analyzed immediately after collection using the capillary thermal desorption unit coupled with a GC/MS. Data was evaluated using non-parametric statistical tests. Background effects were evaluated analyzing indoor air of the places where the breath samples were taken. Results show significant differences in the concentration of the whole population between smokers and non-smokers for the VOCs studied. The evaluation by gender did not show significant differences between the levels detected in male and female smokers for any of the evaluated compounds. When considering the effect of daily consumption of cigarette, a significant but weak correlation was found between the number of cigarettes smoked and the levels of the compounds. Best results as a biomarker were obtained for benzene and 2,5-dimethylfuran, as these two compounds were the only ones that showed significant differences when considering only light smokers. Xylene isomers and toluene were only effective biomarkers for recent exposure to tobacco smoke: they are able to determine the smoking status only after 45 minutes of the last cigarette smoked. Although benzene is useful for light smokers and for a long period after smoking (12-13 h), its applicability is also limited. Overall, the best results are obtained for 2,5-dimethylfuran, which gives significant differences between smokers and non-smokers after more than 48 h without smoking. Moreover, it is the only compound that is able to detect passive smokers. The method allowed us to evaluate the effect of smoking controlled substances in its use as smoking biomarker. 2,5-dimethylfuran was always detected in individuals who smoke drugs combined with tobacco, but this compound was not detected in those individuals who only smoke cannabis, without mixing with tobacco.

Considering that this compound could be used for assessing passive smoking, the next approach was to evaluate 2,5-dimethylfuran for determining ETS contamination in public premises and its effect on passive smokers working in these premises (publication 3). Field study was carried out on 56 premises: smoking was allowed on 41 and forbidden on 15. Breath samples of non-smoking employees were collected to evaluate the effect of ETS in passive smokers. Background effect was also evaluated analyzing outdoor air samples surrounding the smoking and non-smoking premises. Statistical significant differences were obtained between smoking and non-smoking premises and also between smoking premises and outdoor air for the target VOCs.

Comparing non-smoking premises and outdoor air, only significant differences were obtained for benzaldehyde, 2-ethyltoluene and ethylbenzene. The best results were obtained for 2,5-dimethylfuran. Moreover, this is the only compound that can be used as a qualitative marker of ETS contamination. Indoor/outdoor ratios for smoking premises confirmed that ETS is the most significant source of contamination in these environments. Premises evaluated could be classified in two groups, bar/cafés and restaurants. Significant differences were obtained between these groups, variability and mean values were higher for bar/cafés. Other parameter considered in this study was the seasonal variability. Two groups of samples were obtained, one during end of summer/beginning of autumn and a second group during winter. All the parameters evaluated such temperature, indoor relative humidity and all the VOCs except ethylbenzene and 2-ethyltoluene present significant differences between different seasons. Main reason of these results is that in winter windows and doors are closed and a heater system is working; in summers windows and doors are usually opened are there is an air change ratio between indoor and outdoor. The hypothesis is that air change/extraction has the largest effect on the levels of VOCs measured, which agrees with studies developed by other authors.

The possibility to perform very sensitive analysis in short periods of time permitted to evaluate daily variations of indoor air contaminated in different premises. Concentrations of VOCs vary considerable during the day and its maximum concentration obtained agrees with their activity: for café/restaurant maximum concentrations is in the morning when people go to breakfast but concentration decrease during lunchtime due people is eating; for regular cafés the major peak is obtained at after lunchtime when people go to have a coffee and a cigarette. Finally, the breath of passive smokers working in smoking premises was evaluated. Breath samples were taken every 1.5 h and were evaluated for 2,5-dimethylfuran. The compound was not detected during the 3 first hours of working in the premises, but it was detected in consecutive samples over a period of 4 h. These results also confirm 2,5-dimethylfuran as a breath biomarker of exposure to ETS for passive smokers. The results obtained in this study give a new approach for indoor air quality. Benzene on its own cannot be used as a marker for smoking as it has other different sources of origin, but correlates with 2,5-dimethylfuran in smoking premises.

Another study was developed to evaluate the contamination in the laboratories of our faculty and the effect in the workers' breath (publication 1). Acetone, ethyl acetate, hexane and methylene chloride were evaluated due they are major solvents used in the laboratories evaluated. Levels of these compounds in working ambient air were evaluated during all the worksheet. In the morning, concentrations were low because during the night nobody was working there; then concentration increase during all the day except at midday during lunch time when they slightly decrease. Breath of workers from these laboratories and workers from surrounding laboratories was also collect. The same contaminants could be identified in all cases, which indicate poor safety conditions, but concentrations of employees working in contaminated laboratories were significantly higher than those for the other workers.

NTDs were also evaluated for the analysis of gas samples. Firstly, the NTDs were evaluated for the same type of samples examined with the in-house micro-trap (breath samples for smoker and non-smokers, indoor air and outdoor air). Results obtained did not show significant differences with those obtained with the first methodology developed. However, the comparison of the two developed methodologies indicates that the in-house micro-trap has a better performance when working with a large volume of gas sample. For breath samples, alveolar breath sampling was compared with mixed expiratory breath sampling, but 2,5-dimethylfuran was only detected in the case of mixed expiratory sampling. It is attributed to the low concentration of this compound in the breath, which requires a large volume of sample. The volume of alveolar breath collected in every expiration process is not enough to collect an amount of this compound that can reach the detection limit of the method. The in-house micro-trap has lower pneumatic restrictions (larger i.d.) than the NTD, which allows sampling at higher flow rates (i.e., significant reduction in the sampling time), and higher amounts of sorbent materials can be used, which gives larger breakthrough volumes. Moreover, the in-house micro-trap is more robust than the NTDs and can be used without significant variations in sensitivity and precision for much more samples (it has been applied for more than 1000 samples when the best NTD only has been useful for ~200 samples).

Liquid samples

Other application evaluated with the NTD methodology is the analysis of VOCs from liquid samples applying the head-space sampling. It has been evaluated for the analysis

of natural and waste water samples (publication 5) and for the analysis of a complex biological fluid, such as blood (publications 6 and 7).

In the case of water samples (publication 5), three urban WWTPs were evaluated, together with different mineral, natural and tap waters. The results obtained are in agreement with those reported in the literature and confirmed the fact that all the WWTPs only received domestic wastewaters, with no industrial inputs. It was found that the levels of VOCs at the effluent of the plants represent a significant reduction of these compounds except for benzene, which showed similar levels at all sampling points in all the WWTPs. These results confirm the applicability of the proposed NTD methodology for the analysis of liquid samples.

In the case of blood samples (publication 6), the NTD sampling at different cycles was used to evaluate blood samples for smokers, non-smokers and former-smokers for several target compounds. It was determined that the percentage of extraction can be calculated if a quantitative adsorption of all VOCs is produced in each sampling cycle. Therefore, the use of 20 cycles results in the collection of ~70% of the VOCs present in the gaseous phase of the vials with the phase ratio proposed. The need to dilute the samples to avoid matrix effects results in relatively large LODs for the adequate quantification of all VOCs, but this problem can be overcome by decreasing the phase ratio and analyzing a larger volume of blood samples.

Conventional SPME was also evaluated for blood samples and the results were used to validate the NTD method (publication 7). Equivalent results were obtained with both methods, which confirmed the applicability of the NTD method. The NTD method seems to be more robust than SPME.

4.3 General conclusion

The overall results obtained suggest that the new NTD methodology is appropriate for both liquid and gaseous samples. These new needle traps are very robust, yield very good sensitivity and does not require any extra and sophisticated instrumentation.

In the case of gaseous samples, NTDs have the limitation that only small sample volumes can be analyzed without significant instrumentation problems due to the limited sampling flow achieved with these devices. When VOCs at very low concentration have to be analyzed (in the range of few $ng \cdot m^{-3}$) the in-house micro-trap performs better as large sample volumes have to be collected. The main problem associated with the use of the micro-trap is that it is not field portable and samples have to be collected in sampling bags, which increases the possibility of artifacts formation.

For the analysis of VOCs in liquid samples, the NTD methodology works perfectly and gives equivalent results to those obtained with other conventional solvent-free methodologies, such as SPME and purge-and-trap.

4.4. Future trends

Once the applicability of the new micro-trap methodologies has been demonstrated, the future work in this field should be directed to the use of these methodologies in real exposure studies. A first step will be the study of correlations between VOC levels detected in breath and blood samples from the same exposed and non-exposed individuals. Positive correlations will confirm the applicability of breath analysis as an adequate, simple and non-invasive methodology to determine the exposure to volatile contaminants. Taking into account the results obtained, blood samples would be better analyzed using the cHS-NTD methodology. In the case of breath samples, the in-house micro-trap seems to be the most appropriate analytical methodology.

It also would be interesting redirect the research to clinical applications. Once the applicability for finding reliable biomarkers of exposure has been demonstrated, it would be interesting to study possible disease biomarkers. In this sense, a study in collaboration with the neurology unit of the University Hospital Dr Josep Trueta of Girona is currently being developed to assess the real smoking activity of different individuals that are participating in a clinical study that evaluates the effect that smoking habits can have on stroke.

5. Conclusions

According to the results obtained in the present thesis, the main conclusions are:

1. The developed in-house concentrator/injector coupled to a GC/MS instrument gives simplicity, high sensitivity, and is a powerful and robust methodology for the analysis of VOCs in breath and atmospheric samples at sub-ppbv levels.

2. The in-house micro-trap has been successfully applied in the analysis of air samples from different environments and for the analysis of breath samples of people in contact with these environments.

3. A study developed on 204 smoking and non-smoking volunteers concluded that 2,5-dimethylfuran is a very selective breath biomarker of the smoking status. This compound has permitted to detect the contamination coming from environmental tobacco smoke in passive smokers.

4. The evaluation of environmental tobacco smoke contamination in public premises indicated that 2,5-dimethylfuran is an appropriate and effective marker of ETS contamination. This compound was only detected in environments where people have smoked and cannot be attributed to other sources of contamination, as traffic or coffee aroma.

5. Needle microextraction traps have been developed to obtain a portable device for VOC determination in gas and aqueous samples. This is a simple, relatively inexpensive, field-portable, and robust device that can be introduced directly to a conventional GC injector without requiring any additional instrumentation.

6. The evaluation of the needle traps performance has demonstrated their high efficiency and sensitivity, which permits to obtain limits of detection in the range of $ng \cdot m^{-3}$ with small volumes of gaseous samples.

7. The combination of the needle trap devices and headspace sampling techniques gives an improved method sensitivity for the analysis of liquid samples, allowing LODs in the range of $ng \cdot L^{-1}$ to be reached.

8. Different sampling procedures have been evaluated for HS-NTD analysis of liquid samples, being conventional static head-space sampling and dynamic sampling using various sorption cycles the most effective taking into account the combination of sensitivity and simplicity in the instrumentation required.

9. The NTD and SPME techniques have been evaluated and compared for the analysis of VOCs in blood samples in order to study whether the inhaled exogenous compounds found in breath samples can reach body fluids. Both methods gave equivalent results in terms of sensitivity, but the new NTD methodology is more robust and simple to use.

10. The in-house microtrap have demonstrated to be more adequate for gas samples when large volume of sample have to be collected as it presents lower pneumatic restrictions. This procedure has the drawback that the samples have to be previously collected in a sampling device and analyzed as soon as possible to avoid artefacts from the sampling storage.

11. The needle traps are powerful devices for the analysis of volatile compounds from liquid samples and also in gas samples when small amounts of samples are evaluated.

6. Acknowledgments

Aquesta memòria més enllà del contingut purament científic és un recull de vivències i experiències viscudes durant quatre anys intensos de recerca al costat dels meus companys i companyes, tant becaris com doctors, i del meu director de tesis Juan Manuel Sánchez. Es per això que en primer lloc m'agradaria agrair al meu director la seva constant implicació en el nostre projecte i l'esforç dedicat a que tots aquests anys de recerca arribessin a bon port. Fruit de tot això n'ha sortit aquest llibre i el conjunt publicacions englobades en aquest treball. També voldria esmentar particularment la doctora Enriqueta Anticó i la meva companya i amiga Anna Godayol que, com molts sabeu, formen part del nostre grup treball i en molts moments ens hem ajudat mútuament a vèncer petits entrebancs, trobar solucions i seguir endavant. També m'agradaria donar les gràcies als membres del LEQUIA, Maria Martínez i Ester Vega, que en diverses ocasions han col·laborat amb nosaltres oferint-nos part del seu material i equip de laboratori i per això crec que també mereixen un lloc en aquest apartat.

M'agradaria agrair també a les meves companyes de laboratori el seu suport al llarg d'aquest quatre anys; de fet no és just parlar de companyes ja que realment el meu sentiment és d'amistat. Especialment vull agrair a la Carme i l'Ester el fet que elles sempre estiguessin allà quan hi havien de ser i m'han ajudat a passar tant els bons com els mals moments. Amb elles he compartit els meus anys de recerca quasi be des de l'inici i em passat un munt d'experiències divertides i d'altres de no tant, però que han donat lloc a la creació d'una bona amistat que espero que segueixi així independentment del camí que segueixi cadascuna. També vull anomenar de nou l'Anna, la Raquel, l'Aida i la Dolors les quals han estat també sempre presents. Totes juntes hem passat per moltes anècdotes divertides, molts sopars, alguns viatges, casaments i també moments de tensió; totes aquestes històries estaran sempre al meu record. Finalment faltaria mencionar la meva Laureta, amb la qual hem compartit tres mesos molt divertits i ens els quals ens em ajudat molt mútuament, gràcies.

Finally, I would like to thank W. Miekisch and J. Schubert for my wonderful stay in Rostock during three months. They taught me a lot of things related to breath analysis, which were completely unknown for me at that moment and helped me in my following research during my next two PhD years. Also I would like to mention here my mates Phillip, Ulrike and Julianne who make my stay there really comfortable and I will have them always in my mind.

Per acabar donar les gràcies a tota la resta de companys Clàudia, Eva, Mònica I., i la resta de membres d'àrea de la facultat i del parc per haver estat sempre allà.

També m'agradaria nombrar la meva família i el suport de la meva germaneta Olga.

Moltes gràcies a tots.

7. Annexes

In this section I would like to add two more papers related with the present Thesis.

The first is a paper written in Catalan, which is a summary of all the work done developing the needle trap devices. This paper will be published in the 2012 issue of the "Revista de la Societat Catalana de Química". This is an invited paper because I was awarded with the prize of the best presentation in the field of Analytical Chemistry in the annual conference of "Young Researchers of the Catalan Society of Chemistry" held in Mallorca in February 2012.

The second is a review sent to the journal "TRAC, Trends in Analytical Chemistry" that covers all the aspects devoted to the sampling collection and preconcentration tecnhiques in breath analysis. It is a part of the introduction section in the present Thesis.

Annex A. **New methodologies for the analysis of volatile compounds using needle traps. Applications to breath, atmospheric and water analysis.**

Mònica Alonso, Anna Godayol, Enriqueta Anticó, Juan M. Sánchez.

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SCQ

Noves metodologies d'anàlisi de compostos volàtils mitjançant trampes d'agulla. Aplicació a l'anàlisi d'alè, atmosfèrica i d'aigües.

New methodologies for the analysis of volatile compounds using needle traps. Applications to breath, atmospheric and water analysis.

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Rebut: xx/xx/xx Acceptat:xx/xx/xx

Resum. S'ha desenvolupat una nova tècnica de preconcentració per a compostos volàtils basada en trampes d'agulles. Les trampes d'agulla consisteixen en agulles d'acer inoxidable farcides amb un o varis adsorbents, el que permet la preconcentració dels analits que flueixen pel seu interior. S'han estudiat els diferents paràmetres que afecten al procés de sorció/desorció (dimensions de les agulles i de la cambra de vaporització, temperatura de l'injector, temps sense divisió de flux, efecte memòria i estabilitat d'emmagatzematge). En el cas de mostres líquides, on cal acoblar les trampes agulles amb la tècnica d'espai de cap, s'han avaluat quatre modalitats de pressa de mostra, tant actives com passives. La metodologia mes adequada en quant a simplicitat i sensibilitat és la pressa de mostra de l'espai de cap emprant varis cicles de pressa de mostra d'un volum petit i fix. Una vegada trobades les millors condicions d'anàlisi, el mètode ha estat validat tant per mostres gasoses com líquides. Els resultats obtinguts indiquen que les trampes d'agulla són una nova metodologia vàlida per a l'anàlisi de mostres gasoses (p.e., alè i ambientals) i líquides.

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Paraules clau: trampes d'agulla, espai de cap, anàlisi d'alè, anàlisi atmosfèrica, anàlisi d'aigües.

Abstract. A new preconcentration technique has been developed for the analysis of volatile compounds based on the use of needle traps. These traps are based on stainless steel needles filled with one or more adsorbents, which allows the preconcentration of the analites inside the trap by passing a gas flow through the needle. The parameters affecting the sorption/desorption process have been assessed (e.g. needle and liner dimensions, injector temperature, splitless time, memory effects, and stability inside the needle). For liquid samples, four different sampling methodologies were studied, including passive and active sampling methods. The best results, considering the simplicity and sensitivity, are obtained by sampling the headspace volume using various cycles of a small and fix volume. Once the best conditions of analysis have been found, the method has been validated for gas and liquid samples. The results obtained show that needle traps are a good analytical methodology for the analysis of breath, environmental and liquid samples.

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Keywords: needle traps, headspace, breath analysis, atmospheric analysis, water analysis.

Introducció

Els compostos orgànics volàtils, coneguts habitualment sota les sigles angleses VOCs, són una de les principals famílies de contaminants atmosfèrics. La determinació de la contaminació ambiental per VOCs s'ha destacat durant anys per l'efecte que presenten com a precursors a la generació d'ozò, conjuntament amb els òxids de nitrógen. Avui dia, és àmpliament reconegut que els VOCs formen una de les principals famílies de contaminants atmosfèrics i són components clau en mostres ambientals, industrials i biològiques [1-4]. Aquest compostos són nocius per a la salut humana i la principal problemàtica que presenten és que els mecanismes pels quals s'incorporen a l'organisme i actuen de forma nociva són poc coneguts. Es coneixen efectes neurotòxics, organotòxics i carcinogènics a nivells d'exposició alts [5]. A nivells d'exposició mitjans i baixos es poden produir irritacions sensorials. No obstant, a nivells d'exposició baixos els efectes són pràcticament desconeguts degut a la baixa concentració dels compostos i la dificultat per ser analitzats [6]. Tot i la falta d'evidències de risc per a la salut als nivells habitualment detectats en ambients no industrials, alguns VOCs son carcinogènics (p.e. benzè), genotòxics o poden ser alergènics, pel que poden tenir efectes negatius sobre la salut humana [7]. En general, els riscs per a la salut associats a l'exposició a VOCs han anant augmentat a mesura que s'ha incrementat el consum de productes derivats del petroli.

La majoria de les metodologies analítiques actuals per a l'anàlisi de VOCs han estat desenvolupades per analitzar la contaminació en ambients exteriors i no són adequades per determinar la pol·lució en ambients interiors degut al seu cost, mida i quantitat d'aire que desplacen [8]. Cal desenvolupar noves metodologies analítiques que permetin assolir límits de detecció (LODs) inferiors a 1 $\mu g \cdot m^{-3}$ [5,9]. Actualment encara hi ha pocs estudis on es mesuri directament l'exposició

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resultant en ambients poc contaminats [10,11].
Una de les metodologies més innovadores que s'ha proposat darrerament per suplir aquests dèficits són les trampes d'agulla farcides amb adsorbents. La principal característica d'aquestes trampes és l'elevat factor de preconcentració que es pot assolir utilitzant un volum de mostra molt reduït [11]. Les principals avantatges de les trampes d'agulla són que es tracta d'una tècnica lliure de solvents, els temps de pressa de mostra i anàlisi són reduïts, tenen un gran potencial com a tècnica de cribatge (*screening*), són robustes, el procés de presa de mostra i desorció és simple (no es requereix cap instrumentació addicional), són fàcils d'automatitzar i tenen capacitat per realitzar la presa de mostra *on-site*. Tot i això, també presenten alguns inconvenients, com són la limitada capacitat de mostra ja que la quantitat d'adsorbent és petita i pot provocar una saturació ràpida de la trampa, i el fet que les zones d'elució són lleugerament disperses.
Els objectius del present estudi són el disseny de trampes d'agulla farcides amb adsorbents que permetin obtenir elevats factors de preconcentració dels compostos d'interès i la seva aplicació a mostres d'alè, atmosfèriques (tant d'ambients interiors com exteriors) i d'aigües de depuradores.

Experimental

Materials

Els adsorbents utilitzats són Tenax TA, Carbopack X i Carboxen 1000 (Supelco, Bellefonte, PA, USA). A la Taula 1 es pot veure un resum de les seves principals característiques.

Taula 1. Adsorbents utilitzats a les trampes d'agulla i les seves principals característiques.

Adsorbents	Estructura química	Temperatura límit (ºC)	Àrea superficial (m^2/g)	Densitat (g/cm^3)	Mida de malla	Rang de compostos
Tenax TA	Polímer porós	350	35	2.5	60/80	C7-C26
Carbopack X	Carbó negre grafititzat	400	240	0.44	60/80	C3-C5
Carboxen 1000	Tamisos moleculars	400	1200	0.44	60/80	C2-C5

Els compostos avaluats amb les trampes d'agulla són els que es mostres en la Taula 2. S'han estudiat un total de 22 analits en tres tipus diferents de mostres: alè, atmosfèriques i aigües.

Trampes d'agulla: tipus de suport i preparació.

Suport per a les trampes

Les agulles per preparar les trampes d'adsorció es troben assequibles comercialment en diferents formats de diàmetre, tant intern com extern. S'han avaluat agulles de calibre 20 (20G: 0.91 mm o.d., 0.60 mm i.d.) i calibre 22 (22G: 0.71 mm o.d., 0.41 mm i.d.). Independentment de la dimensió de l'agulla seleccionada, aquestes poden presentar configuracions diferents pel que fa a la forma de la punxa i la ubicació del forat. S'han seleccionat dos tipus diferents per al seu estudi, les de punta bisellada amb forat a l'extrem (punta tipus 2) i les de punta cònica amb forat lateral (punta tipus 5). Totes les agulles van ser obtingudes de Hamilton (Bonaduz, Suïssa), amb una longitud de 51 mm en tots els casos.

Taula 2. Llistat dels compostos orgànics volàtils estudiats. S'ha marcat amb una creu els compostos avaluats en cada tipus de mostra.

			MOSTRES		
	Compostos	Masses específiques	Alè	Ambientals	Aigües
1	Diclormetà	84, 86			X
2	Cloroform	83, 85			X
3	Benzè	78	X	X	X
4	2,5-dimetilfuran	96	X	X	
5	1,2-diclorpropà	63, 112			X
6	Toluè	91, 92	X	X	X
7	Clorbenzè	112, 114			X
8	Etilbenzè	91, 106	X	X	X
9	p-xilè	91, 106	X	X	X
10	o-xilè	91, 106	X	X	X
11	Estirè	103, 104	X	X	
12	Propilbenzè	91, 120			X
13	2-clorotoluè	91, 126			X
14	4-clorotoluè	91, 126			X
15	Benzaldehid	106	X	X	
16	2-etiltoluè	105, 120	X	X	X
17	n-butilbenzè	91, 134			X
18	Acetofenona	105, 120	X	X	
19	1,2-diclorobenzè	146, 148			X
20	1,2,4-triclorobenzè	180, 182			X
21	Naftalè	128			X
22	1,2,3-triclorobenzè	180, 182			X

Preparació de les trampes

Per a la preparació de les trampes amb agulles tipus 2 s'introdueix inicialment un espiral metàl·lic que es posiciona al cos de l'agulla a una distància predeterminada en funció de la quantitat d'adsorbent que s'hagi d'ubicar a l'interior de l'agulla; a continuació s'introdueixen els adsorbents amb l'ajut d'una bomba de buit; finalment es col·loca un segon espiral metàl·lic o reïna epoxi a la punta de l'agulla per fixar-ho tot a l'interior. En el cas d'agulles tipus 5, no s'utilitza reïna epoxi a la punta. A la Figura 1 es mostra un esquema d'aquestes agulles.

Anàlisis per GC/MS

La separació cromatogràfica dels components es realitza amb una columna de 30 m de longitud Zebron-5 ms amb 0.25 mm i.d. i 0.25 µm de gruix de film (Phenomenex, Torrance, CA, USA) per a les mostres gasoses, i amb una columna de 30 m de longitud TR-Meta.VOC amb 0.25 mm i.d. i 1.5 mm de gruix de film

(Teknokroma, Barcelona, España) per a les mostres líquides. S'utilitza un cromatògraf de gasos Focus GC (Thermo Scientific, Waltham, MA, USA) acoblat a un espectròmetre de masses (DSQ II, Thermo Scientific).

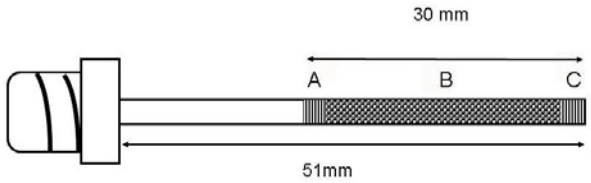

Figura 1. Esquema d'una trampa d'agulla. A: espiral metàl·lic; B: sorbents; C: reïna epoxy (agulles de punta 2) o espiral metàl·lic (agulles de punta 5).

Les cambres de vaporització (liners) de 1 i 3 mm de diàmetre intern (diàmetre extern de 8.0 mm i 105 mm de longitud) s'obtenen de Restek (Bellefonte, PA, USA). La temperatura de l'injector es manté a 300ºC per tenir una desorció ràpida i quantitativa dels compostos d'interès.

El programa de temperatures del forn consisteix en una temperatura inicial de 40ºC que es manté durant 2 min, seguit d'una rampa de 15ºC/min fins a 250ºC on s'hi manté durant 2 min (mostres líquides), o bé d'una rampa de 10ºC/min fins a 225ºC on s'hi manté durant 2 min (mostres gasoses). S'utilitza heli com a gas portador amb un flux a l'entrada constant de 0.8 mL/min. L'anàlisi per MS es realitza en mode d'escombratge de tots els ions (full-scan) en un rang de 40-200 uma. Es va utilitzar ionització d'impacte electrònic a 70 eV i la línia de transferència es va mantenir a 230ºC. La informació cromatogràfica s'adquireix amb el software Xcalibur (v.14, Thermo Electron).

Resultats i discussió

Avaluació de diferents materials com a suport per fixar els adsorbents a l'interior de les trampes

Primerament es va avaluar l'efecte que el tipus de material utilitzat per fixar els adsorbents a l'interior de les agulles té sobre la reproductibilitat dels resultats i els blancs obtinguts. Els primers experiments es van iniciar utilitzant agulles de punta tipus 2 i reïna epoxi ja que era la tècnica mes àmpliament utilitzada en estudis previs [13-20]. S'ha comprovat que aquest sistema de preparar les trampes presenta dos inconvenients importants. En primer lloc, la reïna epoxi conté una gran quantitat de compostos semi-volàtils en la seva composició que són difícils d'eliminar durant l'etapa de condicionament i segueixen apareixent en els blancs, fins i tot després de 24 hores de condicionament a 300ºC, de manera que poden interferir amb compostos que presentin temps de retenció similars. La segona problemàtica associada a l'ús de reïna epoxi és la dificultat per controlar la quantitat que se'n diposita a l'extrem de l'agulla. Això dóna lloc a una variabilitat elevada en els fluxos que es poden obtenir amb les agulles, anant des d'un bloqueig total de l'agulla degut a una quantitat excessiva de reïna, fins a fluxos de 40 ml·min^{-1}.

L'ús d'espiral metàl·lic en els dos extrems del llit d'adsorbents elimina el problema de la contaminació i simplifica el procés de condicionament: 2 hores a 300ºC és suficient per condicionar les trampes i obtenir blancs nets. A més, la substitució de les agulles de punta tipus 2 per les de tipus 5 elimina problemes associats a la obturació de les agulles provocades per petites peces de sèptum que es poden despendre durant les injeccions al GC i que tenen lloc més sovint en el cas de les agulles amb forat bisellat a l'extrem.

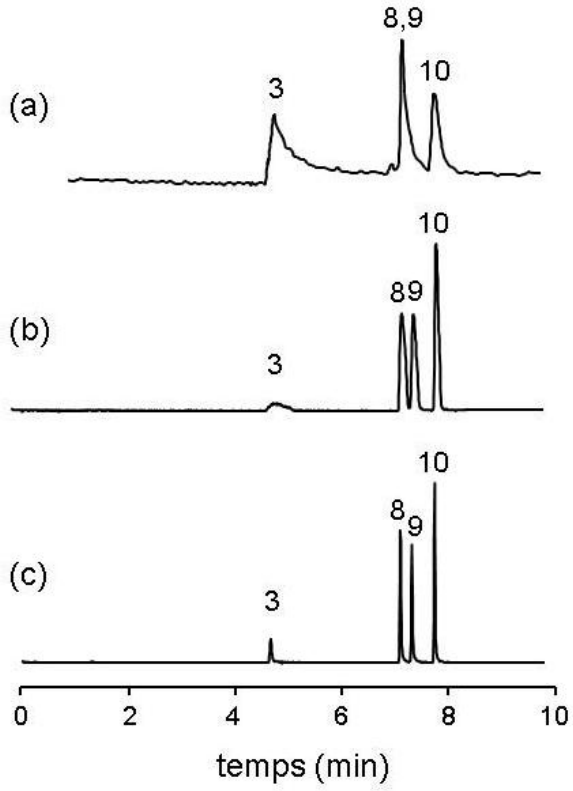

Figura 2. Forma dels pics i resolució obtinguda en la determinació de VOCs utilitzant agulles i cambres de vaporització (liners) de diferents dimensions. (a) agulla 20G i liner de 3 mm i.d.; (b) agulla 22G i liner de 3 mm i.d.; (c) agulla 22G i liner de 1 mm i.d. La numeració dels pics corresponen als números dels compostos a la Taula 2.
Condicions experimentals: sorbent, Carbopack X; temperatura injector, 300ºC.

Amb l'ús d'agulles de forat lateral (tipus 5), la variabilitat de fluxos ve donada només pel farciment dels adsorbents a l'interior de les agulles. Tot i que aquest procés és manual i es podria millorar significativament una vegada automatitzat, els fluxos que s'obtenen oscil·len entre 35 i 55 ml·min^{-1}, sense que tingui lloc en cap cas l'obturació total de les trampes. Tenint en compte aquestes consideracions, es recomana treballar amb agulles de punta tipus 5 i evitar l'ús de reïnes epoxi [21].

Dimensions de les trampes d'agulla i de la cambra de vaporització (liner)

S'han avaluat agulles de calibre 20 i 22. Les agulles de calibre 20 tenen l'avantatge que al presentar un diàmetre

intern més gran l'empaquetament i preparació de les trampes és més senzill. No obstant, com més gran és el diàmetre intern, més lenta és la transferència de calor a l'interior de l'agulla cap als adsorbents, el que dóna lloc a una desorció més lenta dels compostos i a l'obtenció de bandes amples als cromatogrames (Figura 2a). Aquest problema no és tan acusat amb les agulles de calibre 22 i les bandes d'injecció que s'obtenen són prou estretes per obtenir separacions eficients en els cromatogrames (Figura 2b).

Una de les grans avantatges de les trampes d'agulla és que el procés de desorció tèrmica és equivalent al que té lloc amb la microextracció en fase sòlida (SPME) i es produeix directament a l'interior de l'injector del GC sense la necessitat de cap instrumentació suplementària. Cal obtenir fluxos linears elevats a l'interior de la cambra de vaporització al voltant de l'agulla per desplaçar ràpidament els compostos desorbits de l'injector cap a la columna cromatogràfica com una banda d'injecció estreta. Per aconseguir aquest efecte, cal reduir el diàmetre de la cambra de vaportització utilitzada. La utilització de cambres amb 1 mm i.d. dóna lloc a una millora significativa de les amplades de pic i de la resolució cromatogràfica (Figura 2c) [21] .

L'expansió del flux de desorció que s'obté a l'incrementar la temperatura al cos de l'agulla fins a la temperatura de l'injector s'ha demostrat que és suficient per donar lloc a un flux a l'interior de l'agulla que transporta tots els analits cap a la columna cromatogràfica [16,21]. En el cas d'agulles de calibre 20 aquest flux és encara insuficient i és necessari l'ús d'un gas auxiliar per ajudar a transportar ràpidament els compostos desorbits cap a la columna. Tenint en compte els resultats obtinguts, es recomana treballar amb agulles de calibre 22 i cambres de vaporització de 1 mm i.d.

Comportament de les trampes d'agulla

Temperatura del injector

S'han avaluat diferents temperatures de treball de l'injector, cobrint el rang de 200 a 300ºC (Figura 3). A les temperatures més baixes avaluades (fins a 225ºC) s'observa la formació de cues per a tots els compostos degudes a una desorció lenta que provoca que aquests entrin com a bandes d'injecció excessivament amples a la columna. A partir de temperatures de 250ºC en el cas del Tenax i 280ºC en el cas del Carbopack X i Carboxen 1000 no s'observa la formació de cues i s'obté una bona separació dels compostos. Tenint en compte aquests resultats, es recomana treballar a una temperatura de desorció màxima de 300ºC. Temperatures més elevades no són adequades degut a què els adsorbents polimèrics tenen un límit d'estabilitat tèrmica al voltant d'aquesta temperatura i que estudis previs han demostrat que la descomposició dels compostos tèrmicament més làbils (principalment els terpens) no és significativa fins a uns 300ºC, però s'incrementa exponencialment a partir d'aquesta [22,23].

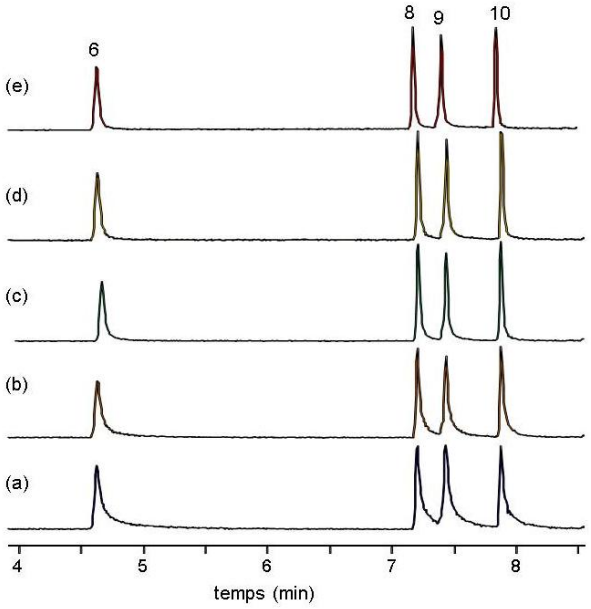

Figura 3. Cromatograma mostrant la forma dels pics i la resolució obtinguda a la separació d'una barreja de VOCs a diferents temperatures de desorció. Les temperatures de l'injector (desorció) van ser 200 (a), 225 (b), 250 (c), 280 (d) i 300ºC (e). La numeració dels pics corresponen als números dels compostos a la Taula 2. Sorbent: Tenax TA.

Temps sense divisió de flux (splitless)

Igual que en el cas de la SPME, amb les trampes d'agulla cal utilitzar una injecció en mode sense divisió de flux (*splitless*) per assegurar la desorció completa i reproduïble dels compostos retinguts a la trampa. S'ha avaluat el temps de *splitless* necessari per evitar una pèrdua considerable dels compostos estudiats. Per als compostos més volàtils estudiats (benzè, 2,5-dimetilfurà i toluè) el temps de *splitless* necessari per obtenir desorcions quantitatives va ser de 15 segons. Altres compostos amb volatilitats intermèdies, com l'etilbenzè i els xilens, van requerir temps entre 30-45 segons, mentre que els compostos menys volàtils (2-etiltoluè, benzaldehid i acetofenona) van necessitar un mínim de 60 segons. Per tant, es recomana un temps de *splitless* de 1 minut per assegurar la completa desorció dels compostos menys volàtils i assegurar que no hi hagi pèrdues.

Flux de pressa de mostra

La pressa de mostra amb trampes d'agulla es pot realitzar tant de forma manual, acoblant l'agulla a una xeringa, o de forma automàtica amb una bomba de xeringa o de buit. La pressa de mostra automàtica dóna millor precisió que la manual, pel que és recomanable utilitzar un sistema automàtic quan es treballa amb trampes d'agulla.

Es van avaluar fluxos de pressa de mostra d'entre 5 i 53 $ml \cdot min^{-1}$. Es va observar que a fluxos >15 $ml \cdot min^{-1}$ les variabilitats que s'obtenen són excessives (RSD>20%). Aquest fet s'associa a una adsorció incomplerta dels analits a la trampa. Un dels principals inconvenients de

les trampes d'agulla és que el seu diàmetre és petit, això comporta que la velocitat de flux lineal a l'interior de la trampa és elevada i el temps de contacte dels analits amb els adsorbents és baix. Per a un flux de 20 ml·min^{-1} el corresponent flux linear a l'interior d'una agulla de calibre 22 és d'uns 250 cm·s^{-1}, que correspon a un temps de residència dels analits en una trampa de 10 mm de tan sols 0.004 s. En aquestes condicions els analits no tenen suficient temps per quedar completament retinguts i una part d'aquests no es retenen a la trampa. Els resultats obtinguts indiquen que calen fluxos de treball <15 mL·min^{-1} per obtenir coeficients de variació inferiors al 15%.

Contaminació creuada

Un paràmetre important a tenir en compte quan es treballar amb sorbents és la possibilitat de contaminació creuada deguda a desorcions incompletes en anàlisis prèvies. S'avalua aquest efecte fent passar una quantitat de gas amb estàndards dels analits estudiats a través de les trampes d'agulla i analitzant aquesta mostra. Una vegada desorbits els compostos, es guarda la trampa a 4ºC durant 72h i es torna a analitzar passat aquest temps. Els cromatogrames que s'obtenen són completament nets, indicant que en les condicions de treball utilitzades la desorció dels compostos és quantitativa i no dóna lloc a efecte memòria.

Estabilitat d'emmagatzematge

Una de les aplicacions potencials de les trampes d'agulla és com a eina per mostrejar *on-site*. Per poder dur a terme aquesta pràctica cal determinar prèviament l'estabilitat dels analits a les trampes. Es van passar mostres de patrons de concentració coneguda i es van conservar a 4ºC per períodes de 24 i 48 h. Els resultats obtinguts es van comparar amb els valors que s'obtenen analitzant el mateix patró immediatament després de ser mostrejat. Les recuperacions obtingudes per a tots els compostos i temps d'emmagatzemament van ser entre el 91 i el 110%, confirmant que les trampes d'agulla es poden conservar per períodes de fins a 48 h entre la pressa de mostra i l'anàlisi.

Validació per a mostres gasoses

El mètode és lineal per a tots els compostos avaluats en el rang de 0.01 a 10 ng. Els límits de detecció es troben en el rang de 0.004 a 0.010 ng. Si considerem un volum de mostra de 1 L, els LODs corresponen a concentracions entre 4 i 10 ng·m^{-3}.

La repetibilitat per a diferents trampes i adsorbents ha donat en tots els casos valors inferiors al 10%.

Anàlisi de mostres líquides

Per a l'anàlisi de mostres líquides la millor opció és l'acoblament de les trampes d'agulla amb la tècnica de l'espai de cap (HS, *headspace*) de manera que els compostos volàtils passen a la fase gas a l'interior d'un vial segellat i posteriorment són recollits de la fase vapor amb les trampes d'agulla. En aquest treball es comparem quatre metodologies diferents.

a. HS estàtic recollint un volum de la fase gas amb la trampa d'agulla (HS-NTD). Després de 50 min d'equilibració a 50ºC es mostregen 4 mL de l'espai de cap a 2 mL·min^{-1} [24].

b. HS estàtic amb difusió passiva de la fase gas per la trampa d'agulla (pHS-NTD). Es deixa la trampa d'agulla connectada al vial durant tot el temps d'equilibració, de manera que té lloc una difusió passiva dels compostos volatilitzats per la trampa degut a la sobrepressió que es va generant dins el vial per l'increment de la temperatura [24].

c. HS actiu amb la trampa d'agulla (PT-NTD). Es deixa la trampa d'agulla dins el vial i es fa passar una purga de nitrogen a 6 mL·min^{-1} per afavorir el transport dels compostos de la fase líquida a la fase gas [24].

d. HS dinàmic utilitzant varis cicles de pressa de mostra (cHS-NTD). Es col·loca la trampa d'agulla al vial i es mostreja un volum fix aplicant varis cicles de pressa de mostra. A cada cicle es fa passar per la trampa 1 mL de la fase gas i es retorna posteriorment el volum de gas extret cap al vial per mantenir les condicions de pressió [19].

HS-NTD

La pressa de mostra amb HS-NTD va ser avaluat a dues temperatures diferents (35 i 50ºC). A temperatures baixes la precisió que s'obté és millor, però a temperatures altes hi ha una millora de la sensibilitat per als compostos estudiats. S'ha de tenir en compte que l'increment de la temperatura pot donar lloc a una retenció no quantitativa per a compostos molt volàtils quan s'utilitzen aquest tipus de sorbents. Per tant, és recomanable realitzar la pressa de mostra a la temperatura més baixa possible per tal d'evitar pèrdues, tot i que això comporta un increment en el temps necessari per arribar a l'equilibri entre les dues fases al vial i disminueix el percentatge de compostos que poden difondre cap a la fase gas.

pHS-NTD

La pressa de mostra amb pHS-NTD va ser avaluada segons el temps d'equilibri i la temperatura. Aquesta metodologia es basa en la difusió passiva dels compostos volàtils per la trampa d'agulla, el qual és un procés lent. Els resultats obtinguts mostren que passats 150 minuts d'equilibració encara no s'assoleix l'equilibri. Aquests resultats suggereixen que, tot i la seva simplicitat instrumental, no és un mètode útil ja que el temps és massa llarg per ser utilitzat en anàlisis rutinàries. A més, l'efecte de la temperatura és encara més acusat que en el cas anterior degut als llargs temps de contacte dels compostos amb l'adsorbent. Quan es mostreja a temperatures més elevades hi ha una desorció lenta dels compostos més volàtils que dóna lloc a diversos processos d'adsorció i desorció dins el llit d'adsorbents. En aquestes condicions s'observen pics

amb cua i espatlla pels compostos més volàtils. Aquest efecte és més acusat com més volàtil és el compost.

PT-NTD

Igual que en els casos anteriors, a temperatures elevades s'obtenen valors de repetibilitat excessius (RSD>17%). La gran avantatge d'aquesta metodologia és que els temps de pressa de mostra es redueixen significativament si es compara amb els altres mètodes i que la sensibilitat és superior a les altres opcions avaluades (LODs en el rang de les unitats de pptv). S'ha observat el mateix mecanisme d'adsorció/desorció descrit amb pHS-NTD. Per als compostos més volàtils s'observa trencament a temperatures al voltant de 50ºC. Un inconvenient que presenta aquest sistema és la necessitat de desenvolupar una instrumentació més complexa per reduir la variabilitat deguda al flux de gas de purga.

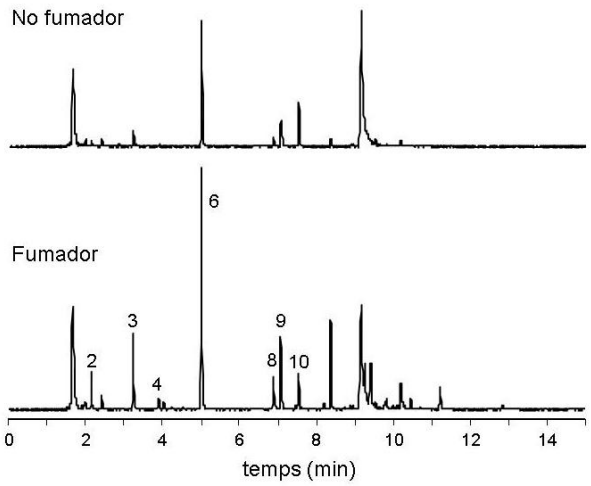

Figura 4. Cromatogrames de mostres d'alè d'un fumador i un no fumador. La numeració dels pics corresponen als números dels compostos a la Taula 2.

cHS-NTD

Aquesta metodologia de pressa de mostra conjuga la simplicitat instrumental de HS-NTD amb el fet que es pot combinar amb diferents cicles per poder incrementar el percentatge de compostos extrets. S'ha comprovat que tot i treballar a temperatures baixes (30ºC) es pot assolir la retenció quantitativa de tots els compostos volàtils presents a la mostra incrementant el número de cicles. S'ha desenvolupat un model matemàtic que permet determinar el percentatge d'analit extret en cada cicle i el número de cicles que calen per assolir un percentatge d'extracció determinat (sempre i quan l'adsorció sigui quantitativa en cada cicle) [25]. Amb aquesta metodologia s'obtenen LODs en el rang de les pptv de forma relativament simple.

Tenint en compte totes les consideracions descrites anteriorment, HS-NTD i cHS-NTD són les tècniques més simples i robustes per a l'anàlisi de volàtils en mostres líquides.

Aplicacions de les trampes d'agulla

La metodologia desenvolupada s'ha utilitzat per a l'anàlisi de diversos tipus de mostres: alè, aire ambiental, i mostres líquides.

En el cas de les mostres d'alè, s'ha comprovat que les trampes d'agulla són útils per analitzar aquesta matriu i permeten detectar compostos que es troben a nivell sub-ppbv utilitzant poc volum de mostra. S'ha pogut detectar la presència de 2,5-dimetilfuran en fumadors, un compost que s'ha mostrat com a un biomarcador molt selectiu i sensible de l'hàbit fumador d'una persona [26] (Figura 4). Es pot considerar que la nova metodologia de trampes d'agulla millora respecte d'altres ja existents degut a la seva simplicitat instrumental i portabilitat al lloc de pressa de mostra.

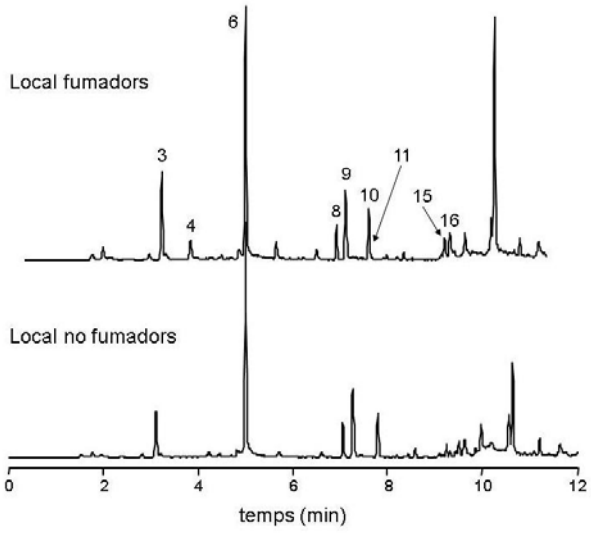

Figura 5. Cromatogrames obtinguts a l'anàlisi de l'aire interior d'un local on es permetia fumar al seu interior i un altre de no fumadors. La numeració dels pics correspon als números dels compostos a la Taula 2.

El segon tipus de mostres analitzades són mostres ambientals en locals de fumadors i no fumadors per tal de comprovar si s'obtenen resultats equivalents als obtinguts en estudis previs [27] i es pot proposar aquesta nova metodologia com a alternativa simple. Igual que en el cas de les mostres d'alè, es pot detectar el 2,5-dimetilfuran en els locals de fumadors i es comprova la seva absència en locals de no fumadors (Figura 5).

El darrer grup de mostres analitzades són les d'aigües procedents de plantes depuradores (Figura 6). La metodologia de les trampes d'agulla, tot i el seu fonament exhaustiu [12], permet assolir límits de detecció equivalents als que s'obtenen amb SPME (metodologia no exhaustiva i que, en principi, s'espera que sigui més adient quan es disposa de pocs volums de mostra). En la comparació amb SPME [25], s'ha pogut comprovar que les trampes d'agulla ofereixen la mateixa sensibilitat que SPME, però son més robustes i més adequades quan la pressa de mostra s'ha de fer *on-site*.

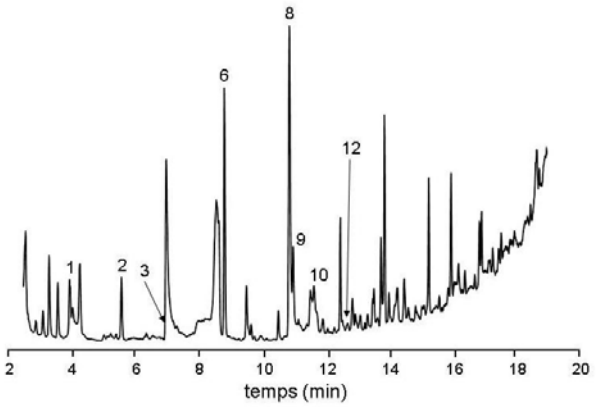

Figura 6. Cromatograma d'una mostra d'aigua de depuradora analitzada per la tècnica HS-NTD. La numeració dels pics correspon als números dels compostos a la Taula 2.

Conclusions

Els estudis portats a terme amb trampes d'agulla han mostrat que aquesta nova metodologia no només és una alternativa a les metodologies convencionals, com SPME, sinó que presenten una millor robustesa, aspecte molt important a tenir en compte per anàlisis rutinaris, i, a més, són adequades per la pressa de mostra on-site, una de les principals limitacions de la tècnica SPME. El principal inconvenient de les trampes d'agulla radica en la seves restriccions neumàtiques que limiten els fluxos màxims de pressa de mostra que es poden assolir, aspecte molt important quan es volen analitzar mostres gasoses d'ambients poc contaminants on es requereix mostrejar un volum considerable de mostra per assolir els nivells mínims detectables. No obstant, en aquells casos on no es requereixi un volum gran de mostra, com a l'anàlisi de mostres líquides per espai de cap, les trampes d'agulla són perfectament adients. La simplicitat de pressa de mostra permet una anàlisi ràpida i sensible de VOCs en mostres aquoses, biològiques i ambientals.

Agraïments

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Anna Godayol és llicenciada en Química per la Universitat de Girona des de l'any 2009. Va cursar el màster interuniversitari en Tècniques Cromatogràfiques Aplicades (UJI, URV, UdG). Actualment es troba cursant els estudis de doctorat a la Universitat de Girona amb una beca FI. La seva línia de recerca és el desenvolupament de mètodes per a la determinació de compostos orgànics volàtils i fragàncies en aigües de depuradora i l'estudi de la seva contribució a la percepció de males olors.

Enriqueta Anticó és llicenciada en Ciències i doctora en Química per la Universitat Autònoma de Barcelona. Va realitzar una estada post-doctoral a la ETH-Zurich (Suïssa, 1 any) on va desenvolupar diferents sistemes de sensors òptics. La seva recerca es focalitza en els camps dels sensors, en l'estudi de sistemes de membrana per al transport selectiu d'espècies metàl·liques i en la caracterització de vins i suros. Darrerament treballa en el desenvolupament de mètodes de determinació de compostos orgànics volàtils relacionats amb defectes sensorials i males olors. Actualment és professora titular d'universitat a la Universitat de Girona.

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Annex B. **Analytical Challenges in Breath Analysis and its Application in Exposure Monitoring.**

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TRAC, Trends in Analytical Chemistry, submitted

Abstract

There is an increasing interest in the use of breath analysis for monitoring human physiology and exposure to toxic substances or environmental pollutants. This review focuses on the current status of the sampling procedures, collection devices and sample enrichment methodologies used for exhaled breath vapor analysis. The different parameters affecting each of the above steps are discussed taking into account the requirements for breath analysis in exposure assessments and the need to analyze target compounds at sub-ppbv levels. Finally, a summary of the practical applications of exposure analysis that have been made over the last two decades is given.

Keywords

Breath analysis; sampling bags; canisters; sorbent trap; SPME; VOC; Exposure

1. Introduction

In the ancient Greece physicians already knew that the specific odor of exhaled breath could be associated with certain diseases. However, breath analysis has had few practical applications to date. Interest in the analysis of volatile organic compounds (VOCs) in breath has increased significantly since the early 1970s, when Pauling et al. [1] reported a gas chromatography method for the analysis of breath samples.

Breath analysis has the great advantage of being a non-invasive technique when monitoring the physiology of a person or exposure to toxic substances or environmental pollutants. Compared to blood or urine analysis, breath analysis is easier to perform and repeat, sampling is less likely to be perceived as unpleasant, and result interpretation is simpler as breath is a less complex matrix. Moreover, biomarkers present in breath can be detected faster than using blood and urine analysis, potentially permitting a quicker reaction against a specific problem.

Breath analysis can be used in two important fields: (i) clinical diagnosis to analyze volatile compounds generated in the organism and eliminated through exhaled breath (endogenous compounds) and (ii) exposure analysis in order to have a fast and accurate knowledge of the levels of inhaled VOCs that can reach the blood stream and may produce harmful effects (exogenous compounds). Clinical diagnosis has received the greatest interest due to its potential to detect a disease state in a simple and non-invasive manner. This application has already been extensively reviewed [2-5] and is beyond the scope of this review.

Exposure assessment is of great interest in the determination of toxic substances in indoor environments as people can be exposed to a range of indoor pollutants that may have adverse effects on health. Exhaled breath has been analyzed to determine personal exposure to solvents and other VOCs [6-21]. Most information on VOC toxicity is based on exposure in industrial environments that typically have high levels of pollutants or has been established from animal and controlled studies with high concentrations. Levels in most indoor environments are below the exposure limits required to demonstrate measurable health impacts [22]. Although there is no evidence

of a health risk at the low levels normally detected in homes, some VOCs are well established carcinogens or may be allergenics and so may have adverse effects on human health. If we also take into account the fact that there have been few epidemiological studies in these conditions, there is insufficient data to elucidate the possible relationship between VOC exposure in non-industrial environments and their effect on human health, even when contact is repeated and prolonged [10].

One of the main problems associated with the analysis of exposure in non-industrial environments is the low concentration of contaminants. Problems in quantifying indoor exposure also arise because many advanced technologies developed for measuring outdoor pollution are not suitable for indoor use due to cost, size and the amount of air they displace. Taking into account the levels usually detected in these conditions, analytical methodologies that can reach detection limits <1 $\mu g \cdot m^{-3}$ are required. Moreover, the volume of breath samples is more limited than air samples. More sensitive methods are therefore needed to achieve appropriate detection limits, which allow target compounds to be detected at the levels at which they are expected to be found.

Exhaled breath vapor is only formed by volatile compounds. The main fraction (> 99%) is composed of a mixture of nitrogen, oxygen, carbon dioxide, water vapor, and inert gases. The remaining fraction (< 100 ppmv) is formed by a mixture of hundreds of VOCs in a wide range of concentrations (ranging from few ppmv to pptv) [3,4]. The main VOCs present in a healthy person's breath are acetone, isoprene, methanol, and ethanol, which are produced in core metabolic processes. All other VOCs are present at very low levels, from a few ppbv to sub-pptv.

2. Sampling procedures

One of the main problems when dealing with breath analysis is the limited volume of sample that can be obtained. Moreover, breath needs to be collected under careful conditions that include monitoring of the breathing [23]. The average total lung capacity of an adult human male is about 6 liters of air, but only a small amount of this capacity is used during normal breathing. In each expiration, almost 500 mL of breath is expired [3,7]. The first portion is "dead space air", which comes from the mouth, trachea and

bronchi and so does not involve a gaseous exchange between air and blood. The remaining fraction is “alveolar air”, which comes from the lungs and so does include a gaseous exchange between air and blood. Exhaled breath is a mixture of both dead space and alveolar air.

Preliminary EPA-sponsored studies in the 1980s relied upon a spirometer and the collection of a 40 L volume of breath [24]. This method allowed the detection of low levels of VOCs but was cumbersome and presented many drawbacks. More recent EPA studies only collect 1 L breath samples. The volume of breath sample that is usually collected currently ranges from a few milliliters when VOCs are directly retained into a sorbent device [15,25-28] to one liter when breath is collected in a gas sampling container [6,7,9,12-14,17,19,20,29-31]. To collect more than half a liter of sample it is necessary either to use forced-expiratory sampling or to collect samples from tidal breathing over several expirations. Forced-expiratory sampling is a common sampling methodology used in different studies [30,31]. This procedure is very simple to perform and does not require complex instrumentation. This methodology has been proposed to obtain steady and representative alveolar air but it has many drawbacks: (i) it is highly dependent on the volunteer’s cooperation and effort, (ii) breath-holding with the lung full or partially emptied gives different results, and (iii) there is no control of the volunteer’s breathing. Despite its widespread use in non-clinical studies, this should not be recommended as a sampling procedure for quantitative analysis.

Sampling by collecting different exhalations during tidal breathing would seem to be the most reliable methodology. Notwithstanding, breathing patterns are irregular and random fluctuations in breathing frequency and intensity are always present [32]. It is therefore necessary to collect breath samples from a series of cycles in order to obtain a representative sample. Samples should be obtained during conditions of normal ventilation, which requires introducing the volunteer to the procedure and encouraging the adoption of a relaxed natural and regular breathing profile.

The large amount of variables indicated and the fact that many different sampling methodologies are used for exhaled air makes it difficult to compare results [10]. It is therefore desirable to find a standardized system to allow comparison [23]. Moreover, a standardized and reproducible breath sample is required for quantitative analysis to

avoid the proportion of alveolar to tidal air varying from sample to sample. The only way to obtain reliable and comparable results is to normalize samples at alveolar concentration levels [23,33].

3. Breath collection devices

Since the end of the 20th century different methods for the direct reading (real time analysis) of breath samples, such as laser spectrometry, selected ion flow tube (SIFT), atmospheric pressure ionization (API), proton transfer reaction (PTR), ion mobility spectrometry (IMS), and sensors have appeared with promising results [2,5,8,9]. Unfortunately, these methodologies require complex, non-portable and expensive instrumentation, which limits its applicability in exposure analysis.

Indirect methods involving collection devices to obtain and transport the samples are less expensive and, at the moment, seem to be the most appropriate methodology for obtaining on-site breath samples. Therefore, the sampling, transport and storage of exhaled breath are critical steps in the whole analytical process. The preservation of the original sample composition is a challenge for gas compounds as losses (e.g., by diffusion), adsorption (e.g., in the surface of the containers) and reactions can occur leading to artifact formation. Thus, the selection of the most appropriate container is essential in breath sampling. Samples can be collected using different devices such as canisters, sampling bags and sorbent materials.

Canisters are used for collecting breath samples [6,7,9,13,14,16,29] but they have the disadvantage of being expensive, needing to be evacuated before sampling and requiring sophisticated equipment for cleaning. Some authors have suggested that passivated stainless steel canisters are extremely durable for breath storage and many VOCs remain stable within these canisters for periods of 30 days or longer without any significant degradation [29]. On the other hand, Batterman et al. [34] evaluated the stability of some aldehydes and terpenes in electropolished canisters and found that recoveries for all terpenes and most aldehydes evaluated dropped substantially within the first hour, followed by a more gradual decrease later.

Glass bulbs can also be used for breath sampling [8,15,19]. However, they are fragile, require silanization to deactivate the interior glass surface, and must be evacuated before sampling. Moreover, loses of volatile compounds have also been observed when glass bulbs are used as containers, although at lower rates than with polymer bags [35]. In some cases, polymeric chambers [25,26,36,37] have been used to collect breath samples. Unfortunately, no information about losses and stability has been recorded. Some losses of volatile compounds are to be expected due to the polymeric structure of the chamber walls.

The most common methodology for breath collection is to use polymer sampling bags due to the ease with which they can be manipulated, their reduced cost and the possibility for them to be reused. These bags must be made of inert materials to avoid both diffusion and reactions between the compounds and the bag. The most common material used is Tedlar® [12,17-20,30,38-42] but other materials such as Teflon®, FlexFoil®, and Nalophan® [38] are also used. Prior to being used for the first time or being reused, bags must be thoroughly cleaned by flushing with pure inert gas to remove adsorbed compounds. This step plays a crucial role in the storage of gas samples [38,42]. Unfortunately, all commercial polymers suffer from diffusion and adsorption of volatile compounds [20,38-40,42], and temperature and storage time have a significant effect on the integrity of the gas samples [41]. Although Tedlar® bags are the most common choice for breath analysis, they also present the most significant background contamination. When Tedlar®, Teflon®, FlexFoil® and Nalophan® polymers were compared [38], only Tedlar® polymer emitted contaminants in blank tests. The main contaminants detected in Tedlar® bags are N,N-dimethylacetamide and phenol, which are both solvents that are used in the production of the film [41,42]. Other contaminants that have been detected in these bags are carbonyl sulfide and carbon disulfide [38]. It is usually recommended that breath samples should be analyzed as soon as possible after sampling.

A common commercial device for breath sampling is Bio-VOC [11,43-45]. This device is based on the collection of the last 100-150 mL of an expired sample. Immediately after finishing sampling, a valve is opened and the collected breath is transported through an appropriate sorbent material in order to retain the VOCs. The gas sample

only remains in the container for a few seconds with this device and no losses are expected.

Different direct sampling methodologies have recently been developed to integrate sampling and pre-concentration into one single step, which can avoid the problems related to storage in containers. These methodologies are based on the direct collection of target VOCs on a sorbent material, which presents better stability and permits longer storage times. A modified holder connected to a solid-phase microextraction fiber (SPME) has been developed [37,46]. The use of hydrophobic membranes to eliminate water vapor and impurities followed by pre-concentration in a sorbent trap has also been proposed [25,26]. The use of a device called SnifProbe which is based on a small length of capillary or porous-layer open tubular column for sample collection, has been proposed [47]. An adaptive breath sampler to collect breath directly in a sorbent tube is another option [32]. Most recently, needle trap devices (NTDs) have been described [27,28,33,48].

4. Sample enrichment

The low concentrations of VOCs in breath samples make it necessary to employ a pre-concentration technique before analysis. There are two main methodologies for this purpose: solid-phase microextraction (SPME) and concentration on solid sorbents.

4.1. Solid-phase microextraction (SPME)

Different procedures are followed in SPME (Table 1) [10,35,37-40,46,49-51]. Sometimes the fiber is inserted into the container containing the total volume of breath collected for a predetermined period of time [35,39,40,49] and at others a fixed and small volume of the sample is transferred inside a sealed vacuum headspace vial before inserting the SPME fiber into the vial [50,51]. The sensitivity of SPME is not as dependent on the volume of the sample as conventional concentration on solid sorbents. LODs are commonly in the low ppbv range when SPME is applied to breath samples (Table 1). Unfortunately, this limits the applicability of SPME when target compounds have to be detected at lower levels. For example, 2,5-dimethylfuran, a promising breath biomarker for determining smoking status or continuous contact to environmental

tobacco smoke, has to be detected at the low pptv range in breath samples in order for detection to be possible some hours after contact with tobacco smoke [12,20,48].

Table 1. Summary of the principal studies using SPME as preconcentration technique and devoted to exposure analysis.

Coatings	**Target VOCs**	**LOD**	**Sampling collection device**	**Ref.**
CAR/PDMS	Isoprene	6 ppbv (SPME) 0.4 ppbv (sorption)	8 L Tedlar bag SPME inside bag, 10 min at 40ºC	39
PDMS/DVB	Acetone	0.05 ppbv	3 L Tedlar bag (max. storage 6 h) SPME inside bag, 4 min at 40ºC	40
PDMS	Tetrachoroethylene	0.3 $mg \cdot m^{-3}$	125 mL glass bulb (exposed 1 min)	49
PA PDMS CW/PDMS PDMS/DVB	Ethanol Acetone Isoprene	6 $nmol \cdot L^{-1}$ 2 $nmol \cdot L^{-1}$ 0.3 $nmol \cdot L^{-1}$ (PDMS/DVB coating)	Fiber directly to mouth (10 s)	46
PDMS CW/DVB PDMS/DVB DVB/CAR/PDMS	benzene	2 ppbv	Fiber directly to mouth (30 s)	37
CW/PEG	2-aminoacetophenone	50 $pmol \cdot mol^{-1}$	1 L glass bulb (24 h fiber)	35
CAR/PDMS	Acetone Acetonitrile Benzene n-butane Dimethylsulfide Furan 2-methylfuran Isoprene Limonene Toluene	2 ppbv 15 ppbv 0.05 ppbv 5 ppbv 4 ppbv 2 ppbv 2 ppbv 0.2 ppbv 2 ppbv 0.1 ppbv	3 L Tedlar (20 mL vials, 10 min 37ºC)	50
CAR/PDMS	43 VOCs	0.7-17 ppbv	3 L Tedlar (20 mL vials, 10 min 37ºC)	51

It has been found that the water content of a sample has a significant effect on the SPME sorption process when direct analysis of breath is performed [46]. For those coatings where absorption is the dominant process, extraction efficiency is not affected

by the water content of the sample. However, there is a significant change in the extraction efficiency in the case of adsorption mechanism based coatings due to the competition with water molecules for the active sites of the sorbent material. Calibration standards should be prepared at the same relative humidity (RH) as samples to avoid quantification mistakes [39,46].

4.2. Concentration on solid sorbents

Concentration on solid sorbents followed by thermal desorption is the most frequent method for the analysis of VOCs in breath samples [6,7,9,11-19,29-32,47]. Sorbent traps present the advantages that they can be prepared on a micro-scale and coupled on-line with a GC system allowing near real-time measurements, and the sorbent configuration can be easily changed to adapt to different compounds.

LODs obtained by sorbent trap techniques strongly correlate with the volume of sample analyzed. Thus, LODs obtained applying this technique decrease significantly compared to SPME limits when a large volume of sample can be collected. The use of a micro-trap allows LODs in the low pptv range with samples volumes of up to 1 L to be reached [12,19].

The most common and simple sorption trap is based on a single adsorbent. In the case of exposure analysis, the most common sorbent used is Tenax [11,15,19]. However, the wide range of VOCs present in breath has the result that no single sorbent is capable of adsorbing all the compounds present in breath samples, and a multicomponent sorbent is necessary to complete the screening and determination of VOCs [17,30].

The sorption and desorption behavior of VOCs in carbon-based sorbents is important as they determine the injection plug width and the ability to perform quantitative studies. An important source of error when sorbent traps are used is the formation of artifacts caused by degradation reactions of both adsorbed analytes and the adsorbent itself during storage of adsorbent tubes [52]. This effect is more important when very low concentrations of target compounds are expected. The high temperatures needed for the quantitative desorption of the trapped compounds can lead to the thermal decomposition of some compounds [53] (Figure 1). The degradation problem is more important with

conventional desorption equipment as a second pre-concentration stage is necessary to refocus the solutes in the analytical column. This is frequently done by cryogenic trapping, which can also result in analyte loss and the formation of artifacts. The sorbent material itself can generate artifacts by degradation [17].

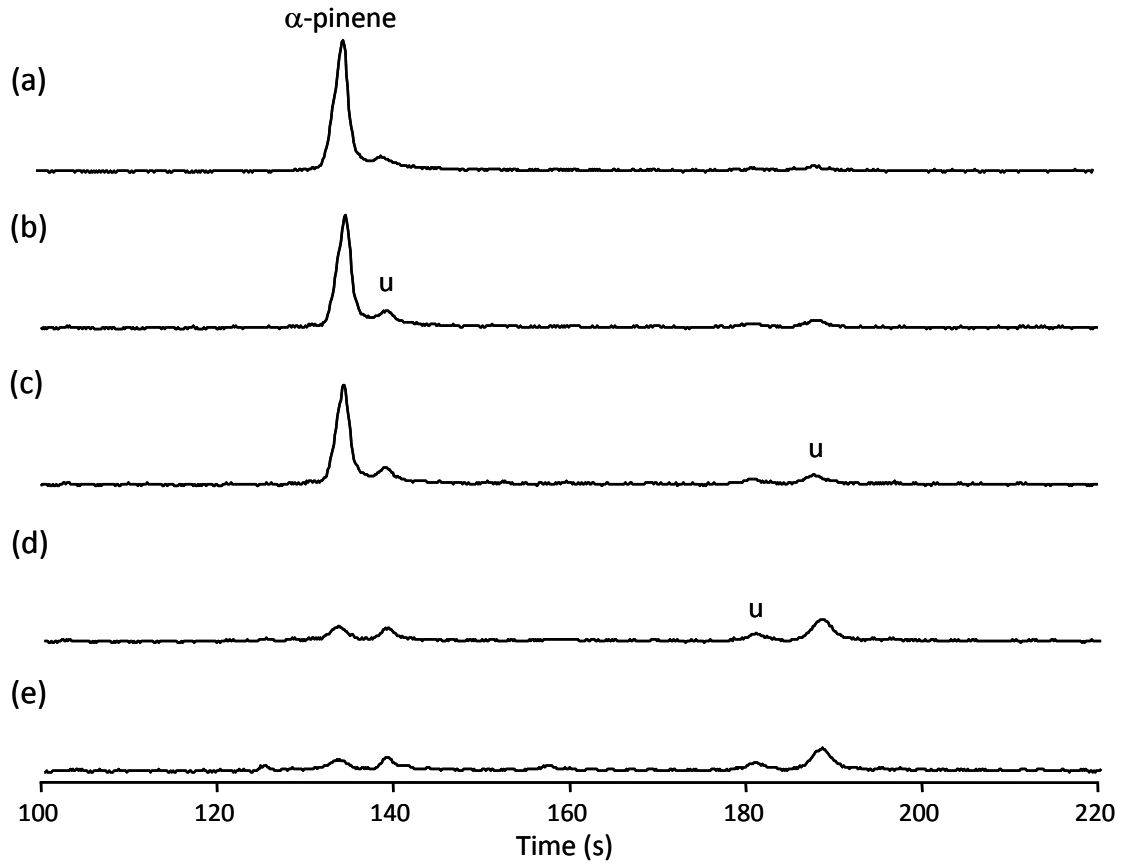

Figure 1. Chromatograms showing the thermal degradation of α-pinene when the temperature applied to the sorbent trap for desorption is increased. The sorbent trap was heated to 200ºC (a), 250ºC (b), 300ºC (c), 350ºC (d), and 380ºC (e). (u: unknown).

In order to simplify the desorption process and to solve decomposition problems, different in-house capillary traps have been developed. These micro-traps eliminate the need for a second cryofocusing stage and allow near real-time measurements [17,19,30,31]. The configuration of the micro-traps allows much greater concentration factors than those obtained with conventional thermal desorption instruments, which also results in a smaller amount of sample being required to reach LODs in the pptv range [17,19,30,31]. NTDs represent a further improvement in capillary traps for breath analysis [27,28,48]. These devices allow direct thermal desorption inside a GC injector (equivalent to SPME) and yield large enrichment factors.

For the sorption process, it is important to take into account the water intake of the sorbents as this can affect the quantitative analysis of VOCs. Graphitized carbon blacks and porous organic polymer adsorbents allow a high percentage of water vapor in the sample to pass through the traps during sampling without significant loss of the target compounds. Unfortunately, if highly volatile compounds are on the target list, strong adsorbents (carbon molecular sieves) are required in order to retain them and large amounts of water are retained at the same time. The simultaneous trapping of water vapor can cause various problems: (i) the accumulation as ice during cryogenic pre-concentration, (ii) a reduction in the adsorption efficiency during sampling on solid adsorbents, (iii) the possible loss and transformation of organic trace gases in the water/ice matrix, (iv) freeze out of water on the trap or in the GC column during cryogenic oven cooling can plug the trap or the column and interrupt the carrier gas flow, and (v) a large water background can also cause shifts in the retention times and pose problems during detection, especially in the case of an MS detector [54].

Different options have been proposed to limit the water vapor problem [54]. One option is to pass the sample flow through a trap containing a drying agent or a membrane (e.g. Nafion). The membrane allows water to permeate through it but also permits other light polar volatile compounds to pass through, resulting in losses of highly volatile compounds [55]. Another simple option is to dry the sample with a dry inert gas after the sample concentration is completed. In this case, there are also limitations due to the possible loss of VOCs or the introduction of contaminants [54,55]. Another alternative is to heat the adsorbent during sampling, but this results in most volatile compounds not being quantitatively retained by the sorbent [30] (Figure 2). The simplest way to reduce the water problem consists in the reduction of the volume of sample so as to reduce the amount of water vapor in the sample to below the thresholds for the proper use of the analytical instrumentation [54]. This option is only available, however, in those cases where small amounts of breath samples are collected.

5. Applications in exposure analysis

Studies found in the literature can be grouped in five categories: (i) simulations in controlled chambers [13,43], (ii) swimming [6,16,36,44,56], (iii) petrol services and

mechanics [14,15,19,37,57,58], (iv) solvents and volatile compounds in the workplace [11,45,59,60], and (v) active and passive smoking [9,12,17,19,20,21,31,48,61,62]. Most of the studies are focused on finding reliable exposure biomarkers.

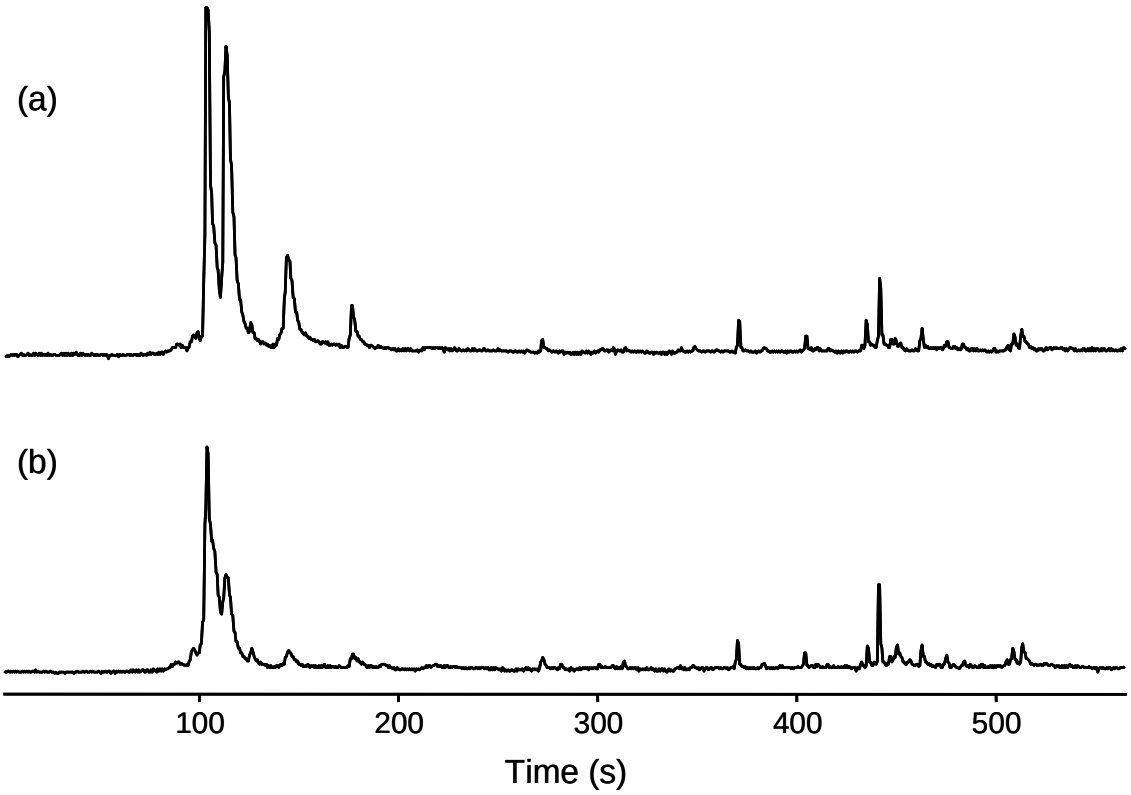

Figure 2. Effect of the temperature applied during the sorption process in the analysis of a breath sample (750 mL exhaled breath). Three-bed trap containing Carboxen 1000, Carbopack X and Carbopack B as sorbent materials. As can be seen, there is a significant decrease in the peak heights for the most volatile compounds (compounds appearing at retention times <200 s) when the trap was heated at 40ºC during the sampling process (b). Sampling at 22ºC (a) yields better sensitivity for the most volatile compounds. Less volatile compounds (r.t. > 200 s) are not significantly affected by the change in the trap temperature during the sorption process.

5.1. Simulations in controlled chambers

These studies try to simulate conventional exposure situations in order to investigate whether breath measurements can be used as a surrogate for blood measurements. The main drawback is that controlled chambers are designed to assess exposure at levels that are equivalent to the threshold limit values, and results cannot be extrapolated to non-exposed people.

Exposure to trichloroethene levels was evaluated from controlled inhalations at high levels for 24 hours [13]. A model was used to predict blood levels from breath elimination curves and blood/breath partition coefficients. The results obtained gave a mean ratio of blood level calculated:measured of 0,98. The study concluded that about 78% of thrichloroethene entering the body was metabolized, stored or excreted through routes other than exhalation.

Exposure to trimethylbenzene was performed in a laboratory controlled atmosphere facility [43]. A rapid absorption of trimethylbenzene into the blood stream was observed, which is largely produced by inhalation. Elimination was determined through the analysis of exhaled breath and a metabolite in urine, and it was found that some trimethylbenzene was not eliminated via breath or urine.

5.2. Swimming

Trihalomethanes (THMs) are important contaminants in indoor and outdoor swimming pools and also in domestic water activities. They are formed as a result of the combination of residual organic matter and chlorine-based disinfection products used in water supply systems. Lindstrom et al. [6] collected breath samples from swimmers before, during and after a 2 hour training period. They suggest that the dermal route of exposure was even more important than the inhalation. Other studies [44,56] have also found that dermal uptake for these compounds is significant. It was found that haloketones are less permeable through skin than chloroform [36].

A significant increase in the breath levels of THMs was observed during bathing and showering [16]. However, other domestic water use activities, such as washing clothes or dishes, did not result in a significant increase in the breath levels even though these activities led to a significant increase in the indoor air levels.

5.3. Petrol services and mechanics

Benzene, toluene, ethylbenzene, and xylene isomers (BTEXs) are common compounds in petrol products. Benzene levels in the exhaled breath of people exposed to petrol vapors are always higher than in volunteers who are not exposed [14,15,19,37,58].

These studies also found large variability in breath benzene levels for all groups evaluated, but this variability was significantly higher in the case of exposed participants. Exhaled toluene and xylenes also showed significant correlations with concentrations found by personal monitoring devices [57]. Therefore, exhaled breath levels of benzene, toluene and xylenes have been proposed as suitable for use as biological exposure indices for petrol station workers. It has been found that naphthalene elimination from the body takes place faster than in the case of benzene [19].

5.4. Solvents and volatile compounds in the workplace

Occupational exposure to benzene was evaluated in workers of a benzene production plant during their entire work shift [59]. Significant differences for alveolar and blood benzene levels were obtained between exposed and non-exposed workers. Benzene alveolar retention of around 55% was suggested. Workers from different occupations have also been evaluated [45] and higher concentrations were detected after work shifts.

Scheepers et al. [11] analyzed alveolar breath and personal exposure to BTEXs of primary school children from two different zones. They found that industrial activity made a relatively small contribution to exhaled BTEXs. Other factors, such as smoking habits, petrol services and traffic, and the use of consumer products, seem to have a greater influence on exposure to benzene and toluene.

Thrall et al. [60] developed a field-portable breath analysis system to measure selected solvents in exhaled air. Benzene and toluene were evaluated in workers from an incinerator, and trimethylbenzene, hexane and methylene chloride were determined from employees in a waste repackaging facility. The system developed has great potential for exposure analysis.

5.5. Active and passive smoking

The last category evaluated is focused on studies devoted to tobacco smoking, exposure to environmental tobacco smoke (ETS) and passive smoking. Buszewski et al. [62] analyzed 56 VOCs in the alveolar breath of non-smokers and active smokers.

Acetonitrile, furan, 3-methylfuran, 2,5-dimethylfuran, 2-butanone, octane and decane were only found in smokers and passive smokers. Berkel et al. [61] identified four VOCs as biomarkers of recent exposure to cigarette smoke: 2,5-dimethylhexane, dodecane, 2,5-dimethylfuran, and 2-methylfuran. Gordon et al. [9] evaluated the breath profiles of benzene, 1,3-butadiene and 2,5-dimethylfuran from smokers and passive smokers after smoking cigarettes in an small unventilated room. All three target VOCs were identified in the breath of non-smokers after exposure, so demonstrating their contamination by ETS.

2,5-dimethylfuran has been found to be a biomarker of smoking status independently of the smoking status [12,17,19,21,31,48]. The evaluation of ETS contamination on public premises also confirmed this compound as a robust biomarker of ETS contamination [20]. The compound was also detected in the breath of non-smoking employees working on smoking premises after a few hours of the beginning of their work shift.

6. Conclusions

The non-invasive nature of breath analysis and the simplicity of the breath matrix compared to other conventional biological matrices have spurred the use of breath analysis in exposure assessment in recent decades. However, despite the improvements that have been achieved in its application, the technique is still far from being accepted for routine analyses. Further development of the sampling collection devices and the sampling mechanisms is required in order to facilitate the taking of reliable and reproducible samples. With regards to the analysis, portable devices need to be developed that will enable simple and robust analysis of VOCs at sub-ppbv levels for the accurate determination of VOCs in the exhaled breath of non-exposed people. New technologies based on micro-traps and needle traps may well be able to help in solving this problem.

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