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LOS NANOCUERNOS Y LOS NANOCONOS DE CARBONO COMO OBJETOS Y HERRAMIENTAS EN NANOCIENCIA Y NANOTECNOLOGÍA ANALÍTICAS

CARBON NANOHORNS AND CARBON NANOCONES AS OBJECTS AND TOOLS IN ANALYTICAL NANOSCIENCE AND NANOTECHNOLOGY

Juan Manuel Jiménez Soto
Tesis Doctoral
Córdoba, Junio 2012

TÍTULO: Los nanocuernos y los nanoconos de carbono como objetos y herramientas en nanociencia y nanotecnología analíticas

Carbon nanohorns and carbon nanocones as objects and tools in analytical nanoscience and nanotechnology

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Trabajo presentado para aspirar al
Grado de Doctor en Ciencias

EL DOCTORANDO,

IMENEZ

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CERTIFICAN: Que la Tesis Doctoral “LOS NANOCUERNOS Y LOS NANOCONOS DE CARBONO COMO OBJETOS Y HERRAMIENTAS EN NANOCIENCIA Y NANOTECNOLOGÍA ANALÍTICAS” ha sido desarrollada en los laboratorios del Departamento de Química Analítica de la Universidad de Córdoba y en el Dipartimento di Scienze Chimiche e Farmaceutiche de la Università degli Studi di Trieste, y que, a nuestro juicio, reúne todos los requisitos exigidos a este tipo de trabajo.

Y para que conste y surta los efectos pertinentes, expiden el presente certificado en la ciudad de Córdoba, 29 de Junio de 2012.

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TÍTULO DE LA TESIS: LOS NANOCONOS Y NANOCUERNOS DE CARBONO COMO OBJETOS Y HERRAMIENTAS EN NANOCIENCIA Y NANOTECNOLOGÍA ANALÍTICAS

DOCTORANDO: Juan Manuel Jiménez Soto

INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

El doctorando Juan Manuel Jiménez Soto cursó brillantemente los estudios del Máster en Química Fina Avanzada, obteniendo excelentes calificaciones en las asignaturas del mismo. El trabajo fin de Máster se publicó en la revista Journal of Chromatography A, situada en el primer cuartil del área de conocimiento.

La temática de la Tesis se encuadra en una línea de investigación puntera, como es la Nanociencia y la Nanotecnología analíticas. En este sentido, se ha trabajado con unas nanopartículas de carbono novedosas, como son los nanocuernos y nanoconos de carbono para los que no existían aplicaciones en el área de la Química Analítica en general y de la (micro)extracción en particular en el momento de iniciar el trabajo experimental.

La realización de la investigación recogida en la Memoria que se presenta, ha permitido al doctorando adquirir además una sólida formación analítica, adiestrándose en el manejo de técnicas de separación cromatográficas, de fraccionamiento en flujo, espectroscopía Raman, microscopía de barrido y transmisión, técnicas de extracción miniaturizadas basadas en el uso de las anteriormente citadas nanoestructuras cónicas de carbono. Asimismo, se han estudiado distintas familias de compuestos en muestras de agua de distinta procedencia, lo que ha permitido completar la formación integral del doctorando en el ámbito analítico. Todo ello ha dado lugar la publicación de 7 artículos científicos, 5 de ellos ya publicados en revistas del primer cuartil del área de Química Analítica, y dos capítulos de libros, uno de ellos ya publicado. También han sido fruto de esta Tesis Doctoral ocho comunicaciones a Congresos nacionales e internacionales, seis de ellas orales/flash.

La estancia realizada en el grupo de investigación del Prof. Prato (Universidad de Trieste, Italia) durante el desarrollo de la Tesis Doctoral ha completado de forma satisfactoria dicha formación, profundizando en el conocimiento de las técnicas empleadas para la caracterización de las nanopartículas de carbono.

Por todo ello, consideramos que la investigación desarrollada y recogida en esta Memoria, reúne todos los requisitos necesarios en cuanto a originalidad, innovación y calidad, y autorizamos la presentación de la Tesis Doctoral de Don Juan Manuel Jiménez Soto.

Córdoba, 29 de junio de 2012
Firma del/de los director/es

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UNIVERSIDAD D CÓRDOBA

Fdo.: Miguel Valcárcel Cases

Fdo.: Mª Soledad Cárdenas Aranzana

Mediante la defensa de esta Memoria de Tesis Doctoral se pretende optar a la obtención de la Mención de “Doctorado Internacional” habida cuenta de que el doctorando reúne los requisitos para tal mención:

1. Cuenta con los informes favorables de dos doctores pertenecientes a instituciones de Enseñanza Superior de países distintos a España.
2. Uno de los miembros del tribunal que ha de evaluar la Tesis pertenece a un centro de Enseñanza Superior de otro país distinto a España.
3. Parte de la defensa de la Tesis Doctoral se realizará en una lengua distinta de las lenguas oficiales en España.
4. El doctorando ha realizado una estancia de cuatro meses en el Dipartimento di Scienze Chimiche e Farmaceutiche de la Università degli Studi di Trieste (Italia), que ha contribuido a su formación y permitido desarrollar parte del trabajo experimental de esta Memoria.

Agradezco al Ministerio de Educación, Cultura y Deporte la concesión de una beca de Formación de Profesorado Universitario (FPU) que ha hecho posible mi dedicación a este trabajo durante los últimos cuatro años.

Índice

Objeto / Aim

La presencia de la Nanociencia y la Nanotecnología en diversas áreas científico-técnicas es incuestionable. “Nano” se ha convertido en los últimos años en un prefijo presente en un elevado número de artículos, libros y material científico en general. La Química Analítica no ha sido ajena a este impacto y ha ido incorporando de forma sistemática las distintas nanopartículas y/o nanomateriales a sus procesos de medida, tanto como analitos objeto de determinación, como herramientas para el desarrollo de nuevas metodologías caracterizadas por una reducción del tamaño de la muestra sometida a análisis así como de los dispositivos e instrumentos, permitiendo así la obtención de información a escala nanométrica.

Probablemente han sido los nanotubos de carbono las nanopartículas base para las propuestas nanotecnológicas debido a sus excepcionales propiedades físico-químicas. Pero desde su descubrimiento, han sido muchos los esfuerzos investigadores orientados a la síntesis y caracterización de nuevas nanopartículas de carbono. Entre ellas, los denominados nanocuernos de carbono monocapa (*single-walled carbon nanohorns*, SWNHs) se han revelado como un nanomaterial muy atractivo en nanotecnología debido a su potencial aplicabilidad en el diseño de electrodos, medios de almacenamiento de hidrógeno y metano y lubricantes sólidos. Por otra parte, los nanoconos y discos de carbono son otras nanopartículas de carbono que, en base a su tamaño, forma y composición resultan ser un material igualmente relevante en el ámbito de la Química Analítica.

Teniendo en cuenta lo anteriormente expuesto, el objetivo principal de la Tesis Doctoral que se presenta en esta Memoria es avanzar en el conocimiento de los nanocuernos y nanoconos de carbono y evaluar su potencial en el ámbito de la nanotecnología analítica. Con este propósito global, los objetivos específicos de la investigación a desarrollar son los siguientes:

- Caracterizar tanto los nanocuernos como los nanoconos de carbono, con vistas a obtener sólidos con propiedades diferenciadas y útiles para el desarrollo de nuevas herramientas analíticas.
- Proponer nuevas herramientas y procesos analíticos en los que intervengan estos nanomateriales cónicos de carbono, centrándose en la puesta a punto de nuevos sistemas de (micro)extracción en fase sólida para el análisis de matrices ambientales. Las metodologías propuestas aportarán un valor añadido a las ya existentes en términos de propiedades analíticas básicas: sensibilidad, selectividad y precisión. Con esto se pretende llevar a cabo estudios experimentales para evaluar las diferentes interacciones entre las nanoestructuras y los analitos modelos seleccionados en cada caso.
- Incorporar los nanocuernos y nanoconos de carbono a sistemas cromatográficos y electroforéticos con vistas a mejorar la separación y resolución de las técnicas.
- Contribuir con nuevas aproximaciones en análisis medioambientales.

The presence of Nanoscience and Nanotechnology in various scientific and technical areas is unquestionable. "Nano" has become in recent years a prefix present in a large number of articles, books and scientific material in general. Analytical Chemistry has not been left out this impact and a variety of nanoparticles and/or materials have been incorporated in a systematic way, both as analytes object of determination and as tools for the development of new methods characterized by a reduction in both, the size of the sample under analysis and the devices and instruments, thereby obtaining information at the nanoscale level.

Carbon nanotubes can be considered the starting point for nanotechnological proposals mainly due to their unique physicochemical properties. But since their discovery, there have been many research efforts aimed at the synthesis and characterization of novel carbon nanoparticles. Among them, the so-called single-walled carbon nanohorns (SWNHs) have emerged as an attractive nanomaterial in nanotechnology because of their potential applicability in the design of electrodes, storage devices of hydrogen and methane and solid lubricants. In addition, carbon nanocones and disks are found to be a material with characteristics and additional benefits because of their size, shape and composition.

Based on the foregoing, the primary aim of the Doctoral work described in this Report is to advance in the knowledge of the single-walled carbon nanohorns and nanocones and evaluate their potential in the field of analytical nanotechnology. The specific objectives to be fulfilled in this respect are as follows:

Aim

- *To develop new functionalization and purification methods for both, carbon nanohorns and nanocones, in order to obtain solids with different properties and useful for the development of new analytical methods.*
- *To propose new tools and analytical processes including these new carbon nanomaterials, focus on the design of new (micro)solid-phase extraction systems for the analysis of environmental matrices, with an added value to the existing ones in terms of analytical properties: sensitivity, selectivity and accuracy. These experimental studies would evaluate the different interactions between the nanostructures and the target analytes.*
- *To incorporate carbon nanohorns to chromatographic and electrophoretic systems in order to improve the separation of analytes and thus the chromatographic/electrophoretic resolution.*
- *To contribute with new approaches in environmental analysis.*

Introducción

Nanopartículas cónicas de carbono en Química Analítica

Conical carbon nanoparticles in Analytical Chemistry

Nanopartículas cónicas de carbono en Química Analítica

Conical carbon nanoparticles in Analytical Chemistry

ABSTRACT

Conical carbon nanoparticles are a particular form of open carbon nanomaterials. Their typical structure follows from the presence of pentagonal disclinations within a seamless graphene sheet. As a result, two main carbon nanostructures are obtained: carbon nanocones and carbon nanohorns. They were discovered after fullerenes and carbon nanotubes and since then, they have been extensively studied, although their analytical applications are still to be exploited. This contribution compiles the main aspects of conical carbon nanoparticles, including their synthesis and properties as well as the functionalization procedures which are different from those proposed for other carbon nanoparticles. Finally, the most relevant applications developed up to date are commented on.

Key words: Carbon nanocones/disks, carbon nanohorns, synthesis, properties, functionalization, analytical applications

Invited contribution to Encyclopedia of Analytical Chemistry
Submitted June 28th, 2012.

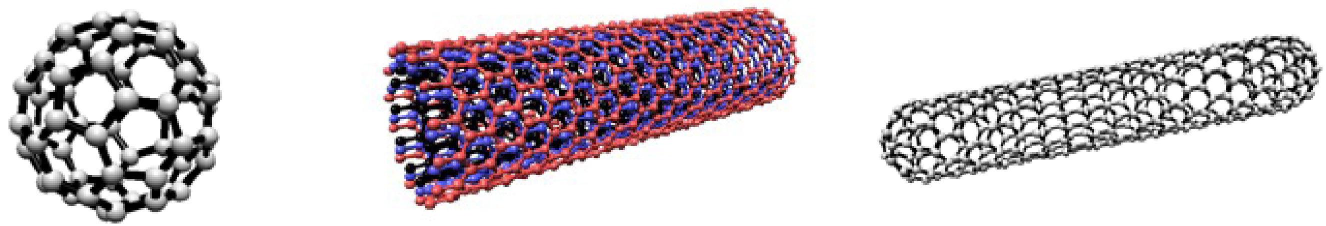
1. Introduction

Carbon is unique among elements and its allotropic forms have been extensively studied. Graphite and diamond were the two well-understood structures until the discovery of fullerenes (Figure 1-a) in 1985 by Smalley et al.(1) Everything started with a series of experiments on the vaporization of graphite, where a gas-phase carbon cluster C_{60} was the predominant species in the mass spectra. They concluded that a closed cluster containing exactly 60 carbon atoms would be responsible for such responses. Their discovery marked the beginning of a new era in the carbon science which would inevitably affects the developments in other scientific and technological fields, including Analytical Chemistry.

Carbon nanotubes were the next nanostructure discovered by Iijima in 1991.(2) They consist of tiny cylinders of graphene closed at each end with caps containing six pentagonal rings. Depending on the synthetic process followed, carbon nanotubes with several concentric layers or with a single graphene layer are obtained. The formers are called multi-walled carbon nanotubes (MWNTs) (Figure 1-b) while the second are single-walled carbon nanotubes (SWNTs) (Figure 1-c). MWNTs range in length from a few tens of nanometers to several micrometers and in outer diameter from about 2.5 to 30 nm. SWNHs have very small diameters, typically 1 nm, and are curled and looped rather than straight. Moreover, they are much more homogeneous than MWNTs and contain fewer structural defects. Several procedures have been proposed to obtain either SWNTs or MWNTs, which clearly influences the properties of the resulting nanomaterial.(3) Carbon nanotubes are by far the most studied carbon nanoparticles because of their exceptional mechanic, electric/electronic, magnetic and superficial properties. Their applicability in different scientific and technological areas has pushed significant advances in these application fields, which has also influenced the evolution in other areas such as Analytical Chemistry.

The outstanding properties of nanostructured carbons have stimulated an intensive research for novel structures. Thus, carbon nanocones (Figure 1-d) are the fifth allotropic form of carbon synthesized in 1997, although the first reference to these nanoparticles dated from 1994.(4) They consist of a rolled graphene sheet with a conical apex defined by the presence of one to five pentagonal rings, which also conditioned the angle of the nanocone. Next, in 1999, Iijima et al. discovered an open-tubular structure, single-walled carbon nanohorns (Figure 1-e), similar to SWNTs but with larger diameter (2-5 nm) and a length of ca. 50 nm.(5) However, the most remarkable structural difference is their horn-shaped tip, resulting from the presence of five of pentagons in the graphene sheet, which also affects to their properties. Moreover, they are also capable of forming stable aggregates of ca 80-100 nm in diameter.

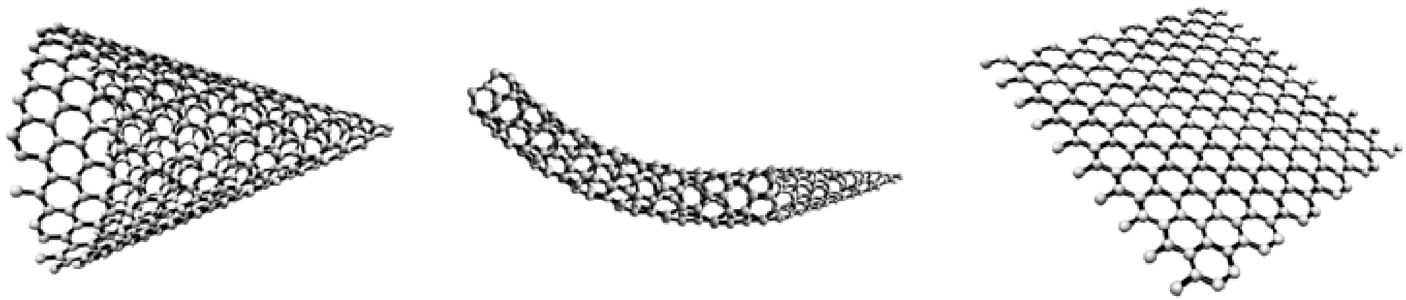
Finally, in 2004, the basic planar structure of these carbon nanoparticle, graphene (Figure 1-f), was isolated and studied.(6)



a) Fullerene

b) Multi-walled carbon nanotube (MWNT)

c) Single-walled carbon nanotube (SWNT)



d) Carbon nanocone

e) Single-walled carbon nanohorn (SWNH)

f) Graphene

Figure 1. Schematic representation of the nanostructures cited in the text.

During these two decades, the advances in nanoscience have appeared very fast, mainly due to the deep study of the synthetic process and the isolation and characterization of some of the subproducts obtained. In this regard, the characterization studies have overlapped with the advances in the applicability of the discovered nanostructures, being the evolution in both fields very impressive. However, the absence of a deep characterization of the nanostructures in the first steps limited the reproducibility of the initial tools developed.

This contribution is specifically devoted to the conical carbon nanoparticles such as carbon nanocones and carbon nanohorns. The latter have been much more studied, characterized than the former and thus, with much more possible applications. The topics afforded in the following sections are synthesis and properties, functionalization approaches, and finally, their applications in the (bio)analytical field.

2. Synthesis of carbon nanocones and nanohorns

Many theoretical studies have been conducted to elucidate why the carbon nanohorns and nanocones acquire their characteristic conical shape.

Graphene is an open, flat, two dimensional structure composed of carbon atoms organized in a network of hexagons attached to each other. This is possible due to sp^2 hybridization of the carbon atoms present in this sheet. However, this structure can generate other three-dimensional ones which may become open (nanocones and nanohorns) or closed (fullerenes and nanotubes).

The conical shape of carbon nanocones and nanohorns results from the modification of the network of hexagons of the graphene sheet by including one or more pentagons.

The inclusion of pentagons in the graphene structure produces a deformation, and the graphene losses a portion with an angle of 60° for each pentagon added. In order to maintain the continuity of the structure, it should be folded, resulting in a conical shape. The angle formed by this new conical structure will depend on the degree of deformation of the original sheet, that is, on the number of pentagons present. The carbon nanocones may have an angle of 112.9°, 83.6°, 60°, 38.9° or 19.2° based on the presence of 1, 2, 3, 4 or 5 pentagons respectively. For a completely closed structure (fullerene or nanotube) it is necessary to include 12 pentagons in the graphene. This can be explained by Euler's theorem, from which, many of the graphene-derived structures can be interpreted.(7)

Finally, another factor to be considered in the formation of conical carbon nanocones is hybridization, i.e., the ability of carbon to change from sp^2 state to sp^3 state and viceversa. This ensures that the structure is flexible and stable. In addition, this property of carbon makes it possible the transformation of graphene into other spatial shapes, which will present exceptional properties.

In 1994, H.V. Balaban et al.(8) reported a brief theoretical study which explained the possibility to find a new conical nanostructure from different dispositions of carbon atoms which form the hexagonal lattice of graphite. In fact, their studies were inspired by the structure of fullerene, which is composed of an organized mixture of pentagons and hexagons. At the same time, Terrones presented an article where transforming an ordinary graphite in a graphite cone by modifications of the Gaussian curvatures was mathematically explained.(9)

2.1. Carbon nanocones

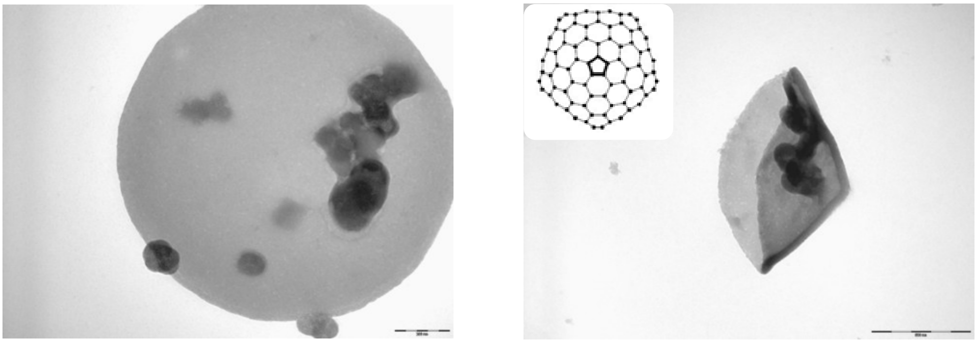
M. Ge and K. Sattler(4) were the first to synthesize nanometer-sized coal cones by vapor condensation on a substrate of carbon graphite. For this purpose, a carbon film was heated at high temperature in ultra high vacuum conditions ($2x10^{-8}$ Torr) to generate carbon vapor. Then, it was cooled to -30 °C and the cones were deposited on a quartz crystal film and analyzed using scanning tunneling microscopy (STM). Cones of up to 24 nm in length and with a base diameter of 8 nm were observed. They presented an apex angle on the cone of ca. 19° corresponding to the presence of 5 pentagons in the structure and, according to the authors, it was possible to find closed cones at the base with spherically shaped cap. In addition, other nanostructures such as islands and tubules were also found.

Three years later, Krishnan et al. proposed a novel technique for the synthesis of carbon nanocones.(10) They conducted synthesis experiments using the pyrolysis of hydrocarbons in a carbon arc, also called Kvaerner Carbon Black & Hydrogen Process (CB&H).(11) Briefly, it consists of using an industrial-scale carbon-arc plasma generator (torch configuration) where a continuous flow of hydrocarbons (in this case, heavy oil) was introduced at 50 to 150 kg/h under a reactor pressure of 2-3 bar. Subsequently, a plasma with a temperature of ca. 2000 °C was generated. The temperature was determined by the level of graphitization of the final product. After this process, a carbonaceous solid material was obtained together with other subproducts that were recirculated into the reactor.

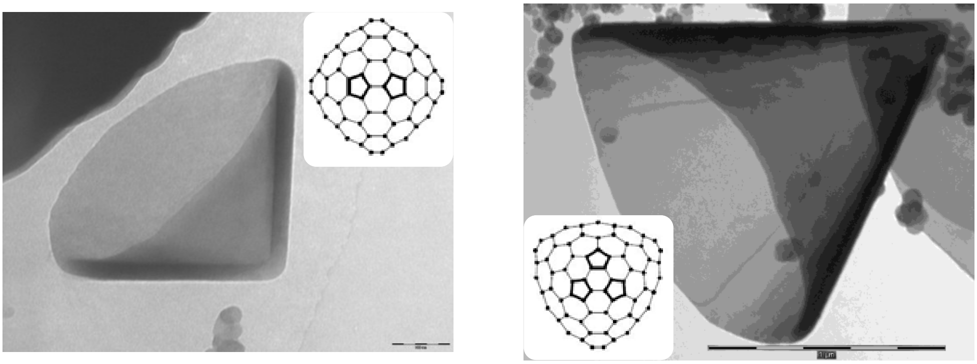
The analysis and characterization by transmission electron microscopy (TEM) of the carbonaceous product obtained revealed that the sample consisted of graphitic microstructures which presented structural imperfections due to deformations in the graphitic sheet with an angle multiple of 60°. As it was previously indicated, it corresponds to the number of effective pentagons introduced for producing such imperfections. The authors identified the five predicted types of conical structures resulting from the presence of a variable number (1 to 5) of pentagons in the structure. Furthermore, the authors found laminar structures with a disk shape which did not present any imperfection. They were considered as a variety of nanocones without the presence of pentagons in their structure. Figure 2 shows the six carbon nanostructures identified.

Structural weight distribution of the sample was found to be: 70% disks, 20% nanocones and the remaining 10% corresponded to carbon nanotubes and amorphous carbon.

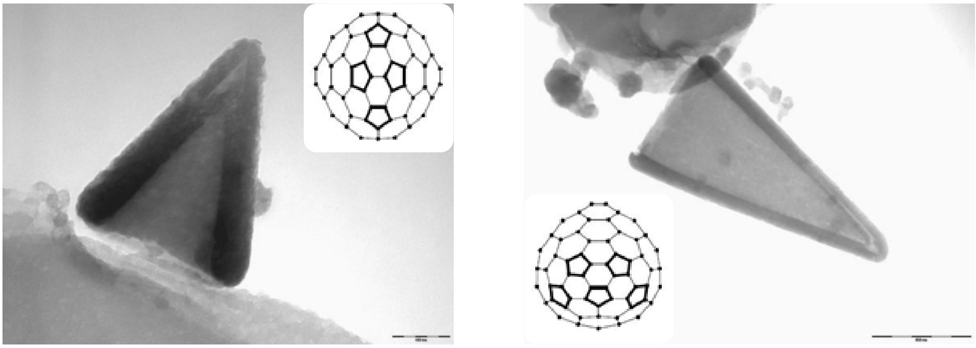
Finally, the existence of naturally formed carbon nanocones was discovered by Jaszczak et al.(12) In this case, carbon nanocones grew stacking on the surfaces of millimeter-sized polycrystalline spheroidal aggregates of graphite and had an overall height from less than 1 µm to 40 µm. Moreover, these cones had a greater distribution of angles that form the vertices, suggesting that nucleation of the pentagon defects is not the only factor determining graphite cone morphologies. The energetic layer to layer



0 pentagons – Carbon disk　　1 pentagon – 112.9°



2 pentagons – 83.6º　　3 pentagons – 60º



4 pentagons – 38.9º　　5 pentagons – 19.2º

Figure 2. Relationship between the number of pentagons and the opening angle of the carbon nanocones. Adapted from reference 70.

interactions between the graphene sheets also play an important role in determining micro- and nano-scale carbon structures.

2.2. Single-walled carbon nanohorns

Three methodologies have been proposed for the synthesis of single-walled carbon nanohorns, namely: synthesis by CO_2 laser and Nd:YAG laser ablation of carbon and synthesis by arc discharge ignited between carbon rods.

Single-walled carbon nanohorns were initially synthesized by Iijima et al. by CO_2 laser ablation of graphite.(5) The procedure was performed as follows: a piece of graphite (50 mm in length, 30 mm diameter, with 99.9% purity and no metal catalysts in the composition) was placed inside the synthesis chamber under an Ar atmosphere. Then, it was rotated on its axis at 6 rpm and the beam of a CO_2 laser irradiated the surface by a high energy source with a wavelength of 10.6 μm, at intensity of 5 kW/cm^2, with a variable pulse from 10 ms to continuous light and a beam diameter of 10 mm, so that a fresh target surface was continually exposed to the laser beam. Under these conditions, a black powder was obtained at a production rate of 10 g/h. It was characterized by TEM, scanning electron microscopy (SEM), Raman spectroscopy, electron energy loss spectroscopy (EELS) and X-ray diffraction, and resulted to be a structurally homogeneous product (95% purity) without inclusions of other carbon structures.

The synthesis procedure provides spherical structures with an approximate diameter of 80 nm. These structures were composed of small spherical individual particles (carbon nanohorns) with tubular forms and their vertex forming a conical-shaped with angles of 20º due to the inclusion of five pentagons in the network of hexagons which form the structure. The average diameter of the tubular portion of nanohorn was 2-3 nm, which means that they are larger than single-walled carbon nanotubes (1.4 nm). The nanoparticles can form stable aggregates due to the existence of van der Waals interactions between the individual carbon nanohorns, acquiring spherical dahlia-shaped aggregates.

The synthesis of SWNHs using continuous Nd:YAG laser ablation by single pulses was proposed by Puretzky and co-workers(13) obtaining a production rate of single-walled carbon nanohorns of 10 g/h approximately. The procedure was conducted in a quartz cylindrical chamber under an Ar atmosphere pre-heated at 1150 °C. Subsequently, a Nd:YAG (λ = 1064 µm) laser beam irradiated the graphite target. The selected power was 600 W and the frequency 500 Hz, which gave a maximum power density applied to the graphite sheet of 10 kW. The authors estimated the temperature reached in the surface of the graphite layer in ca. 3700 °C, which qualitative assesses the effectiveness of the ablation performed, proving pulsed laser of 10 ms the best conditions for the most effective ablation. It was also reported that when short pulses of laser were applied, SWNHs aggregates were more compact, reducing the interstitial spacing. Finally, the authors proposed that SWNHs are formed by sublimation of small particles with the mobility diameters of 15-20 nm from the nanohorn soot at temperatures >950 °C.

The synthesis of SWNHs by arc discharge was carried out for the first time in 2004 by Yamaguchi et al.(14) In a preliminary step, a direct current with a rate repetition of 1 Hz (duration 0.5 s) was applied to a carbon rod with 5 mm of diameter. Then, the carbon rod was kept to ca. 1 mm spacing of the spark and proceeded to the synthesis by applying an arc current of 120 A and a voltage between electrodes of 15 V with pulses of 30 s, resulting in a electrical power density on the surface of the carbon rod of ca. 9 kW/cm^2. This was carried out under air atmosphere at atmospheric pressure and preheating both the rod and the synthesis chamber at 1000 °C to reduce the presence of amorphous carbon in the final product. Subsequently, a black powder was collected and characterized by TEM, SEM and Raman spectroscopy. The aggregates obtained following this procedure showed average diameters of ca. 110 nm. The higher value compared with that obtained using CO_2 laser ablation was attributed to the temperature gradient of the synthesis chamber and the carbonaceous substrate during the growth of SWNHs, which was much more pronounced in the case of the arc discharge synthesis.

Furthermore, Wang et al. used a new variant of the synthesis process of SWNHs through the arc discharge.(15) It was carried out by immersion of the graphite electrodes (99.99% carbon) in liquid nitrogen and applying a voltage and electric current of 34 V and 50 A respectively. Using this technique, MWNTs, amorphous carbon and SWNHs aggregates were obtained with a production rate of 17 g/h. According to the authors, the SWNHs produced using this method were structurally similar to those produced by Iijima et al.(5)

Regardless the synthetic procedure followed, the presence of a catalyst is not required to obtain SWNHs. This mean that, in addition to a high purity of the material obtained (usually higher than 90%), it is transition metal free, which will affect to the magnetic and electronic properties, as it will be commented on in the following section.

3. Properties of conical carbon nanoparticles

3.1. Properties of carbon nanocones

There are few references dealing with the systematic study of the properties of carbon nanocones. In addition, to the electric and magnetic properties, several considerations about structural and mechanical characteristics have been published. They are commented on in this subsection.

Wei and co-workers investigated the mechanical properties (elasticity and plasticity) of carbon nanocones using a simulation model when they were subjected to different stresses.(16) According to the authors, the opening angle of the vertices of nanocones plays a very important mechanic role since the greater the angle, the greater the failure strength and the nanostructures present a smaller maximum strain under stress. Compared to the SWNTs, the nanocones have an earlier tensile rupture and higher failure strength. In addition, the authors were able to see that the carbon nanocones had not obvious elastic limit. The Young's moduli, a parameter that is directly associated with length changes experienced by a material when it is subjected to the action of tensile or compressive loads, was calculated by the authors and they found that this parameter decreased with increasing the radius or height of the nanocones.

The morphology and structure of the walls of the carbon nanocones was studied by Naess and co-workers.(17) They concluded that the temperature at which each nanocone was subjected individually during the synthesis process will strongly influence its final structure. Using techniques such as

TEM, synchrotron X-ray and electron diffraction, they could verify that the walls of nanocones are composed by several layers of a component such as graphite with a noncrystalline covering. Furthermore, the authors noticed that the nanocones have a well-defined atomic structure, but sometimes the symmetry of the structure can be broken and nanocones with two apexes appeared. In this case, Naess et al. proposed the use of these nanoparticles in the preferential adsorption and storage of hydrogen.

Helgesen et al.(18) characterized carbon nanocones by microscopic analysis (SEM, TEM and atomic force microscopy, AFM) and they concluded that carbon nanocones have structures similar to those already predicted by other authors. Indeed, they suggested that the carbon nanocones/disks are composed of several superimposed graphene layers, with an approximate thickness between 10 and 20 nm, although in some cases they can reach 60 nm thickness. Furthermore, these authors conducted experiments to measure electron spin resonance (ESR) which were compared with those of carbon nanotubes and graphite. The differences found in the shift of the ESR peak of carbon nanocones with respect to the other two nanomaterials allowed the authors to conclude that carbon nanocones have electrical properties different to those exhibited by carbon nanotubes and graphite. The same conclusion was confirmed by Raman spectroscopy measurements.

Different studies about the electrical properties of carbon nanocones agree that the valence orbitals will depend on the topology of nanocones, which will determine the curvature of the nanostructure.(19,20) As it is the case with carbon nanohorns, this fact allows the electron density to be concentrated at the tips of the carbon nanocones, thereby creating places with higher reactivity.

Shenderova et al.(21) studied the shape and electronic properties of carbon nanocones and they found that the nanocones which present only one pentagon in their apex are most suitable for emitting tunneling electrons when an electric field is applied to them. Therefore, carbon nanocones have been proposed for applications where they were assembled on flat surfaces to use their electronic properties such as in small-scale lithography, since in this case, the electron emission would be highly localized in the nanocone apex. Furthermore, the authors propose that carbon nanocones can be attached to diamonds to be used as probe tips.

In a theoretical study, Qu et al.(22) evaluated the electronic states of carbon nanocones and their field emission properties. They concluded that the nanocones with three pentagons exhibited a higher field emission current. The authors could verify that the angles formed by the cones are very important because the energy of formation and cohesion of nanocones depends on this feature, while other electronic properties such as the work function, local density of states, redistribution of the charge, and field emission pattern will depend on the position of the pentagons at the vertices. The importance of the position of the pentagons in the structure of the carbon nanocones already reported by Charlier et al.(23), who studied the electronic properties of these nanoparticles using both tight-binding and *ab initio* calculations.

Other studies of electrical and magnetic properties of carbon nanocones were made by Svåsand et al.(24) and Kováč et al.(25) To investigate the electrical properties, the carbon nanocones were dispersed in silicone oil and subjected to low AC electric fields which were enough to form fibrillar structures, although it was determined that these structures were relatively weak due to the high conductivity they presented. To study the magnetic properties of nanocones, they were dispersed in a ferrofluid and later there were analyzed by magnetorheological measurements.(24) This dispersion was subjected to low magnetic fields and an increase in viscosity, due to hydrodynamic effects produced by the interaction between carbon nanocones, was observed.

3.2. Properties of single walled-carbon nanohorns

3.2.1. Porosity and adsorption capacity

An important property of the SWNHs is their high surface area, a common characteristic of carbon nanoparticles. However, this surface area can be increased by increasing the number of pores present in the structure of the SWNHs by heating the solid under O_2(26-30), CO_2(31) or air atmosphere(32), or by strong chemical oxidation using H_2O_2(33,34), H_2SO_4(35) or HNO_3.(36,37) By way of example, Figure 3 shows the images of SWNHs after different thermal treatments. In this regard, Murata et al.(38) performed measurements to determine the porosity of SWNHs and they concluded that the as-grown SWNHs have a surface area of 330 m^2/g and a pore volume of 0.2 mL/g, while after heating the SWNHs at 693 K in oxygen atmosphere, both surface area and pore volume increases, reaching values of 1300 m^2/g and 0.9 mL/g, respectively. This feature directly affects to the adsorption properties of SWNHs which were evaluated by calculating the N_2 adsorption isotherms at 77 K. From this study they also identified the preferential adsorption sites of SWNHs, which are from highest to lowest adsorption capacity: a) the interstitial sites between the individual SWNHs (with spaces around 0.4 nm); b) the inner surface of the walls of SWNHs and; c) the central region of the hollow space inside the SWNHs.

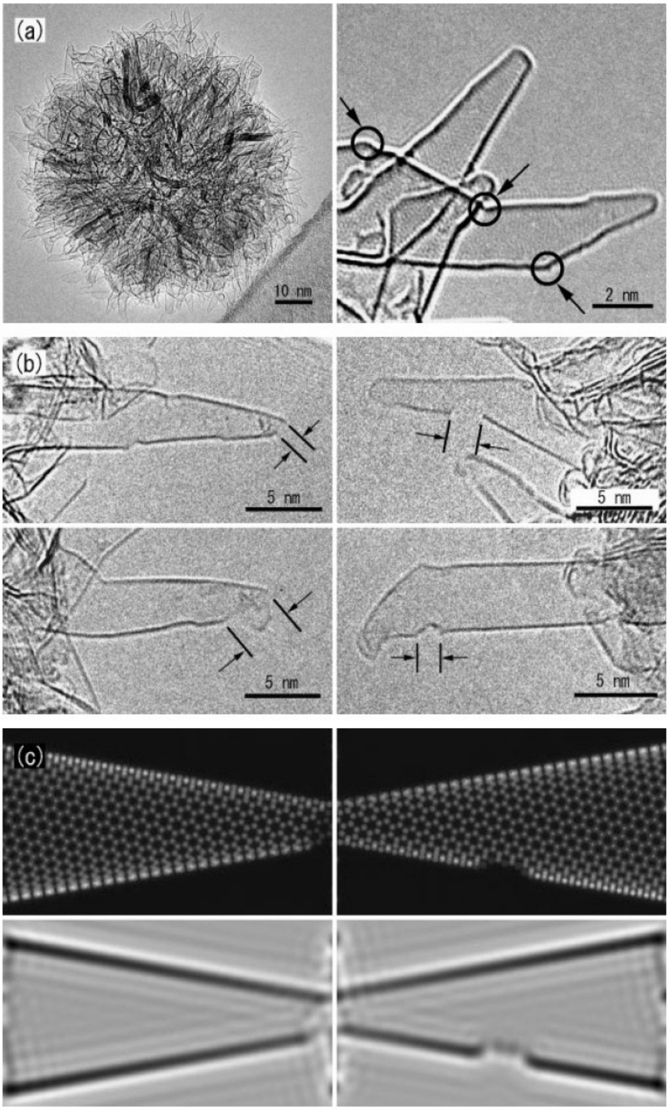


Figure 3. Micrograph of SWNHs before (a) and after (b) thermal treatment. (c) and (d) are simulations by atomic models for the structures of the path at the tip (left) and the sidewall (right) of the SWNHs. From reference 30.

Fan et al. also studied the influence of the gradient temperature used during the oxidation of the SWNHs.(32) They used a low gradient (1 ºC/min or less) and a fast gradient (4 ºC/min) in an air atmosphere (21% oxygen). By different adsorption studies, they concluded that the quantity of material adsorbed on the oxidized SWNHs generated by slow combustion was larger than that of oxidized SWNHs with the holes opened by quick combustion.

Later on, Pagona et al. demonstrated that the edges of the open holes on the walls of the SWNHs are preferential functionalization sites by generating oxygenated functional groups such as carbonyl groups(26) and/or carboxylic groups.(39) They can act as precursors for the immobilization of other functional molecules such as porphyrins(28) or Pd nanoparticles.(29) Few years later, through a theoretical study, it was found that the largest area of reactivity of SWNHs is located at the tips, due to the highest curvature and strain.(40)

Finally, it should also be noted that once the pores are created following any of the proposed strategies, they can be closed by heating at 1200 °C under Ar atmosphere.(41) The holes in the tips have a greater tendency to be closed than those found on the walls.

3.2.2. Electronic/Electric properties

As it was explained before, the carbon nanohorns are characterized mainly by two features: their nanometric size and conical structure. The combination of these characteristics influences the electrical properties of these nanostructures. These properties were studied by Berber and co-workers by analyzing the atomic orbitals of the SWNHs.(42) Among the various investigations carried out by these researchers, it should be highlighted the study of the positioning of the 5 pentagons in the tip of the SWNHs which gives their characteristic conical shape. Moreover, the behavior when they were subjected to disintegration by high temperatures was also investigated.

The authors were able to distinguish 6 different arrangements of the five pentagons existing in the conical tips of SWNHs by scanning tunneling spectroscopy due to different local electron densities at the vertices, and they concluded that, despite the differences in the location of the pentagons, tips always maintained an angle of ca. 20°. Furthermore, a net transfer of electrons to the pentagonal ring of the tips was detected by scanning tunneling microscopy images. The thermal stability of the SWNHs was corroborated in the range 2000-4000 K. When the temperature was increased to 5000 K, SWNHs were broken preferentially in areas where the pentagons were located.

Bandow and co-workers studied the electrical response of SWNHs chemically doped with Li, K and Br.(43) After a characterization by Raman spectroscopy and conductivity measurement, a comparative study between pure and doped SWNHs was carried out. The authors concluded that an abnormally small charge transfer occurs when the nanostructures are doped with these substances.

Bonard et al. also tested the electrical properties of SWNHs.(44) To this end, SWNHs were self-supported on a ceramic film and subjected to different voltages to study their electrical behavior. According to these experiments, the SWNHs showed high current capabilities and, although they were less efficient than other emitters, SWNHs presented a turn-on field below 4 V/mm that makes them good candidates for field emission applications that do not require high current densities.

The increase in electronic density present on the tips of SWNHs was also investigated by Kolesnikov et al. using the continuum gauge field-theory model.(45) As mentioned above, this increase in electronic density is a consequence of the existence of pentagonal defects in the structure which results in the localized electron state at the Fermi level (a true zero-mode state).

As it was discussed in the previous section, the structure of SWNHs can be modified by heat treatment at high temperatures. When this occurs, their electrical properties are also modified. In this regard, Urita et al. analyzed the electrical behavior of SWNHs and oxidized SWNHs when they are subjected to the adsorption of O_2 and CO_2.(46) After characterization by high resolution-TEM, the SWNHs and oxidized SWNHs were evaluated by nitrogen adsorption isotherms at 77 K and it was found that the temperature dependence of the electrical conductivity indicates that the SWNHs are semiconductor. However, a difference in the conductivity was detected when oxidized SWNHs adsorbed CO_2 or O_2, observing an increase in conductivity when CO_2 was adsorbed and a decrease in the adsorption of O_2. According to the authors, this happened due to the annihilation of pentagons in the single graphene wall by oxidation.

3.2.3. Magnetic properties

Magnetic properties of SWNHs are closely related to the electrical ones. Garaj et al. studied the magnetic properties of SWNHs by ESR.(47) For this purpose, different ESR measurements were carried out in a temperature range between 5 and 300 K. The resonance spectra obtained, showed two different electronic systems. On the one hand, high surface disordered graphene sheets and large diameter cones due to the graphene are a two-dimensional structure, yielding a unique temperature-activated paramagnetic susceptibility. Furthermore, it was found that within aggregates dahlia-shaped SWNHs there is a high structural disorder.

A year later, Bandow et al. deepened in the knowledge of the magnetic properties of SWNHs by ESR studies and static magnetic susceptibility measurements.(48) Through these studies, the authors demonstrated the existence of at least one unpaired electron spin, which may come from the tip of the nanostructure caused by an alteration due to the inclusion of 5 pentagons. The small diamagnetism presented by SWNHs might reduce the diamagnetism that should be present for the sp^2 hybridation of the carbon structure.

Imai et al. carried out spectroscopic studies of ^{13}C nuclear magnetic resonance of the surface of the SWNHs(49) and it was found that these carbon nanoparticles are composed of two distinct components: (a) the surface of carbon nanohorn and (b) the graphite which is inside the SWNHs aggregates. Both were differentiated through chemical shift (124 ppm and 116 ppm respectively) and by the spin-lattice relaxation behavior (faster for the first and much slower for the second). In addition, these researchers proposed that the electronic properties of both components indicate that SWNHs are nonmetallic. Through the studies, the authors stated that the SWNHs exhibit similar behavior to those produced in semiconducting materials with paramagnetic centers, and such behavior comes from structural defects where the highest electron density was found.

4. Functionalization of conical carbon nanoparticles

The solubility of SWNHs in aqueous media and common organic solvents is very limited. This fact reduces their use in biomedical, clinical or environmental applications. Several alternatives have been described in the literature in order to overcome this shortcoming. As it is the case with carbon nanotubes, they can be classified in two groups: (i) the use of surfactants or (ii) functionalization, both aimed at reducing their hydrophobicity and thus increase the solubility in polar media. Concerning the use of surfactants, Zang et al. used sodium dodecylbenzenesulphonate to isolate individual SWNHs(50) in contrast to sodium dodecyl sulphate, which preferably solubilized the aggregates.(51) However, the functionalization of SWNHs by means of different strategies is by far the most reported approach. It can be explained taking into account the simplicity of the majority procedures, in contrast with the complexity of those reported for the carbon nanotubes. To the best of our knowledge, only one reference about a theoretical study of the functionalization of carbon nanocones has been reported by Trzaskowski et al.(52) Therefore, this section will present only those alternatives described for SWNHs. The functionalization strategies have been divided in two main groups: a) those in which the functional groups are introduced by π-π interactions, without altering the surface of the nanostructure; and b) those involving the opening of nanowindows on the SWNHs. The relevant resulting modified SWNHs are shown in Tables 1 and 2.

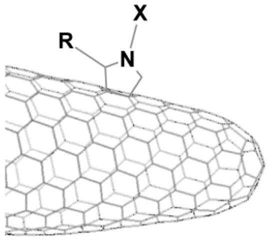
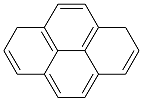
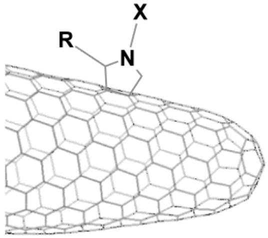
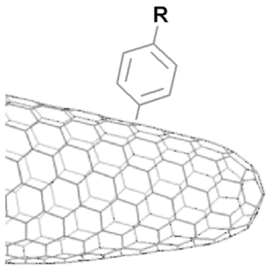
4.1. Covalent functionalization of SWNHs

Tagmatarchis et al. solubilized functionalized SWNHs via 1,3-dipolar cycloaddition with azomethine ylides. For this purpose, the walls of the SWNHs are modified by introducing fused substituted pyrrolidine rings.(53) By adding hydrophobic oligoethylene glycol functionality, the SWNHs were soluble in polar organic media, such as dichloromethane, chloroform or acetone, while simple alkyl units in the α-aminoacid allows the dispersion in toluene and dimethylformamide. The solubility in water was achieved by introducing NH_3^+ groups on the surface. The final concentration obtained ranged from 1 to 2 mg/mL, which is considerably lower than that obtained using other alternatives.(54)

Almost simultaneously, Cioffi et al. proposed 1,3-dipolar cycloaddition to introduce two different compounds by the reaction between pristine SWNHs and a suitable aldehyde and aminoacid.(55) Further derivatization with amine triflate salt makes the SWNHs partially soluble in water and methanol. The solubility can be increased by using larger polyethylene glycol chains.

Following the same strategy, Pagona et al.(40) introduced an oligoethylene chain or aromatic chromophores on the SWNHs to make them soluble in dimethylformamide (DMF), toluene, dichloromethane and tetrahydrofuran (THF). On the other hand, the functionalization with saturated alkyl chains yielded SWNHs only soluble in DMF and toluene. After a rigorous characterization of the products by infrared and UV-Visible spectroscopies, ^{1}H-NMR and HR-TEM, they concluded that the population of the organic units added during the chemical modification process was higher near the conical tip of the SWNHs, where the highest curvature and strain exist.

Table 1. Selected covalent modification of SWNHs

Structure	Functional groups	Ref.
	**1** **R** = H **1** **X** = $(CH_2CH_2O)_2(CH_2)_2NH_3^+CF_3COO^-$ $(CH_2)_4NH_3^+CF_3COO^-$ $(CH_2)_2NH_3^+CF_3COO^-$ **2** **R** =  **2** **X** = $(CH_2CH_2O)_2(CH_2)_2NH_3^+CF_3COO^-$ $(CH_2)_4NH_3^+CF_3COO^-$ $(CH_2)_2NH_3^+CF_3COO^-$	**[53]**
	**1** **R** = H **1** **X** = $(CH_2CH_2O)_2(CH_2)_2NH_3^+CF_3COO^-$ $(CH_2)_4NH_3^+CF_3COO^-$ $(CH_2)_2NH_3^+CF_3COO^-$ **2** **R** = $-C_6H_{13}$ **2** **X** = $-CH_3$	**[55]**
	**R** = NO_2 COOH $CH_2(CH_2)_2CH_3$ $CONH(CH_2CH_2O)_2CH_2CH_2NH_3$	**[56]**

Pagona et al. proposed the in-situ generation of aryl diazonium compounds on the SWNHs walls.(56) Then, four different aniline derivatives were immobilized by maintaining the mixture under continuous agitation at 60 ºC for 18 h. The products obtained showed different solubilities in toluene, DMF, THF, methanol chloroform and water.

SWNHs can also be functionalizated with porphyrin ($H_2P_4^+$) in order to make them soluble in water.(57) The process involves the sonication of pristine SWNHs added to an aqueous solution of H_2P4^+ for 3 min and a further stirring during 20 h. The solution was filtered through PTFE filter. The microscopic and spectroscopic analysis of the product confirmed the immobilization of the porphyrin on the SWNHs via π- π stacking interaction.

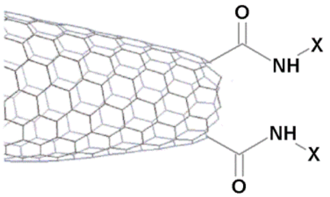
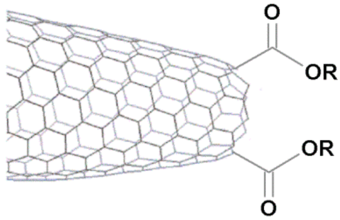
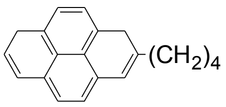
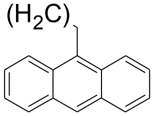
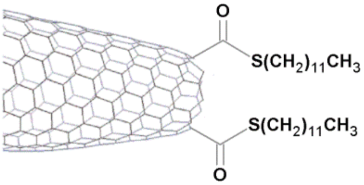
Analogously, a standard condensation reaction between amino-terminated functionalized SWNHs and a carboxylic acid derivative of coumarin was carried out.(58) The resulting hybrid material was found to be soluble in DMF, methanol, CH_2Cl_2 and toluene.

4.2. Opening nanowindows on the surface

In a previous section of this article, the possibility of increasing the porosity of the SWNHs following different approaches has been commented on. Also, the chemical activity of the opened nanowindows was also demonstrated. This reactivity is the basis of the different surfaces modification of SWNHs dealing within this subsection.

Pagona et al. proposed the opening of nanowindows on the tips of the SWNHs by thermal oxidation at 580 ºC an O_2 atmosphere (0.1 MPa, 10 min).(26) The process generated carboxylic groups which were further converted in acyl chloride (SWNH-COCl). The higher reactivity of this derivative facilitated the formation of SWNHs functionalized with short or long hydrophobic alkyl chains, pyrene or anthracene groups or even masked active groups suitable for further organic reactions. The solubility values depend on the final or intermediate derivative obtained.

Table 2. Selected functionalization of opened windows SWNHs

Structure	Functional groups	Ref.
	**X** = $(CH_2)_{11}CH_3$ $(CH_2)_4NHBoc$ $(CH_2)_2(OCH_2CH_2)_2NHBoc$	**[26]**
	**R** = $(CH_2)_{11}CH_3$  	**[26]**
		**[26]**

Murakami and coworkers described a procedure to endow SWNHs with dispersibility in aqueous media using an amphiphatic polymer-drug complex (PEG-DXR).(27) First, the pristine SWNHs were oxidized at 580 ºC for 10 min under an O_2 atmosphere and then, a second heating step at 400 ºC for 2 h under ultrahigh vacuum (ca 1 x 10^{-7} Torr) was carried out. This step allows the binding of the SWNHs with the polymer-drug complex PEG-DXR. The derivative obtained is useful to introduce SWNHs in the clinical field as a drug delivery element due to the biocompatibility of the PEG-DXR.

Alternatively, a porphyrin ($H_2P_4^+$) can react with the carboxylic groups, giving a functionalized solid soluble in CH_2Cl_2, THF and toluene affording stable black solutions for several weeks.(28)

Following this research line, Miyawaki et al. reported that the reactivity of the hole edges can be easily controlled through an appropriate choice of solvents.(59) The authors corroborated that the carboxyl, carbonyl or the hydroxyl groups generated on the SWNHs surfaces can form anhydrides or lactones through dehydration by immersion in water or other organic solvents such as methanol. The functional groups on the hole edges were eliminated by a thermal treatment at 1200 ºC under an H_2 atmosphere for 3 h through a substitution of these groups by hydrogen. However, when the same treatment was carried out under Ar atmosphere, the holes tended to be closed.(41) This procedure was more efficient on the cone tips than on the walls of the SWNHs. The closing of the pores was corroborated by a decrease in the pores volume of SWNHs.

Light-assisted oxidation with hydrogen peroxide was used by Zhang et al. to introduce carboxyl groups on the hole edges created on the walls of the SWNHs.(33) These functional groups were further modified with bovine serum albumin (BSA) through diimide-activated amidation, resulting in a hydrophilic product which is readily dispersed in aqueous medium. The final product was characterized by TEM and laser confocal microscopy, TGA, FT-IR spectroscopy and DLS. The authors also observed that the BSA-modified light-assisted oxidation-SWNHs were incorporated into cultured mammalian cells via an endocytosis pathway. The same functionalization process was followed by Sandanayaka and coworkers to immobilize molecules of phthalaticianine (ZnPc) on the SWNHs oxidized with hydrogen peroxide. The final complex was useful for electron- and energy-transfer mechanisms employed for the photosynthetic model and photodynamic therapy.(34)

A different functionalization method was developed by Shu et al.(60) SWNHs were functionalized with carboxylic groups by a solvent-free high-speed vibration milling method. For this purpose, pristine SWNHs were mixed with succinic acid acyl peroxide and the resulting mixture was placed in a stainless steel capsules and shaken vigorously for 90 min. The solid obtained was soluble in water and acetone for several months. In addition, the functionalized SWNHs may be used in biotechnological applications such as drug and gene delivery systems. Huang et al.(61) used the same approach to obtain nanoparticles-supported liposomes which can be a promising platform for drug delivery, vaccine development and biomedical imaging. The characterization analysis showed that the SWNHs were encapsulated by liposomes with an average size of ca. 120 nm.

SWNHs were thermally fluorinated by heating the nanoparticles at 573 K and further reacted with fluorine gas in a temperature gradient from 303 to 473 K for 24 h and 1 atm.(62) This process changed the C-C boundary to C-F ones. The treatment also generated pores on the SWNHs surface, which were higher at higher temperature. An increase in the temperature also changed the semi-ionic nature of the C-F boundary to a covalent one.

Microwave energy can also be used for SWNHs functionalization. Yoshida and Sano oxidized pristine SWNHs through microwave irradiation of the solid at 150 W for 10 s.(63) The procedure increased the dispersability of the oxidized SWNHs up to 25 times in comparison with the pristine SWNHs. This methodology can be used to deposit platinum nanoparticles at the tips of the SWNHs due to the introduction of oxygen-containing groups after the microwave treatment.

Rubio et al. prepared a mixture of pristine SWNHs with glycines and aldehydes, such as α-amino acid, which was subjected to a microwave irradiation for 1 h. Then, isoamyl nitrite was added and a condenser was attached. The mixture was sonicated for 90 min and the final product obtained presented the typical functional groups of a 1,3-dipolar cycloaddition and it was soluble in polar organic solvents such as CH_2Cl_2, DMF and o-DCB.(64)

Finally, microwave assisted functionalization was also used by Economopoulos et al. to incorporate malonyl moieties on the SWNHs surfaces.(65) Karousis et al. also used this energy to obtain functionalized SWNHs via (2+1) nitrines cycloaddition, which permits further stabilization of Au nanoparticles on the surfaces.(66)

5. Applications of conical carbon nanoparticles

5.1. (Micro)extraction techniques

The SWNHs present an extensive surface area and horn interstices, which provide them with an enhanced adsorption capacity as regards other carbon nanostructures. Moreover, the surface area can be increased by opening sidewall nanowindows which permits the infiltration of small molecules (e.g. fullerenes or iron nanoparticles), which increases the sorptive surface area. Moreover, the oxidation conditions influence the size of the nanowindows, allowing the preparation of different molecular sieves by controlling the operational conditions. The sorbent capacity of single-walled conical nanoparticles was first demonstrated by their storage capacity. This property can also been exploited not only for material storage and further progressive delivery, but also for the isolation and preconcentration of analytes in miniaturized extraction techniques.

The reduction of dimensions in any sample treatment is accompanied by the use of more efficient tools as they must be capable of performing equally or better using less material or a reduced size device. In this context, nanomaterials in general, and carbon nanoparticles in particular, have demonstrated their usefulness in miniaturized solid-phase extraction (SPE) techniques.

Zhu et al. were the first in demonstrating the potential of single-walled carbon nanohorns in SPE using 4-nitrophenol as model compound using a glass carbon electrode modified with single-walled carbon nanohorns. This allows the monitorization of the adsorption process.(67)

Taking into account the better dispersability of SWNHs compared with other carbonaceous nanoparticles and the stability of the aggregates, they would be an outstanding sorbent in dispersive micro solid phase extraction techniques aimed at sensitivity enhancement. This fact has been demonstrated for the extraction of polycyclic aromatic hydrocarbons(68) and triazines(69) from waters. The key-factor in both applications was the dispersion of the nanomaterial. The carbon nanohorns were dispersed in an aqueous media in order to maximize the compatibility with the sample and to reduce the potential negative influence of an organic medium in the analytes extraction through increasing their solubility in the aqueous matrix. Taking into account these considerations, the SWNHs were functionalized via microwave irradiation to facilitate the dispersion. In comparison with carbon nanocones and carbon nanotubes, the efficiency of oxidized SWNHs was better under the same experimental conditions.

Carbon nanocones have been used as sorbent packed in conventional SPE cartridges(70) and immobilized on a fiber in solid-phase microextraction (SPME).(71) Carbon nanotubes and carbon nanohorns cannot be used as sorbent packed in cartridges because of two different reasons: carbon nanotubes aggregate after liquid phases (either the sample, the conditioning reagent or the eluent) flows through the cartridge. This results in a low efficiency and irreproducible preconcentration/elution processes. On the other hand, the small size of the SWNHs makes impossible their packing in the cartridge as they tend to stick in the inner walls.

Carbon nanocones do not share any of these negative connotations as their tendency to aggregation is very low and they are easily packed at the bottom of the SPE cartridge. As it was stated before, commercial carbon nanocones contain a 10% (w/w) of amorphous carbon which results in an irreversible and irreproducible interaction with the analytes. Therefore, the commercial mixture was subjected to a thermal treatment (450 °C, 20 min) to remove almost quantitatively the percentage of amorphous carbonaceous material. This fact was experimentally corroborated by preparing different cartridges packed with the raw solid and after different thermal treatments, being the signal obtained with the former ca. 30% lower than with the others. Moreover, the sorbent capacity of carbon nanocones was compared with that provided by MWNTs for the preconcentration of chlorophenols. It was observed that up to three sequential extractions were necessary to achieve the quantitative elution of the analytes from the carbon nanotubes in comparison with one aliquot of 200 µL of hexane to complete chlorophenols elution from the carbon nanocones cartridge. This could be explained by the lower aggregation tendency of the cones which facilitates both the retention and elution steps.

Concerning the use of carbon nanocones as a coating in SPME, its potential has been evaluated for the determination of volatile organic compounds (toluene, ethylbenzene, xylene isomers and styrene) in waters under the headspace modality to minimize the potential degradation of the fiber. The carbon nanocones were immobilized on a stainless steel by means of an organic binder. The resulting fiber coating presented an average thickness of 50 µm through the 35 mm length. The retained compounds were thermally desorbed in 10 mL glass vials at 110 °C. The limits of detection were between 0.15-0.5 ng/mL with an average precision of ca. 7%. The analytical features were better than those provided by commercial fibers used for the same analytical problem.

5.2. Eletrophoretic separations

The usefulness of capillary electrophoresis for analytes separation has increased with the number of modalities developed. Micellar electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) are among the most used. In both cases, a specific element is added to the background electrolyte or is immobilized in the inner wall of the capillary in order to enhance or even achieve the electrophoretic separation of a given family of compounds.

As far as MEKC is concerned, the nature of the pseudostationary phase determines the separation of the analytes. In this regard, micelles, microemulsions of surfactant coated oil droplets, cyclodextrines, proteins, liposomes, and more recently nanoparticles, have been used to extend the applicability of the technique.(72) Carbon nanoparticles have been successfully employed in this context. They should be previously dispersed with a surfactant in order to reduce their aggregation, thus allowing an additional interaction element into the micelles. However, the concentration of the nanoparticles in the background electrolyte is limited by their influence in the baseline stability. While carbon nanotubes and fullerenes have demonstrated their potential in MEKC, the behavior of carbon nanohorns is not so clear.(51) This fact can be explained taking into account the lower size of the carbon nanohorns compared with the nanotubes and fullerenes, which results in a lower synergic effect to the micelles.

CEC uses a capillary which can be packed with a given sorbent or it can be immobilized in the inner surface of the capillary. In this case, the interaction can be established through π-π interactions, van der Waals forces with the nanoparticles or their pores or even with the polar groups resulting from the functionalization of the nanoparticles. The particular electronic distribution of carbon nanohorns will favor the interaction of polar compounds in the conical end while the central part would retain less polar analytes. The results obtained were compared with those provided by the bare and immobilized carboxylated-SWNTs capillaries for the separation of hydrosoluble vitamins.(51) The capillaries immobilized with the nanoparticles showed good separation efficiencies and higher retention factors than those provided by the bared fused silica capillary. However, the carbon nanohorns showed an improved resolution as the five analytes were completely separated and none of them migrated with the electrosmotic flow. This improved performance is ascribed to the double interaction of the analytes with the carboxylic groups of the nanoparticles via electrostatic interactions and the π-π interactions of the compounds and the dahlia flower like superstructures owing to their large surface area. Among the analytical features, the excellent precision between runs, days and capillaries is the most remarkable as it benefits the robustness of the procedures.

5.3. Carbon nanohorns for material storage

One of the first uses proposed for carbon nanohorns was their use as storage units for gases or other materials. Murata et al. were the first in demonstrating the capacity of SWNHs to store methane even at room temperature.(38) The advantage of this nanomaterial in comparison with carbon nanotubes is its capability to form stable aggregates. The adsorption occurs at the interstitial spaces among SWNHs and in the case of o-SWNHs it also takes place internally and in the edges of the nanowindows.

The storage of methane is achieved by generating a highly porous network of SWNHs by repetitive compressing of the nanomaterial to form single-walled carbon nanoporous rigid structures. The space between adjacent SWNHs in the spherical aggregates is large enough to accommodate small gas molecules.(73) The SWNHs aggregates can also adsorb liquid ethanol ca. 3.5 times that a super high surface area carbon MACSORB.(74) This higher capacity was obtained without the need of any special activation or conditioning steps, which are mandatory for other carbonaceous material.

The adsorption ability of SWNHs can be enhanced following different procedures. One of them involves the use of a charge transfer by doping the solid with lanthanide (La, Eu, Er, Lu) nitrates.(75) The mechanism of this procedure relies on the distortion of the electron distribution through the graphene sheet which results in a non polar-polar interaction with the analyte. Indeed, the addition of europium nitrate to SWNHs increases the adsorption of methane ca. 50% as compared with as-grown SWNHs. The adsorption capacity can be regulated by introducing some additional molecules which could help to stabilize the compound on the SWNHs surface. This fact has been demonstrated for benzene, whose adsorption on SWNHs and oxidized-SWNHs has been studied in a dry atmosphere and in a water saturated atmosphere. As a result, it was observed that the benzene can form a π-hydrogen bonding complexes with the water molecules retained on the nanowindows opened on the nanoparticles. The benzene adsorbed inside the SWNHs was not affected by the presence of water.(76)

5.4. Miscellaneous

In addition to material storage, the stable dahlia SWNHs aggregates can also be used as support for catalysts, such as Pt.(77) The authors were capable of homogeneously dispersing Pt particles of ca. 2 nm in size, reaching up to 40 wt% content. The Pt catalyst supported SWNHs can be used in electrodes of polymer electrolyte fuel cells, with a current density larger than that of a carbon black one. It has been successfully employed in liquid phase oxidation of ethanol.(74)

The large surface area of SWNHs allows their use as carriers in controlled drug delivery systems. Moreover, the size of the aggregates fits the dimensions needed to permeate the biological systems. So, they can penetrate through the damaged vessels in tumor tissues. In this context, the slow release is critical in drug delivery to minimize the losses before reaching the target tissue/cells. Murakani et al. evaluated the potential of pristine and oxidized SWNHs for the in vitro binding and release of dexamethasone.(78) The authors used oxidized SWNHs as they were able to adsorb up to 6 times more DEX than as-grown SWNHs. The adsorption of the drug was carried out by mixing oxidized SWNHs suspension with the DEX solution, both prepared in water:ethanol 1:1. The mixture was incubated overnight, although the equilibrium was reached in ca. 1 h. The adsorbed amount was 200 mg of DEX per gram of nanoparticles when a 0.5 mg/L of DEX was used. Concerning the release profile of DEX-oxidized SWNHs in different media, the authors concluded that the drug is slowly released into PBS (ca. 50% within two weeks), while in cell culture medium two release phases, fast (less than 50% in 8 h) and slow (50% in 24 h), were observed. The authors suggest that it would be the presence of some hydrophobic compounds present in the culture medium responsible for an apparent increase of the solubility of DEX in this medium. These results confirm the possibility of using SWNHs as novel drug delivery system without significant side effects.

A similar conclusion was achieved by Ajima et al, who used oxidized SWNHs as anticancer drug carrier.(79) In this case, cisplatin was encapsulated inside the SWNHs aggregates by nanoprecipitation. The release rate was similar to that calculated for DEX, although in this case it was the same in PBS or cell culture media.

6. Conclusions

This contribution reviews the state of the art of conical carbon nanoparticles, specifically carbon nanocones and carbon nanohorns. These particular nanostructures differ from carbon nanotubes in their open tubular structure and the non-homogeneous electronic density through the graphene sheet, which is higher in the conical apex. This fact conditions some of their properties and hence their chemistry. The applicability of carbon nanohorns seems to be higher than that of carbon nanocones, for which two applications have been described up to date. SWNHs present as additional characteristic their ability to form stable aggregates with diameters between 80-100 nm which is believed by several authors to be the responsible for the majority of their applications. The main advantage of SWNHs as sorbent or storage media is their synthetic process, which is catalyst-free and exhibits a pore graphitic structure.

In the Analytical Chemistry context, SWNHs and carbon nanocones can be used for the development of miniaturized extraction tools taking advantage of their exceptional sorbent properties and their higher compatibility with aqueous and organic solvents after their easy functionalization (especially SWNHs). The continuous evolution of the research in this field would result in the discovery of new nanoparticles and the development of applications in different scientific and technological areas with outstanding properties.

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Herramientas analíticas

En el desarrollo experimental de la presente Tesis Doctoral se han empleado diferentes herramientas analíticas como: nanoestructuras de carbono; analitos objeto de análisis; disolventes orgánicos; materiales de laboratorio; muestras; instrumentos analíticos entre los que se encuentran sistemas cromatográficos, electroforéticos y equipos de microscopía; así como aparatos necesarios para llevar a cabo los distintos procesos analíticos. En esta sección de la Memoria de Tesis Doctoral se enumeran dichas herramientas y se profundiza en sus características y aspectos más relevantes de su utilización.

Nanoestructuras de carbono

Las nanoestructuras de carbono empleadas para la realización de esta Tesis Doctoral han sido:

– Nanoconos/discos de carbono, adquiridos a la empresa nórdica n-Tec (Oslo, Noruega). Su síntesis consiste en la descomposición de hidrocarburos mediante antorcha de plasma, a una temperatura de 2.000 ºC. El producto resultante contiene un 20% en peso de nanoconos, un 70% de discos y un 10% de carbón amorfo. Como se deduce de su nombre, los nanoconos de carbono presentan una disposición cónica adquirida debido a la inclusión de un número de pentágonos definido en su vértice que van de 1 a 5, lo cual hace que presenten un ángulo característico en el mismo, de tal modo que, cuando el nanocono presenta 1, 2, 3, 4 o 5 pentágonos en el vértice, la estructura adquirirá un ángulo de 113º, 84º, 60º, 39º o 19º respectivamente. Del mismo modo, se puede afirmar que la ausencia de pentágonos en el entramado hexagonal de la estructura da lugar a la creación de discos de carbono. En relación al tamaño de estas nanopartículas, los nanoconos de carbono vienen definidos por una longitud que oscila entre 300 y 800 nm, un diámetro máximo en la base del cono de 1 µm y un grosor de pared entre 20 y 50 nm. Los discos de carbono presentan un diámetro de entre 0.8 y 3 µm y un grosor similar a los nanoconos de carbono. Un dato adicional de estas nanopartículas es la estabilidad térmica en atmosfera de aire, la cual está garantizada hasta una temperatura de 500 ºC.

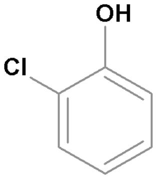
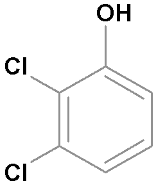
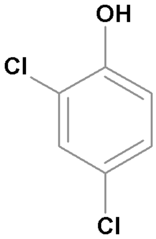
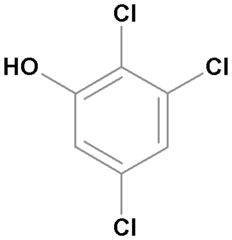
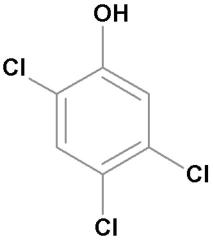
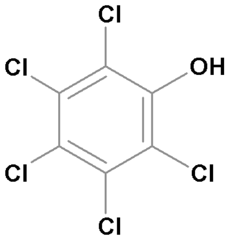
- Nanocuernos de carbono de pared simple (SWNHs). Estas nanopartículas fueron suministradas por dos compañías diferentes: Mer Corporation (Tucson, Arizona, EEUU) y Carbonium S.r.l. (Padova, Italia). Los nanocuernos de carbono de pared simple, al igual que los nanoconos de carbono, pueden ser considerados como nanoestructuras con forma cónica pero, a diferencia de las anteriores, los nanocuernos de carbono tienen una forma cilíndrica (similar a los nanotubos de carbono) con una terminación cónica en uno de sus extremos. Dicha terminación cónica se debe a la presencia de 5 pentágonos en el vértice, lo cual le confiere una abertura de ángulo de 20º. El resto de su estructura se compone de un entramado de hexágonos similares a los existentes en una lámina de grafeno. Los nanocuernos de carbono presentan una longitud aproximada de 40 a 50 nm, con un diámetro creciente que va desde 1-2 nm en el vértice a 4-5 nm en su extremo más ancho. Generalmente, este tipo de nanoestructuras se encuentran formando agregados estables con forma de dalia los cuales presentan unos diámetros medios de 60-80 nm. Una particularidad de estas nanopartículas es la ausencia de catalizadores metálicos en su síntesis, lo cual hace que se obtengan nanocuernos de carbono libres de impurezas.

- Nanotubos de carbono de pared simple (SWNTs), adquiridos a Sigma-Aldrich (Madrid, España). La pureza del material es superior al 90%. Sus longitudes varían entre 0.5 y 2 µm y sus diámetros externos en la estructura cilíndrica están entre 1 y 2 nm.
- Nanotubos de carbono de pared simple carboxilados (c-SWNHs), suministrados por Cheap Tubes Inc. (Brattleboro, Vermont, EEUU). La pureza del material es superior al 95% y las dimensiones de los mismos oscilan entre 10-30 nm de longitud y 20-30 nm de diámetro externo. El contenido de grupos carboxilo es del 3.8% en peso.
- Nanotubos de carbono de pared múltiple (MWNTs), proporcionados por Sigma-Aldrich (Madrid, España). Estos nanotubos de carbono tienen una longitud que varía entre 0.5 a 200 µm y un diámetro interno entre 5 y 10 nm. En su síntesis se obtiene un producto con una pureza superior al 95%.

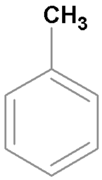
Analitos

Los analitos utilizados para llevar a cabo la investigación fueron de pureza analítica o superior. A continuación se enumeran agrupados por familias:

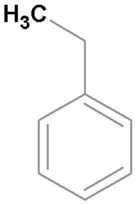
- **Clorofenoles.** Como su nombre bien indica, se trata de fenoles que tienen enlazados covalentemente uno o más átomos de cloro. La mayoría de los clorofenoles tienen un número diferente de isómeros posicionales. En esta Tesis Doctoral se seleccionaron los siguientes: 2-clorofenol; 2,3-diclorofenol; 2,4-diclorofenol; 2,3,5-triclorofenol; 2,4,5-triclorofenol; y pentaclorofenol. Estos compuestos se emplean habitualmente como plaguicidas, herbicidas y desinfectantes.

 2-clorofenol	 2,3-diclorofenol	 2,4-diclorofenol
 2,3,5-triclorofenol	 2,4,5-triclorofenol	 Pentaclorofenol

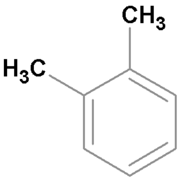
- **Compuestos volátiles monoaromáticos.** En este grupo de analitos se incluyen el tolueno, el etilbenceno, los isómeros orto-, meta- y para- xileno y el estireno. Son compuestos orgánicos volátiles (*VOCs*) que aparecen en el petróleo y sus derivados como la gasolina. Se encuentran como contaminantes en compartimentos ambientales como suelos y aguas subterráneas próximas a lugares de almacenamiento de carburantes derivados del petróleo. Algunos de ellos pueden tener efectos dañinos sobre el sistema nervioso central. El estireno se emplea en la fabricación de materiales como el caucho y diferentes materiales plásticos, como el poliestireno, formado por largas cadenas de este compuesto.



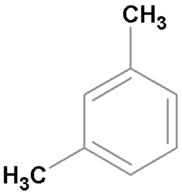
tolueno



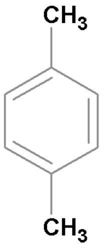
etilbenceno



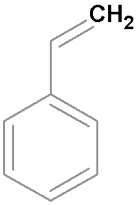
o-xileno



m-xileno

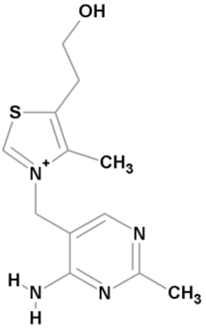


p-xileno

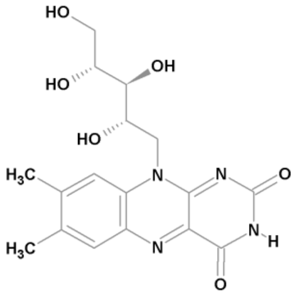


estireno

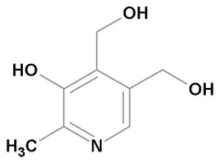
- **Vitaminas hidrosolubles.** De las cinco vitaminas seleccionadas, cuatro de ellas pertenecen al grupo de vitaminas B: vitamina B_1 o tiamina, vitamina B_2 o riboflavina, vitamina B_6 o piridoxina, y vitamina B_{12} o cianocobalamina. La quinta vitamina empleada fue la vitamina C o ácido ascórbico. Todas ellas pertenecen al grupo de vitaminas solubles en aguas, las cuales son coenzimas o precursores de coenzimas necesarias para muchas reacciones químicas del metabolismo. A diferencia de las vitaminas liposolubles, éstas no se almacenan en el cuerpo, por lo que es necesario un aporte continuo de las mismas. El exceso de vitaminas hidrosolubles se excreta por la orina, por lo que generalmente no tienen efecto tóxico por elevada que sea su ingesta, aunque se podría sufrir anormalidades en el riñón por no poder evacuar la totalidad de líquido.



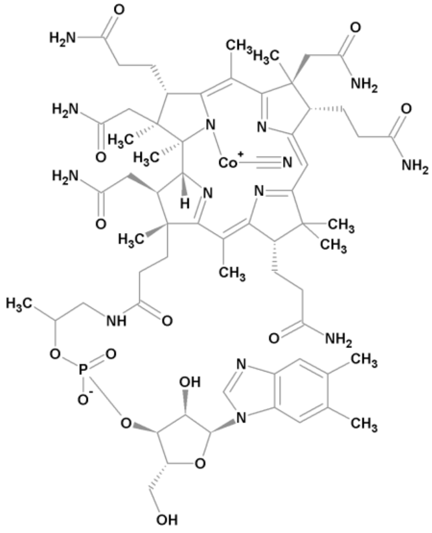
vitamina B_1 o
tiamina



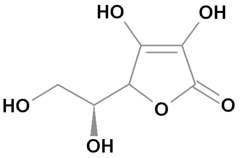
vitamina B_2 o
riboflavina



vitamina B_6 o
piridoxina

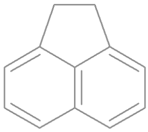


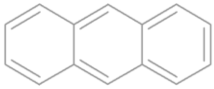
vitamina B_{12} o
cianocobalamina

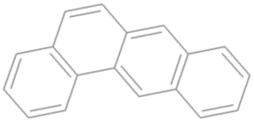


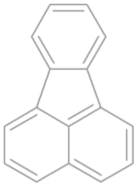
vitamina C o
ácido ascórbico

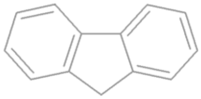
- **Hidrocarburos Policíclicos Aromáticos (*PAHs*).** Estos compuestos están formados por anillos aromáticos simples condensados, y no contienen heteroátomos ni presentan sustituyentes. Estos analitos se suelen encontrar en el carbón y en el petróleo y sus derivados. Como contaminantes, han despertado preocupación debido a que algunos compuestos se han identificado como carcinógenos, mutágenos y teratógenos. Entre una amplia variedad de *PAHs* se seleccionaron como analitos los siguientes: acenafteno, antraceno, benzo(a)antraceno, fluoranteno, fluoreno, naftaleno, fenantreno y pireno.

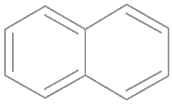

acenafteno

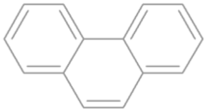

antraceno

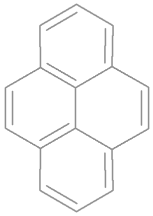

benzo(a)antraceno


fluoranteno

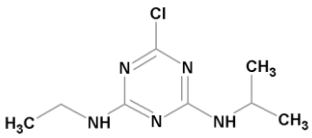

fluoreno


naftaleno

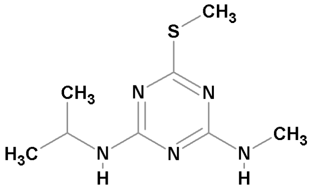

fenantreno


pireno

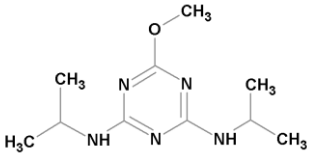
- **Triazinas.** Las triazinas son compuestos cuya estructura consta de un anillo heterocíclico, similar a un anillo de benceno, pero con tres átomos de nitrógeno sustituyendo a tres átomos de carbono. Esto hace que existan tres isómeros de la triazina, en función del lugar que ocupen dichos átomos de carbono. Las triazinas pueden tener diferentes usos, pero en concreto, para la realización de la investigación se seleccionaron algunas de las triazinas que se emplean como herbicidas, como son: atrazina, desmetrin, prometon, prometrin, propazina, secbumeton, simazina, simetrin, terbumeton, desetil terbutilazina y terbutrin.



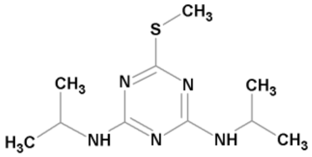
atrazina



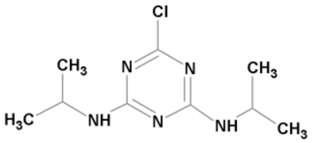
desmetrin



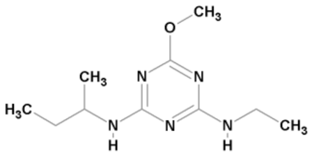
prometon



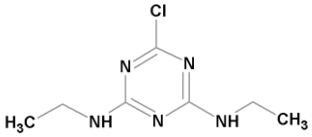
prometrin



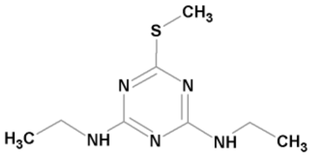
propazina



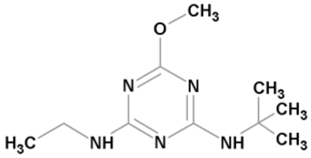
secbumeton



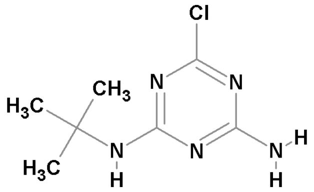
simazina



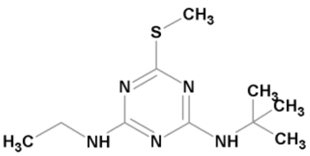
simetrin



terbumeton



desetil terbutilazina



terbutrin

Disolventes orgánicos

Durante el desarrollo de esta Tesis Doctoral se han empleado diferentes disolventes orgánicos, adquiridos con una pureza analítica o superior en Panreac (Barcelona, España), los cuales se enumeran a continuación:

- 1-octanol
- 1,2-dicloroetano
- acetato de etilo
- acetona
- acetonitrilo
- dimetilformamida
- etanol
- n-hexano
- metanol

Se han empleado con diferentes finalidades, entre las que cabe destacar las siguientes:

- Preparación de disoluciones estándar de los analitos.
- Eluyentes en los procesos de extracción en fase sólida.
- Preparación del pegamento orgánico para recubrir una fibra de acero inoxidable para su posterior uso en microextracción en fase sólida.
- Preparación de rejillas de cobre para su uso en microscopía electrónica de transmisión.

Otros reactivos

- Ácidos, como ácido clorhídrico (35% de pureza), ácido nítrico (60% de pureza), ácido sulfúrico (95-98% de pureza) y ácido bórico.
- Sales, como hidróxido sódico, sulfato sódico anhidro y cloruro sódico
- Terpineol, dibutil ftalato y etilcelulosa para la fabricación del pegamento orgánico necesario para inmovilizar los nanoconos/discos de carbono sobre la fibra de acero inoxidable.
- Nitrógeno, de pureza 5.0.
- 3-aminopropil trietoxisilano (APTS), 1-3, diciclohexil-carbodiimida y glutaraldehido para la inmovilización de nanocuernos y nanotubos de carbono en el interior del capilar de sílice fundida.
- Tensioactivos como dodecil sulfato sódico (SDS) o dodecil bencenosulfonato sódico (NaDDBS) empleados para la dispersión de las nanopartículas de carbono.

Materiales de laboratorio

- Cartuchos comerciales de extracción en fase sólida de 1 y 3 mL de capacidad.
- Fritas de polipropileno de 20 µm de tamaño de poro.
- Rejillas de cobre de 3 mm de diámetro para la visualización de muestras en el microscopio electrónico de barrido.
- Fibra de acero inoxidable de 35 mm, como soporte de inmovilización de nanoconos/discos de carbono en microextracción en fase sólida.
- Fibra hueca microporosa de polipropileno con un espesor de pared de 200 µm, un diámetro interno de 600 µm, un tamaño de poro de 0.2 µm y una porosidad del 75%, sobre la cual se inmovilizaron los

nanocuernos de carbono oxidados para llevar a cabo el proceso de microextracción en fase sólida en fibra hueca.

- Capilar de sílice fundida de 57 cm de longitud para inmovilizar en su interior la fase estacionaria empleada en electrocromatografía.
- Filtros de membrana de nitrato de celulosa de 0.45 µm de tamaño de poro.
- Filtros desechables de Nylon de 0.45 µm de tamaño de poro y con un diámetro interno de 13 y 25 mm.
- Filtros del tipo JVWP04700 y JHWP02500 de 0.1 y 0.45 µm de tamaño de poro, respectivamente.
- Frascos de vidrio ámbar de 20, 60 y 125 mL.
- Viales de vidrio de 5, 10 y 20 mL con tapones magnéticos con septum de silicona.
- Insertos de vidrio de 200 µL de volumen.
- Viales eppendorf.
- Crisoles de porcelana.
- Vidrio de reloj.
- Plato cerámico para su uso en el horno mufla.
- Mortero.
- Kitasato.
- Guantes de látex y máscara de protección para el rostro.
- Contenedores de plástico de diferentes tamaños para la correcta gestión de residuos de laboratorio.

Muestras

Para llevar a cabo el estudio/aplicación de la capacidad sorbente y el potencial de separación de las nanopartículas de carbono utilizadas en esta Memoria de Tesis Doctoral, se seleccionaron muestras de agua de diferente procedencia. En este caso se optó por muestras de agua de pozo, agua de grifo, agua de rio/arroyo, agua de piscina y agua mineral embotellada. En todos los ensayos realizados no se hallaron los analitos de interés, por lo que fueron fortificadas con la familia de analitos correspondiente en cada estudio. Posteriormente, las muestras se almacenaron en frascos de vidrio color ámbar sin espacio de cabeza y a 4 ºC, analizándose en los cinco días siguientes a su recogida, según el estudio realizado. En el caso de las muestras de agua mineral embotellada, éstas se mantuvieron en su envase original hasta su análisis.

Instrumentos

Para la realización de esta Tesis Doctoral se han utilizado diversos instrumentos y aparatos. En lo que a los instrumentos se refiere, se podría hacer una simple clasificación dividiéndolos en dos grandes bloques: los instrumentos empleados para la identificación y cuantificación de analitos, y los instrumentos usados para la caracterización de los diferentes materiales sorbentes empleados.

Atendiendo a esta categorización, los instrumentos empleados en el análisis cualitativo y cuantitativo de los analitos fueron:

- Cromatógrafo de gases acoplado a un espectrómetro de masas con o sin automuestreador de espacio de cabeza.
- Electroforesis capilar con detector UV-Visible.

Para la caracterización de las nanopartículas de carbono empleadas se utilizaron las siguientes técnicas de análisis:

- Sistema de fraccionamiento en flujo con campo de flujo (FlFFF) equipado con un detector UV-Visible y un detector multiángulo de dispersión de luz en tándem.
- Microscopio electrónico de transmisión.
- Microscopio electrónico de barrido.
- Equipo de espectroscopía de infrarrojo.
- Equipo de espectroscopía Raman.
- Equipo de análisis termogravimétrico.

Sistemas cromatográficos

En el análisis de los clorofenoles y los compuestos volátiles monoaromáticos se empleó un cromatógrafo de gases HP6890 de Agilent (Palo Alto, California, EEUU). Este equipo ofrece una alta versatilidad en su configuración ya que está diseñado para ser acoplado a diferentes detectores como pueden ser: un detector de conductividad térmica, de ionización en llama o espectrómetro de masas. En esta Tesis Doctoral se ha empleado el acoplamiento cromatografía de gases - espectrometría de masas.

Tanto el inyector como el horno del cromatógrafo de gases se programaron en términos de temperatura, presión y flujo, para realizar los análisis bajo las condiciones óptimas, en función de la familia de analitos a determinar. Tanto en el análisis de clorofenoles como en el caso de los compuestos volátiles monoaromáticos se seleccionó una columna capilar de sílice fundida HP-5ms (Supelco, Madrid, España) de 30 metros de longitud y

0.25 mm de diámetro interno, recubierta en su interior por una fase estacionaria de 0.25 µm de espesor de película y compuesta por un 5% de fenilpolisiloxano y un 95% de metilpolisiloxano. Como gas portador, se seleccionó helio de pureza 6.0 (Air Liquide, Sevilla, España) a un caudal de 1.4 mL/min que se fijó mediante un regulador digital de presión y flujo.

El cromatógrafo de gases se acopla mediante una línea de transferencia fijada a 250 ºC a un espectrómetro de masas HP5973 de Agilent (Palo Alto, California, EEUU), el cual se compone de una fuente de ionización de impacto electrónico, configurada para ionizar las moléculas procedentes del cromatógrafo de gases con una energía de ionización de 70 eV a una temperatura de 200 ºC. De esta manera, los espectros de masas obtenidos pueden compararse directamente con las diferentes bibliotecas estandarizadas de espectros existentes, lo cual favorece el proceso de identificación de los analitos monitorizados. Además, este espectrómetro está equipado con un cuadrupolo como analizador de masas. Tanto en la determinación de clorofenoles como en la de compuestos volátiles monoaromáticos, el cuadrupolo se programó para llevar a cabo la selección de masas en modo *full scan*. El rango de masas seleccionado para el análisis de los clorofenoles y los compuestos volátiles monoaromáticos fue de 50-200 uma y de 40-500 uma, respectivamente. Este espectrómetro de masas está equipado con un detector multiplicador de electrones el cual se programó con una frecuencia de barrido de 1.276 scan/s (para clorofenoles) y 1.360 scan/s (para compuestos volátiles monoaromáticos).

Tanto los parámetros instrumentales como los datos y cromatogramas obtenidos mediante el cromatógrafo de gases - espectrómetro de masas se adquirieron y procesaron mediante el programa informático G1701BA Standlone Data Analysis (Agilent Technologies), instalado en un ordenador con procesador Intel Pentium 4.

Para llevar a cabo la determinación de los compuestos volátiles monoaromáticos mediante microextracción en fase sólida, se hizo uso de un automuestreador de espacio de cabeza MPS2 (Gerstel, Mülhein an der Ruhr, Alemania). Este dispositivo está compuesto por un módulo de inyección en el que se encuentra una jeringa de gases de 2.5 mL que se mantuvo a una temperatura de 110 ºC; dos bandejas donde se colocan los viales con las muestras a analizar; un brazo robotizado para transportar tanto los viales como el módulo de inyección, y un horno que permite agitar y calentar las muestras para la generación del espacio de cabeza.

La separación de hidrocarburos policíclicos aromáticos y triazinas se llevó a cabo en un cromatógrafo de gases Varian CP-3800. En este caso, se optó por la inyección manual de 2 µL de extracto orgánico que contenía los analitos mediante una microjeringa de 5 µL (Hamilton Co, Nevada, EEUU). La temperatura de inyección se fijó en 250 ºC y se seleccionó una división de flujo de 1:10. La columna capilar empleada para la separación de los analitos fue similar a la empleada en el caso anterior. El gradiente de temperatura de trabajo fue distinto en función de la familia de analitos a separar. El gas portador empleado en este equipo fue helio de pureza 6.0 (Air Liquide, Sevilla, España), a un caudal de 1.4 mL/min ajustado mediante un regulador digital de presión y flujo.

El detector del equipo fue un espectrómetro de masas Varian 1200 MS/MS acoplado al cromatógrafo de gases mediante una línea de transferencia que se mantuvo a una temperatura de 280 ºC. La ionización de la muestra se realizó mediante impacto electrónico (70 eV) a una temperatura de 250 ºC. Este equipo dispone de un analizador de masas de triple cuadrupolo, pudiéndose llevar a cabo una segunda ionización de tipo químico, aunque en esta Tesis Doctoral, el espectrómetro de masas se utilizó en su modalidad de cuadrupolo simple. Los cromatogramas se adquirieron en el modo SIM (*selected ion monitoring*) estableciéndose en cada caso los valores de *m/z* más convenientes para cada analito en sus correspondientes ventanas temporales. Todo ello se llevó a cabo mediante el programa informático MS Workstation de Varian, que también controlaba las variables instrumentales del equipo.

Sistemas electroforéticos

Para llevar a cabo la separación de vitaminas solubles en agua se utilizó un equipo de electroforesis capilar Beckman Coulter (Palo Alto, California, EEUU) P/ACE 5500 CE provisto de un detector UV-Visible de diodos en fila. Los componentes principales del equipo son: una lámpara de deuterio fijada a una longitud de onda de 254 nm, capilares de sílice fundida (75 µm de diámetro) con una longitud efectiva desde el inyector hasta el detector de 50 cm (57 cm en total), electrodos de platino y un automuestreador. Este equipo está dotado de un sistema de refrigeración el cual impide que la muestra se vea alterada por el efecto de las altas temperaturas. El control del equipo y la toma y tratamiento de datos se hizo con el software P/ACE Station (Beckman Coulter). Con este equipo se consiguió la separación de cinco vitaminas hidrosolubles utilizando dos metodologías electroforéticas diferentes: cromatografía electroforética micelar usando una fase pseudoestacionaria con nanopartículas de carbono (nanotubos y/o nanocuernos) recubiertas de tensioactivos; y electrocromatografía capilar usando las nanopartículas de carbono como fase estacionaria.

Fraccionamiento en flujo con campo de flujo (FlFFF)

En el Capítulo 1 de esta Tesis Doctoral se aborda la separación por tamaños de los agregados formados por nanocuernos de carbono, así como la purificación de nanoconos y discos de carbono. Para llevar a cabo esta tarea se recurrió a un sistema de fraccionamiento en flujo con campo de flujo asimétrico (FlFFF) equipado con un detector UV-visible y un detector multiángulo de dispersión de luz en tándem. Los elementos del equipo utilizado se comentan a continuación. Como mecanismo para el control de flujos líquidos se empleó una bomba isocrática Agilent Technologies 1200 series (Agilent, Tokio, Japón). Dicho sistema estaba conectado a una cámara (*channel*) (Eclipse AF4, Wyatt Technology, Dernbach, Alemania) donde se llevaba a cabo la separación de las nanopartículas de carbono. Esta unidad consiste en una cámara de 145 mm de longitud (*short channel*) equipada con un espaciador de 350 μm de espesor y una membrana de celulosa regenerada de 10 kDa de tamaño de poro (Nadir membranes, Wiesbaden, Alemania). En esta cámara tiene lugar la separación de las nanopartículas y/o agregados en función de su forma y tamaño. Las muestras se inyectan a través de una válvula de 6 vías que conectaba el bucle de inyección con la unidad de separación de muestras. Una vez separadas las nanoestructuras, la detección de las mismas se llevó a cabo mediante un espectrofotómetro UV-Vis Agilent Technologies 1200 series (Agilent, Tokio, Japón) fijado a una longitud de onda de 422 nm, el cual fue acoplado en tándem a un detector de dispersión de luz de triple ángulo modelo miniDAWN TREOS (Wyatt Technology, Dernbach, Alemania) equipado con un láser que actuaba a 658 nm y con 3 detectores dispuestos en diferentes ángulos. Todos estos componentes se controlaron a través del programa informático Astra 5.3.4.15 (Wyatt Technology, Dernbach, Alemania) mediante el cual se recogieron y procesaron las señales analíticas resultantes.

Equipos de microscopía

La caracterización de las nanopartículas de carbono empleadas a lo largo de esta Tesis Doctoral se llevó a cabo mediante microscopía electrónica de barrido y de transmisión. Los equipos utilizados fueron: un microscopio electrónico de barrido (SEM) JEOL JSM 6300 (Isaza, Alcobendas, España) con una capacidad de aumento de entre 70x y 300.000x, mediante el cual se caracterizó la fibra de SPME recubierta de nanoconos y discos de carbono, y se pudieron visualizar los nanocuernos de carbono inmovilizados sobre la fibra hueca; un microscopio electrónico de transmisión PHILIPS CM-10, el cual permitió obtener imágenes aumentadas de 18x a 450.000x, empleado en la caracterización de formas y tamaños de las nanopartículas; y un microscopio electrónico de transmisión PHILIPS EM 208 mediante el cual se obtuvieron imágenes con un rango de aumentos de 25x a 200.000x.

Otros instrumentos de caracterización

Además de estos instrumentos, en el ámbito de la caracterización de las nanopartículas de carbono se empleó la técnica de análisis termogravimétrico (TGA) utilizando para ello un equipo TGA Q500 (TA Instruments, Eschborn, Alemania) en el cual se hicieron las medidas equilibrando inicialmente las muestras a 100 ºC y aumentando la temperatura mediante una rampa de 10 ºC/min hasta alcanzar los 1.000 ºC, todo ello bajo una atmósfera de nitrógeno.

Además, para comprobar el grado de funcionalización de los nanocuernos de carbono se utilizó un equipo de espectroscopía Raman modelo Invia (Renishaw, Turín, Italia) acoplado a un microscopio óptico (50x). Los análisis se realizaron mediante el empleo de dos láseres de He-Ne con diferentes longitudes de onda: 532 nm y 633 nm. También se hizo uso de un espectrómetro UV-Vis-NIR modelo Cary 5000 (Varian, Milán, Italia) para la caracterización de los nanocuernos y nanoconos de carbono.

Aparatos

Durante el desarrollo del trabajo experimental realizado en esta Tesis Doctoral, se emplearon los siguientes aparatos:

- Microondas de uso domestico (AEG, Estocolmo, Suecia). Este microondas está equipado con un magnetrón de 2450 MHz y una potencia máxima de 800 W. Se empleó para llevar a cabo la funcionalización de las nanopartículas de carbono.
- Microondas monomode de uso científico CEM-Discover (Bérgamo, Italia) equipado con un pirómetro de infrarrojos, el cual se usó bajo irradiación continua para la oxidación de los nanocuernos de carbono.
- Agitador Vortex (Heidolph, Merida, España) para la homogenización de muestras.
- Agitador magnético (Velp Científica, Milán, Italia) para la agitación de las muestras acuosas y dispersiones empleadas en los diferentes procesos de microextracción.
- Baño de ultrasonidos (Branson 3510E, Connecticut, EEUU), para la dispersión y homogenización de muestras y nanopartículas de carbono.

- Baño de ultrasonidos (Branson 2510, Connecticut, EEUU), fijado a una frecuencia de 40 KHz, para la dispersión y homogenización de muestras y nanopartículas de carbono.
- Estación de vacío VacElut-20 (Scharlab, Madrid, España), constituida por una cámara con paredes de vidrio con una tapa de polietileno de 20 posiciones, dotada de una gradilla para situar los tubos en los que se recoge el eluido de cada cartucho. Este dispositivo tiene un sistema de control de presión mediante el cual se monitoriza el grado de vacío existente en la cámara de extracción.
- Centrífuga Centronic BL-II (J.P. Selecta, Barcelona, España), con una velocidad máxima de 13.500 rpm.
- Horno mufla Heron modelo HD-150, con una temperatura máxima de 1200 ºC.
- pHmetro Crison micropH 2000 (Crison, Barcelona, España).
- Balanza analítica Cobos AI-220CB (Cobos, Barcelona, España) con un intervalo de medidas de 0.01 a 220 g, y un error de 0.0001 g.
- Balanza analítica OHAUS Explorer (OHAUS, Nänikon, Suiza) que puede realizar medidas de masas desde 0.01 a 110 mg, con un error de 0.0001 g.
- Cámara de secado con convención natural Binder ED53, la cual puede alcanzar una temperatura máxima de 300 ºC.

Bloque I

Nanoconos y nanocuernos de carbono como objetos en Nanociencia y Nanotecnología Analíticas

La calidad ha sido, es y será un concepto inherente a la Química Analítica. La obtención de información analítica de calidad supone un reto cuando se habla en términos de Nanociencia y Nanotecnología. De ahí que la inclusión de la calidad en el contexto de la Nanociencia y Nanotecnología Analíticas sea de vital importancia por tres razones principales:

a) para garantizar resultados satisfactorios en el campo de la investigación básica, es decir, en Nanociencia;
b) para hacer efectiva la información analítica obtenida mediante la investigación básica y ponerla a disposición del desarrollo y la innovación en el campo de la Nanotecnología, obteniéndose una continuidad productiva en la I+D+i;
c) para asegurar que el desarrollo y la aplicación nanotecnológica se lleva a cabo con nanoestructuras y/o nanodispositivos bien caracterizados, con una pureza idónea y unas dimensiones dentro de los límites nanométricos, entre otras propiedades.

Por ello, la Química Analítica pone en juego todo su potencial cuando se trata de llevar a cabo tanto la determinación como la caracterización de nanopartículas.

A continuación se comentarán brevemente los fundamentos de las técnicas analíticas empleadas para el desarrollo de la investigación correspondiente a este capítulo agrupándolas en técnicas espectroscópicas, gravimétricas, microscópicas y de separación.

Espectroscopía Raman

La espectroscopía Raman es la técnica analítica que posiblemente proporcione más información sobre la composición de las nanopartículas de carbono, pudiendo llegar a caracterizar estructuras de carbono que van desde el grafeno hasta el diamante. Dicho potencial se basa en el estudio de los átomos que componen las nanopartículas, así como de los enlaces que establecen entre sí. Atendiendo a esto, mediante esta espectroscopía es posible detectar cambios locales en la hibridación existente en las paredes de nanopartículas de carbono, como por ejemplo, los nanotubos de carbono [1,2]. Estas nanoestructuras de carbono, en su forma pura, presentan una hibridación sp^2 (propia del grafeno). Sin embargo, cuando se someten a procedimientos de funcionalización covalente, la estructura del enlace de sus carbonos pasa de un estado sp^2 a un estado sp^3. Estos cambios de hibridación se pueden detectar mediante el análisis a través de espectroscopía Raman [3].

La determinación del grado de funcionalización de las nanoestructuras de carbono se lleva a cabo principalmente mediante la medida de las intensidades de las bandas D y G de los espectros Raman, las cuales se observan entre 1300 y 1400 cm^{-1} (banda D) y alrededor de los 1600 cm^{-1} (banda G). Diferentes autores están de acuerdo en que existe una correlación entre el grado de funcionalización de dichas nanoestructuras y la intensidad de la banda D, de tal modo que, cuanto mayor sea la intensidad de dicha banda, mayor es el porcentaje de funcionalización de la nanopartícula [4,5]. De hecho, existe un índice (relación I_D/I_G) que correlaciona la intensidad de la banda D con la intesidad de la banda G, la cual se emplea como referencia.

Análisis termogravimétrico

El análisis termogravimétrico (TGA) es una potente herramienta mediante la cual es posible correlacionar la pérdida de peso del nanomaterial con el grado de funcionalización del mismo [6].

La técnica se basa en la medida de la pérdida de peso que sufre un compuesto sólido (o una mezcla de compuestos sólidos) cuando se somete a un aumento progresivo de la temperatura. De este modo, los compuestos más volátiles (o las partes más volátiles de un compuesto) pasarán antes al estado gaseoso. Dicha transición se corresponde con una pérdida de peso del material sólido, la cual se monitoriza y representa en una curva de pérdida de peso.

Esta técnica puede servir como complemento a la espectroscopía Raman ya que se puede relacionar la correspondencia entre la pérdida de peso y el grado de funcionalización obtenido mediante los análisis de espectroscopía Raman (índice I_D/I_G).

Microscopía electrónica de transmisión

La microscopía electrónica de transmisión (TEM) se basa en el empleo de un haz de electrones, el cual se proyecta sobre la muestra. Una parte de estos electrones rebotan o son absorbidos por la muestra y otros la atraviesan formando una imagen aumentada de la misma. Para ello, la muestra debe ser ultrafina y disponerse sobre unos soportes especiales llamados rejillas o *grids*.

El microscopio en sí se compone de un cañón donde se produce un haz de electrones que se enfoca hacia la muestra mediante una serie de lentes magnéticas. Tanto la muestra como todos los dispositivos por los que pasa el haz de electrones han de encontrarse en condiciones de vacío ya que las moléculas de aire podrían desviar dichos electrones y distorsionar las imágenes. Además, es necesario que el microscopio esté equipado con un sistema de visualización que puede ser una pantalla de fósforo (donde se puede observar directamente la imagen amplificada) o un sistema de registro de imágenes tales como una película fotográfica o un sensor CCD para la toma y almacenamiento de imágenes.

Mediante la microscopía electrónica de transmisión se pueden obtener imágenes aumentadas hasta 100.000x. Por ello, esta técnica ha sido y es ampliamente utilizada en aplicaciones físicas, químicas y biológicas.

Fraccionamiento en Flujo mediante Campo de Flujo (*Flow-Field Flow Fractionation*, FlFFF)

Esta técnica permite llevar a cabo una separación y medida de tamaños de partículas, y guarda una cierta relación con la cromatografía (concretamente, cromatografía de exclusión por tamaños). Si bien es cierto que ambas técnicas comparten el mismo fin (lograr la separación de compuestos), el procedimiento mediante el cual se obtiene dicha separación difiere claramente entre ellas.

En los últimos años, el uso de la técnica de Fraccionamiento en Flujo mediante Campo (FFF) se ha incrementado en ámbitos de la ciencia como son la investigación farmacéutica [7], biomédica [8] o el campo medioambiental [9]. Esto se debe a su capacidad para separar y caracterizar muchos compuestos tales como proteínas [10], células [11,12], polímeros, polisacáridos y otros compuestos supramoleculares [13] y, cómo no, nanopartículas de diferente formas y composición [14].

Las bases teóricas de la técnica de FFF fueron propuestas por Gidding en 1966 [15]. Existen diferentes modalidades de FFF, entre la que destaca la de Fraccionamiento en Flujo mediante Campo de Flujo (FlFFF), en el que se emplea un flujo líquido para la separación de los componentes. Dicha modalidad presenta una serie de ventajas tales como: (i) el amplio rango de tamaños de partícula que abarca, pudiendo analizar compuestos que van desde 1 nm hasta 50 µm; (ii) la compatibilidad de las disoluciones de transporte con la matriz de la muestra; (iii) y la posibilidad de llevar a cabo una caracterización en línea con un amplio rango de detectores (UV-Visible, fluorescencia, plasma acoplado por inducción–espectrometría de masas y otros de dispersión de radiación. Además, se puede emplear otras técnicas fuera de línea, como TEM o Microscopía de Fuerza Atómica (AFM).

En este capítulo se plasman los resultados del estudio de caracterización de las nanopartículas de carbono empleadas en esta Tesis Doctoral. Se ha realizado la separación por tamaños de los nanocuernos de carbono, así como la purificación de nanoconos de carbono mediante el empleo de la técnica de FlFFF con detectores UV-Visible y *multi-angle light scattering* (MALS). Posteriormente, las fracciones obtenidas se analizaron por microscopía electrónica de transmisión para contrastar los resultados obtenidos.

La funcionalización de los nanocuernos de carbono de pared simple (*SWNHs*) se llevó a cabo empleando energía de microondas que introduce en su estructura grupos funcionales oxigenados [16]. Tras una pormenorizada optimización de dicha funcionalización, se procedió a la caracterización de estas nanopartículas mediante espectroscopía Raman. Para ello, se realizaron medidas de SWNHs puros y funcionalizados bajo diferentes condiciones. Además, se llevaron a cabo diferentes análisis termogravimétricos (TGA) para corroborar el grado de funcionalización de dichas nanopartículas. Este procedimiento de funcionalización fue necesario para lograr una dispersión homogénea y estable de nanocuernos de carbono en agua, y de esta manera hacerlos compatibles con los requerimientos necesarios para ser empleados en la técnica de separación por tamaños de FlFFF con detectores UV–Visible y MALS. Posteriormente, las fracciones obtenidas fueron analizadas mediante TEM.

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Capítulo 1

Purification, separation and diameter determination of modified single-walled carbon nanohorns using the flow field flow fractionation technique

Purification, separation and diameter determination of modified single-walled carbon nanohorns using the flow field flow fractionation technique

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ABSTRACT

For the first time, using the flow field-flow fractionation technique (FlFFF) we report herein the separation and characterization of aqueous dispersion of oxidized and functionalized SWCNHs in presence of SDS based on the diameter of the aggregates. FlFFF proved to be the method of choice for the determination of the SWCNTs diameters by using UV-Vis absorption and triple angles light scattering detectors. Different diameters were obtained by collected fractions, which were subjected to extensive TEM investigation. In addition, for each modified SWCNHs, this technique found to be also efficient for the separation of functionalized and non-functionalized SWCNHs.

* Submitted to Carbon

1. Introduction

Discovered for the first time in 1999 by Iijima et al [1-3], single-walled carbon nanohorns (SWCNHs) are a new horn-shaped single-walled material with a conical tip. Prepared in considerable amounts with good yield (75%) and high purity (95%), through CO_2 laser ablation of a graphite target at room temperature and under argon atmosphere, the SWCNHs appears as spherical aggregates of tubules so called dahlia-like shaped. Each individual SWCNH has a diameter of 1-2 nm (at the tip) and 4-5 nm (in the tubule-body part), a length of 40 nm, a cone angle of 20° and the dahlia-like aggregates have a diameter of about 80-100 nm. Besides their shape and size, one of the most important differences between single-walled carbon nanotubes (SWCNTs) and SWCNHs is that there is no use of metal catalyst in their preparation giving them a high purity. Due to this advantage, the SWCNHs have been used in different fields of application: methane storage [4], hydrogen–deuterium separation [5], fuel-cell electrodes [6], hydrogen generation from methane with steam [7], capacitors [8], drug delivery [9-11], and biomedical applications [12]. Physical-chemical properties and chemical functionalization of SWCNHs have been reported on several reviews and articles [13-17]. The solubility of the SWCNHs in an organic solvent or in aqueous media depends on the covalent and non-covalent (π-π stacking interactions) chemical modifications making them easy to handle, compatible with other materials and thus, the solution can be studied in detail. In spite of their high purity, SWCNHs aggregates may have different diameters, which makes it rather difficult to obtain homogeneous and reproducible materials. We focus our effort on the separation and characterization based on the diameters of the SWCNHs, using the field-flow fractionation technique, so-called FFF, which was introduced by J. Calvin Giddings in 1966 [18]. FFF is an elution based chromatography-like method in which the separation is carried out in a single liquid phase and suited for separation of macromolecules, micro-and microsized analytes [19-21]. An external field is applied perpendicularly to the direction of the sample flow through an empty and thin ribbon-like channel. A laminar parabolic flow takes place into the channel, with flow velocity increasing from near zero (channel walls) to a maximum (center of the channel) (Fig. 1). Various perpendicularly fields of force can be used (e.g. thermal [22], electrical [23], sedimentation [24], magnetic [25]) interacting with a specific physicochemical property of the analyte. In our case, we used a flow as a field of force (FlFFF) to achieve the separation according to the size and the shape of the SWCNHs aggregates.

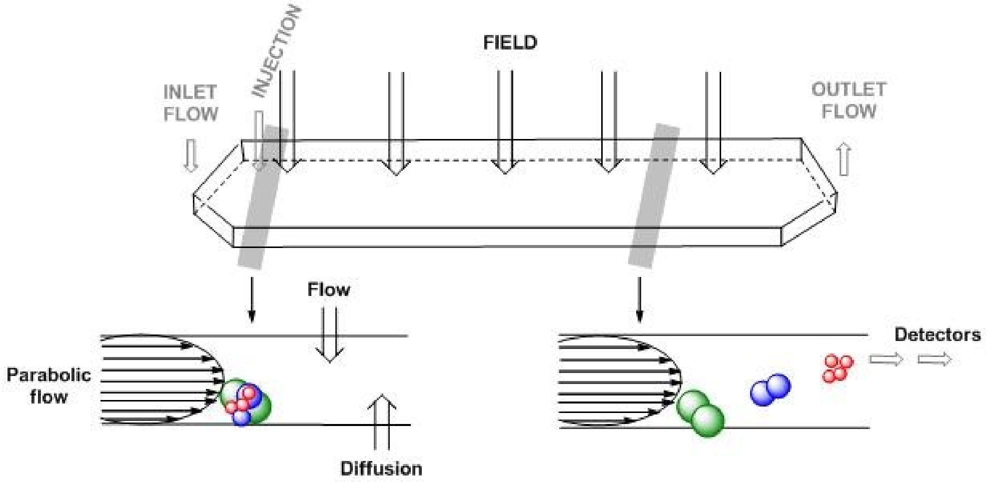

Fig. 1 - Schematic representation of a FlFFF channel cut-out.

Separation and length determination of CNTs are already described in the literature. FlFFF proved to be the method of choice for the analysis of oxidatively shortened single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs) [26], water soluble and functionalized MWCNTs [27] and aqueous dispersion of high purity pristine SWCNTs and MWCNTs in the presence of sodium dodecylsulphate (SDS) [28,29].

Towards the aim to obtain homogeneous and reproducible materials, we herein report for the first time the separation and characterization of aqueous dispersion of oxidized and functionalized SWCNHs (*ox*-SWCNHs and *f*-SWCNHs respectively, Fig. 2) in presence of SDS using the FlFFF technique coupled with UV-Vis and light scattering detectors. Separations were based on the diameters of the SWCNHs aggregates. Each collected fraction was subject to intensive TEM investigation and compared to various fitting method (Zimm, Debye and Berry) of the FlFFF. In addition, this technique seems to be a good method to separate modified SWCNHs from the pristine material, less dispersed and forming aggregates of higher diameter.

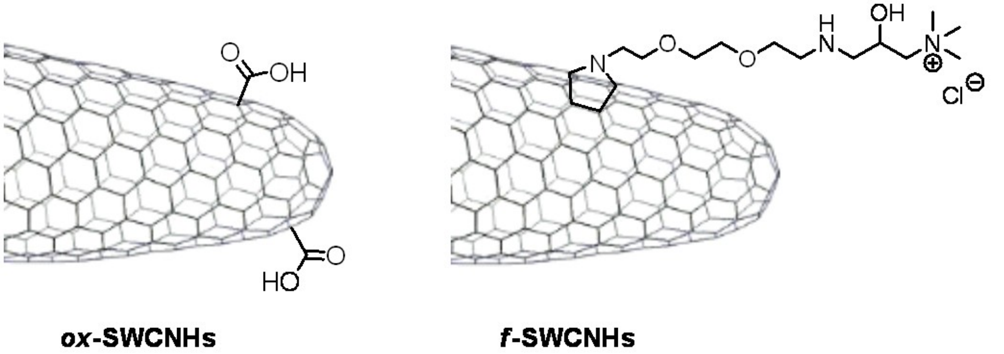

Fig. 2 - Modified SWCNHs

2. Experimental Section

2.1. *Chemicals*

Ammonium hydroxide solution (28-30% NH_3 in water, ACS reagent) used for pH adjustment, and sodium dodecyl sulfate (SDS, technical grade) were purchased from Sigma-Aldrich. The water was Milli-Q plus 18 MΩ cm (Millipore System). Filters used were JVWP04700 (0.1 µm) and JHWP02500 (0.45 µm) for the mobile phase and for the filtration of SWCNHs respectively (Millipore, Vimodrone, Italy). SWCNHs were produced by Carbonium S.r.l., (Padova, Italy) by direct graphite evaporation in Ar flow according to patented methods and used without purification [30-31].

2.2. *Instruments*

Continuous microwave irradiation was carried out in a CEM-Discover monomode microwave apparatus, with infrared pyrometer. The sonication energy was applied by using a Branson 2510 ultrasonic Cleaner (40 KHz). TGA was recorded on a TGA Q500 (TA instruments), under N_2, by equilibrating at 100 °C, and following a ramp at 10 $°C·min^{-1}$ up to 1000 °C (approximately 1 mg of each compound). Raman spectra were recorded with an Invia Renishaw microspectrometer (50x) and a laser source at 532, 633 or 785 nm. The optical properties were carried out by UV-Vis-NIR spectroscopy with a Cary 5000 spectrophotometer using a 10 mm path length quartz cuvettes. TEM measurements were performed on a TEM Philips EM208, using an accelerating voltage of 100 kV. Samples were prepared by drop casting from a dispersion onto a TEM grid (200 mesh, nickel, carbon only). *Fractionation:* Experiments were performed on an asymmetrical flow field-flow fractionation system (Eclipse AF4, Wyatt Technology, Dernbach, Germany). The short channel length was 145 mm and was equipped with a spacer of 350 µm of thickness and with a regenerated cellulose membrane (10-kDa cut-off, Nadir membrane). Flow rates were controlled with an Agilent Technologies 1200 series isocratic pump (Agilent, Tokyo, Japan). Detection chain consisted in a variable wavelength ultraviolet/visible spectrophotometer (UV-Vis) Agilent Technologies 1200 series (Agilent, Tokyo, Japan) tuned at 422 nm and a triple angles light scattering detector (miniDAWN TREOS, Wyatt Technology, Dernbach, Germany) equipped with a laser of 658 nm and 3 detectors disposed in different angles. Data from both detectors were collected and treated with Astra 5.3.4.15 software (Wyatt Technology, Dernbach, Germany).

2.3. Preparation of the samples

After chemical modification of the SWCNHs, non-filtered aqueous dispersions of oxidized and functionalized SWCNHs in the presence of SDS were prepared in order to obtain the ideal dispersion not physically modified by dispersion treatment. 3×10^{-4} mol·L^{-1} SDS concentration in aqueous NH_3 solution (pH = 9-10) was found to be a good concentration under the critical micellar concentration (CMC) able to dispersed these nanomaterials. Samples were prepared by adding 1.0 mg of modified SWCNHs in 1 mL of SDS solution and diluted to 0.01 mg·mL^{-1}. The suspension obtained was then sonicated for 10 minutes at room temperature.

2.4. Fractionation conditions

The mobile phase was an aqueous NH_3 solution (pH = 9-10) without SDS. For all injections, the detector-flow rate was adjusted at 2.0 mL·min^{-1}, the injection-flow rate during the focus at 0.3 mL·min^{-1} and the cross-flow varied from 0.8 to 1.5 mL·min^{-1}. 100 µL of a non-filtered aqueous dispersion of modified SWCNHs were injected *via* an injection valve. Each run was replicated three times in order to prove the reproducibility of the separation.

3. Results and Discussion

3.1. Modified single-walled carbon nanohorns

The two modified single-walled carbon nanohorns represented in Fig.2, were used for the separation and diameter determination study. *ox*-SWCNHs were obtained in one step after oxidation of the pristine material using microwave irradiation as describes in the literature [32]. *f*-SWCNHs were prepared and characterized according to a similar procedure reported by some of us [33]. This procedure was based on a three steps synthesis involving the 1,3 dipolar cycloaddition with the corresponding phthalimid *N*-protected amino acid and, after deprotection of the phthalimid group, the reaction between the free amino group and the glycidyltrimethylammonium chloride in order to introduce positive charges. Both techniques, Raman spectroscopy and thermogravimetric analysis (TGA) were used to confirm the modification of the SWCNHs. In addition, prior to FlFFF separation, TEM analysis was done on the two modified SWCNHs showing diameter sizes from 26 nm to 131 nm for the *ox*-SWCNHs and from 19 to 162 nm for the *f*-SWCNHs. Average diameters have been also calculated and are 66 nm and 78 nm respectively (Fig. 3).

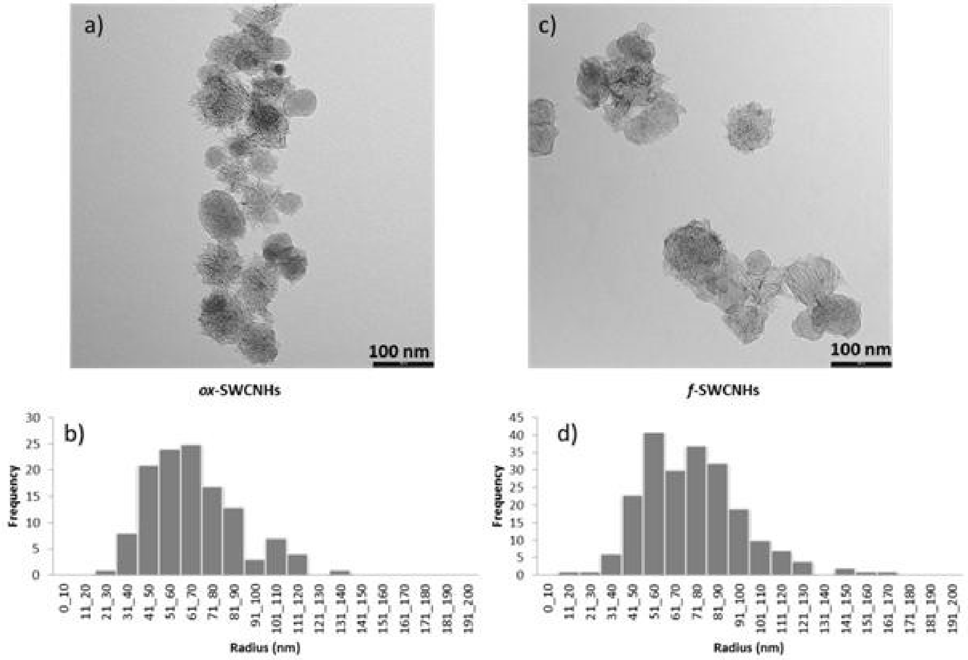

Fig. 3 - TEM images and radius distribution of *ox*-SWCNHs (a and b) and *f*-SWCNHs (c and d).

3.2. Optimization of the separations

Aqueous NH_3 (pH = 9-10) was chosen as the mobile phase due its good compatible with the two detectors and with the component of the channel. 10 KDa membrane regenerated cellulose and a 350 μm thickness spacer were used for all the analysis. The 350 μm spacer allows the modified SWCNHs peak to be well separated from the void volume even if the analysis duration is not so short and the peak is wide with a slight splitting signal. All samples were prepared using SDS as a surfactant at 0.086 $mg{\cdot}mL^{-1}$ in an aqueous NH_3 solution (pH = 9-10) in order to obtain a stable and homogeneous suspension, necessary for FlFFF separation and were sonicated only for 10 minutes to not damaged the modified SWCNHs. The surfactant was not present into the mobile phase and at this concentration, no signals coming from the formation of the micelles were observed. Several samples of modified SWCNHs of different concentrations were prepared from 0.01 to 1 mg·mL^{-1} Up to 0.1 mg·mL^{-1}, even in the presence of surfactant, the sample was not enough well dispersed to be injected into the channel. From 0.01 to 0.1 mg·mL^{-1}, good dispersions and reproducible analysis were obtained (Fig. S1, Supporting information). 0.1 mg·mL^{-1} was chosen for all the FlFFF separation in order to have enough material to be detected and measured by TEM microscopy. Using these parameters, different cross-flow rates were tested over the range of 0.8-1.5 mL·min^{-1} where each fractionation peak was well separated from the void peak as shown in Fig. 4. Cross-flow of 1.0 and 1.5 mL·min^{-1} were chosen for the separation of modified SWCNHs. All optimized conditions are summarized in Table 1.

Table 1 - Optimal fractionation conditions.

FlFFF parameters	**Optimal conditions**
Mobile phase	NH_3 aq. (pH = 9-10)
Elution-flow	2.0 mL·min^{-1}
Cross-flow	1.0 mL·min^{-1} or 1.5 mL·min^{-1}
Injection-flow	0.3 mL·min^{-1}
Injection volume	100 µL
Concentration of the sample	0.1 mg·mL^{-1}
Membrane	Regenerated cellulose
Cut off of the membrane	10 KDa
Spacer	350 µm

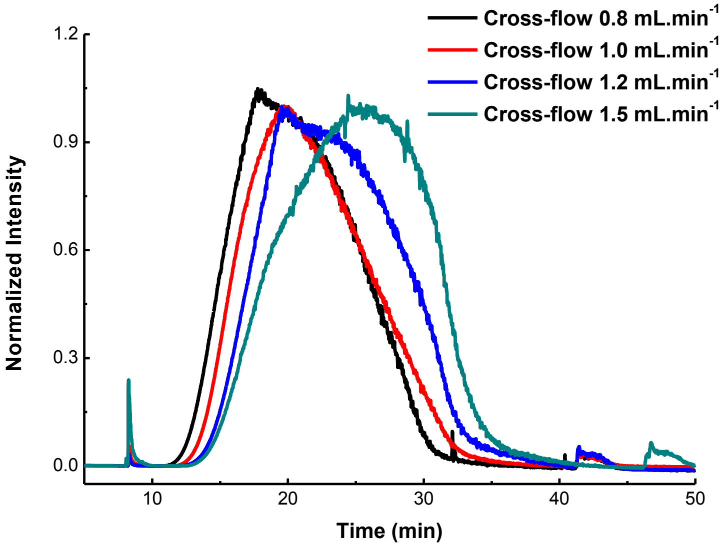

Fig. 4 - Typical fractograms of *ox*-SWCNHs according to different cross-flow rates (operating conditions: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}).

3.3. Fractionation and size characterization via TEM microscopy

Two different cross-flow (1.0 and 1.5 mL·min^{-1}) were used for the separation of the *ox*-SWCNHs. 5 and 6 fractions respectively of the wide peak of the *ox*-SWCNHs were collected at the detector outlet, reduced to 1 mL and subjected to extensive TEM investigation. Minimum, maximum and average radius were determined for each fraction containing SWCNHs and compared to the one determined *via* FlFFF analysis. Data obtained for each fraction are summarized in Table 2.

Using 1.0 mL·min^{-1} as a cross-flow, 5 fractions were collected from 11 to 17, 17 to 23, 23 to 29, 29 to 35 and 35 to 41 minutes respectively. Fractions 1 to 4 were containing SWCNHs whereas in the fraction 5, no absorbing species were observed through TEM analysis in accordance to the very low intensity signal (Fig. S2, Supporting information). Fractions 1 to 3 showed an increase of the average radius from 44.3 to 70.1 nm (Table 2 and also Fig. 5). The average radius of the fourth fraction was not following the same behavior (67.1 nm). In addition, in this fraction, a large radius range of SWCNHs was observed (minimum ≅ 30 and maximum ≅ 160 nm). This can be due to the aggregation of the non-functionalized SWCNHs, less soluble and not well dispersed and can be confirmed by the TEM images showing strong aggregates (Fig. 5).

With a cross-flow of 1.5 mL·min^{-1} (Fig. 6), *ox*-SWCNHs were observed in all the collected fractions (from 12 to 17, 17 to 22, 22 to 27, 27 to 32, 32 to 37 and 37 to 42 minutes respectively, Table 2) with an increasing of the radius. Fractions 1 and 6, in accordance to the low intensity (Fig. S2, Supplementary material) were containing very low quantities of SWCNHs. Here again, the last fraction was not following the same behavior and strong aggregates have been observed by TEM analysis. In the case of the *f*-SWCNHs (Fig. 7), separation was made using a cross-flow of 1.0 mL·min^{-1}. 5 fractions were collected and all fractions were containing SWCNHs. Same conclusion can be made for these *f*-SWCNHs, an increase of the average radius was observed in fractions 2 to 4, from 64.1 to 93.0 nm. Even if the average radius of the last fraction is similar to the fourth one, TEM images show very strong aggregates and a large range of diameter once again due to the non-functionalization of the SWCNHs.

Table 2. Values of radius (minimum, maximum and averages) given by FlFFF and TEM analysis.

Fractions		Radius *ox*-SWCNHs (nm) (cross-flow mL.min^{-1})		Radius *ox*-SWCNHs (nm) (cross-flow 1.5 mL.min^{-1})		Radius *f*-SWCNHs (nm) (cross-flow 1.0 mL.min^{-1})	
		TEM	**FlFFF**[a]	**TEM**	**FlFFF**[a]	**TEM**	**FlFFF**[a]
1	Minimum	15.2	-[c]	-[b]	-[c]	-[b]	-[c]
	Maximum	104.8					
	Average	44.3					
2	Minimum	38.1	47.7	25.6	45.2	36.4	86.2
	Maximum	132.6	86.4	111.4	77.0	106.5	108.0
	Average	70.1	68.9	55.8	63.7	64.1	91.9
3	Minimum	32.1	86.4	35.4	77.0	44.2	108.0
	Maximum	166.8	142.8	133.5	89.3	94.9	147.2
	Average	70.1	107.6	71.33	82.9	73.9	126.3
4	Minimum	30.0	-[c]	40.9	89.3	49.4	147.2
	Maximum	159.7		127.3	155.1	131.0	196.6
	Average	67.1		75.0	111.6	93.0	171.8
5	Minimum	-[b]	-[c]	32.2	-[c]	63.7	-[c]
	Maximum			157.8		144.1	
	Average			96.5		91.1	
6	Minimum	-[d]		38.0	-[c]	-[d]	-[d]
	Maximum			110.2			
	Average			69.4			

[a] Determined with the Berry fitting, [b] no presence or very low amount of SWCNHs to be representative, [c] out of the range of the FFF fitting, [d] No fraction.

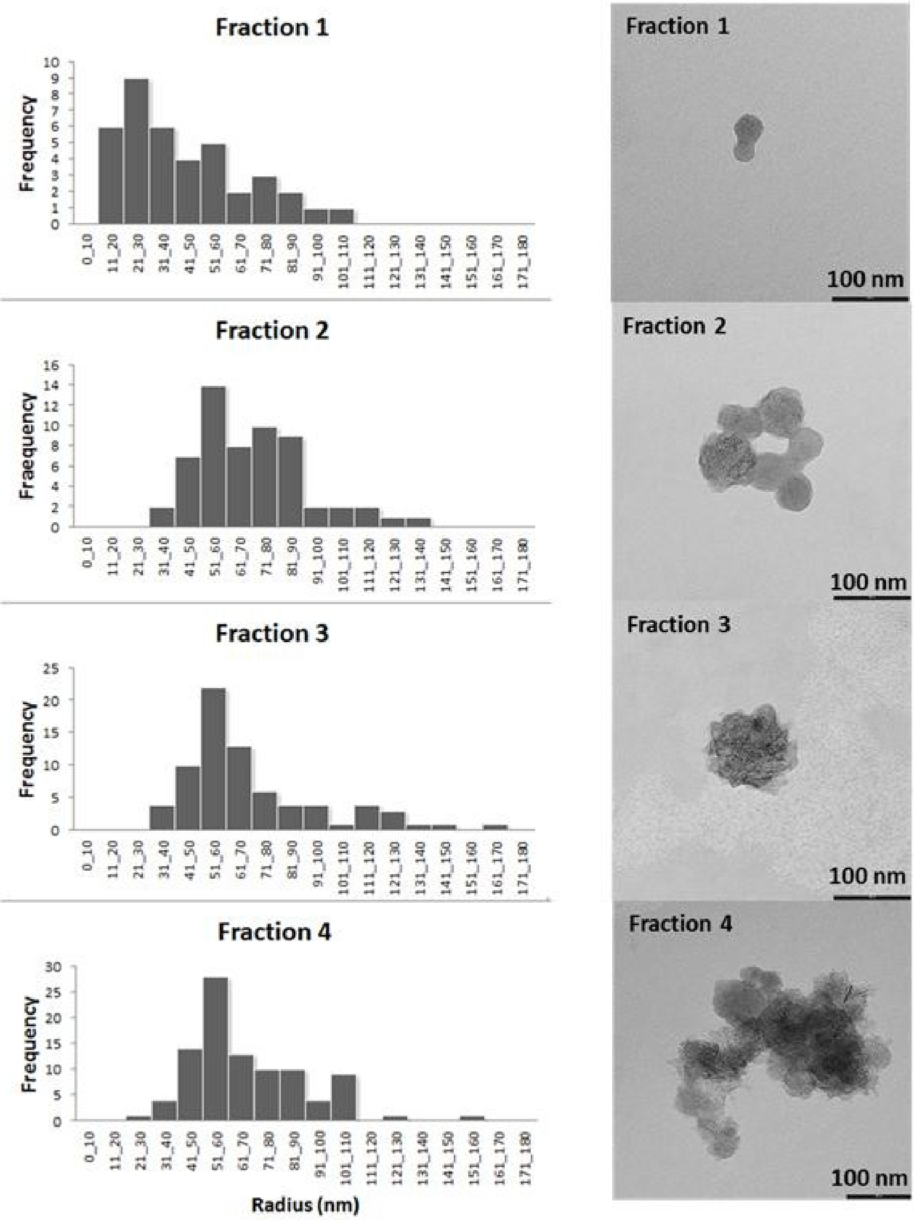

Fig. 5 - Histograms and representative TEM images of the four fractions containing *ox*-SWCNHs (operating conditions: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}, cross-flow 1.0 mL·min^{-1}).

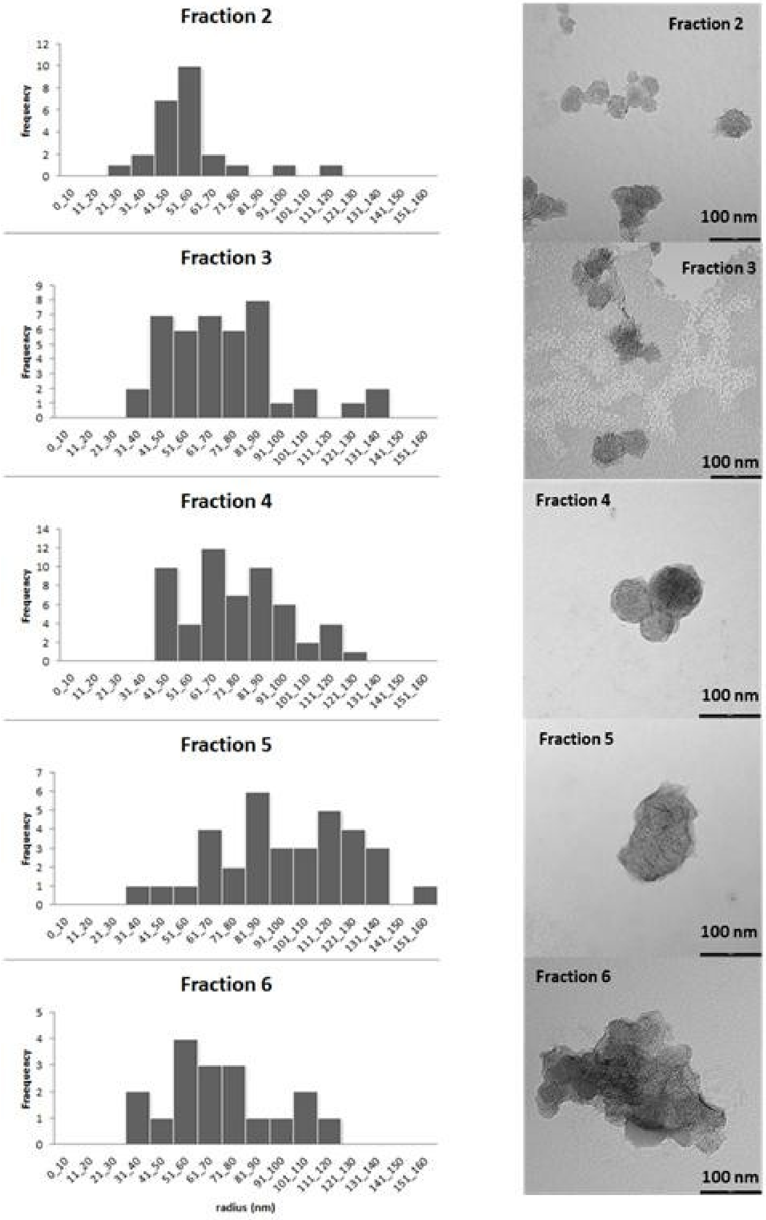

Fig. 6 - Histograms and representative TEM images of the five fractions containing *ox*-SWCNHs (operating conditions: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}, cross-flow 1.5 mL·min^{-1}).

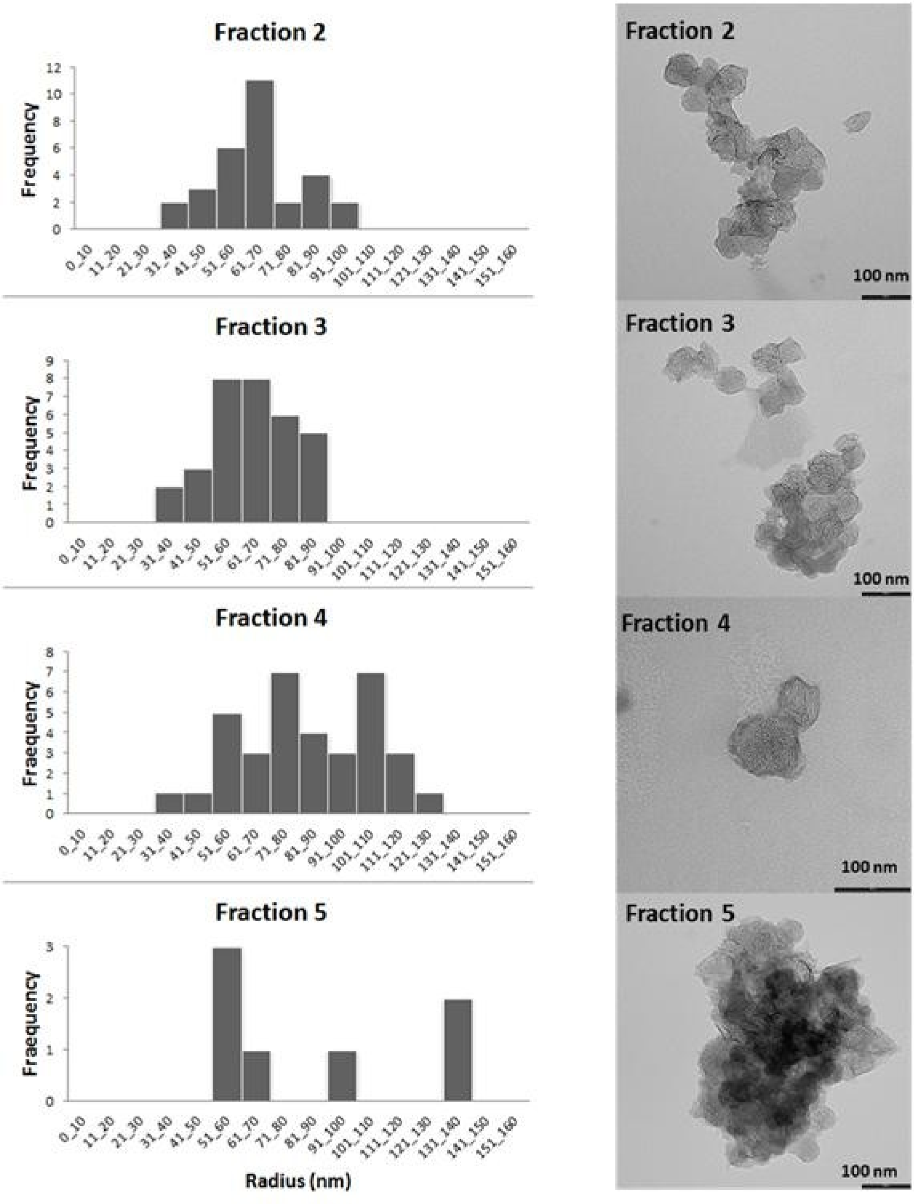

Fig. 7 - Histograms and representative TEM images of the five fractions containing *f*-SWCNHs (operating conditions: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}, cross-flow 1.0 mL·min^{-1}).

3.4 Size characterizations via FlFFF: fitting methods

An Astra software package can be used for calculating the molecular weight (Mw, Mn, Mz) and the radius of gyration (Rg) from the extrapolation of the light scattered to zero-angle at each “slice” according to the Zimm, Debye and Berry methods. The properties for these procedures can be set before running the experiment or can be modify after to see the effects of changing the settings. The Zimm model uses the K*c/R(θ) formalism and should be used for molecules that have RMS radii smaller than 50 nm but for large (>50 nm) molecules, it often produces a negative molar mass and should not be used. In general, Zimm method is the most popular method for analysis of light scattering data and works well for mid-sized molecules and is used for the size determination of CNTs [26-29]. The Debye model uses the R(θ)/K*c formalism and gives better results over a wider range of molar mass, including the very large (greater than ~106 Daltons or ~100 nm RMS radius). Concerning the Berry model, it uses the √K*c/R(θ)) formalism and can be useful, in combination with deleting high angle data, when analyzing molecules with RMS radii greater than 50 nm. Fig. 8 represents typical FlFFF fractograms with their corresponding radius distribution for each modified-SWCNHs using the Zimm, Berry and Debye fitting. Data coming from these fitting are compared to the one obtained after TEM analysis and are summarized in Table 2. Results suggest that for the modified SWCNHs and using a triple angles LS detector, the Berry method is the best fitting method. That is in accordance to the use of this model in the case of molecules with RMS radii greater than 50 nm.

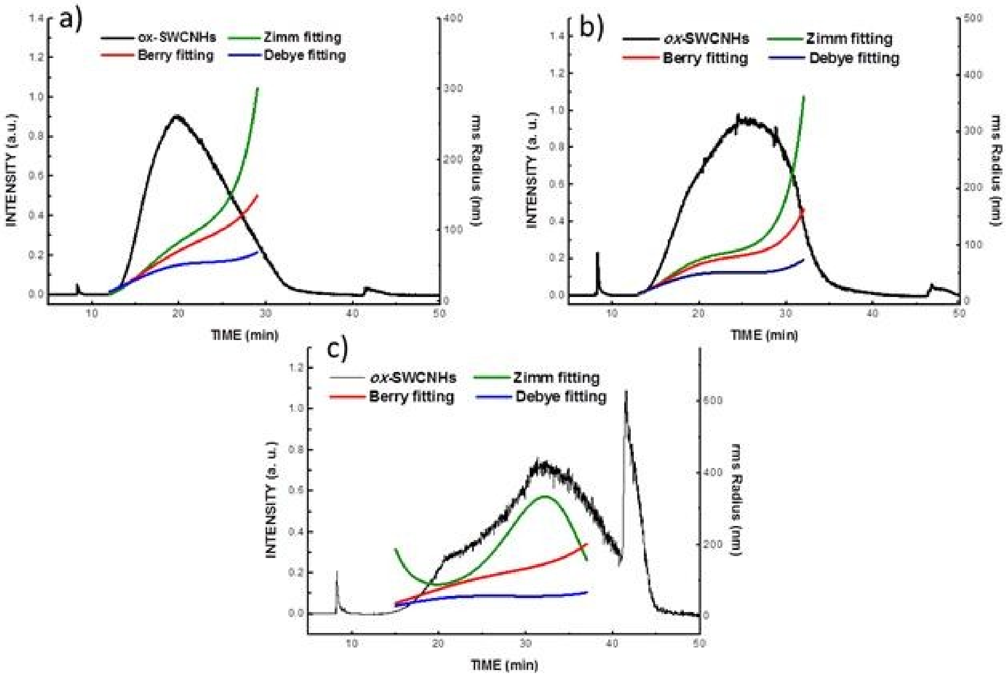

Fig. 8 - Typical fractograms of modified SWCNHs and their corresponding Zimm, Berry and Debye fitting (operating condition: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}): a) *ox*-SWCNHs, cross-flow 1.0 mL·min^{-1}, b) *ox*-SWCNHs, cross-flow 1.5 mL·min^{-1} and c) *f*-SWCNHs, cross-flow 1.0 mL·min^{-1}.

4. Conclusions

In this work, FlFFF technique coupled to UV-Vis absorption and triple angles light scattering detectors can be successfully used to provide purification, size separation and determination of modified SWCNHs. One of the most important points to obtain high repeatability and reproducibility of the separation is the solubility of the sample which can be obtained with the use of a surfactant. In addition, TEM analysis have been proven to be a method of choice to study all collected fractions and to confirm the separation of the modified SWCNHs according to their increasing radius. As an analytical tool, using the adequate fitting method, FlFFF is also capable of estimating the diameter distribution of the SWCNHs. Possible scale-up of the separation/purification procedure to study the influence of SWCNHs diameter on biological properties is under scrutiny.

Acknowledgements

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Supporting information

Captions to Figures

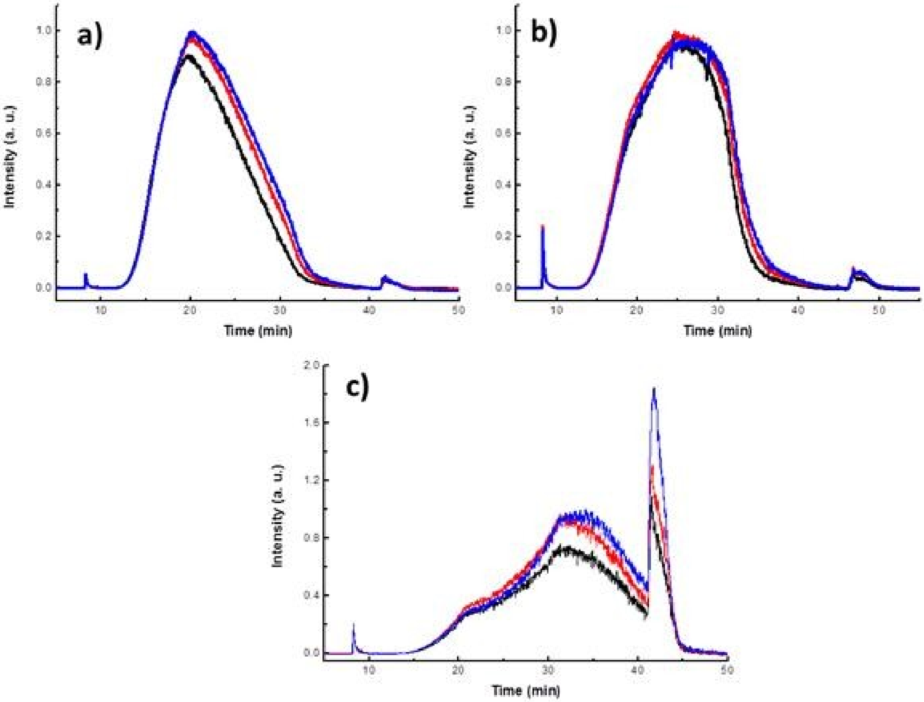


Fig. S1 – Typical fractograms of modified SWCNHs (operating condition: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}): a) *ox*-SWCNHs, cross-flow 1.0 mL·min^{-1}, b) *ox*-SWCNHs, cross-flow 1.5 mL·min^{-1} and c) *f*-SWCNHs, cross-flow 1.0 mL·min^{-1}.

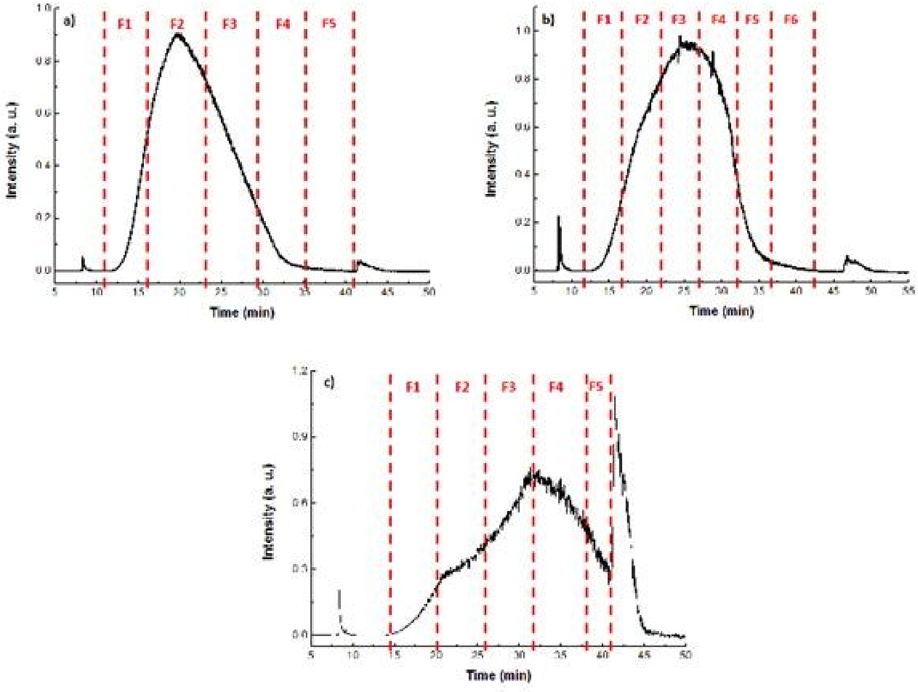


Fig. S2 – Time fractograms of modified SWCNHs (operating condition: regenerated cellulose membrane with 10 KDa membrane cut-off, 350 µm spacer thickness, injected volume 100 µL, detector-flow 2.0 mL·min^{-1}): a) *ox*-SWCNHs, cross-flow 1.0 mL·min^{-1}, b) *ox*-SWCNHs, cross-flow 1.5 mL·min^{-1} and c) *f*-SWCNHs, cross-flow 1.0 mL·min^{-1}.

Bloque II

Nanoconos y nanocuernos de carbono como herramientas en Nanociencia y Nanotecnología Analíticas

El empleo de nanopartículas supone actualmente más del 50% de las aplicaciones analíticas basadas en la Nanotecnología. Dichas nanopartículas se están utilizando con vistas a explotar las características (ópticas, eléctricas, térmicas, magnéticas, químicas, y otras) excepcionales de la nanomateria para el desarrollo de nuevos métodos, o para mejorar los ya existentes incrementando las propiedades analíticas supremas, básicas y/o complementarias.

Las nanoestructuras de carbono presentan unas magníficas propiedades que pueden aprovecharse en el ámbito analítico con diferentes fines, entre los que destacan la mejora de las propiedades analíticas anteriormente mencionadas, así como el desarrollo de nuevas metodologías que serían difícil de implementar sin tener en cuenta la componente nanotecnológica para, de esta manera, abordar los nuevos y desafiantes requerimientos de información (bio)química de calidad. Para ello es necesario resaltar las propiedades más relevantes que entran en juego cuando se utilizan las nanopartículas de carbono como herramientas en Nanociencia y Nanotecnología Analíticas. Dichas propiedades se podrían agrupar en:

- Propiedades superficiales. Tienen una repercusión crucial en el uso de las nanopartículas de carbono en Química Analítica. Al tratarse de nanoestructuras, la superficie específica (relación superficie/volumen) es muy alta, lo cual potencia su capacidad de interacción con los analitos. Asimismo, conviene resaltar que estas propiedades superficiales favorecen la tendencia a la agregación que, como se verá más adelante, es una importante limitación para su empleo como herramientas en el proceso analítico.

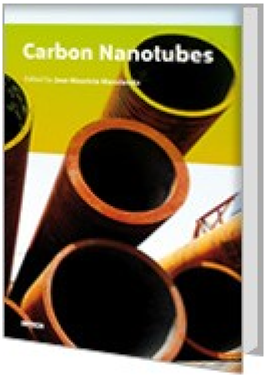
- Reactividad química. Como consecuencia de la gran superficie específica y la elevada densidad electrónica, la reactividad superficial se ve también muy reforzada. Por una parte las nanoestructuras de carbono pueden unirse covalentemente a diferentes grupos orgánicos e inorgánicos, dando lugar a un gran número de derivados estables, lo cual incrementa su versatilidad en el uso como herramientas analíticas y, por otra parte, se favorece la formación de enlaces π-π con una gran variedad de moléculas de carácter aromático, que es la base de los procesos de adsorción, muy explotados desde el punto de vista analítico tanto para el tratamiento de muestras como para el desarrollo de nuevas fases en electroforesis capilar.

En este bloque de la Memoria de Tesis Doctoral se abordará el empleo de las nanopartículas cónicas de carbono en técnicas de (micro)extracción y separaciones electroforéticas.

Capítulo 2

Solid phase (micro)extraction tools based on carbon nanotubes and related nanostructures

Capítulo 2



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Solid phase (micro)extraction tools based on carbon nanotubes and related nanostructures

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1. Introduction

The evolution of Analytical Sciences has been marked by several turning points which have changed the analytical laboratories. They have defined a variety of stages dominated by one or two key trends in Analytical Chemistry with clear influence in the "products" yielded in those years. This evolution started with the massive use of instruments in 1950s and continues with the growing importance of miniaturization and simplification in recent years. Nowadays, the analytical trends are also influenced by the developments in other scientific and technological areas. Concretely, nanotechnology can be considered as a new, increasingly important trend in science in general and in Analytical Chemistry in particular. The outstanding properties of the nanoparticles and nanostructured materials have opening up a wide variety of challenging possibilities in this area. It should be expected that the evolution of this analytical trend will be markedly affected by the nanotechnological developments.

Nowadays there are a large variety of nanoparticles commercially available or easily synthesizable in the laboratory such as gold nanoparticles, quantum dots and carbon nanoparticles, among others. Carbon nanotubes (CNTs) have received special research attention since their discovery on account of their unique and outstanding properties which results in a wide variety of applications in different scientific and technological areas. Carbon nanotubes can be considered as a graphene sheet that has been rolled up to form an extended tube–like structures [1]. They range between a few tens of nanometers and several micrometers in length with an outer diameter from ca. 2.5 to 30 nm. They can consist of a single graphene sheet (singlewalled carbon nanotubes, SWCNTs) or a series of concentric layers (multiwalled carbon nanotubes, MWCNTs).

Since their discovery in 1991 by Iijima [2], carbon nanotubes have been the target of theoretical and practical research with the aim of dilucidating their outstanding properties at the nanoscale level. The main difficult in this context is the marked tendency to aggregation of this nanomaterial, which makes difficult to distinguish between supramolecular assemblies and isolated carbon nanotubes properties. Apart from their thermal stability, excellent mechanical and electric properties, as well as their highest tensile strength among known materials, three characteristics which will play a crucial role in their sorption capacity can be highlighted [3]:

- They present nonpolar bonds and high length to diameter ratio. This result in a low water and commonly used organic media solubility and high aggregation tendency in these media as result. This negatively affects the effective surface area available for interactions with analytes.
- They can be covalently functionalized. Although they are not especially reactive, they can incorporate hydroxylic or carboxylic groups on their side walls under strong chemical conditions. It permits the immobilization of carbon nanotubes on solid supports such as controlled pore glass, silica or steel.
- They can establish non–covalent interactions with analytes which include hydrogen bond, π–π stacking, dispersion forces, dipole–dipole interactions and hydrophobic effect.

2. Sorbent properties of carbon nanotubes

As it has been commented on, the hydrophobic surface of carbon nanotubes permits the establishment of strong interactions with organic compounds. Indeed, several examples can be found in the literature dealing with the use of carbon nanotubes as effective sorbents in water treatment as regards octadecyl silica (RP–C_{18}) or activated carbon [4,5]. Recently an excellent critical review dealing with the adsorption mechanisms of organic chemicals on carbon nanotubes has been published [6]. The authors stated in their article that the chemical adsorption on carbon nanotubes cannot be easily described using a single adsorption model. It can be ascribed to the fact that the presence of surface defects, functional groups or even interstitial regions can generate preferential adsorption sites. Readers interested in a detailed explanation on these mechanisms are referred to the article by Pan and Xing. Initially, the hydrophobic interactions between the carbon nanotubes surface and the organic analytes were considered as the sole mechanism for adsorption. If this is so, the interactions can be predicted by means of the octanol–water distribution constant, K_{ow}. However, further investigations demonstrated that it was not possible to establish an explicit relationship between the adsorption coefficient of the organic compounds and the corresponding K_{ow} value [7,8]. Three additional mechanisms have been proposed to explain the adsorption of the analytes on the carbon nanotubes such as the π–π interactions that can be established between the aromatic moiety of the molecule and the π–system of the nanotubes; the hydrogen bonds with the functional groups of the nanostructures; and electrostatic interactions due to the residual charge on the nanotubes surface [9,10]. As several factors affect these interactions, the relative contribution of each mechanism cannot be easily attributed.

Probably, one of the variables that will determine the availability of the adsorption equilibrium is directly related to the carbon nanotubes properties, viz: aggregates and functional groups. Moreover, the chemical environmental conditions (e.g. pH, ionic strength and surfactants) can also affect the analyte–CNTs adsorption.

The insolubility of carbon nanotubes in common aqueous and organic media results in the formation of aggregates of different sizes and stability. As a result, the superficial sites available for analytes interaction decreased as the interstitial and inner pores adsorption is sterically hindered. The procedure selected for the synthesis of the carbon nanotubes can also influence the adsorption capability of the material as residues of amorphous carbon and metal catalyst could also block the inner pores [11].

Different procedures have been described to reduce or even eliminate these negative effects on analytes adsorption. The sorbent capacity of surfactant coated carbon nanotubes has been deeply studied in the literature [12]. This evaluation was conducted using toluene and n–undecane as model compounds for the calculation of the corresponding solid–liquid and liquid–liquid isotherms. In order to obtain relevant conclusions, solid–liquid single component isotherms were calculated under three different experimental conditions: solid multiwalled carbon nanotubes; surfactant coated carbon nanotubes and surfactant media (sodium dodecyl sulphate, SDS). In all cases, the concentration of the nanoparticles and surfactant were maintained constant. For the liquid–liquid isotherms, the distribution of the analytes between an organic solvent (heptane) and an aqueous phase containing solid nanotubes, surfactant coated nanotubes and surfactant was considered.

The behaviour observed for the solid–liquid and liquid–liquid isotherms was analogous. The authors concluded that the maximum extractant capacity for toluene corresponds to dispersed nanotubes, with negligible contribution of the SDS solution. This fact permits one to assess that the whole adsorption can be attributed to the presence of the carbon nanoparticles. If benzene is considered instead of toluene as analyte, the enrichment factor obtained is lower which can be explained by the fact that the presence of methyl group in the toluene molecule would facilitate the adsorption on the MWCNTs surface due to an additional interaction with the hydrocarbon chain of the surfactant. On the contrary, the n–undecane extraction was maximum with the surfactant, with the lowest yields obtained with non–dispersed carbon nanotubes. The hydrocarbon molecules are located in the core of the micelles, and when surfactant coated carbon nanoparticles are used, there are fewer sites available, which reduce the extraction efficiency. However, the n–undecane can substitute the carbon nanoparticle from the micelles as the stability of the suspension is lower, providing an intermediate extraction capability.

From the above, it can be concluded the adsorption properties of carbon nanotubes are affected by the nanoparticles state, being possible to increase this property by the use of surfactant, which clearly reduces the natural aggregation tendency of these nanoparticles, increasing the active surface of CNTs.

Adsorption of organic compounds on carbon nanotubes can also be affected by the synthesis procedure and further purification step. The raw material is mainly hydrophobic and in general, the adsorption can be increased after heating the carbon nanotubes due to the removal of amorphous carbon and oxidation of residual catalyst moieties. The increasing amount of oxygenated groups will lead to a higher interaction with polar compounds due to an increased hydrophilicity of the carbon nanotubes. Other functional groups such as –OH, –COOH and –CO can be added by acid treatment (typically a mixture of HNO_3 and H_2SO_4). The presence of these moieties can be used for carbon nanotubes immobilization on a variety of surfaces. The presence of isolated nanostructures can increase the adsorption through a reduced aggregation. Finally, the hydrophobicity of the carbon nanotubes can be restored by using extreme heating conditions (viz 2200 ºC). Depending on the conditions used, the most appropriated carbon nanotubes state can be selected for the given application. The next sections are devoted to the reported applications of carbon nanotubes and related materials in solid–phase (micro) extraction.

3. Carbon nanoparticles in solid–phase extraction

Solid–phase extraction (SPE) is a well–known, worldwide used sample treatment technique. Its favorable features as regards analytes preconcentration factors, interferents removal, solvent consumption, sample processing time, automation and potential sorbent reusability, justifies its use against its counterpart liquid–liquid extraction. Moreover, the variety of sorbents currently available permits the preconcentration of analytes of different polarity and even charged species. Among the new sorbents proposed for SPE, carbon nanostructures and more specifically fullerenes and carbon nanotubes have been successfully used in this context [13]. The applicability of these nanoparticles in this separation technique relies on the sorbent properties described in the previous section of this chapter. Here, the main applications of carbon nanostructures in SPE will be presented. The selected examples have been classified according to the extraction unit in which the nanoparticles can be presented, namely, commercial cartridges and minicolumns. Furthermore, their used immobilized on a solid support and dispersed with surfactants will be commented on. Carbon nanotubes have been used packed in conventional SPE cartridges for the determination of a variety of organic compounds in different matrices. The isolation and preconcentration of organic pollutant from environmental samples is the most reported application of this configuration. By way of sample, Table 1 lists analytical information related to these references.

The first sign of the adsorption capacity of carbon nanotubes was revealed by Long et al. [14] when they proposed their use for dioxin removal. Two years later, the first analytical application of packed carbon nanotubes was reported [15]. The authors employed a commercial polypropylene SPE cartridge from which the sorbent was replaced by 500 mg of multiwalled carbon nanotubes. The upper and low frits were maintained at both ends to hold the nanotubes in place during the extraction. The cartridge was evaluated for the enrichment of bisphenol A, 4–n–nonylphenol and 4–tert–octylphenol from waters. The high affinity of the carbon nanotubes for the endocrine disruptors is demonstrated by the large volume of sample that can be preconcentrated (up to 1 L) without analytes losses. Moreover, the low volume of organic eluent required (2.5 mL of methanol) permits limits of detection as low as 0.018 ng mL^{-1} (for 4–n–nonylphenol). The analytical figures of merit provided by the nanostructured material resulted better than those of RP–C_{18} and XAD–2 for these compounds. The applicability of carbon nanotubes to solve environmental–related analytical problems was extended in the following years by determining chlorophenols [16], herbicides [17–19] and dichlorodiphenyltrichloroetane and metabolites [20] in waters. In all cases, multiwalled carbon nanotubes were preferred to singlewalled carbon nanotubes as the presence of concentric sheets resulted in an enhanced interaction with the analytes. The amount of sorbent used can be reduced to 100 mg and in some of the applications, the cartridge can be reused up to 200 times without efficiency loss [18]. The eluent used was different depending on the analyte and, in some cases the use of mixtures is needed. Moreover, the

Table 1. Main analytical features of the procedures developed using carbon nanotubes packed cartridges.

Sample	**Analyte**	**Amount of sorbent**	**Sample volume**	**Eluent**	**Detection technique**	**References**
Environmental waters	4–n–nonylphenol 4–tert–octylphenol	500 mg MWCNTs	500 mL	MeOH, 2.5 mL	HPLC–FL	Cai et al., 2003
Environmental waters	Chlorophenols	300 mg MWCNTs	200 mL	MeOH, pH 10, 6 mL	HPLC–UV	Cai et al., 2005
Environmental waters	Atrazine Simazine	100 mg MWCNTs	500 mL	ACN, 4 mL	HPLC–DAD	Zhou et al., 2006a
Environmental waters	Cyanazine Chlortoluron Chlorbenzuron	100 mg MWCNTs	250 mL	CH_2Cl_2, 5 mL	HPLC–UV	Zhou et al., 2007a
Waters	Sulphonylurea Herbicides	100 mg MWCNTs	500 mL	ACN 1% acetic acid, 12 mL	HPLC–DAD	Zhou et al., 2007b
Waters	Dichlorophenyltrichloroetane and metabolites	100 mg MWCNTs	500 mL	CH_2Cl_2, 5 mL	HPLC–UV	Zhou et al., 2006b
Pork	Barbital, amobarbital and phenobarbital	250 mg MWCNTs	10 mL	Acetone:Ethylacetate 3:7 (v:v), 5 mL	GC/MS/MS	Zhao et al., 2007
Urine	Antidepressants	30 mg MWCNTs	10 mL, diluted in water 1:1	ACN:acetic acid 97:3, 2 mL	HPLC–UV	Cruz–Vera et al., 2008
Virgin olive	Pesticides	30 mg c–SWCNTs	3 mL, diluted in water 1:1	Ethylacetate, 0.5 mL	GC–MS	López–Feria et al., 2009

eluent volume required for quantitative elution can be as high as 6 mL which is an inconvenience for sensitivity. An evaporation–redissolution step can be used to overcome this limitation.

In addition to waters, the use of commercial cartridges packed with MWCNTs has been extended to the determination of barbiturates in porks [21], antidepressants in urine [22] and pesticides in virgin olive oil [23]. In the last two articles, the amount of carbon nanotubes was reduced to 30 mg and, despite the matrix complexity, the cartridge reusability was demonstrated by the authors by the mere inclusion of a washing step between samples.

Recently, a novel carbon nanostructure, carbon nanocones, has been proposed for its use in SPE cartridge [24]. The commercial product contains 20%wt carbon nanocones, 70%wt carbon disks and 10%wt amorphous carbon. The presence of the amorphous solid results in irreversible interactions with the analytes and lower recovery values, as result. This negative effect can be reduced by maintaining the commercial solid at 450 ºC for 20 min. This treatment dramatically reduced the amorphous carbon content with negligible influence on the nanostructured material. In the model application developed for the determination of chlorophenols in waters, 20 mg of the thermally treated sorbent were packed in a 3–mL commercial SPE cartridge. Limits of detection as low as 0.3 ng mL^{-1} were achieved by preconcentrating 2 mL of water sample and eluting the retained compounds with 200 µL of hexane. As compared with carbon nanotubes, the conical nanostructures showed to be more efficient in the preconcentration process as similar analytical features were obtained with lower sorbent amount. This fact can be explained taking into account that the carbon nanocones show lower aggregation tendency than the carbon nanotubes and, therefore, the effective surface available for analytes interaction is higher with the former.

Solid–phase extraction has also been implemented in a continuous fashion by using flow system configurations. This approach permits the miniaturization and automation of the whole process with the inherent advantages. Fullerenes have been extensively used in this context. The general configuration used is depicted in Figure 1.

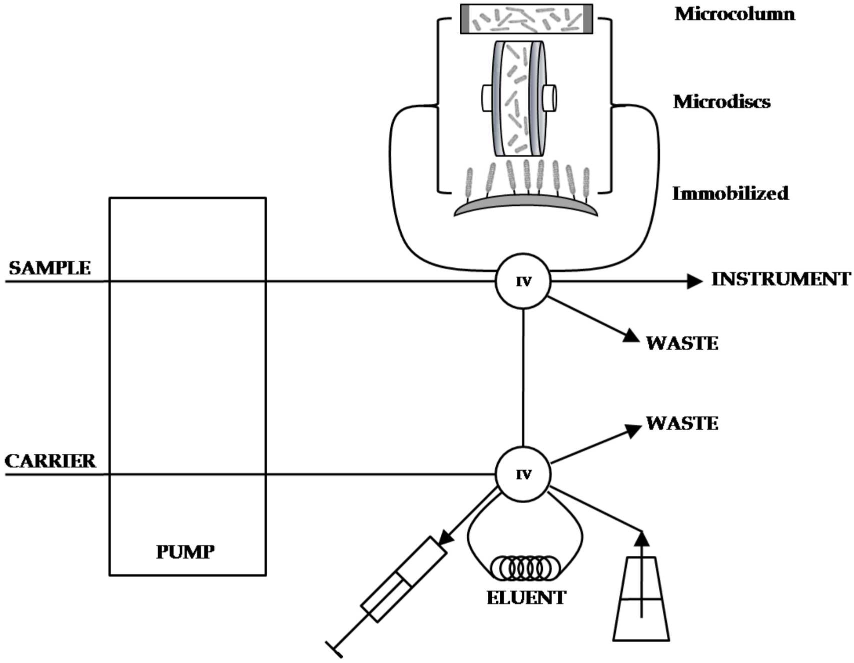

Fig. 1. Flow configuration for the use of carbon nanotubes as sorbent in solid phase extraction procedures.

As it can be seen, an amount of ca. 80 mg of fullerene C_{60} are packed in a PTFE minicolumn fitted with cotton wool beds at both ends to prevent sorbent losses. The column is placed in the loop of an injection valve to allow the preconcentration of large volumes of sample. Once the sample has been completely aspirated into the system, a small plug of eluent (in the microliter range) was passed through the C_{60} column for analytes elution. As the continuous unit is on–line coupled to the atomic absorption spectrometer, a transient analytical signal is obtained when the eluent, containing the analytes, reach the flame. By using this configuration, a large variety of metals and their alkyl derivatives have been determined in waters. Table 2 summarized the main applications developed in this context. As it can be seen, the adsorption capacity of fullerene C_{60} for organic compounds is rather limited while they show great potential retaining organometallic compounds. The study carried out by Ballesteros et al. [25] also pointed out that the efficiency of the extraction process decreased when the polarity of the analytes increased. Despite this behaviour, some references dealing with the determination of organic molecules using fullerenes as sorbent have been reported. Serrano et al. used fullerene C_{60} for the extraction of benzene, toluene, ethylbenzene and xylene isomers from waters [26]. In this case, the preconcentration step was followed by the gas chromatographic–mass spectrometric determination of the volatile organic compounds. The sensitivity and precision of the method were better than those obtained with conventional sorbents such as RP–C_{18} and Tenax TA. Moreover, the selectivity was also enhanced as fullerenes were not affected by the low pH selected for retention. As far as metallic species is concerned, they required previous derivatization to form neutral chelates that could be effectively retained on fullerenes. In order to obtain discriminated information on the sample composition, a chromatographic separation can be implemented after the analysis of the sample by an atomic technique to estimate the total concentration of the target metals in the sample. Such is the

Table 2. Representative examples of the applicability of carbon nanotubes and fullerenes in continuous flow separation techniques.

Sample	Analyte	Sorbent	Eluent	Derivation reaction	Instrumental technique	Reference
Environmental samples	Cd, Mn, Ni	MWCNTs	HNO_3 0.1 M	–	ICP–AES	[31]
Waters	Cu	MWCNTs	HNO_3 0.5 M	–	FAAS	[32]
Egg and pork	Sulfonamides	MWCNTs	Mobile phase MeOH:H_2O 22:78	–	HPLC–UV	[33]
Waters	Pb (II)	c–MWCNTs	HNO_3 1 M	–	FAAS	[34]
Waters	BTEXs	Fullerene C_{60}	Ethyl acetate	–	GC–MS	[26]
Waters	Pb	Fullerene C_{60}	IBMK	–	FAAS	[35]
Wheat flour	Co	Fullerene C_{60}	IBMK	–	ETAAS	[36]
Rainwaters	Pb and alkyllead derivates	Fullerene C_{60}	IBMK	Diethylditiocarbamate complexes	FAAS	[27]

case of the determination of lead and alkyllead compounds in rainwaters [27] or organometallic compounds in environmental samples [28].

Reducing the dimensions of the extraction units for carbon nanotubes was also aimed at minimizing the aggregation of the nanoparticles which negatively affects to the retention efficiency. Therefore, Suárez et al. proposed the package of 6 mg of MWCNTs in the minicolumn, which was further located in the loop of the injection valve. It permits the retention and elution processes to be carried out in opposite direction thus reducing the compacting of the sorbent. This approach has been used for the determination of tetracycline residues in waters [29]. In this case, the minicolumn presents a larger diameter, (9 mm i.d.) than that reported for fullerenes, which also helps to reduce the overall pressure in the system. A similar configuration has been on–line coupled to a liquid chromatography for the simultaneous determination of 10 sulfonamides in egg and pork [30].

Suárez et al. used immobilized carboxylated single-walled carbon nanotubes (c–SWCNTs) on inert controlled pore glass and used the combined solid as sorbent in SPE [37]. An accurately weighed amount of 15 mg of the combined solid was packed in a PTFE tube (1.2 mm i.d.) which was placed in a continuous flow device. The automated system was used for the determination of non steroidal anti–inflammatory drugs (NSAIDs) in urine samples. The authors compared the adsorption capacity of the same amount of immobilized c–SWCNTs, SWCNTs and MWCNTs for tetracyclines and NSAIDs [Figure 2]. The best results were obtained with the immobilized sorbent, with the additional consideration that the amount of nanoparticles is lower than in the other cases as the weighed material referrers to both, the c–

SWCNTS and the inert glass. It can be ascribed to the fact that the immobilization process results in a vertical alignment of the c–SWCNTs on the particle surface, which clearly enhanced the molecular interactions of the analytes with the nanotube surface.

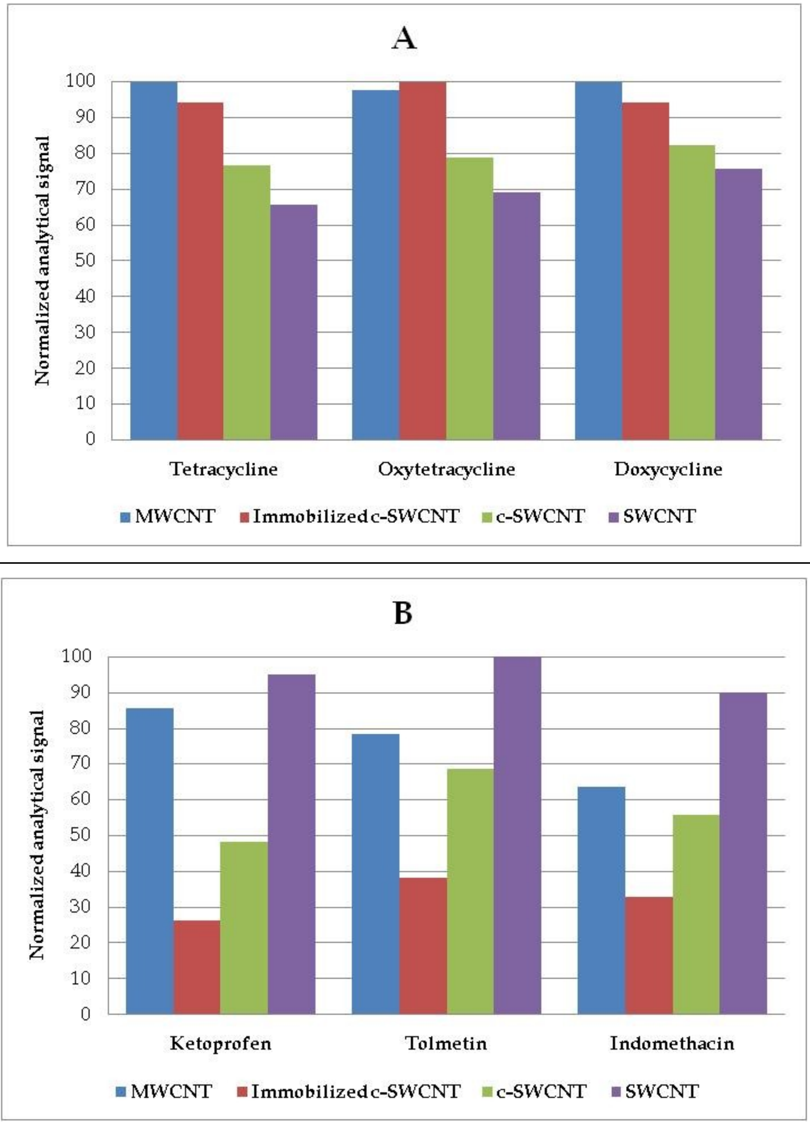

Fig. 2. Comparison of sorption capacity of different types of carbon nanotubes.

The determination of inorganic species using carbon nanotubes requires the previous derivatization of the nanoparticles to introduce functional groups (carbonyl, carboxylic and hydroxyl) that allows the interaction with the metallic species. Such is the case of the extraction of cadmium, manganese, and nickel [31], Cu (II) [32] or Pb (II) [34] from waters. In these applications, the pH of the sample resulted to be a critical variable as it will define the surface charge of the sorbent, which should be negative to interact with the cations. The microcolumns of variable dimensions and containing and average sorbent amount of 50 mg were inserted in a continuous flow configuration that can be on–line coupled to the flame atomic absorption or inductively coupled plasma atomic emission spectrometers.

As it was indicated in the previous section, surfactants have been reported to disperse carbon nanoparticles in aqueous media, facilitating their use for analytical purposes. The aqueous suspension obtained is stable under optimal conditions (the amount of nanoparticles and surfactant concentration being critical) as the nanoparticles are located inside the micelles. The surfactant coated carbon nanoparticles have been used as pseudophase for liquid–liquid extraction [12]. The interactions between the organic compounds and the dispersed carbon nanotubes differ from that observed for the raw material. As no aggregates exist in the dispersed phase, more adsorption sites are available for retention, which has a positive effect on extraction. This methodology has been successfully applied for the extraction of benzene, toluene, ethylbenzene and xylene isomers from olive oil samples with a clearly sensitivity and selectivity enhancement in comparison with the direct headspace analysis [38]. The procedure is schematically depicted in Figure 3.

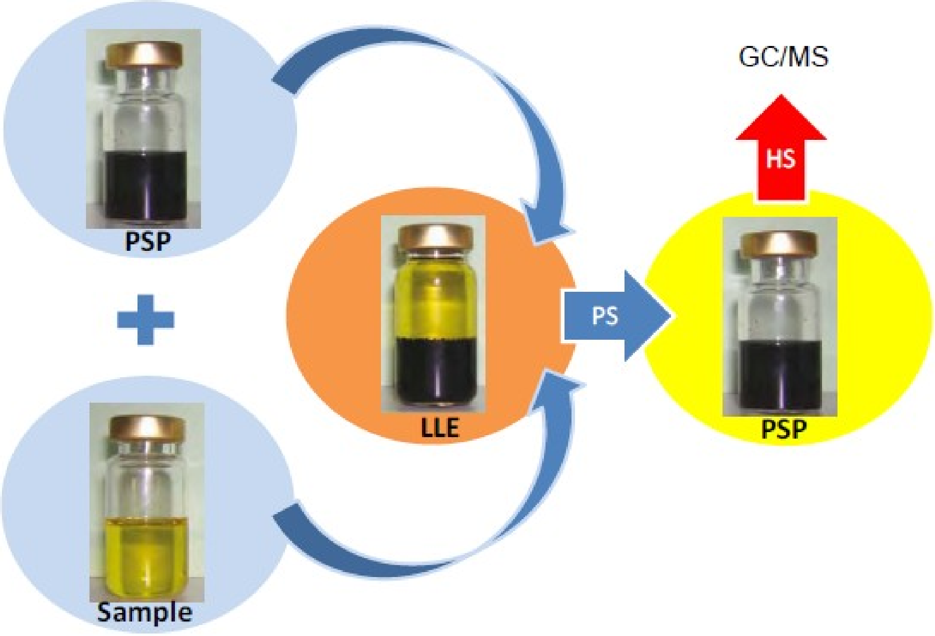

Fig. 3. Analytical procedure for the liquid–liquid extraction (LLE) of BTEXs from olive oil samples using surfactant coated nanotubes as pseudophase (PSP).

Finally, and considering the thermal stability of the carbon nanotubes, they can be used as preconcentration units on–line coupled to a gas chromatograph for retention of low molecular weight compounds (methyl–ethyl ketone, toluene and dimethyl methyl phosphonate) from a vapour stream by maintaining the unit at low temperature. For desorption, the temperature was rapidly increased and the analytes directly transferred to the injection port of the instrument [39].

4. Solid–phase microextraction based on carbon nanoparticles

Solid–phase microextraction (SPME) appeared as an evolution of the solid phase extraction. It was introduced by Arthur and Pawliszyn in 1990 [40] and it was conceived as an alternative to traditional methods for sample preparation. SPME overcomes the main drawbacks of classical preconcentration techniques, regarding the number of steps involved, extraction time or the consumption of toxic organic solvents. Solid–phase microextraction has gained importance in recent years because it is an efficient and fast methodology which combines in a single step, the sampling, preconcentration, matrix removal and injection in the chromatographic system. Owing to its favorable features, SPME has widely been applied in various fields including environmental, food, natural products, pharmaceutical, biology, toxicology and forensic [41].

SPME is based on the distribution of the analytes between the sample (or its headspace) and the stationary phase immobilized on the solid support. The analytes transference starts when the coated fiber is placed in contact with the sample and it is considered that the extraction is complete when the equilibrium between the sample matrix and fiber coating is reached [42].

The SPME extraction process can be carried out in three ways: direct–immersion, headspace configuration, and membrane–protected approach [42]. In the direct immersion method (DI–SPME), the coated fiber is completely immersed in the sample matrix (liquid or gas) and the analytes are directly transferred to the extractant phase under continuous stirring. In DI–SPME, the selectivity is provided by the type of fiber coating, the main limitation being the potential fiber damage due to irreversible interaction with endogenous matrix compounds. In headspace mode (HS–SPME), the analytes are release from the sample matrix to its headspace for further interaction with the coating of the fiber. The selectivity of this approach is higher and the fiber lifetime longer, although it is limited to the preconcentration of volatile analytes. The membrane–SPME approach permits the isolation/enrichment of polar compounds from aqueous samples by protecting the polar probe by means of a hydrophobic membrane. It can expand the applicability of the SPME with the additional selectivity enhancement provided by the membrane [42]. Once the analytes have been preconcentrated on the fiber coating, the next step is their desorption for qualitative and quantitative analysis. The thermal desorption is the preferred choice taking into account that the compounds are separated by gas chromatography. In this case, the analytes are transferred to the chromatographic column by heating the fiber in the injector of the instrument. It reduces the analytes dilution with the favorable effect on the sensitivity. For the HPLC coupling, the analytes are eluted by means of the proper organic solvent/mobile phase.

The type of coating used in the SPME fiber plays a crucial rol in the extraction/desorption process, since the efficiency of this process will depend on the distribution constant between the analytes and the stationary phase. In addition, it will provide some selectivity to the SPME process towards the analytes versus other matrix compounds. For this reason, a wide variety of materials are used as coatings for SPME fibers. The most common commercially available are: polydimethylsiloxane (PDMS), polyacrylate (PA), divinylbenzene (DVB), Carbowax (CW) and Carboxen. Despite their advantages, they also present some drawbacks regarding the instability at high temperatures (e.g 240–280 ºC), which results in the presence of artifacts in the chromatogram, carry over owing to incomplete desorption or shorter lifetime of the fiber. Furthermore, the organic nature of the coatings are incompatible in some cases with the use of organic solvents under which the commercial fibers are unstable and have swelling, which reduces or limits their applicability in liquid chromatography. In addition, commercial fibers have poor reproducibility, are fragile and expensive. Therefore, the development of novel coatings for SPME fibers is welcome. The carbon nanostructures have emerged in the last decade as a robust alternative to commercial coatings on account of their excellent mechanical, electrical and chemical properties. Both, fullerenes and carbon nanotubes as well as their derivatives have been used in this context using different immobilization processes. The most relevant reported applications are summarized in Table 3 and they will be commented on.

Carbon nanotubes have been immobilized on the SPME fiber by physical deposition technique or dipping technique, which consists in the immersion of the uncoated fiber into a solution of the sorbent material for a short period of time, and subsequent solvent removal by evaporation. Wang et al. [43] used a fused silica fiber without the polyimide protective layer as probe support. It was then immersed in a dimethylformamide solution containing MWCNTs at a concentration of 20 mg mL^{-1}. The organic solvent was further removed by heating at 160 ºC. The process was repeated until the desired coating thickness was reached, in this case, ca. 40 µm. Later, the fiber was subjected to a sintering process by heating at 80 °C for 30 min, further increasing the temperature to 280 ºC for 4 h, under a helium stream.

Table 3. Selected applications on the use of carbon nanoparticles as active components in SPME.

Sorbent (film thickness, µm)	Analytes(1)	Sample	LOD (ng L^{-1})	Precision (% RSD)(2)	Recoveries (%)	Reference
MWCNT (40)	PBDEs	River water and milk	3.6–8.6	6.9–8.8 (n=4)	90.0–119.0	[43]
MWCNT (35)	PBDEs	Water	1.1–16.2	5.0–9.1 (n=5)	74.2–98.7	[44]
c–MWCNT (20)	Phenols	River and wastewater	5.0–50.0	0.97–7.96 (n=3)	70.83–148.10	[45]
SWCNT (50)	OCPs	Lake and wastewater	0.19–3.7	3.5–13.9 (n=5)	44.7–111.0	[46]
SWCNT (50)	Oxygenated ethers	Human urine	10.0	4.8–9.6 (n=6)	90.0–95.0	[47]
c–MWCNT/ MWCNT (50)	Phenols	River and wastewater	0.25–3.67	1.52–6.38 (n=5)	86.13–118.64	[48]
MWCNT (12.5)	Polar aromatic compound	Water	30.0–570.0	4.4–7.5 (n=7)	83.3–106.0	[50]
MWCNT	Aromatic hydrocarbons	Petrol station wastewater	90.0–390.0	4.8–9.6 (n=6)	93.8–106.1	[51]
c–MWCNTs (3)	BTEX	Water	–	1.8–2.5 (n=6)	–	[52]
c–SWCNT (25)	Phenols	Sea and tap water	900.0–3800.0	0.7–3.2 (n=3)	87.5–102.0	[53]
MWCNT (40)	Phenols	Water	1.0–65.9	2.7–6.5 (n=6)	87.7–115.5	[54]
Polymeric fullerene (33)	BTEXs, NPCs and PAHs	Water	80.0–1200.0	1.52–6.80 (n=6)	–	[55]
Hydroxyfullerenes (30)	PCBs, PAHs and PAAs	Water	0.013–125.0	1.1–8.9 (n=6)	88.2–94.0	[56]

(1) PBDEs: Polybrominated Diphenyl Ethers; BTEX: Benzene, Toluene, Ethylbenzene and Xylenes; PCBs: Polychlorinated Biphenyl; OCPs: Organochlorine Pesticides; PAHs: Polycyclic Aromatic Hydrocarbons; PAAs: Polar Aromatic Amines; NPCs: naphthalene congeners.

(2) RSD: relative standard deviation.

Finally, the fiber was assembled on a modified syringe to be used as SPME device for the determination of polybrominated diphenyl ethers (PBDEs) in milk and water samples by gas chromatography (GC) with electron–capture detection (ECD). The SPME process was carried out by direct immersion of the fiber in the samples and the thermal desorption in the gas chromatograph injector at 295 ºC. The fiber–to–fiber reproducibility expressed as relative standard deviation (RDS) ranged between 8 to 16 %, using three fibers coated with the same procedure. Furthermore, the fiber could be used up to 100 times with negligible loss of performance. Also, a comparison with other coatings, namely activated carbon and poly (5% dibenzene–95% dimethylsiloxane) (PDMS–DB) was carried out following the same coating and SPME procedures. The results were compared through the enrichment factor (EF), defined as the ratio between the peak area after extraction and the peak area of the direct injection of a standard solution. The comparison pointed out that the MWCNTs coating reached enhancement factors 4 to 4.5 times higher than activated carbon and PDMS–DB. In the same way, Tiang and Feng [44] carried out a similar process for SPME fiber coating with MWCNTs for the determination of polybrominated diphenyl ethers (PBDEs) in water samples. In this case, the extraction process was carried out using the DI–SPME (20 ºC, 45 min) and HS–SPME (100 ºC, 45 min) approach. As a result, the analytical response obtained with the HS–SPME was better (between 2–4 times) with cleaner chromatograms and longer fiber lifetime. Moreover, the precision (RSD, n=5) ranged between 5.0% and 9.1%. Also, the MWCNTs fiber was compared with the SE–54 coating and it was obtained a sensitivity 3–5 times higher for the MWCNTs. It can be ascribed to the large specific surface and strong adsorption capability of the nanostructured material. In this context it should also be mentioned the work carried out by Liu et al. [45] in which, oxidized MWCNTs were immobilized on a fused silica capillary previously silanized. MWCNTs were functionalized by acid attack ($H_2SO_4/HNO_3 = 3/1$) and subsequently dispersed in an aqueous solution with SDS (1%). The fused silica capillary was immersed in the solution of MWCNTs at 70 °C for 4 h and then, dried in an oven at 120 ºC. As in the previous cases, this process was repeated until reaching the desired thickness (in this case, 20 µm) and then the fiber was subjected to a sintering process (280 °C, 12 h) under a nitrogen stream. The extraction of selected analytes was made by DI–SPME and subsequent thermal desorption in the injector of the chromatograph. From this work it is remarkable the precision of the method (RSD, n = 3) which ranged between 0.97% and 7.96%.

Fullerenes and carbon nanotubes have also been immobilized on the fiber following the epoxy–glued solid sorbent or pasting with adhesives procedure. The coating is accomplished by means of an organic binder. For this aim, both the carbon nanoparticles and the organic binder components are dissolved in a solution in which the support is immersed as many times as necessary to achieve the desired thickness of the coated fiber. Then, the organic solvent is removed and a sintering process is carried out. This simple and expeditious procedure results in a very temperature resistant fiber. The main drawback is the progressive loss of sorbent when the fiber is in contact with organic solvents. In addition, the fiber coating process results in a lower reproducibility between fibers than the physical deposition. Notwithstanding this, the procedure has been successfully used for a variety of applications. Lü et al. [46] immobilized SWCNTs on a SPME fiber using an organic adhesive composed of terpineol, ethylcellulose and dibutyl phthalate. Once the fiber was coated, it was used to extract eight organochlorine pesticides in lake and wastewater samples by DI–SPME mode. The precision of the method ranged between 3.5% and 13.9%. Furthermore, a comparison between the SWCNTs fiber and a commercial PDMS one in terms of both thermal stability and lifetime demonstrated that the SWCNTs fiber was better in all instances as it was not affected at temperatures higher than 350 ºC and its extraction efficiency remained constant for ca. 150 extractions. This can be explained by the strong adhesion of the coating on the substrate through the organic binding. Rastkari et al. [47] also used the same compounds to immobilize SWCNTs on a SPME fiber to determine methyl tert–butyl ether, ethyl tert–butyl ether and methyl tert–amyl ether in human urine. Moreover, a similar methodology was implemented by Liu et al. [48], who used an epoxy resin to immobilize both raw MWCNTs and functionalized MWCNTs on the fiber. In this work, the desorption method used should be highlighted as it used static desorption mode. For this aim, once the analytes were adsorbed on the SPME fiber, it was placed in contact with an aliquot of 70 μL of acetonitrile:water (70:30 v:v) at room temperature. Then, 5 μL of the solution containing the desorbed analytes was injected in a HPLC–UV for analysis. The authors demonstrated that the carboxylated–MWCNTs coating provided better responses than raw MWCNTs, Alumina N, Carbograph, PDMS–DVB and CW–TPR. It can be ascribed to the favorable effect of the oxidation process. On the one hand, the acid treatment increases the surface area, which improves the adsorption capacity and the sensitivity of the whole procedure. On the other hand, the functionalized CNTs contain oxygenated groups (COOH, CO, OH) on their surface, which makes the CNTs more hydrophilic, enhancing the affinity for polar molecules. These authors used the same sorbent immobilization technique to fix carboxylated MWCNTs onto a fused silica capillary, which was further used for in–tube SPME for the determination of substituted aniline compounds in water samples [49]. The methodology of immobilization by organic glue has also been used by Xiao et al. [55] to fix polymeric fullerenes on a SPME fiber. In this case the immobilization was carried out by dissolving the fullerenes in a solution of heptane containing 5% photosensitive reagent (dicumyl peroxide, DCP) and then, the paste was immobilized by UV radiation. With this modification, the reproducibility between fibers was better than 4% (as relative standard deviation). The coating of CNTs with organic glue or by epoxy resin was also used by Chen et al. [50] who used Nafion as the polymer to immobilize MWCNTs on the fiber for the determination of polar aromatic compounds in natural water samples. Similarly, Adomaviciute et al. [51] employed an epoxy glue to deposit CNTs on a modified SPME fiber to determine traces of aromatic hydrocarbons in petrol station wastewaters.

Another technique widely used in the preparation of SPME fibers is the sol–gel approach. In this case, an effective chemical bond is established between the coating and the fiber surface. It clearly improves the thermal stability and resistance of the SPME fiber to organic solvents. However, the reproducibility of the coating procedure is not satisfactory since the method is subjected to a wide number of variables such as pH, temperature and reaction time, concentration and nature of catalyst and sintering step. The sol–gel technique has been used by Jiang et al. [52] to prepare SPME fibers containing CNTs as sorbent. In order to avoid the insolubility of CNTs in some organic solvents, they were functionalized as CNTs–OH and CNTs–COOH derivatives. The steps needed were as follows: first, a stainless steel wire (length 20 cm) was treated with acetone, NaOH and HCl to remove surface impurities. Secondly, the functionalized CNTs were dissolved in a mixture of methyltrimethoxysilane (MTOS), distilled water and poly(methylhydrosiloxane) (PMHS). Later on, the solution was stirred by ultrasound and then trifluoroacetic acid (TFA) was added to form a stable sol solution. To improve the reproducibility in the coating process of the fiber, Jiang et al. used a mechanical device which controls the rate at which the fiber was immersed into the sol–gel solution. In this way, a uniform coating with a controlled thickness was obtained. The fiber was then cut into pieces of 2 cm and it was fixed on a commercial SPME device using epoxy glue. Subsequently, the fiber coated with the sol–gel solution was subjected to a drying process at room temperature for 24 h. Finally, the fiber was conditioned at 50–260 °C under a nitrogen atmosphere for 6 h. The repeatability and reproducibility between fibers was evaluated with an aqueous solution containing BTEX, which were extracted by HS–SPME, obtaining values of repeatability (% RSD, n=6) and reproducibility (% RSD, n=5) better than 2.5% and 6.5%, respectively. In terms of reproducibility between fibers, the sol–gel method significantly improves the previously proposed techniques, as a long fiber was initially prepared, and several working fibers were obtained by cutting the long one into small pieces. Also, the sol–gel–CNT fiber showed a high stability with the temperature, being possible its use at 350 ºC. Moreover, a study on the stability of the fiber versus organic solvents such as methanol or acetonitrile was carried out, obtaining a good durability of fiber in these media. Not only carbon nanotubes have been immobilized by the sol–gel technique. This methodology has also been used by Yu et al. [56] to deposit hydroxyfullerenes on a fused silica capillary, which was further used for the determination of PCBs, PAHs and polar aromatic amines in water samples by HS–SPME coupled with GC–ECD/FID. The fiber can be used for nearly 200 times, while commercial fibers can be typically used for ca. 40–100 times.

Moreover, the coating was stable against temperature (even to 360 ºC) and solvents because of the properties of fullerene and the chemical binding between the coating and the fiber surface. Electrochemical deposition is a technique widely used in the field of ceramic materials. It is based on the production of thin film coatings by deposition of charged particles, previously dispersed in an organic solvent, to which an electric field is applied through an electrode. This generates rigid and homogeneous layer on the electrode, which is used as a support of SPME fiber. This is why a support with metallic characteristic such as a steel, aluminum, nickel or titanium fiber is required. The main advantages of using this technique are its simplicity and low cost, easy thickness control, high coating uniformity and compatibility with the use of CNTs. Li et al. [53] proposed the use of electrophoretic deposition for the coating of functionalizated SWCNTs in a SPME fiber. The c–SWCNTs were dispersed in DMF by ultrasonic agitation. Subsequently, two Pt wires (one used as a cathode and another as anode) were immersed in the solution of c–SWCNTs and separated at a distance of 1 cm. A d.c. 40 V was applied between two electrodes for 10 s and the c–SWCNTs were immobilized on the Pt used as anode. Finally, the wire was removed from solution and dried at 120 ºC for solvent evaporation. Using this methodology, a homogeneous fiber of 25 μm in thickness was obtained. This SPME fiber was used for the extraction of phenols from water samples by DI mode. Despite the good precision of the method, obtaining RSD values for a single fiber of 0.7–3.2%, the lifetime of the fiber decreased significantly after 80 analyses, which is shorter than that obtained with the organic binder [46]. In a similar manner, Du et al. [54] covered a Pt fiber with a composite of MWCNTs and polyaniline (PANI) by electrochemical deposition for the determination of phenolic compounds in lake water samples. For this purpose, a mixture of

MWCNTs–PANI was prepared and a Pt wire (which served as a support for fixing the sorbent) was immersed in the supernatant. Subsequently, an electric current varied between –0.1 and 0.9 V at a scan rate of 50 mV s^{-1} was applied to the electrode. During electrochemical polymerization, the electrode surface was darkening due to the formation of a thin film of sorbent material. To complete the coating process, the resulting fiber was conditioned under a stream of nitrogen to a temperature of 300 ºC for 2 h. The fiber thickness was 40 µm, test by scanning electron microscopy (SEM).

5. Concluding remarks

Analytical Chemistry has benefit from the advances in other scientific areas such as nanotechnology. The discovery, synthesis and characterization of nanoparticles and nanostructured materials with outstanding properties can be identified as one of the most relevant inputs in this context. Among them, carbon nanoparticles (nanotubes, fullerenes and nanocones) have been proved to positively contribute to the development of analytical methods with enhanced properties. Their use as sorbent material in different approaches such as conventional SPE procedures, automated flow configurations, pheudophases in liquid–liquid extraction or SPME fibers, has permitted to improve the sensitivity and selectivity of the underlying methods. The reported research has also been capable of reducing or eliminate the negative aspect related to the aggregation tendency of the carbon nanotubes mainly. Although environmental waters have been the most analyzed matrix, some other applications dealing with the analysis of foods and biological samples allows one to advance the future proposal of new approaches in which the carbon nanoparticles, raw or functionalized, will play a crucial role.

6. References

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Capítulo 3

Evaluation of carbon nanocones/disks as sorbent material for solid–phase extraction

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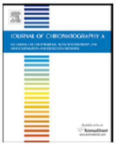

Evaluation of carbon nanocones/disks as sorbent material for solid–phase extraction

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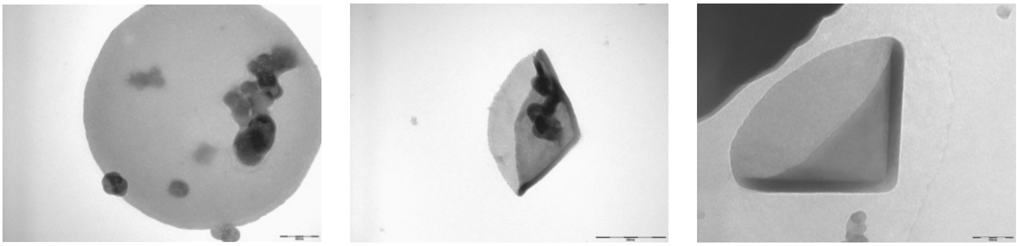
ABSTRACT

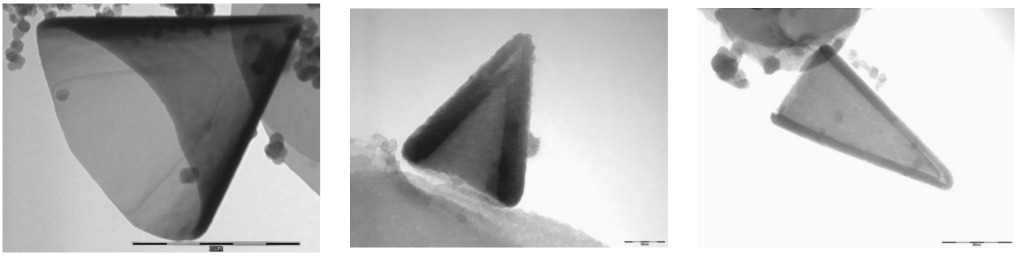
The potential of carbon nanocones/disks as sorbent material in solid–phase extraction (SPE) procedures has been evaluated. For this aim, a model analytical problem, the determination of chlorophenols in water samples, was selected. An accurately weighed amount of 20 mg of purified carbon nanocones/disks was packed in 3 mL commercial SPE cartridges. Once conditioned, up to 8 mL of water samples can be preconcentrated without analyte losses. The chlorophenols were eluted by using 200 µL of hexane. Aliquots of 2 µL of the organic extract were injected in the gas chromatograph–mass spectrometer for separation and quantification. The purification of the commercial nanocones/disks to reduce the presence of amorphous carbon has been successfully achieved by heating the carbon nanocones/disks at 450 ºC for 20 min. Detection limits of chlorophenols were in the range 0.3–8 ng mL^{-1} by using 2 mL of sample. Moreover, excellent average recovery values (98.8–100.9%) have been obtained after the analysis of water samples from different nature. Finally, the performance of the carbon nanocones/disks as sorbent material has been compared with that of multiwalled carbon nanotubes, providing the former better results under the experimental conditions assayed.

1. Introduction

Carbon nanostructures have been one of the hot research topics in last years. Fullerenes, discovered in 1985 [1] were the first new allotropic carbon form and they can be now considered as a well characterized nanostructure which can be purchased with a high purity grade and comparable properties among suppliers. Fullerene and related materials (derivatized or functionalized nanostructures) have been used in analytical chemistry exploiting their sorption properties in both chromatographic and non–chromatographic techniques [2]. Carbon nanotubes were discovered in 1991 by Iijima [3] and belong to the fullerene family of carbon allotropes. They are tubular shaped nanostructures consisting of one (single–walled carbon nanotubes) or more (multiwalled carbon nanotubes) graphene sheets rolled up into a nanoscale tube end–capped with a half of a fullerene molecule. Their outstanding chemical, physical and electric properties have attracted considerable attention of scientists and a wide variety of analytical applications have been developed in the last decade [4]. Carbon nanocones are the fifth allotropic form of carbon synthesized in 1997 [5] although they were first accidentally identified in 1994. The material found after the decomposition of hydrocarbons consisted of 20 wt% carbon nanocones and 70 wt% flat carbon disks [6], being the rest amorphous carbon. Geometrically, they consist of curved graphite sheets with one or more pentagonal rings, defining a conical apex, which is extended by a rolled–up graphene sheet into a larger conical structure [7]. Theoretically, only five discrete pentagonal disclinations can define the tip of the carbon cone (six pentagons yield a nanotube), which results in five predicted cone angles. This fact has been experimentally checked by our research group after the analysis of the commercial carbon nanocones/disks by transmission electronic microscopy and the micrographs obtained are shown in Figure 1, in which the cone angles have been correlated with the presumable number of pentagonal rings at the cone tips. It is worth noting the unique electronic distribution provided by these pentagonal rings to the carbon nanocones, which results in an enhanced local density at the cone apex. In this sense, conical carbon nanostructures are markedly different to carbon nanotubes and fullerenes.



Disk – 0 pentagons 113º – 1 pentagon 84º – 2 pentagons



60º – 3 pentagons 39º – 4 pentagons 19º – 5 pentagons

Fig. 1. Angles formed at the vertex of the carbon nanocones depending on the number of existing pentagons

Few articles can be found in the literature dealing with the practical potential of carbon nanocones [8–17]. Most of them are theoretical works or experimental research aimed at the characterization of the synthesized material. The limited references on the use of this new carbon nanoparticles can also been ascribed to the recent availability of commercial carbon nanoconos sample. Indeed, the product distributed by n–Tec company is mainly composed by disk–shaped particles which are, up to date, impossible to separate from the nanocones [6].

This article presents a novel practical research involving carbon nanocones/disks as analytical tool in solid–phase extraction (SPE). They have been selected on account of the previously described capacity of similar carbon nanostructures, as carbon nanohorns to storage, deliver and adsorb substances [18,19]. The main aim is therefore to evaluate the performance of carbon nanocones/disks in conventional SPE using the determination of chlorophenols in water samples as model analytical problem.

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade or better. Chlorophenols (2–chlorophenol, 2,3–dichlorophenol, 2,4–dichlorophenol, 2,3,5–trichlorophenol, 2,4,5–trichlorophenol and pentachlorophenol) were purchased from Sigma–Aldrich (Madrid, Spain). Carbon nanocones/disks, high grade were supplied by n–Tec (Oslo, Norway). They consist of 20 wt% carbon nanocones, 70 wt% carbon disks and 10 wt% carbon black. The lengths of the nanocones varied between 300 and 800 nm with a maximum base diameter between 1 and 2 µm. The wall thickness was in the range 20–50 nm. The dimensions of the carbon disks were: 0.8–3 µm diameter, 20–50 nm thickness. The solid was thermally stable up to 500 ºC in air, with an apparent density of 0.12 g cm^{-3}. Multiwalled carbon nanotubes (MWNTs) were obtained from Sigma–Aldrich (Madrid, Spain) with a minimum purity of 95%. They were 0.5–200 µm long and the inner diameter was in the interval 5–10 nm.

Hydrochloric acid, sodium hydroxide, anhydrous sodium sulphate, n–hexane and acetone were obtained from Panreac (Barcelona, Spain). Milli–Q ultrapure water (Millipore, Madrid, Spain) was also used. Stock standard solutions of individual analytes were prepared in acetone at a concentration of 1 g L^{-1} and stored at 4 ºC. Working standard solutions were prepared on a daily basis by rigorous dilution of the stock in ultrapure water. Drinking, swimming pool, water–tank and well water samples were collected in amber glass bottles without headspace and stored in the dark at 4 ºC until their analysis.

2.2. Apparatus

Chromatographic analyses were carried out on a gas chromatograph–mass spectrometer (HP6890–HP5973) from Agilent (Palo Alto, CA) equipped with a quadrupole analyzer and a electron multiplier detector. Chlorophenols were separated on a fused silica capillary column HP5MS (30 m × 0.25 mm i.d.) coated with 5% phenyl–95% methylpolysiloxane (film thickness 0.25 µm) (Supelco, Madrid, Spain). The temperature program of the chromatographic oven began at 60 ºC (1 min), ramped to 220 ºC at 10 ºC min^{-1} and held for 2 min. The injection port was maintained al 225 ºC throughout the experiments. A column split ratio of 1:10 was selected for the manual injection of 2 µL using a 5 µL microsyringe (Hamilton Co., Nevada, USA). Helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.4 mL min^{-1}, regulated by a digital pressure and flow controller, was used as the carrier gas. Electron impact ionization (EI) was used with an ionization energy of 70 eV. The transfer line, detector and quadrupole were maintained at 250, 200 and 230 ºC, respectively. Mass spectra were acquired using the full scan mode, within the interval 40–500 amu at 1.36 scan s^{-1}. Total ion current chromatograms were acquired and processes using G1701BA Standlone Data Analysis software (Agilent Technologies) on a Pentium 4 computer which also controlled the whole system.

A Philips CM–10 Transmission microscopy was also used at 10,500–46,000 magnifications to obtain the micrographs of the carbon nanocones/disks treated at different temperatures and times as well as the commercial raw material.

SPE was performed in a VacElut–20 sample–processing station (Scharlab, Barcelona, Spain) equipped with a vacuum control valve and PTFE cartridge adapters (Varian, Barcelona, Spain). For analyte extraction, 20 mg of carbon nanocones/disks previously treated at 450 ºC for 20 min, were placed in 3 mL commercial SPE cartridges using frits to avoid material losses.

2.3. Purification procedure

In order to reduce the presence of amorphous carbon in the solid packed in the cartridges, the commercial carbon nanocones/disks were subjected to the following thermal process: an accurately weighed amount of 70 mg of solid was placed in a ceramic plate and introduced into a furnace kept at 450 ºC for 20 min. Once cooled at room temperature, a carbon nanocones/disks–packed cartridge was prepared from empty commercial cartridges (PTFE 3 mL). Then, 20 mg of the solid, consisting of purified carbon nanocones and disks were packed into the cartridge. 20–µm polypropylene upper and lower frits were maintained in each cartridge to hold the sorbent packing in place. Then the outlet tip of the cartridge was connected to a model VacElut–20 vacuum pump (Scharlab, Barcelona, Spain) equipped with a pressure control valve.

2.4. Analytical procedure

Extractions of aqueous standards or water samples were performed in laboratory–packed cartridges containing 20 mg of thermally treated carbon nanocones/disks, preconditioned with 5 mL of hexane and equilibrated with 5 mL of ultrapure water at pH 5.0. Then, aliquots of 2 mL of standard or samples, previously adjusted to pH 5.0 were passed through the cartridge at a flow rate of 1 mL min^{-1}. A washing step using 2 mL of ultrapure water at pH 5.0 was included and then, the sorbent was dried. Quantitative elution of the analytes was accomplished with 200 µL of hexane a flow rate of 1 mL min^{-1}. The eluent was collected in an eppendorf vial containing anhydrous sodium sulphate to prevent water from reaching the chromatographic column. Finally, 2 µL of the organic solvent was injected into the gas chromatograph for analysis. Between extractions, the cartridge was sequentially washed with 3 mL of hexane and 3 mL of ultrapure water, pH 5.0. In this way, the cartridge can be reused for ca. 50 analyses.

3. Results and discussion

Solid–phase extraction is widely used for the enrichment and isolation of a variety of organic pollutants from samples of environmental concern. Its favourable features regarding the sample and eluent volumes, which provide very high preconcentration factors, expeditiousness and compatibility with chromatographic separation techniques are in the grounds of its acceptance by analytical laboratories. The use of newly synthesized solid materials as sorbents in SPE is a clear trend in this area, all of them promising better selectivity and efficiency towards the target analytes. Carbon nanocones are a relativity novel nanostructured carbon material whose physical and chemical properties would made them appropriate for use in SPE.

3.1. Thermal stability of the commercial carbon nanocones/disks

The carbon nanocones/disks used were of the highest purity commercially available. The powder consists of a mixture of carbon nanocones (20 wt%), carbon disks (70 wt%) and amorphous carbon (10 wt%). Taking into account the irreversible interactions that can be produced between the amorphous material and the analytes, the first experiments were aimed at reducing the percentage of this fraction in the solid. Moreover, the isolation of the nanocones from the disks was also attempted.

The supplier guarantees the stability of the commercial product up to 500 ºC. Therefore, the influence of the temperature on the solid was evaluated. For this purpose, aliquots of ca. 70 mg of the commercial product were accurately weighed and placed into a ceramic plate distributed in a homogeneous thick film, which facilitated a homogeneous heating of the sorbent. Once prepared, the plate was introduced in a furnace and heated at different temperatures (between 350 and 800 ºC) for variable times (0–90 min).

Figure 2 represents the weight losses with the time at the different temperatures assayed. In addition, micrographs of the thermally treated solid were made in order to evaluate the potential degradation of the carbon nanocones and disks (see Figure 3).

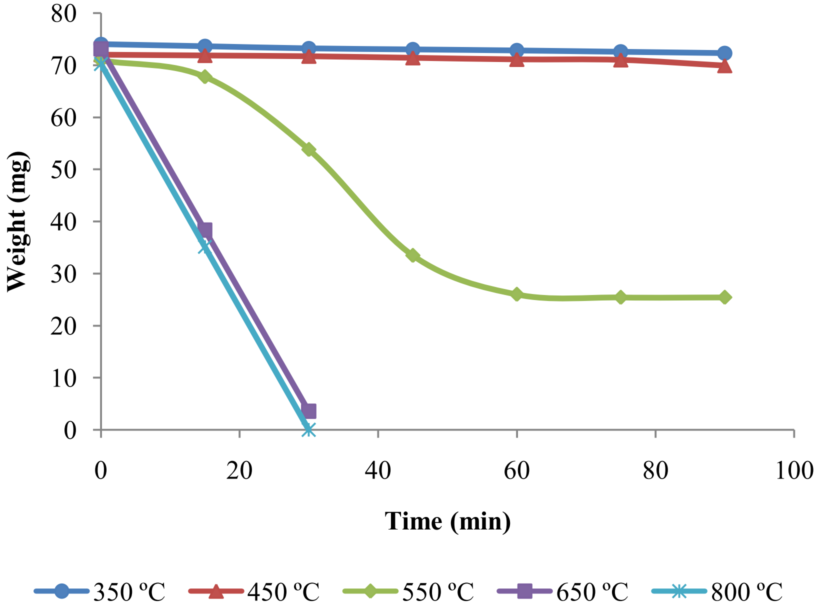
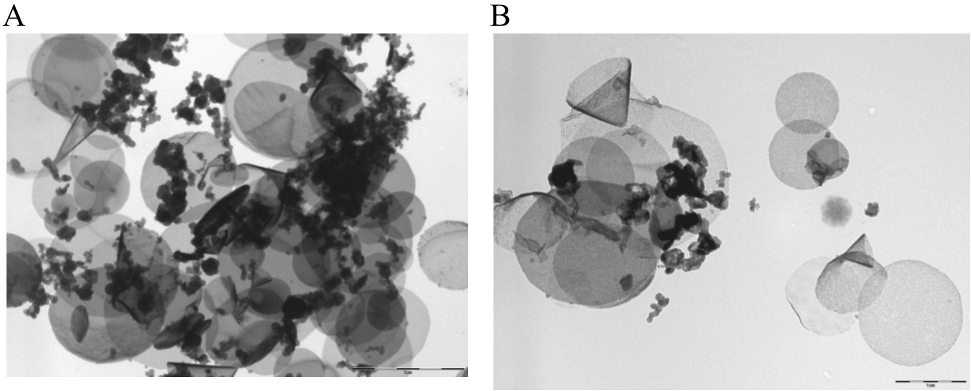


Fig. 2. Weight losses of the carbon nanocones/disks with the time at the different temperatures assayed.

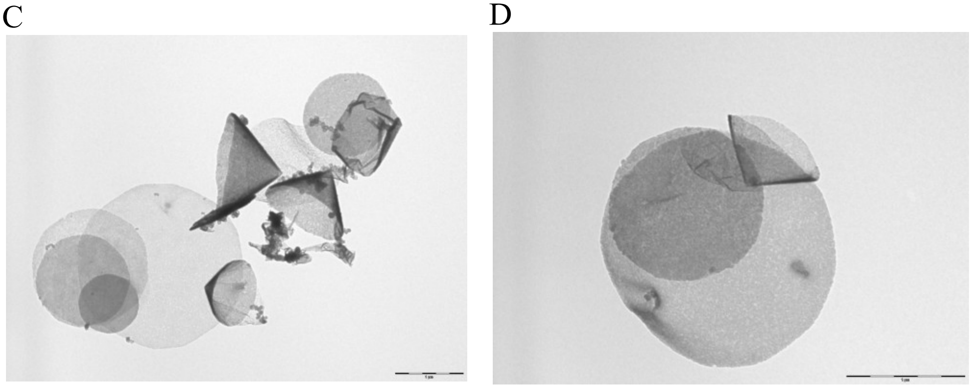
As it can be seen, at temperatures lower than 550 ºC ca. 10% of the solid was lost after 90 min of treatment, which corresponds mainly to the amorphous carbon, as it was corroborated by the TEM (transmission electron microscopy) images (see Figures 3A and B). On the other hand, values higher than 550 ºC produced a complete loss of the solid in ca. 30 min. The moderate weight reduction observed at 550 ºC permitted a more detailed evaluation of the modifications suffered by the nanostructured carbon material.

From this study, it was concluded that the presence of amorphous carbon was reduced with the time, although it was not possible to remove it completely. In addition, the weight losses cannot be specifically attributed to the nanocones or the disks as both nanostructures were observed in the TEM micrographs (see Figures 3C and D). From the microscopic analysis it was also concluded that both, the nanocones and the disks were damaged by the temperature in the border (disks) and the base (nanocones).



Commercial carbon cones (x10500)

Carbon cones treated at 450 ºC for 20 min (x13500)



Carbon cones treated at 450 ºC for 45 min (x13500)

Carbon cones treated at 550 ºC for 75 min (x25000)

Fig. 3. Micrographs obtained for the carbon nanocones/disks after different heating processes. (A) Commercial carbon nanocones/disks (x10,500); (B) carbon nanocones/disks treated at 450 ºC for 20 min (x13,500); (C) carbon nanocones/disks treated at 450 ºC for 45 min (x13,500); and (D) carbon nanocones/disks treated at 550 ºC for 75 min (x25,000).

It has been described that the surface of conical nanostructured carbon materials can be degraded by the effect of the light, microwave energy or heating [20–22]. These superficial defects can generate preferential sites of reaction. In order to evaluate the influence of the above described thermal treatment on the sorbent capacity of the carbon nanocones/disks, four individual cartridges packed with 20 mg of carbon nanocones/disks subjected to different thermal treatment were prepared, namely: (a) raw material; (b) heated at 450 ºC for 20 min; (c) heated at 450 ºC for 45 min; and (d) heated at 550 ºC for 45 min.

In all cases, 2 mL of an aqueous standard solution containing the six chlorophenols at a concentration of 1 µg mL^{-1} were passed through each cartridge, previously conditioned as described in Section 2.4. Aliquots of 2 mL of methanol were used as eluent and 2 µL of the organic extract were injected into the GC–MS for analysis. As no analytes preconcentration takes place, the signals obtained in each case can be compared with those provided by a direct injection of a methanolic standard of the six chlorophenols (1 µg mL^{-1}) into the GC–MS. In this way, the sorbent performance of the different solids packed can be evaluated. In all cases, the chromatographic separation was carried out under the conditions described in Section 2.2. From this study, it was concluded that the worst results in terms of analyte recovery were obtained with the cartridge packed with the raw material (ca. 30% lower), which can be ascribed to the irreversible interactions that were established between the analytes and the amorphous carbon particles. When the carbon nanocones/disks were heated at 450 ºC, a marked improvement of the peak areas were observed with negligible influence of the time. It can be explained not only by the lost of amorphous particles but also by a possible restructuring of the material by annealing. Therefore, heating the sorbent at 450 ºC for 20 min selected as optimum treatment prior to solid package in the commercial cartridge. It should be noted that higher temperatures produced important losses of the solid without improving the sorbent capacity.

3.2. Optimization of the solid–phase extraction procedure

The variables studied in the SPE procedure using heated carbon nanocones/disks included the amount of sorbent, type and volume of eluent, as well as sample pH and volume. All of them were evaluated by using an aqueous standard solution containing the six chlorophenols at a final concentration of 1 μg mL^{-1}. 2 μL of the final organic extract were directly injected into the GC–MS. Several cartridges were packed with variable amounts of carbon nanocones/disks (between 5 and 20 mg). In all cases, 2 mL of the aqueous standard solution were preconcentrated and the analytes eluted with 2 mL of methanol. The peak areas for the chlorophenols assayed increased when increasing the amount of sorbent, being quantitative for 20 mg (e.g. the peak areas were similar to those provided by the direct injection of the methanolic standard prepared at the same concentration). Therefore, it was selected as optimum to study the type and volume of eluent. Three different organics solvents were evaluated for this aim, namely: methanol, hexane and ethyl acetate. However, only methanol and hexane could be quantitatively evaluated as the ethyl acetate produced sorbent losses as the likely result of a partial solubilization of the solid. The recoveries obtained with hexane were ca. 30% higher than those provided by methanol, being the former selected as eluent. The volume of hexane was studied within the interval 0.1–1 mL and it was concluded that quantitative elution was obtained with 200 μL, as a sequential elution with an equal volume of hexane revealed the absence of chlorophenols. The sample pH can affect the retention of analytes on a given sorbent and therefore, this variable was investigated in the range 2–9 by adding appropriate small volumes of dilute hydrochloric acid or sodium hydroxide solutions to the aqueous standards. The results obtained are shown in Figure 4. As it can be seen, the best results were obtained at pH 7, being negligible the influence of this variable for 2,4,5–trichlorophenol and pentachlorophenol within the interval 2–7. For all the analytes studied, the retention was worse under alkaline conditions. In order to facilitate the sample processing, pH 5 was selected as working conditions at the expense of a lower sensibility in some cases. This compromise avoids the use of a buffer solution for the adjustment and thus, sample dilution.

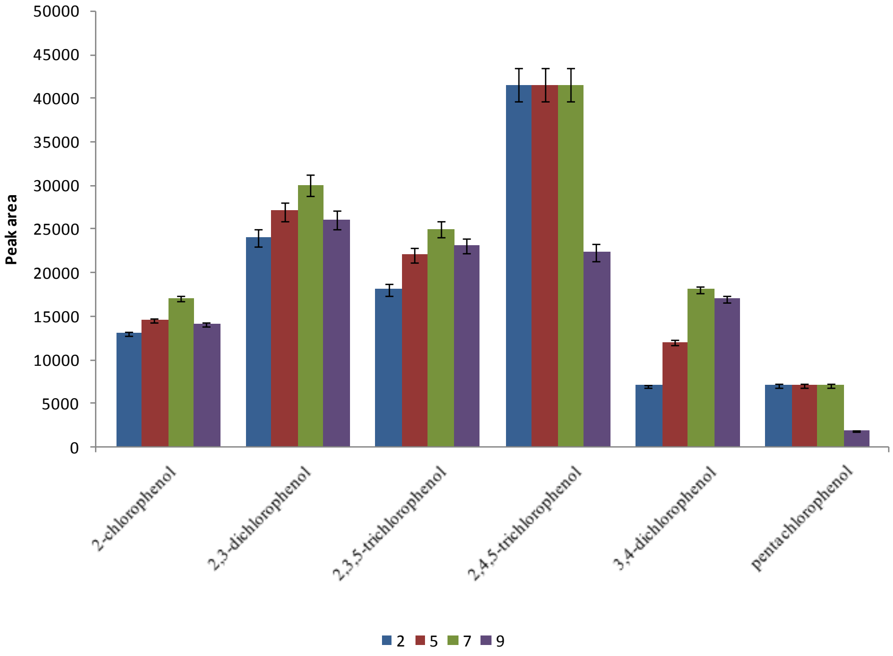


Fig. 4. Influence of the sample pH on the retention of the six chlorophenols selected on the carbon nanocones/disks cartridges. Each aqueous standard was analyzed by triplicate.

The sample volume was optimized within the interval 2–50 mL of aqueous standards containing in all cases 2 μg of each chlorophenol and adjusted to pH 5.0. It was observed that volumes higher than 10 mL produced a decrease in the peak areas of ca. 10%, and therefore, it was fixed as the breakthrough volume.

Finally, the effect of the dimensions (viz. volume) of the cartridge used for sorbent package on the SPE process was evaluated. We compared the results obtained for the six chlorophenols when 20 mg of the thermally treated sorbent were placed in a cartridge of smaller dimensions (1 mL).

The values of peak area for all the compounds were found to be ca. one–third of those obtained with the cartridge of 3 mL. To test whether this was a problem of worse retention or lower elution, sequential elutions with 200 μL of hexane were implemented and the organic fractions were then injected in the gas chromatograph. It was verified that up to 600 μL of eluent were needed to achieve quantitative elution of the analytes. Moreover, we have studied the influence of the volume of a single elution in the 1 mL cartridge within the range 200–800 μL. It was observed that 500 μL were necessary to obtain quantitative elution. It can be attributed to the higher length of the packed material in this cartridge. Since these results lead to a dramatic reduction in the preconcentration factor, the 3 mL cartridges were selected as optimum.

3.3. Comparison with multiwalled carbon nanotubes (MWNTs)

Finally, the performance of multiwalled carbon nanotubes for the concentration of the six chlorophenols selected was evaluated. For this purpose, 20 mg of MWNTs were packed in a 3 mL SPE cartridge and the previously optimized procedure was followed. The results obtained are plotted in Figure 5 versus those provided by the carbon nanocones/disks cartridge. As it can be seen, the peak areas obtained after the injection of 2 µL of the eluent (200 µL of hexane) were ca. 30% of those provided by the carbon nanocones/disks cartridge. The sequential elution with identical volumes of eluent revealed that it was necessary the use of up to 600 µL of hexane to achieve the quantitative elution of the target pollutants from the MWNTs. It results in lower preconcentration factors and subsequent higher detection limits. Cai et al. used MWNTs for the isolation and preconcentration of various chlorophenols in water samples [23]. The optimized method required the use of a cartridge packed with 300 mg of MWNTs and 6 mL methanol (pH 10) for elution, with average recoveries of ca. 85%. These results corroborate the highest volume required for the elution of the analytes from the MWNTs cartridge.

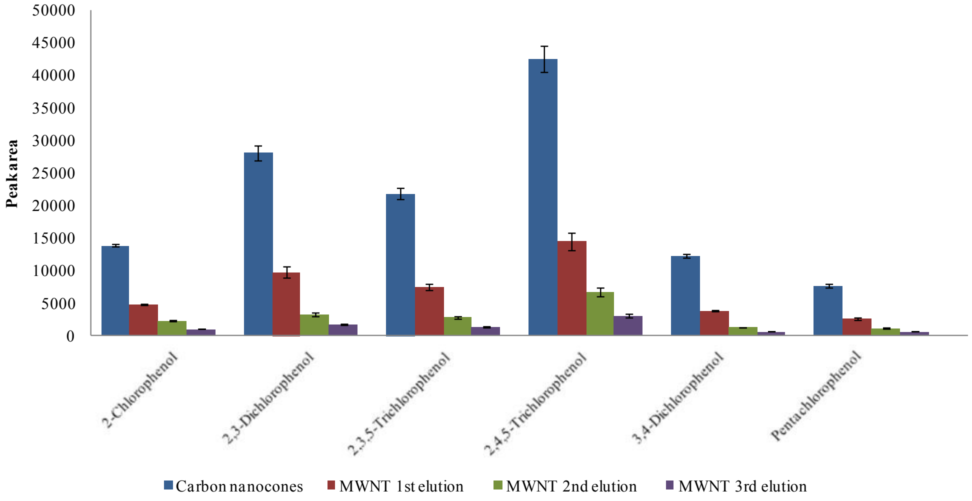


Fig. 5. Comparison of the performance of carbon nanocones/disks and MWNTs (20 mg packed in 3 mL SPE cartridges) for the preconcentration of the six chlorophenols. Each aqueous standard was analyzed by triplicate.

3.4. Analytical figures of merit

Table 1 presents the figures of merit of the proposed method for the chlorophenols selected. Calibration graphs were constructed by analyzing aqueous standards (pH 5.0) containing the analytes at 10 concentration levels in the range 1–1000 ng mL^{-1}. The standards were processed following the optimized SPE procedure and 2 μL of the final organic extract were injected into the GC–MS for analysis. The corresponding equations were obtained by plotting the peak area against the concentration for each chlorophenol. Good linear relationships were observed in all cases ($R > 0.990$). The detection limits were calculated as the concentration providing an analytical signal three times higher than the background noise. The detection limits were in the range 0.3–8 ng mL^{-1} and the quantitation limits varied between 1 and 25 ng mL^{-1}. The precision of the method, expressed as relative standard deviation was calculated under two experimental conditions, namely: (a) using a single cartridge and preconcentrating five individual standard solutions containing the chlorophenols at a concentration of 100 ng mL^{-1} ($n = 5$) and (b) using three independent cartridges packed with the sorbent individually heated and passing three different aliquots of the standard solution at the same concentration ($n = 9$). The relative standard deviation (RSD) average values are also listed in Table 1 and were lower than 6% in all instances.

Table 1. Figures of merit of the proposed method.

	t_R (min)	R	Regression equations	LODs (ng mL^{-1})	Linear range (ng mL^{-1})	RSD (%)	
						1 cartridge (n=5)	3 cartridges (n=9)
2–chlorophenol	5.61	0.997	$y = 121.8\ x + 170.3$	0.3	1 – 1000	1.6	1.7
2,3–dichlorophenol	8.41	0.998	$y = 262.1\ x - 114.2$	0.3	1 – 1000	4.1	5.5
2,3,5–trichlorophenol	10.65	0.992	$y = 269.2\ x + 277.1$	0.3	1 – 1000	3.6	4.3
2,4,5–trichlorophenol	11.01	0.997	$y = 269.2\ x + 102.2$	0.3	1 – 1000	4.6	5.7
3,4–dichlorophenol	11.69	0.990	$y = 32.7x + 92.9$	8	25 – 1000	2.3	4.6
pentachlorophenol	15.85	0.994	$y = 34.8\ x - 39.4$	8	25 – 1000	3.0	6.0

[a] y = peak area and x = concentration in ng mL^{-1}.

3.5. Analysis of water samples

The proposed method was applied to the determination of the six chlorophenols in different water matrices such as drinking, swimming pool, well and tank water collected from different locations. Aliquots of 25 mL of each sample were filtered through a 0.45 µm Nylon filters and adjusted to pH 5.0 prior to the SPE procedure. None of the samples provided positive results for the selected pollutants. In order to confirm that the samples were negative as regards the presence of chlorophenols, the volume of preconcentrated water samples was increased up to 8 mL (breakthrough volume, 10 mL). No signal of any of the six chlorophenols evaluated was found at their characteristics retention times. Therefore, a fortification procedure was used to evaluate the applicability of the method developed. Thus, aliquots of 50 mL of the four water samples were spiked with the chlorophenols at three different concentration levels (10, 100 and 250 ng mL^{-1}).

The spiked samples were stored in the dark for three days, then filtered, adjusted to pH 5.0 and individual aliquots of 2 mL were analyzed (n = 3). The results obtained are listed in Tables 2–5. The average recovery for each matrix ranged from 98.8% (n = 16, tap water) and 100.9% (n = 16, well water). By way of example, Figure 6 shows a typical chromatogram obtained after the analysis of a tap water sample spiked with the six analytes at a concentration of 250 ng mL^{-1}. From this results, it can be followed the suitability of the carbon nanocones/disks evaluated as sorbent for the determination of the selected chlorophenols in waters without interference of endogenous matrix compounds.

Table 2. Recovery values obtained for the six chlorophenols spiked to the drinking water ($n = 3$).

Analyte	Concentration added (ng mL^{-1})	Concentration found ± SD (ng mL^{-1})	Recovery (%)
2–chlorophenol	10	9.4 ± 0.2	94 ± 2
	100	104 ± 2	104 ± 2
	250	254 ± 5	102 ± 2
2,3–dichlorophenol	10	9.9 ± 0.4	99 ± 4
	100	104 ± 4	104 ± 4
	250	254 ± 9	102 ± 4
2,3,5–trichlorophenol	10	9.4 ± 0.4	94 ± 4
	100	101 ± 4	101 ± 4
	250	255 ± 9	102 ± 4
2,4,5–trichlorophenol	10	7.1 ± 0.3	71 ± 3
	100	98 ± 5	98 ± 5
	250	248 ± 12	99 ± 5
3,4–dichlorophenol	100	102 ± 3	102 ± 3
	250	260 ± 6	104 ± 2
pentachlorophenol	100	103 ± 3	103 ± 3
	250	257 ± 8	103 ± 3

SD = standard deviation

Table 3. Recovery values obtained for the six chlorophenols spiked to the swimming pool water (*n* = 3).

Analyte	Concentration added (ng mL^{-1})	Concentration found ± SD (ng mL^{-1})	Recovery (%)
2–chlorophenol	10	10 ± 0.2	100 ± 2
	100	97 ± 2	97 ± 2
	250	248 ± 5	99 ± 2
2,3–dichlorophenol	10	10.3 ± 0.5	103 ± 5
	100	94 ± 4	94 ± 4
	250	247 ± 9	99 ± 4
2,3,5–trichlorophenol	10	10.3 ± 0.4	103 ± 4
	100	103 ± 4	103 ± 4
	250	248 ± 9	99 ± 4
2,4,5–trichlorophenol	10	9 ± 0.4	90 ± 4
	100	101 ± 6	101 ± 6
	250	253 ± 12	101 ± 5
3,4–dichlorophenol	100	104 ± 3	104 ± 3
	250	245 ± 8	98 ± 3
pentachlorophenol	100	98 ± 3	98 ± 3
	250	252 ± 8	101 ± 3

SD = standard deviation.

Table 4. Recovery values obtained for the six chlorophenols spiked to the tank water ($n = 3$).

Analyte	**Concentration added ($ng\ mL^{-1}$)**	**Concentration found ± SD ($ng\ mL^{-1}$)**	**Recovery (%)**
2–chlorophenol	10	10.4 ± 0.2	104 ± 2
	100	104 ± 3	104 ± 3
	250	240 ± 5	96 ± 2
2,3–dichlorophenol	10	9.8 ± 0.5	98 ± 5
	100	104 ± 5	104 ± 5
	250	253 ± 9	101 ± 4
2,3,5–trichlorophenol	10	10.1 ± 0.4	101 ± 4
	100	97 ± 4	97 ± 4
	250	250 ± 9	100 ± 4
2,4,5–trichlorophenol	10	9.9 ± 0.4	99 ± 4
	100	104 ± 6	104 ± 6
	250	248 ± 12	99 ± 5
3,4–dichlorophenol	100	104 ± 3	104 ± 3
	250	245 ± 8	98 ± 3
pentachlorophenol	100	104 ± 3	104 ± 3
	250	245 ± 8	98 ± 3

SD = standard deviation.

Table 5. Recovery values obtained for the six chlorophenols spiked to the well water ($n = 3$).

Analyte	**Concentration added (ng mL^{-1})**	**Concentration found ± SD (ng mL^{-1})**	**Recovery (%)**
2–chlorophenol	10	9.9 ± 0.2	99 ± 2
	100	100 ± 2	100 ± 2
	250	248 ± 5	99 ± 2
2,3–dichlorophenol	10	10.6 ± 0.6	106 ± 6
	100	96 ± 5	96 ± 5
	250	249 ± 9	100 ± 4
2,3,5–trichlorophenol	10	9.8 ± 0.4	98 ± 4
	100	99 ± 4	99 ± 4
	250	257 ± 9	103 ± 4
2,4,5–trichlorophenol	10	10.4 ± 0.4	104 ± 4
	100	104 ± 6	104 ± 6
	250	251 ± 12	100 ± 5
3,4–dichlorophenol	100	101 ± 3	101 ± 3
	250	255 ± 8	102 ± 3
pentachlorophenol	100	103 ± 3	103 ± 3
	250	252 ± 8	101 ± 3

SD = standard deviation.

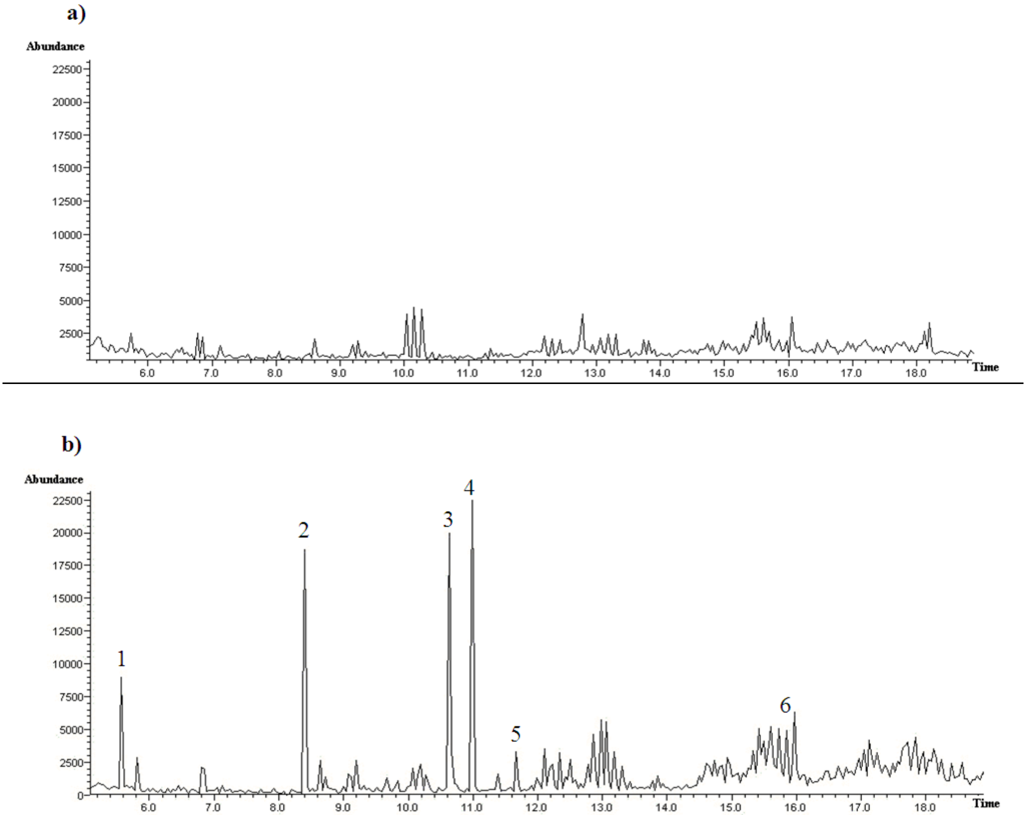


Fig. 6. Typical chromatogram obtained from the analysis of: (A) unspiked tap water sample and (B) tap water sample spiked with the six chlorophenols at the concentration of 250 ng mL^{-1}. Peak: (1) 2–chlorophenol; (2) 2,3–dichlorophenol; (3) 2,3,5–trichlorophenol; (4) 2,4,5–trichlorophenol; (5) 3,4–dichlorophenol; and (6) pentachlorophenol.

4. Conclusions

In this article, the sorbent capacity of a new nanostructured carbon material has been evaluated. By the impossibility of separating the two main components of the commercial product, the combined potential of carbon nanocones and disks has been studied. The percentage of amorphous material has been reduced by thermal treatment of the commercial product by heating the raw material at 450 ºC for 20 min, achieving a marked increase of the sorbent properties in terms of reversibility of the interaction analyte–sorbent. The results obtained for chlorophenols demonstrate the suitability of carbon nanocones/disks to carry out the determination of these pollutants in waters from different sources. It is also noteworthy the excellent values of reproducibility achieved (with and between cartridges) considering the whole procedure, including the thermal treatment of the solid. In comparison with carbon nanotubes, this material presents as advantages: lower price (23 $/g), better characterization of the final product which facilitates the method transfer, and better sorbent features when used in commercial cartridges. The sensitivity of the method can be enhanced by preconcentrating 8 mL of sample and including an evaporation of the eluent and redissolution of the residue in 25–50 μL of hexane.

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Capítulo 4

Carbon nanocones/disks as new coating for solid–phase microextraction

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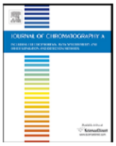

Carbon nanocones/disks as new coating for solid–phase microextraction

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ABSTRACT

In this article, the potential of carbon nanocones/disks as coating for solid–phase microextraction has been evaluated for the first time. The nanostructures were immobilized on a stainless steel needle by means of an organic binder. The fiber coating obtained was ca. 50 µm of thickness and 35 mm in length. The evaluation of the sorbent capacity was carried out through the determination of toluene, ethylbenzene, xylene isomers and styrene in water samples following the headspace sampling modality (15 min, 30 ºC). The fiber was then transferred to a 10 mL vial which was sealed and heated at 110 ºC for 15 min in the headspace module of the instrument to achieve the thermal desorption of the analytes. Then 2.5 mL of the headspace generated were injected in the gas chromatograph–mass spectrometer for analytes separation and quantitation. The detection and quantitation limits obtained for 10 mL of sample were 0.15 and 0.5 ng mL^{-1} (0.6 and 2 ng mL^{-1} for toluene). The optimized procedure was applied to the determination of the selected volatile compounds in waters collected from different locations. The recovery values obtained (average recovery ca. 92%) demonstrated the usefulness of the carbon nanocones/disks as sorbent material in solid–phase microextraction.

1. Introduction

The contamination of environmental compartments such as air, waters, soils and sediments is one of the major current concerns, due to their negative impacts on humans and other living organisms. Among these pollutants, volatile organic compounds (VOCs) have been identified as hazardous substances by the United States Environmental Protection Agency (US–EPA) [1]. Concretely, aromatic hydrocarbons constitute a series of compounds that are emitted into the atmosphere, waters and soils through oil and fuel spills, evaporation, combustion emissions from vehicles, industries as well as industrial and geochemical processes [2–4].

Regardless the instrumental technique used for analytes determination, a preliminary step of extraction and preconcentration of the target compounds is required. For this purpose, a variety of alternatives are available, which include solid–phase extraction (SPE) and liquid–liquid extraction (LLE) [5,6]. However, these techniques have some drawbacks such as the use of large volumes of organic solvents (particularly for LLE), some of which may be toxic and carcinogens. Moreover, they are time consuming and are prone to errors due to the high number of manual manipulations involved. For these reasons, several novel extraction techniques have been recently developed based on single–drop microextraction [7,8], and solid–phase microextraction [9–11], for the analysis of volatile compounds. Among of all these techniques, SPME is gaining acceptance because it combines extraction and preconcentration in a single step, being a solventless technique. SPME was introduced in 1990 by Arthur and Pawliszyn [12] and since then, it has been widely used for the analysis of pollutants, including aromatic hydrocarbons in waters [13]. The fiber coating is extremely important to achieve adequate sensitivity and selectivity, and it should be selected according to target analytes. A wide variety of coatings with different physico–chemical properties are commercially available. Among them, polydimethylsiloxane (PDMS), polyacrylate (PA), carbowax (CAR), divinilbenzene (DVB) and polyethylene glycol (PEG), are the most referred in the literature.

The extraordinary properties of the nanostructured materials have boosted the development of new analytical tools which exploit such outstanding characteristics. Among them, the enhanced sorbent capacity of the nanomaterials in comparison with commercial sorbents, has been used for the development of more efficient analytical processes in which the sorbent is presented in different configurations. In fact, the excellent performance of carbon nanostructures as sorbent material for SPME fibers has been recently demonstrated. It can be attributed to their enhanced sorbent capacity for analytes in different matrices [14]. Single–walled carbon nanotubes (SWNTs) [15–18], multi–walled carbon nanotubes (MWNTs) [19–23] and hydroxyfullerenes [24] have been used as coating for the extraction of different analytes such as phenols, polybrominated diphenyl ethers and polycyclic aromatic hydrocarbons in wastewaters, human urine and milk.

In this article, the usefulness of carbon nanocones/disks as coating in SPME has been evaluated. The nanostructured material was thermally treated in order to enhance its sorbent properties [25]. All the steps involved in fiber preparation have been deeply studied and the final design was evaluated using the determination of volatile organic compounds in waters as model analytical problem.

2. Experimental

2.1. Reagent and samples

All reagents were of analytical grade or better. Toluene, ethylbenzene, o–, m– and p–xylene (individual, pure compounds) and styrene were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solutions of individual analytes were prepared in methanol at a concentration of 1 g L^{-1} and stored at 4 ºC. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in ultrapure water.

Carbon nanocones/disks high grade, were supplied by n–Tec (Oslo, Norway). This solid material is composed of 20 wt% carbon nanocones, 70 wt% carbon disks and 10 wt% amorphous carbon black. The carbon nanocones are characterized by a length between 300 and 800 nm, and a maximum base diameter between 1 and 2 μm. Moreover, the thickness of the wall is in the range of 20–50 nm. The apparent density was 0.12 g cm^{-3} and the thermal stability of carbon nanocones/disks was up to 500 ºC in air. Carbon nanocones/disks were thermally treated at 450 ºC for 20 min in order to reduce the presence of amorphous carbon and thus minimize irreversible interaction with the analytes [25].

Terpineol, dibutyl phthalate, ethyl cellulose and 1,2–dichloroethane were supplied by Sigma–Aldrich (Madrid, Spain) and they were used for the preparation of the coating that was fixed on a removable stainless steel needle purchased from Hamilton (Bonaduz, Switzerland), with 0.36 mm o.d. (gauge 28) and cut to obtain a length of 40 mm. Sodium chloride, methanol (Panreac, Barcelona, Spain) and Milli–Q ultrapure water (Millipore, Madrid, Spain) were also used.

Drinking, well and river water samples were locally obtained and collected in amber glass bottles without headspace and stored in the dark at 4 ºC until analysis.

2.2. Apparatus

Analyses were carried out with a MPS2 headspace autosampler fitted with a 2.5 mL gastight HS–syringe (Gerstel, Mülhein an der Ruhr, Germany) including a robotic arm and an oven for sample heating/headspace generation, coupled to a gas chromatograph mass spectrometer (HP6890–HP5973) from Agilent (Palo Alto, CA, USA) equipped with a quadrupole analyzer and an electron multiplier detector. The target analytes were separated on a fused silica capillary column HP–5MS (30 m × 0.25 mm i.d.) coated with 5% phenyl–95% methylpolysiloxane (film thickness 0.25 μm)(Supelco, Madrid, Spain). The temperature program of the chromatographic oven began at 40 ºC (3min), ramped to 60 ºC at 5 ºC min^{-1}, then increased up to 280 ºC at 20 ºC min^{-1} and held for 2 min. The injection port was maintained at 225 ºC throughout the experiments. Helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.0 mL min^{-1}, regulated by a digital pressure and flow controller, was used as the carrier gas. Electron impact ionization (EI) was used with an ionization energy of 70 eV. The transfer line, detector and quadrupole were maintained at 250, 200 and 230 ºC, respectively. Mass spectra were acquired using the full scan mode, within the interval 50–200 amu at 1.276 scan s^{-1}. Total ion current chromatograms were acquired and processed using G1701BA Standalone Data Analysis software (Agilent Technologies) on a Pentium 4 computer which also controlled the whole system.

A JEOL JSM 6300 scanning electron microscopy (Isaza, Alcobendas, Spain) was also used to obtain the micrographs of the carbon nanocones/disks coated on the stainless steel needle.

2.3. Preparation of the carbon nanocones/disks fiber

The SPME carbon nanocones/disks fiber was prepared as follows: firstly, 500 mg of thermally treated carbon nanocones/disks were dispersed in 25 mL of 1,2–dichloroethane in an ultrasonic bath for 30 min. Then, 2.7 g of terpineol, 0.15 g of ethyl cellulose and 0.15 g of dibutyl phthalate were added as organic binder, being ultrasonicated for 30 min. The mixture was further heated at 90 ºC under continuous stirring to evaporate the 1,2–dichloroethane, a viscous sticky paste being obtained. The stainless steel needle was vertically dipped (3.5 cm) for 30 s into the resulting paste of carbon nanocones/disks. The coated stainless steel needle was dried in the vertical position with a hot air stream for 15 min. This procedure was repeated two times. Then, the fiber was sintered in the injection port of a gas chromatograph for 6 h at 200 ºC under a nitrogen stream.

2.4. Analytical procedure

The extraction procedure consists of three different steps: (a) headspace solid–phase microextraction of the analytes from the water samples; (b) thermal desorption of the extracted analytes from the fiber in the headspace module of the gas chromatograph and, (c) gas chromatographic separation and mass spectrometric detection of the target analytes. The whole procedure is depicted in Figure 1.

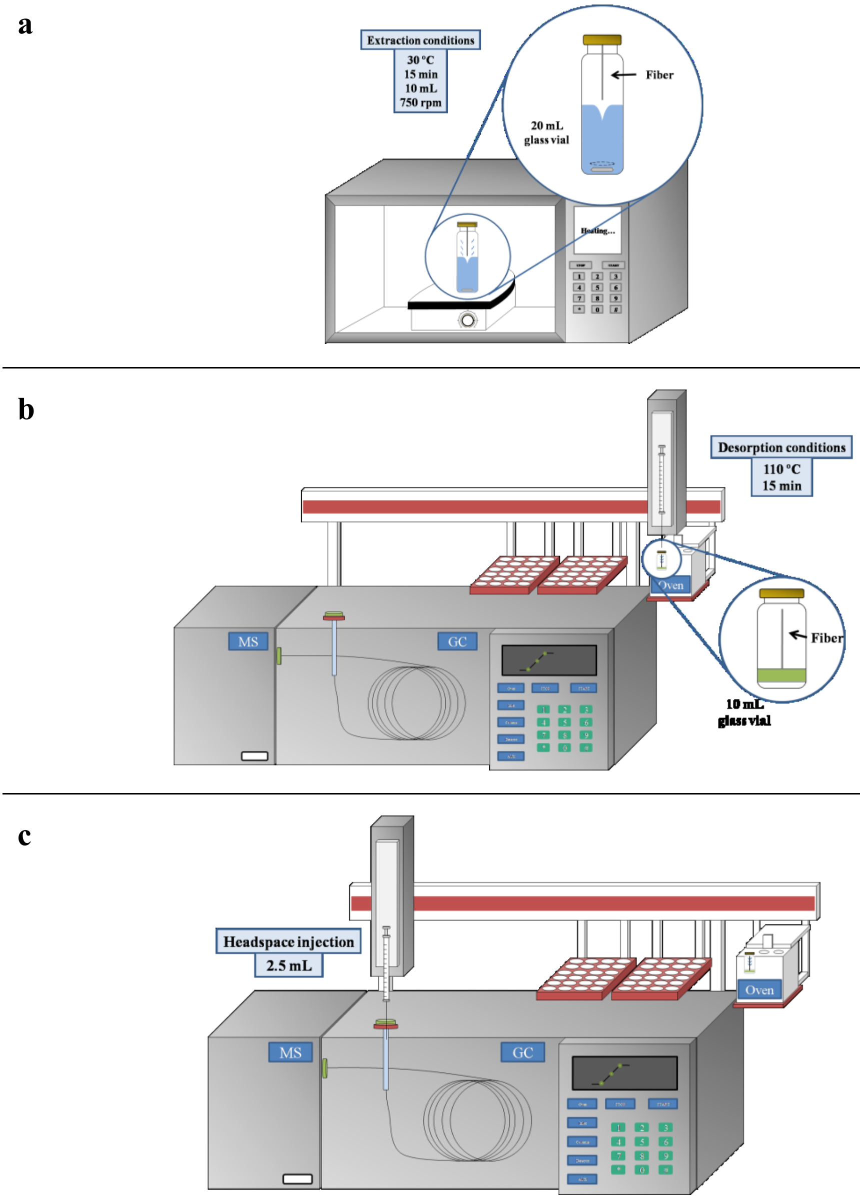

Fig. 1. Schematic representation of the extraction process of monoaromatic hydrocarbons in water: a) headspace solid–phase microextraction of the analytes from the water samples; b) thermal desorption of the extracted analytes in the headspace module of the gas chromatograph and, c) automated injection of 2.5 mL of the headspace in the GC/MS for analytes separation and detection.

Aliquots of 10 mL of water samples or standard solutions containing the six volatile organic compounds were placed in a 20 mL glass vial containing 3.0 g of sodium chloride and fitted with a removable aluminum cap provided with a silicone septum in which the SPME fiber was previously inserted. Once sealed, the fiber was exposed to the headspace of the sample for 15 min at 30 ºC. The sample/standard was magnetically stirred over this period. Then, the SPME fiber containing the extracted analytes was transferred to a 10 mL glass vial which was tightly sealed with a silicone septum for the subsequent thermal desorption (110 ºC, 15 min) of target analytes. Finally, 2.5 mL of the gas homogenized phase of the vial enriched with the six compounds was injected into the gas chromatograph–mass spectrometer for qualitative and quantitative determination.

Between extractions, the fiber was heated at 200 ºC for 5 min under a nitrogen stream in order to remove any traces of the analytes or other impurities that could interfere in subsequent extractions.

2.5. Safety considerations

The organic solvents and analytes used in this work are relatively volatile and slightly toxic and they have been handled using protective gloves and face mask. All the wastes were collected in special bottles for a proper management. No references on the toxicity of carbon nanocones have been described up to date. Notwithstanding this, they were handled under the above described safety considerations.

3. Results and discussion

For the evaluation of the potential of carbon nanocones/disks as active components in SPME coating, the determination of volatile organic compounds in water samples was selected as model analytical problem. According to the literature, these compounds can be determined using the headspace sampling approach. Moreover, thermal desorption of retained analytes can also been accomplished which helps to extend the reusability of the SPME fiber while diminishes coating damage.

3.1. Variables affecting the carbon nanocones/disks coating.

From the existing alternatives described in the literature, the use of organic binder was chosen on the basis of the lower temperature required (400 ºC for carbon nanotubes) which will preserve the integrity of the carbon nanostructured material used in this work. It should be noted that the carbon nanocones were thermally treated to obtain the highest sorption efficiency [25]. The procedure described by Lü et al. for the preparation of a SWCNTs fiber coating [16] was selected and all the variables affecting the synthesis of the sorbent layer (amounts of carbon nanocones/disks, numbers of coating layers and sintering conditions) were optimized for carbon nanocones/disks. The initial (non–optimized) extraction conditions are listed in Table 1. Once extracted, the fiber was transferred to a headspace vial which was further located in the HS module of the GC–MS for the thermal desorption of the analytes in order to preserve the fiber integrity during the optimization process.

Table 1. Variables studied in the HS–SPME procedure

Variable	Initial value	Interval studied	Optimum value
Sample volume (mL)	5	1 – 10	10
Stirring rate (rpm)	500	0 – 1000	750
Extraction temperature (ºC)	30	30 – 60	30
Extraction time (min)	30	10 – 30	15
NaCl Concentration (g L^{-1})	0	0 – 375	300
Desorption temperature (ºC)	90	80 – 120	110
Desorption time (min)	30	10 – 45	15

The amount of carbon nanocones/disks should affect the analytical signal as they are the active element in the sorption process. Therefore, this variable was studied between 50 and 750 mg in 25 mL of 1,2–dichloroethane. Their low aggregation tendency facilitates their dispersion/solubilization in the organic medium. As it can be seen in Figure 2, the analytical signal for all the compounds increased when increasing the amount of carbon nanocones/disks up to 500 mg with a slight decrease over this value, toluene excepted. Therefore, 500 mg were selected to study the number of coating layers on the stainless steel needle. It was observed that the use of two layers of the carbon nanocones/disks paste was enough to obtain a homogeneous sorbent surface.

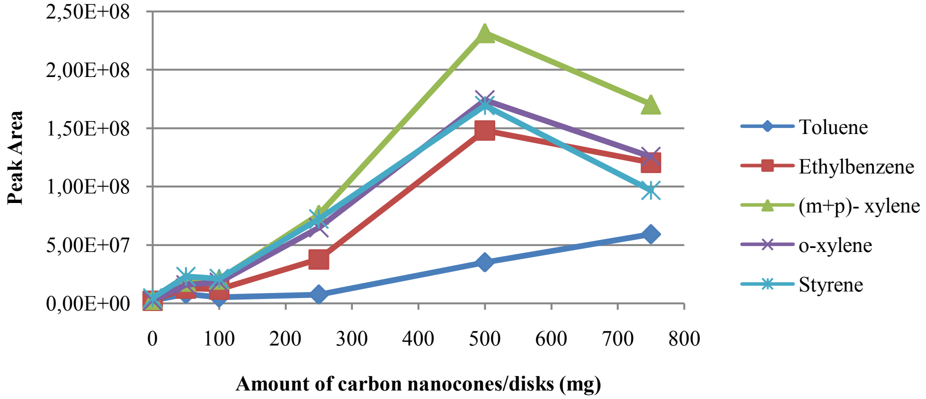

Fig. 2. Influence of the amount of carbon nanocones/disks used in the fiber fabrication on the analytical response obtained for the selected analytes.

The next variable studied was the temperature for fiber conditioning before its first use. This variable is important as it affects the potential interferences from the organic binder during the analyte thermal desorption. In all cases, it was carried out under a nitrogen stream which minimized potential oxidation processes and helped to the removal of the organic compounds released during the heating step. The temperature was studied between 100 and 400 ºC and the time was initially fixed at 2 h. Figure 3 shows the blank chromatograms obtained at different sinterization temperatures (100, 200, 300 and 400 ºC). From the baselines obtained, it seems that when the fiber is subjected to temperatures higher than 200 ºC, some artifacts appear in the chromatograms which can be ascribed to a potential degradation of the reagents used during the coating step. The signals obtained at lower temperatures revealed an incomplete sintering of fiber. Moreover, this variable was also studied in term of selectivity and reproducibility of the fiber towards the model compounds. In all cases, the analyses were carried out by duplicate and using two fibers (n = 4). Based on both criteria, the optimum temperature was 200 ºC and it was maintained at this value for 6 h, as no improvement was observed over this value.

Finally, the length of the fiber was evaluated between 10 and 35 mm. A slightly increase on the analytes response was obtained with increasing fiber length and 35 mm was selected. The maximum length was determined by the vials used for the extraction.

The characterization of the carbon nanocones/disks–based fiber was carried out by scanning electron microscopy (SEM). Figure 4 compares the SPME fiber obtained before (a) and after (b) the sinterization step. As it can be seen in Figure 4b, after the conditioning step, the fiber surface was homogenous in thickness and a showed rough texture which improves its adsorption properties. Furthermore, the micrographs (Figure 4c) have permitted an estimation of the coating thickness which was ca. 50 µm. The length of each fiber coated was 3.5 cm.

Once the variables affecting the coating process were optimized, the fitting of the fiber on the autosampler syringe was unsuccessfully attempted, and therefore the use of a commercial syringe was selected. It was observed that after 15–20 injections, a regeneration step of the fiber was required as the coating was damaged. Due to the volatility of the analytes, we chose the thermal desorption by locating the fiber in a 10 mL headspace vials that can be introduced in the headspace module of the instrument. In this way, the process was automatically implemented.

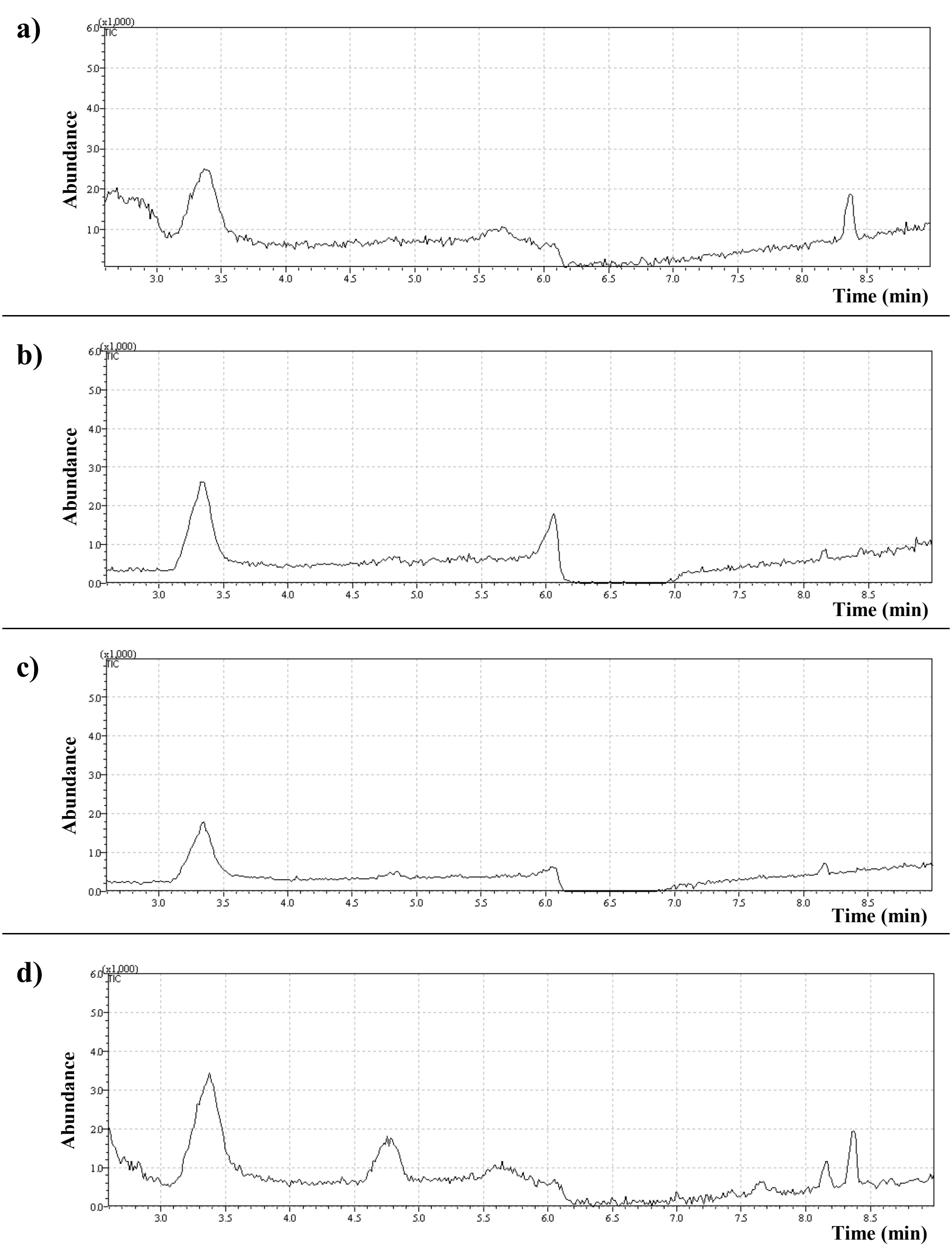

Fig. 3. Blank chromatograms obtained after the sintering of the fiber at different temperatures. a) 400 ºC, b) 300 ºC, c) 200 ºC, d) 100 ºC.

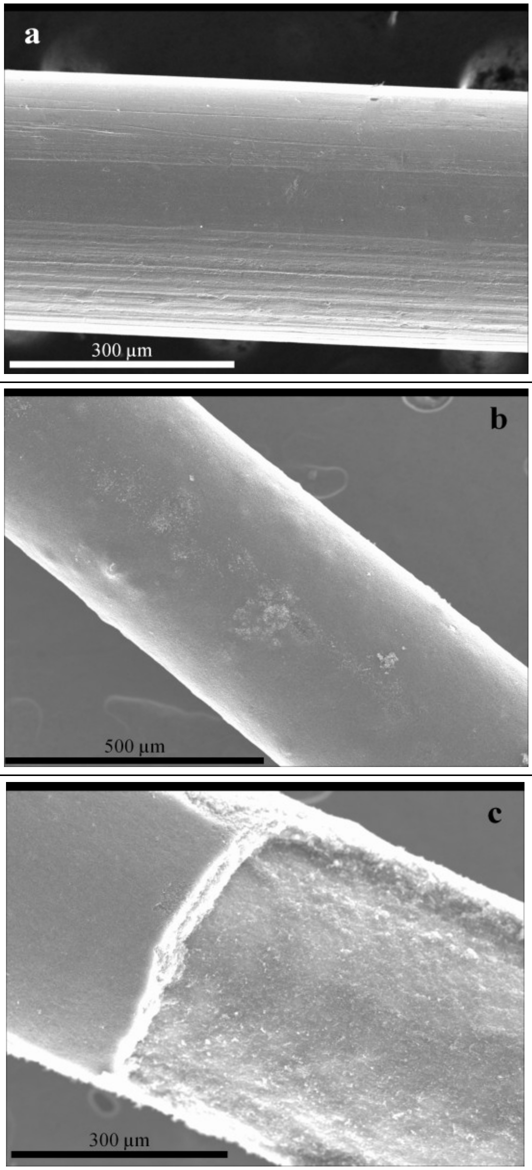

Fig. 4. SEM micrographs of the carbon nanocones/disks uncoated and coated fiber: a) stainless steel needle before the sinterization step (145x); b) fiber coated with carbon nanocones/disks (100x) and; c) details of the depth of the cut in the coating (140x).

3.2. Optimization of the desorption step

Desorption temperature was studied between 80 and 120 ºC. This variable was found to have negligible influence for toluene, as the peak areas remained almost constant through the studied interval which can be attributed to its higher volatility. For the other analytes, a slight increase of the chromatographic peak area was observed in all cases up to a desorption temperature of 110 ºC and remaining constant over this value. Therefore, 110 ºC was fixed to study the influence of the desorption time on the analytes response. As it is indicated in Table 1, this variable was studied between 10 and 45 min being necessary to maintain the fiber for 15 min at the optimum desorption temperature to obtain the maximum response for all the analytes assayed.

3.3. Optimization of the HS–SPME procedure

Before the analysis of water samples, the most relevant variables affecting the analytes extraction and desorption were optimized, including: sample volume, extraction temperature and time, stirring rate, ionic strength, desorption temperature and time. In all cases, an aqueous standard containing the analytes at an individual concentration of 100 μg mL^{-1} was used. The optimized variables, ranges studied and values selected are listed in Table 1. The sample/headspace volume ratio affects the extraction as higher ratios would lead to higher sensitivity since the headspace in the vial decreases with increasing sample volume. In this case, the minimum headspace volume is determined by the length of the fiber (ca. 10 mL in 20 mL vials). Under these conditions, the maximum signal corresponds to the use of 10 mL of sample. Sample agitation is an important kinetic factor as it favors the mass transfer of the analytes in the sample to the headspace of the vial, thus reducing extraction time by enhancing the diffusion of the analytes towards the fiber. The analytical signals for all the analytes increased with increasing agitation speed up to 500 rpm, remaining constant over this value. A stirring rate of 750 rpm was selected to study the extraction temperature and time.

The temperature has a double influence on the HS–SPME. High temperatures favor analytes released to the headspace while decreasing the interaction with the fiber as adsorption is an exothermic process. When we studied the influence of this variable on the analytes signal in the interval 30–60 ºC it was found that the signals remained almost constant up to 45 ºC, with a slight decrease over this temperature. Therefore the lower value of the interval studied, 30 ºC was chosen. Concerning the extraction time, this variable was studied between 10 and 30 min and it was found that the peak areas remained almost constant within the interval for the six compounds assayed, and therefore, 15 min was selected for further studies.

It is well–known that the presence of salts in a given sample has a positive effect on the efficiency of the headspace sampling procedures [26]. The influence of this variable on the proposed HS–SPME method was studied by adding increasing amounts of sodium chloride to the 10 mL of sample (0–350 g L^{-1}). The peak areas for all the compounds sharply increased up to 100 g L^{-1}. Between 100 and 250 g L^{-1} the variations in the analytical signals were less marked, reaching a steady stay over this value. Therefore, a concentration of 300 g L^{-1} was selected to afford the optimization of the desorption step.

3.4. Analytical features of the method

Table 2 summarizes the figures of merit of the HS–SPME method proposed in this article. The corresponding calibration graphs were constructed by using aqueous standards containing the six analytes at concentrations in the range of 0.1–1000 ng mL^{-1}. The standards were processed following the optimized procedure and 2.5 mL of the gas phase generated after the analytes desorption was injected into the GC–MS. For quantification, peak areas of the characteristic m/z fragment ion for each compound (see Table 2) were used as analytical signal. Good linear relationship between the corresponding peak areas and the concentrations were obtained for all the analytes ($R > 0.990$). The detection limits were calculated as the concentration providing an analytical signal three times higher than the background noise and they resulted to be 0.15 ng mL^{-1} (0.6 ng mL^{-1} for toluene) and the quantitation limits were 0.5 ng mL^{-1} (2 ng mL^{-1} for toluene). The precision of the method, expressed as relative standard deviation (RSD), was calculated from five individual aqueous standard prepared at a concentration of 50 ng mL^{-1} and it was lower than 6% for all the analytes (toluene excepted).

3.5. Analysis of water samples

The proposed method has been applied to the identification and quantification of toluene, ethylbenzene, (m+ p)– and o–xylene, and styrene in river, tap and well waters locally collected. After the analysis, none of them gave positive results for selected analytes. Therefore, aliquots of 50 mL of each sample were spiked with the target compounds at two concentration levels, 20 and 250 ng mL^{-1} and they were maintained in amber glass bottles without headspace and in the dark for 3 days before analysis.

Table 2. Analytical features of the proposed HS–SPME using a carbon nanocones/disks coating.

	t_R[a]	Quantification ion	LOD[b]	LOQ[c]	Linear range[d]	RSD[e]
Toluene	4.8	91	0.60	2	2 – 1000	10.7
Ethylbenzene	7.4	91	0.15	0.5	0.5 – 1000	5.7
m + p–Xylene	7.6	91	0.15	0.5	0.5 – 1000	5.0
o–Xylene	8.1	91	0.15	0.5	0.5 – 1000	4.6
Styrene	8.1	104	0.15	0.5	0.5 – 1000	4.2

[a] t_R, retention time in minutes.

[b] LOD, limit of detection in ng mL^{-1}.

[c] LOQ, limit of quantitation in ng mL^{-1}.

[d] Linear range in ng mL^{-1}.

[e] RSD, relative standard deviation (method precision), based on five replicate aqueous standards, each containing 50 ng mL^{-1}.

Then, three independent aliquots of 10 mL were analyzed using the proposed HS–SPME method, and the results obtained are shown in5Table 3. The recoveries were calculated by interpolating the analytical signal obtained for the fortified water sample in the corresponding calibration curve constructing with aqueous standards processes following the same analytical procedure. As it can be seen, the recoveries were acceptable in all cases and they ranged from 76% (styrene, river water) to 104% (ethylbenzene and (m+ p)– xylene, tap water). The recovery for toluene spiked at 20 ng mL^{-1} to the river water sample was an exception of the normal behaviour.

Table 3. Analysis of waters for the determination of toluene, ethylbenzene, xylene isomers and styrene using the carbon nanocones/disks fiber in HS–SPME, n=3.

		River water		Tap water		Well water	
Analyte	**Conc. Added ($ng\ mL^{-1}$)**	**Conc. found ± SD ($ng\ mL^{-1}$)**	**Recovery (%)**	**Conc. found ± SD ($ng\ mL^{-1}$)**	**Recovery (%)**	**Conc. found ± SD ($ng\ mL^{-1}$)**	**Recovery (%)**
Toluene	20	10 ± 1	52 ± 6	15 ± 2	77 ± 8	20 ± 3	98 ± 9
	250	202 ± 22	81 ± 9	255 ± 27	102 ± 11	260 ± 31	104 ± 12
Ethylbenzene	20	16 ± 1	81 ± 5	18 ± 1	91 ± 5	20 ± 1	101 ± 6
	250	222 ± 13	89 ± 5	260 ± 15	104 ± 6	250 ± 14	100 ± 6
m+p–Xylene	20	19 ± 1	95 ± 5	21 ± 1	104 ± 5	21 ± 1	103 ± 5
	250	205 ± 10	82 ± 4	250 ± 13	100 ± 5	258 ± 13	103 ± 5
o–Xylene	20	16 ± 1	81 ± 4	17 ± 1	85 ± 4	20 ± 1	100 ± 5
	250	228 ± 11	91 ± 4	247 ± 12	99 ± 5	256 ± 12	102 ± 5
Styrene	20	15 ± 1	76 ± 3	16 ± 1	79 ± 3	20 ± 1	99 ± 4
	250	233 ± 10	93 ± 4	240 ± 10	96 ± 4	253 ± 11	101 ± 4

In general, the recoveries for all the analytes were lower in this matrix. We attribute this fact to a potential interaction of all the analytes with suspended organic matter, being this effect more marked for toluene.

As an example, Figure 5 shows an extracted chromatogram with m/z 91 (a) and m/z 104 (b) of the six analytes analyzed in a sample of water at a concentration of 250 ng mL^{-1}.

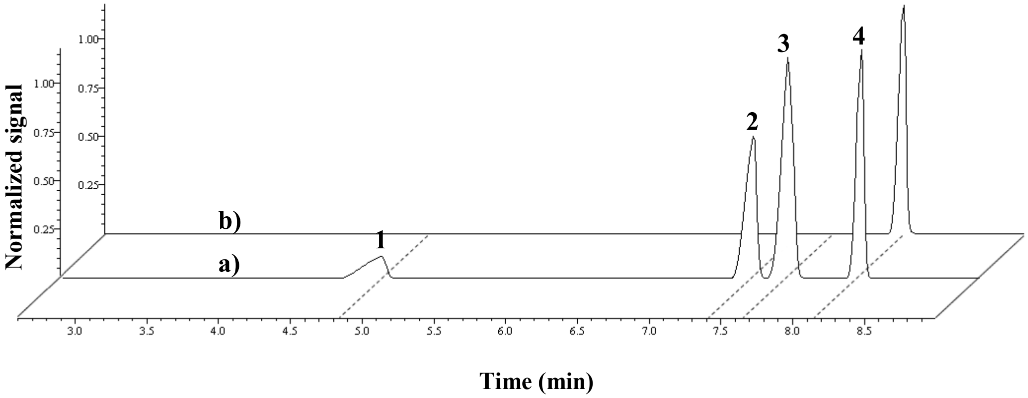

Fig. 5. Typical chromatogram of m/z=91 (a) and m/z=104 (b), obtained from the analysis of a tap water sample spiked with the six analytes at the concentration of 250 ng mL^{-1}. (1) Toluene, (2) Ethylbenzene, (3) (m+p)–Xylene, (4) o–Xylene and (5) Styrene.

4. Conclusions

This article presents for the first time the use of carbon nanocones/disks as novel coating for solid–phase microextraction. The enhanced sorbent capacity of these carbon nanostructures was previously demonstrated by our research group in conventional SPE [25]. The lower aggregation tendency of the carbon nanocones disks simplifies the preparation of the paste for the SPME coating as they are easily dispersed in the organic solvent selected. Because the target analytes are volatile, the headspace approach has been used for the isolation and preconcentration of the compounds from the sample. Furthermore, they were also thermally desorbed from the fiber which also prevent coating damage in the injection port of the gas chromatograph. The data obtained for the synthesis of the SPME fiber demonstrated the robustness of the approach. The proposed method is adequate for its aim as the detection limits are in the low nanogram per milliliter level.

The analytical performance of the proposed method can be compared with that of the SPME procedures reported in the literature for the determination of these compounds in environmental water samples. The main figures of merits are listed in Table 4. The sample volume used in the majority of the cases is higher than that used in the present approach which leads to lower detection limits. In addition, the thermal desorption carried out in the injection port of the gas chromatograph is also of advantage as in our case, a dilution of the extracted analyte during the desorption step has a negative effect on sensitivity. Notwithstanding this, the analytical performance of the proposed approach is good enough to quantify the target compounds in the environmental matrix selected. Further efforts will employ the synthesized fiber in the conventional SPME approach by using alternative immobilization procedures to determine less volatile analytes in order to extent the applicability of these new carbon nanoparticles.

Table 4. Comparison of different fibers reported in the literature for the determination of aromatic hydrocarbons in waters.

Analytes	Sample volume	SPME fiber	Extraction mode	Detection	LOD (ng mL^{-1})	Precision (% R.S.D.)	Recov. (%)	Ref.
toluene, ethylbenzene, xylene isomers and styrene	10 mL	Carbon nanocones/disks	HS	GC–MS	0.15–0.60	4.2–10.7	52–104	Proposed method
BTEX	20 mL	PDMS–DVB	HS	GC–FID	0.02–0.07	2.6–8.5	–	[27]
BTEX and styrene	40 mL	PDMS/DVB/CAR	HS	GC–FID	0.015–0.160	2.7–5.4	68–106	[28]
benzene, toluene, ethylbenzene, o–xylene	25 mL	Carbon nanotubes	HS – DI	GC–MS	–	1.8–2.5	–	[29]
benzene, toluene, ethylbenzene, o–xylene	10 mL	PDMS and PA	HS	MCC/UV–IMS	<4.5 (µg mL^{-1})	7.3–9.4	99–100	[30]
BTEX	5 mL	PP	HS	GC–FID	0.11–0.37	1.2–3.7	–	[31]
benzene, toluene, ethylbenzene and o–xylene	25 mL	PPY	HS	GC–FID	0.02–0.05	3.9–5.1	67–129	[32]

Analytes – BTEX: Benzene, Toluene, Ethylbenzene and Xylene isomers

SPME fiber – PDMS: Polydimethylsiloxane; DVB: Divinylbenzene; CAR: Carboxen; PA: Polyacrylate; PP: Polypropylene; PPY: Polypyrrole

Extraction mode – HS: Headspace; DI: Direct immersion

Detection – GC: Gas Chromatography; FID: Flame Ionization Detection; MS: Mass Spectrometry; MCC: Multi–Capillary Column; UV: Ultraviolet; IMS: Ion Mobility Spectrometry

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Capítulo 5

Oxidized–single walled carbon nanohorns as sorbent for in–porous hollow–fiber solid phase microextraction

Oxidized–single walled carbon nanohorns as sorbent for in–porous hollow–fiber solid phase microextraction

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ABSTRACT

This paper evaluates the potential of oxidized–single walled carbon nanohorns (o–SWNHs) immobilized on the pores of a hollow–fiber (HF) for the direct–immersion solid–phase microextraction of triazines from waters. The fabrication of the device requires the oxidation of the nanoparticles by means of microwave irradiation in order to obtain a homogeneous dispersion in methanol. Then, a porous hollow–fiber is immersed in the methanolic dispersion of the o–SWNHs under ultrasound stirring. This procedure permits the immobilization of the o–SWNHs in the pores of the hollow–fiber. For the extraction, a stainless steel wire was introduced inside the fiber to allow the vertical immersion of the o-SWNHs-HF in the aqueous standard/water sample. The triazines were preconcentrated on the immobilized o–SWNHs maintaining the liquid phase under continuous magnetic stirring at 400 rpm for 15 min. Once complete, the o–SWNHs–HF was transferred to a glass insert for analytes elution using 150 μL of methanol. The solvent was evaporated and the residue reconstituted in 10 μL of methanol for sensitivity enhancement. Gas chromatography–mass spectrometry was selected as instrumental technique. The limits of detection were between 0.05 and 0.1 μg/L with an excellent precision (expressed as relative standard deviation, RSD) between runs (below 10.2%) and between fibers (below 12.8%). Finally, the method was applied to the determination of the triazines in fortified waters, an average recovery value of 90% being obtained.

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1. Introduction

Since the early XX century, the advances in agriculture have kept a close relationship with the synthesis of pesticides for the control and elimination of pests that affect crops. As a result, a wide variety of families of pesticides are currently available. Despite their beneficial action on the crop, the main concern on their use is their persistence in different environmental compartments, including surface and ground waters [1]. Therefore, the control and monitoring of the concentration of pesticides, not only in the commodities but also in waters and soils is mandatory to prevent their negative effects on living things and the environment. Triazines are included among these regulated compounds. Chemically, they comprise different isomers which have in common a substituted heterocyclic ring structure composed of $C_3H_3N_3$ [2]. The United States Environmental Protection Agency (USA-EPA) states that 3 μg/L is the maximum permitted level for atrazine (a representative triazine) [3], while the EU legislation sets the maximum level allowed for a single herbicide at 0.1 μg/L and 0.5 μg/L for the herbicides mixture [4].

The detection and quantitation of triazines at these low concentrations requires the development of analytical methods that include an efficient isolation and preconcentration steps. Apart from conventional liquid–liquid extraction (LLE) [5] and solid–phase extraction (SPE) [6], miniaturized extraction techniques have gained importance taking into account their favorable connotations [7]. In this context, solid–phase microextraction (SPME), which was firstly proposed in 1990 by Arthur and Pawliszyn [8] has been extensively used. SPME employs a fiber (support) containing a sorptive phase which simultaneously performs the isolation and preconcentration of the analytes [9] either by direct–immersion of the fiber in the liquid matrix (DI–

SPME), or through exposure of the fiber to the headspace (HS–SPME) [10]. In order to extent the applicability of the technique, different coatings such as polydimethylsiloxane, Carboxen or divinylbenzene are used. The sorbents can be immobilized on fused silica or metallic supports.

Porous hollow–fibers have been successfully employed in liquid–phase microextraction (LPME). The first approach used a static LPME format using a single drop of organic solvent as extractant phase. The instability of the drop and the low sensitivity of the technique were identified as the main shortcomings of the approach. In 1998, Pedersen–Bjergaard and Rasmussen introduced hollow–fiber LPME [11], which shares the same chemical principle of supported liquid membrane extraction [12]. Hollow fiber–LPME can be performed under the two or three phase format. In the first one, the analytes are extracted from the aqueous phase to the organic phase immobilized on the porous walls and also present inside the lumen of the hollow–fiber. On the other hand, the three–phase format involves the extraction of the analytes from the aqueous donor phase to the aqueous acceptor one located inside the hollow–fiber through the thin film of organic solvent retained on the pores of the fiber. Recently, Es´haghi et al have proposed the so–called carbon nanotubes reinforced hollow–fiber solid/liquid microextraction technique [13] in which the carbon nanoparticles are used as membrane interface in a three–phase format: aqueous (donor phase), organic solvent/nanosorbent (membrane) and aqueous (acceptor phase). The unit was directly immersed in the sample and once the extraction/elution process is completed, the fiber is discarded, thus reducing the risk of cross–contamination. A similar configuration was used for the determination of phenobarbital in wastewater [14]. Further, they have also determined BTEX in hair and wastewater by using a disposable reinforced sol–gel based hollow–fiber SPME with the sorbent injected inside the fiber [15]. A disposable polymer–coated hollow–fiber membrane microextraction was proposed by Basheer et al for the extraction of organochlorine pesticides from waters [16]. The analytes were eluted by using 200 µL of hexane because the polymer was insoluble in this medium.

In this paper, oxidized–single walled carbon nanohorns (o–SWNHs) are evaluated as active sorbent in SPME. For this purpose, they were immobilized on the pores of a hollow–fiber. The main conditions influencing the fiber preparation were studied in order to obtain the most efficient configuration. Next, the parameters affecting to the DI-SPME process such as the sample pH, ionic strength and volume, agitation and extraction time as well as the elution step were optimized. Finally, the o-SWNHs-HF-DI-SPME procedure was applied for the determination of triazines in waters.

2. Experimental

2.1. Reagents and samples

The reagents were of analytical grade or better. The eleven triazines selected (atrazine, desmetryn, prometon, prometryn, propazine, secbumeton, simazine, simetryn, terbumeton, terbuthylazine desethyl and terbutryn) were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solutions of the individual analytes were prepared in acetonitrile (Sigma–Aldrich, HPLC gradient grade) at a concentration of 1 g/L and stored at 4 ºC. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in Milli–Q water (Millipore Corp, Madrid, Spain).

Single–walled carbon nanohorns (SWNHs) were purchased from Carbonium S.r.l. (Padua, Italy). The production of SWNHs was carried out by direct graphite evaporation in Ar flow, and the purity obtained was above 90 wt%. SWNHs form stable dahlia–shaped aggregates. According to the information reported by the manufacturer, these aggregates have an average diameter of 60–80 nm. Individually, the lengths of these SWNHs are in a range 40–50 nm, and the diameter in the cylindrical structure varies between 4–5 nm.

Methanol (Sigma–Aldrich, HPLC gradient grade) was used for triazines elution.

The Q3/2 Accurel polypropylene microporous hollow–fiber membrane (200 μm wall thickness, 600 μm inner diameter, 0.2 μm pore size, 75% porosity) was obtained from Membrana (Wuppertal, Germany).

River, tap and bottled waters were selected for the determination of the target triazines following the proposed o–SWNHs hollow–fiber solid–phase microextraction (o–SWNHs–HF–SPME) approach. Water samples from the Genil River were collected in amber glass bottles without headspace and stored at 4 ºC until analysis. The sample pH was 6.9 and all the aliquots were filtered through a 0.45 μm disposable Nylon filter prior to analysis. Bottled water (500 mL, polycarbonate container) was purchased in local markets. The individual bottles were maintained refrigerated in their original packing until analysis.

2.2. Apparatus

A domestic microwave oven equipped with a magnetron of 2450 MHz and a nominal maximum power of 800 W was used for SWNHs oxidation. An ultrasonic bath model 3510 from Branson (Connecticut, USA) was also used in different steps of the procedure.

Chromatographic analyses were carried out on a gas chromatograph (Varian CP–3800) – mass spectrometer (Varian 1200 MS/MS) working under single quadrupole mode and with an electron multiplier detector. The gas chromatograph was equipped with a fused silica capillary column VF–5ms, (30 m x 0.25 mm i.d.) coated with 5% phenyl – 95% dimethylpolysiloxane (film thickness 0.25 µm) (Supelco, Madrid, Spain) to separate the eleven triazines. The GC oven was programmed as follows: the initial temperature, 40 °C, was maintained for 2 min and then raised to 170 °C at 40 °C/min and further to 200 °C at 2 °C/min. The final temperature, 260 °C, was reached with a ramp of 10 °C/min and maintained for 2 min. The injector temperature was 280 °C and it was used in splitless mode. The injection volume, 2 µL, was measured with a 5 µL microsyringe (Hamilton Co., Nevada, USA). The carrier gas used was helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.0 mL/min, and it was regulated by a digital pressure and flow controller. The transfer line and ionization source were maintained at 280 ºC and 250 ºC, respectively. The ionization mode employed in the mass spectrometer was electron impact (EI) with ionization energy of 70 eV. Mass spectra were acquired using the selected ion monitoring mode (SIM), dividing the analysis time in different time windows, each of them containing the characteristic ion fragments of each triazine. The m/z selected were 186 (from 9.10 to 11.00 min); 200, 201, 210 and 214 (from 11.00 to 12.50 min); 196 (from 12.50 to

13.75 min); 213 (from 13.75 to 15.40 min); 241 (from 15.40 to 16.00 min); and 226 (from 16.00 to 17.00 min) at 1 scan/s. Chromatograms were acquired and processes using MS Workstation (Varian) on a AMD Sempro™ Processor computer which also controlled the whole system.

A JEOL JSM 6300 scanning electron microscopy (Isaza, Alcobendas, Spain) was also used to obtain the micrographs of the hollow fiber before and after the immobilization of the o–SWNHs.

2.3. Preparation of the immobilized o–SWNHs hollow–fiber

The functionalization of SWNHs consisted of the introduction of oxygen–containing functional groups on the surface of these nanostructures [17] and it was carried out as described elsewhere [18]. Briefly, an accurately weighed amount of 5 mg of pristine SWNHs was placed in a glass vial and it was irradiated with microwave energy (800 W, 10 min) using a household microwave oven. After cooling at room temperature, 10 mL of methanol were added. The dispersion was achieved by sonication of the mixture in an ultrasonic bath for 30 min. Then, a hollow–fiber of 25 mm supported on a stainless steel wire was immersed in the dispersion (0.5 g/L) and sonicated during 30 min in order to ensure that o–SWNHs remain uniformly distributed over the pores and surface of the hollow–fiber. Later, the fiber was dried in an oven at 80 ºC during 8 h. The whole procedure is schematically depicted in Fig. 1A. Following this procedure, a hollow–fiber with uniformly immobilized o–SWNHs was obtained. Figure 2 shows the micrographs obtained for the bare hollow–fiber (Fig. 2A) and the hollow–fiber with the o–SWNHs immobilized on their pores (Fig. 2B). Moreover, no losses of nanoparticles

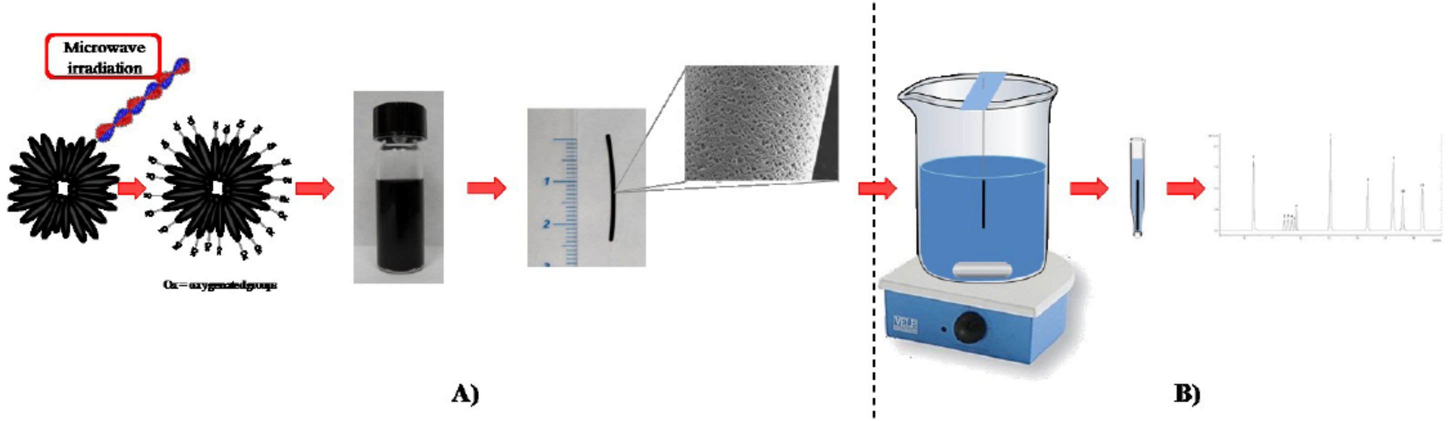

Figure 1. Schematic representation of A) the fabrication of the o-SWNHs-HF and B) de direct immersion solid phase extraction procedure.

were observed during the extractions, which testify to the stability of the final device obtained.

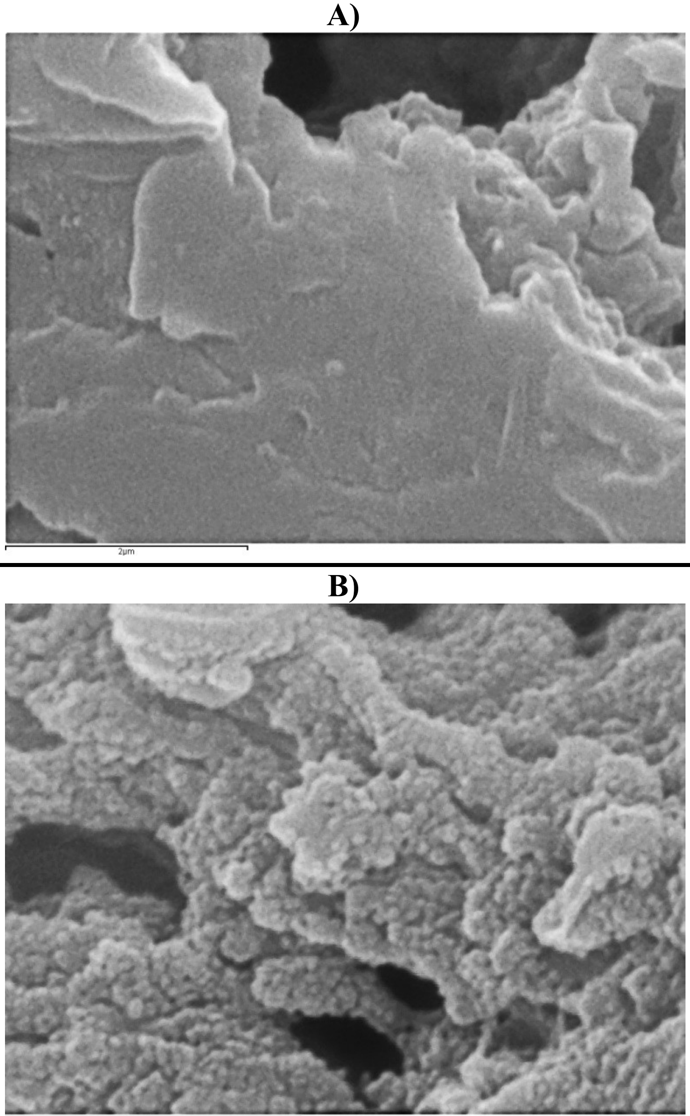

Figure 2. Scanning electron microscopy of a polypropylene hollow fiber before (A, x18000) and after (B, x22000) the immobilization of the o-SWNHs on its pores.

2.4. o–SWNHs hollow–fiber direct immersion solid–phase microextraction procedure

The analytical method proposed for the extraction of the 11 triazines from waters using o–SWNHs hollow–fiber DI-SPME is schematically depicted in Fig. 1B and it consist of the following steps.

Aliquots of 50 mL of the water sample or aqueous standard (pH 6.0) containing the 11 triazines were added to a 75 mL beaker containing a magnetic stir bar and further placed on a magnetic stirrer. For the extraction, the o-SWNHs-HF supported by the stainless steel wire was fixed to a plastic film which was further placed on the top of the beaker. In this way, the extraction unit was vertical and completely immersed in the standard solution/water sample during the extraction. The liquid phase was stirred at 400 rpm for 15 min in order to facilitate the migration of the triazines from the sample to the o–SWNHs immobilized on the pores of the hollow–fiber. After extraction, the o–SWNHs–HF was transferred to a glass insert containing 150 μL of methanol, being the analytes eluted by sonication (5 min). Once completed, the extraction unit was removed, the organic phase evaporated at 80 ºC and the residue redissolved in 10 μL of methanol. Finally, 2 μL of the extract containing the eleven triazines were injected into the GC–MS for separation and detection. Peak areas were used as analytical signal. The o–SWNHs–HF was sequentially conditioned with methanol (5 min) and Milli–Q water (10 min). This procedure permitted the reusability of the fiber for ca. 30 measurements without efficiency losses.

2.5. Safety considerations

The organic solvents and analytes used in this work are relatively volatile and slightly toxic and they should be handled using protective gloves and face mask. All the wastes were collected in special bottles for a proper management. The nanoparticles were handled under the above described safety considerations.

3. Results and discussion

Carbon nanotubes have been extensively used in sorptive extraction techniques [19]. Their outstanding properties in this context have driven the evaluation of other carbonaceous nanoparticles as sorbent materials. Our research group has demonstrated the potential of conical carbon nanoparticles such as carbon nanocones and carbon nanohorns in (micro) solid–phase extraction techniques [18,20–22]. Specifically, o–SWNHs have been used in dispersive micro solid–phase extraction for the isolation and preconcentration of PAHs [18] and triazines from waters [22]. In order to extend the applicability of this nanomaterial, their efficiency immobilized on a porous hollow–fiber has been evaluated using triazines as model family of compounds.

3.1. Preliminary experiments

The first experiments were focused on the design of the extraction unit. Taking into consideration the optimal dispersability of the o–SWNHs in polar media, the functionalized nanoparticles were selected for their immobilization on a porous hollow–fiber, following a SPME configuration. For this purpose, a stainless steel wire was inserted into the hollow–fiber in such a way that only the external surface of the fiber with the sorbent immobilized on the pores was available for the extraction of the analytes. The final assembly stainless steel wire o–SWNHs–hollow fiber was supported on a plastic film that can be placed on the top of the sample vial in order to fix the position of the extraction unit during the extraction.

In order to achieve the highest extraction efficiency of the o–SWNHs hollow fiber for the target analytes, the influence of the experimental conditions affecting to the extraction process was studied. This optimization was carried out by using an aqueous standard, containing the eleven triazines at a concentration of 100 µg/L.

3.2. Optimization of the o–SWNHs hollow–fiber preparation

The fiber used in the preliminary experiments was prepared by dipping a 10 mm polypropylene fiber in a 0.2 g/L dispersion of o–SWNHs in 1–octanol for 30 min under ultrasound agitation. Then, the hollow–fiber with the nanoparticles immobilized on its pores was dried at 80 ºC for 8 h.

First of all, the length of the fiber was studied, and the extraction capabilities of 10 mm and 25 mm hollow–fibers with the o-SWNHs immobilized on their pores were tested. It was observed that the peak areas for all analytes increased ca. 3 times when the longest fiber was used and therefore it was selected for further experiments. Lengths higher than 25 mm were not evaluated because this is the maximum height of the glass insert used in the elution step (150 µL volume).

Several organic solvents (1–octanol, acetonitrile, hexane and methanol) and Milli–Q water were used to disperse the o–SWNHs. Methanol resulted to be the best choice as it provided the most uniform immobilization of the o–SWNHs on the fiber pores which result in a better extraction efficiency of the analytes from the aqueous media and it was selected as optimum.

The influence of the concentration of the o–SWNHs methanolic dispersion on the triazines extraction was evaluated in the interval 0–1 g/L. For all the analytes, the extraction was better when the o–SWNHs were present, which corroborates the sorbent capacity of the carbon nanoparticles. As it can be seen in Fig. 3, the signals increased with the concentration of o–SWNHs reaching a steady state for concentrations higher than 0.5 g/L, being this value selected as optimum.

3.3. Optimization of the extraction step

The pH of the medium can influence the extraction efficiency for those compounds which can exist under different forms in acid or alkaline conditions. As this is the case of the triazines (pK_{as} between 1.6 and 4.6) [1], the effect of the sample pH on the extraction was investigated from 2 to 10 by adding hydrochloric acid or sodium hydroxide to the aqueous standard. Two different behaviors were obtained. Those triazines with lower pK_a values reached the maximum efficiency at pH 4, remaining almost constant until pH 6. However, for triazines with higher pK_a values, the maximum extraction was obtained at pH 6. The decrease in the peak areas observed in alkaline conditions can be attributed to the potential ionization of the oxygenated groups generated during the oxidation of the nanoparticles which would negatively affect to the interactions of the analytes with the sorbent. Therefore, pH 6 was selected as optimum.

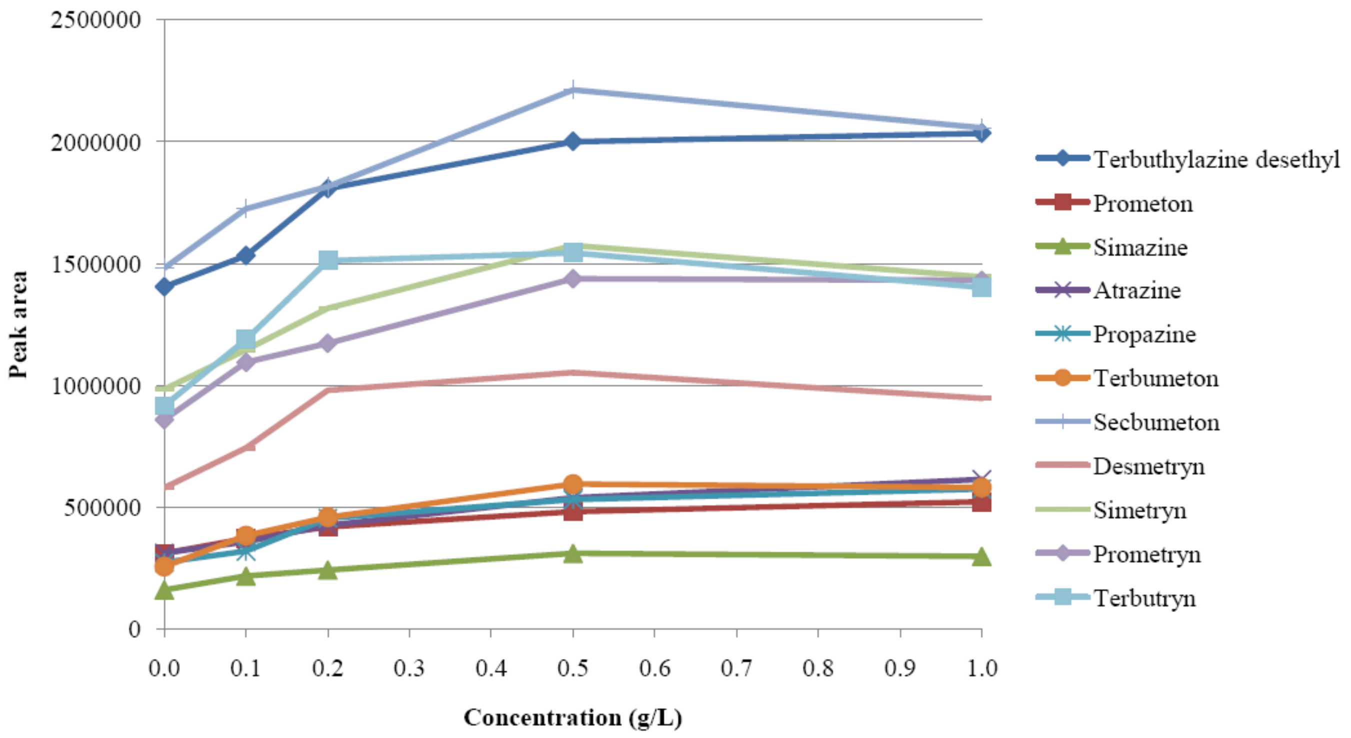

Figure 3. Influence of the concentration of the o–SWNHs dispersion on the extraction of the triazines using o–SWNHs hollow–fiber SPME.

It is well–known that the addition of salt in an extraction procedure may have contradictory effects on the process. On the one hand, it can benefit the efficiency because of the salting–out effect. On the other hand, the inherent increase of the viscosity of the aqueous phase negatively affects to the kinetic of the extraction. In this particular application, a decrease of the peak areas was observed for all the analytes in all the intervals studied (0–375 g/L NaCl), therefore, no salt was added to the samples in order to maintain the efficiency of the extraction.

The next variable optimized was the sample volume. Its influence was studied in the interval 15–100 mL. The analytical signals increased with the sample volume up to 50 mL, with a slight decrease over this value, being 50 mL fixed as optimum. The agitation of the sample during the extraction plays a key role as a fast stirring of the samples increases the extraction rate as it facilitates the contact between the o–SWNHs hollow–fiber and the analytes. In this case, the magnetic stirring of the sample was evaluated from 0 to 825 rpm. As it can be seen in Fig. 4, the peak areas for all the analytes increased up to 275 rpm, remaining constant over this value, with a slight decrease over 550 rpm. Therefore, 400 rpm was fixed to select the optimum extraction time in the interval 0.5–20 min. The results pointed out that this variable positively affects to the extraction up to 10 min, being negligible its influence over this value, and therefore 15 min was selected as optimum to ensure the reproducibility of the measurements.

The optimization of the elution step comprises the eluent and the elution time. It was aided by ultrasounds as it provided better results than the vortex, taking into account the low volume used (150 µL). Several organic

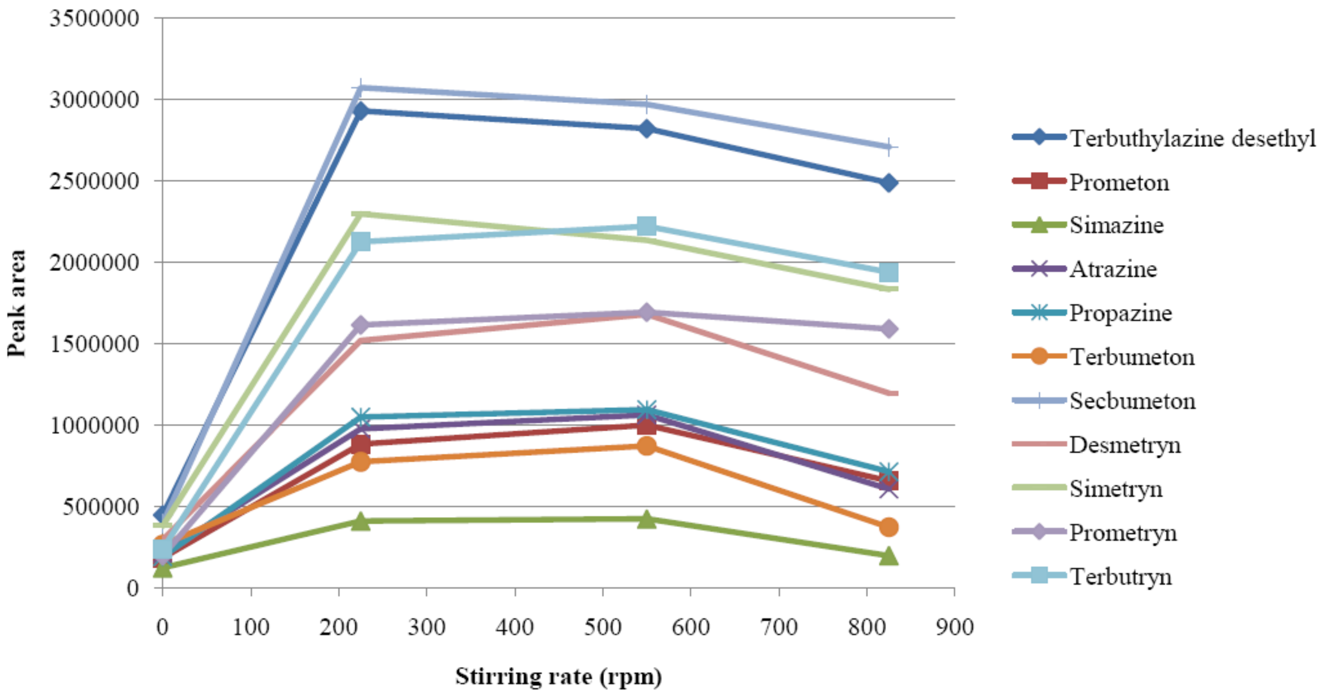

Figure 4. Effect of the sample stirring rate on the extraction of the triazines using the o–SWNHs hollow–fiber SPME.

solvents compatible with the gas chromatographic separation, such as hexane, methanol, acetonitrile and acetone were tested. Methanol was selected because it offered the best results in term of analytes peak areas. Concerning the elution time, this variable was evaluated by sonicating the o–SWNHs hollow–fiber immersed in 150 µL of methanol for different periods of time, between 0.5–10 min. It was observed that 5 min was enough to elute the triazines and therefore, it was selected as optimum. In order to corroborate the quantitative elution of the analytes under these conditions, a sequential elution using a fresh volume of methanol was carried out. The chromatographic analysis of the second elution demonstrates the absence of the target analytes and therefore, 5 min was selected as optimum. Finally, an evaporation–redissolution step was included in order to reduce the final volume to 10 µL, thus increasing the sensitivity of the method.

3.4. Analytical features of merit of the proposed o-SWNHs hollow–fiber direct immersion solid–phase microextraction method

The optimized o–SWNHs-HF DI-SPME method was characterized in terms of sensitivity, linearity and precision. The results obtained are shown in Table 1. Calibration graphs were constructed by analyzing aqueous standards (pH 6.0) containing the eleven triazines at eight concentration levels in the range 0.1 to 250 µg/L. Standards were processed using the optimized approach method and 2 µL of the methanolic extract was injected into the GC–MS for analysis. The corresponding equations were obtained by plotting the peak areas against the concentration for each triazines. Good linear relationships were observed in all cases (R> 0.9990). The detection limits (LODs) were calculated as the concentration providing an analytical signal three times higher than the background noise. They ranged from 0.05 to 0.1 µg/L and quantitation limits (LOQs) were calculated as the concentration providing an analytical signal ten times higher than the background noise, being between 0.15 and 0.35 µg/L. The precision of the method (expressed as relative standard deviation) was calculated under two experimental conditions, namely: (a) using a single fiber and analyzing five individual standard solutions containing eleven triazines at a concentration of 100 µg/L (n=5) and (b) using three different fibers with the o–SWNHs immobilized on their surface according to the optimized procedure and analyzing three aqueous standards containing eleven triazines at the same concentration (n=9). The mean values of relative standard deviations (RSD) are also listed in Table 1 and they were in the range between 5.1 – 10.2% (repeatability) and 8.6 – 12.8% (reproducibility). The enrichment factors for all the analytes, which were obtained by comparing the calibration graphs before and after the extraction process, were in the range from 31 (for terbutryn and secbumetron) and 133 (for terbuthylazine desethyl). These values highlight the good performance of the o-SWNHs-HF DI-SPME method.

3.5. Analysis of water samples

Once optimized and analytically characterized, the proposed o–SWNHs hollow–fiber DI-SPME method was applied to the determination of the target triazines in different samples: river, tap and mineral bottled waters. As a result, none of the analytes were found in any of the matrices. Therefore, aliquots of 200 mL of each sample were enriched with the triazines at two concentration levels: 5 and 100 µg/L and they were left to stand for 5 days. Then, the fortified samples were analyzed using the proposed method and the concentration for each triazine was calculated by interpolating the peak area obtained in the corresponding calibration graph. The recovery values obtained (average of three replicates) are shown in Table 2. As it can be seen, they were acceptable in all instances. By way of example, Fig. 5 shows a typical chromatogram obtained after analyzing a river water sample spiked with the eleven triazines at a concentration of 100 µg/L.

Table 1. Analytical figures of merit of the proposed direct immersion o-SWNHs hollow fiber SPME for the determination of triazines in waters

Analyte	**m/z**	**LOD (µg/L)**	**LOQ (µg/L)**	**Precision (% RSD)**	
				Between runs (n=5)	Between fibers (n=9)
Terbuthylazine desethyl	186	0.06	0.20	6.9	9.1
Prometon	210	0.10	0.35	5.9	8.9
Simazine	201	0.10	0.35	6.5	8.6
Atrazine	200	0.10	0.35	5.5	9.9
Propazine	214	0.10	0.35	10.2	11.3
Terbumeton	210	0.10	0.30	5.1	8.6
Secbumeton	196	0.05	0.15	6.7	9.5
Desmetryn	213	0.08	0.20	8.1	10.7
Simetryn	213	0.06	0.20	8.2	11.6
Prometryn	241	0.08	0.25	8.6	10.6
Terbutryn	226	0.08	0.25	9.9	12.8

LOD: limit of detection
LOQ: limit of quantitation
RSD: relative standard deviation

Table 2. Analytical figures of merit of the proposed direct immersion o-SWNHs hollow fiber SPME for the determination of triazines in waters.

Analyte	Concentration added (µg/L)	River water (%, n=3)	Tap water (%, n=3)	Bottled mineral water (%, n=3)
Terbuthylazine desethyl	5	74 ± 6	88 ± 7	88 ± 7
	100	97 ± 7	88 ± 7	97 ± 7
Prometon	5	82 ± 5	96 ± 6	97 ± 6
	100	99 ± 6	90 ± 6	92 ± 6
Simazine	5	95 ± 7	95 ± 7	99 ± 7
	100	98 ± 7	108 ± 7	102 ± 7
Atrazine	5	78 ± 5	97 ± 6	93 ± 6
	100	94 ± 6	97 ± 6	93 ± 6
Propazine	5	72 ± 8	92 ± 9	71 ± 8
	100	97 ± 10	85 ± 9	96 ± 10
Terbumeton	5	82 ± 4	100 ± 6	76 ± 4
	100	100 ± 5	100 ± 6	96 ± 5
Secbumeton	5	83 ± 6	95 ± 7	89 ± 6
	100	95 ± 7	95 ± 7	91 ± 7
Desmetryn	5	83 ± 7	83 ± 8	91 ± 8
	100	95 ± 8	83 ± 8	98 ± 8
Simetryn	5	77 ± 7	91 ± 8	94 ± 8
	100	99 ± 9	91 ± 8	93 ± 8
Prometryn	5	98 ± 8	88 ± 8	79 ± 7
	100	94 ± 8	79 ± 7	87 ± 8
Terbutryn	5	79 ± 8	75 ± 8	83 ± 8
	100	93 ± 9	70 ± 7	81 ± 8

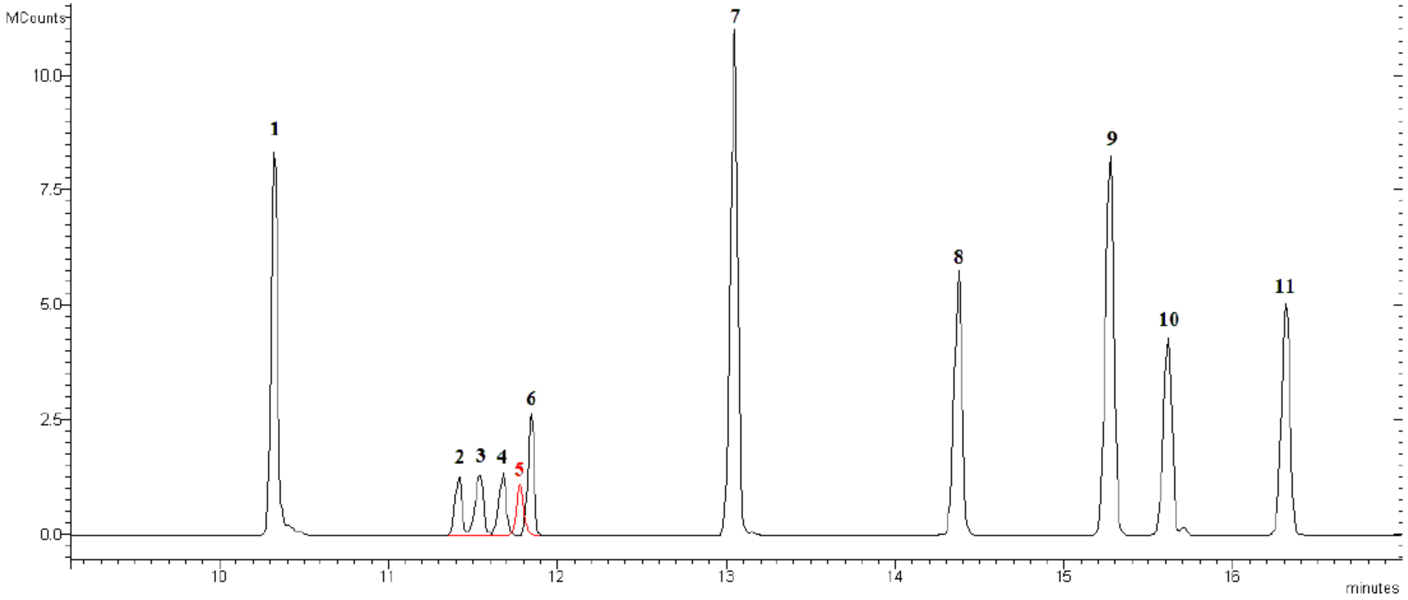

Figure 5. Typical chromatogram obtained for a river water sample spiked with eleven triazines at a concentration of 100 µg/L. Peak: 1, Terbuthylazine desethyl; 2, Prometon; 3, Simazine; 4, Atrazine; 5, Propazine; 6, Terbumeton; 7, Secbumeton; 8, Desmetryn; 9, Simetryn; 10, Prometryn; 11, Terbutryn.

4. Conclusions

This article has demonstrated the sorbent capabilities of o–SWNHs immobilized in the pores of a hollow–fiber. Although this porous membrane has been extensively used in liquid–phase microextraction under different formats, to its applicability in solid–phase microextraction is less reported. The present approach takes advantage of the excellent sorbent properties of the carbon nanoparticles, which were previously evaluated under dispersive micro solid–phase extraction conditions [21,22]. In addition to its favorable analytical features, the described o–SWNHs immobilized hollow–fiber SPME can be reused up to 30 times with negligible changes in extraction efficiency. This fact, together with its simplicity and expeditiousness makes it a valuable tool for the determination of triazines in waters.

Acknowledgements

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Capítulo 6

Evaluation of single–walled carbon nanohorns as sorbent in dispersive micro solid–phase extraction

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Evaluation of single–walled carbon nanohorns as sorbent in dispersive micro solid–phase extraction

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ABSTRACT

A new dispersive micro solid–phase extraction method which uses single–walled carbon nanohorns (SWNHs) as sorbent is proposed. The procedure combines the excellent sorbent properties of the nanoparticles with the efficiency of the dispersion of the material in the sample matrix. Under these conditions, the interaction with the analytes is maximized. The determination of polycyclic aromatic hydrocarbons was selected as model analytical problem. Two dispersion strategies were evaluated, being the functionalization via microwave irradiation better than the use of a surfactant. The extraction was accomplished by adding 1 mL of oxidized SWHNs (o–SWNHs) dispersion to 10 mL of water sample. After extraction, the mixture was passed through a disposable Nylon filter were the nanoparticles enriched with the PAHs were retained. The elution was carried out with 100 μL of hexane. The limits of detection achieved were between 30 and 60 ng L^{-1} with a precision (as repeatability) better than 12.5%. The recoveries obtained for the analytes in three different water samples were acceptable in all instances. The performance of o–SWNHs was favourably compared with that provided by carboxylated single–walled carbon nanotubes and thermally treated carbon nanocones.

1. Introduction

The recent years have witnessed the burst of the nanoscience and nanotechnology in almost all scientific areas. The reason behind such enormous impact is that, on the nanometer scale, the properties of matter can differ from those in the micro and macroworld and even depend not only on the chemical composition and phase but also on the size of the given materials. It envisages relevant advances in imaging, electronics and therapeutics. Nanomaterials are already present in chips, sporting goods, clothing, cosmetics and dietary supplements with numerous forthcoming applications such as pharmaceutical formulations. As nanomaterials are unique because of their nanometer scale, their exceptional properties are maintained as far as the nanometric dimensions prevail [1].

Analytical Chemistry has also taken benefit from the exceptional chemical, electric, optical, thermal or magnetic properties of nanoparticles to simplify and miniaturize the chemical measurement processes in general, and sample treatment in particular [2]. Among the different steps that can be included in the preliminary operations of the analytical process, separation techniques are ubiquitous as they permit the analytes isolation (improving selectivity) and preconcentration (increasing sensitivity) and also compatibility of the media with the instrumental technique used. The miniaturization of the separation techniques and more specifically the liquid and solid phase extraction approaches is a current trend in Analytical Chemistry as it improves the productively related analytical properties in terms of expeditiousness and cost [3,4]. The success of miniaturized extraction techniques relies on their efficiency and it is directly related to the properties of the extractant (liquid phase or sorbent material). In the context of the solid–phase microextraction, several approaches have been proposed in the literature in which the extraction is carried out in a fiber or a stir bar [5,6]. The wide use of these miniaturized approaches grounds on the commercially available devices which are in some cases fully compatible with the analytical instruments, such as the powerful combination between in–fiber SPME and gas chromatography. The sorbents used in the miniaturized approaches must present a high surface area in order to maximize the interaction with the analytes. This requirement is not present in conventional sorbents but it is an outstanding property of nanostructured materials. The high aggregation tendency of such nanoparticles, and most concretely carbon–based ones, clearly limits their applicability in this context because the properties are associated with the nanometric scale and they are diminished when the nanoparticles are aggregated [7].

Dispersive solid phase extraction was proposed in 2003 as a very efficient procedure to increase the selectivity of the analytical processes [8]. It was focused on the determination of pesticides in fruits and vegetables. In the general procedure, the solid is selected to interact with the interferents (pigments, sugars, or lipids) while the analytes remain in the liquid phase. This technique has been recently proposed to improve the sensitivity of the process by adding few amounts of sorbent (in the very low mg range) and once retained the analytes are eluted, thermally desorbed or directly monitored by spectroscopic techniques [9–11]. As far as nanoparticles is concerned, magnetic ones have been proposed in the literature taking into account that after extraction, they can be separated from the liquid media by means of a magnet, which simplifies the process as no centrifugation step is required. It has been used to determine PAHs [12] and UV–filters [13] in water samples.

In this paper, a novel dispersive micro solid–phase extraction (μ–SPE) using carbon nanohorns as active sorbent is presented. The dispersion of the nanoparticles has been deeply studied in order to maximize the efficiency of the extraction. Moreover, the whole procedure was optimized in order to achieve the highest recoveries. Polycyclic aromatic hydrocarbons were selected as model compounds taking into account their aromaticity, which clearly favours the interaction with the carbon nanohorns and also their consideration as priority pollutants. Finally, the performance of carbon nanotubes and carbon nanocones was also evaluated in order to compare their behaviour in the optimized dispersive micro solid–phase extraction conditions.

2. Experimental

2.1. Reagents and samples

All reagents were of analytical grade or better. Polycyclic aromatic hydrocarbons: acenaphthene, anthracene, benzo(a)anthracene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solutions of individual analytes were prepared in methanol at a concentration of 1 g L^{-1} and stored at 4 ºC. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in ultrapure water or methanol (Sigma–Aldrich) as required.

Single–walled carbon nanohorns (SWNHs) were purchased from Carbonium S.r.l. (Padua, Italy). These nanoparticles were produced by direct graphite evaporation in Ar flow with purity above 90%. According to information reported by the manufacturer, SWNHs form dahlia–shaped stable aggregates with an average diameter of 60–80 nm. Individually, each nanohorn has a variable length between 40 and 50 nm and a diameter in the cylindrical nanostructure ranging between 4 and 5 nm.

Carbon nanocones/disks high grade, were supplied by n–Tec (Oslo, Norway). This solid material is composed of 20 wt% carbon nanocones, 70 wt% carbon disks and 10 wt% amorphous carbon black. The carbon nanocones are characterized by a length between 300 and 800 nm, and a maximum base diameter between 1 and 2 μm. Moreover, the thickness of the wall is in the range 20–50 nm.

Single–walled carbon nanotubes and single–walled carbon nanotubes functionalized with carboxyl groups (c–SWNTs) were also used. The raw SWNTs were purchased from Sigma–Aldrich (Madrid, Spain). They present an approximate purity of 90%, an external diameter of 1–2 nm and variable length of 0.5–2 μm. Functionalized SWNTs were supplied by Cheap Tubes Inc. (Brattleboro, USA) with a purity exceeding 95%. These c–SWNTs present an outer diameter on 20–30 nm and lengths from 10 to 30 μm approximately.

River, tap and bottled water were selected for the determination of the target PAHs following the proposed dispersive micro–solid phase extraction approach. Bottled water was purchased in local markets and they were packed in 500 mL plastic containers. They were maintained refrigerated in their original packing until analysis. Water samples for the Genil River were collected in amber glass bottles without headspace and stored at 4 ºC until analysis. The sample pH was 6.9 and all the aliquots were filtered through a 0.45 μm disposable Nylon filter prior to analysis.

2.2. Apparatus

Functionalization of SWNHs was carried out using a household microwave oven equipped with a magnetron of 2450 MHz with a nominal maximum power of 800 W as marketed. For the complete dispersion of the oxidized SWNHs (o–SWNHs), a centrifuge and an ultrasonic bath, both from Selecta (Barcelona, Spain) were employed. A Vortex agitator (Heidolph, Mérida, Spain) was also used.

Chromatographic analyses were carried out on a gas chromatograph (Varian CP–3800)–mass spectrometer (Varian 1200 MS/MS) equipped with triple quadrupole analyzer and an electron multiplier detector. PAHs were separated on a fused silica capillary column VF–5 ms (30 m × 0.25 mm i.d.), coated with 5% phenyl – 95% dimethylpolysiloxane (film thickness 0.25 μm) (Supelco, Madrid, Spain). The temperature program of the chromatographic oven began at 60 ºC (2 min), ramped to 240 ºC at 35 ºC min^{-1} and then to 300 ºC at 15 ºC min^{-1}. This final temperature was held for 5 min. The injection port was maintained at 250 ºC throughout the experiments. A column split ratio of 1:10 was selected for the manual injection of 2 μL using a 5 μL microsyringe (Hamilton Co., Nevada, USA). Helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.4 mL min^{-1}, regulated by a digital pressure and flow controller, was used as the carrier gas. Electron impact ionization (EI) was used with an ionization energy of 70 eV. The transfer line and ionization source were maintained at 280 ºC and 250 ºC, respectively. Mass spectra were acquired using the selected ion monitoring mode, recording the following fragment–ions: 128 (from 5.1 to 6.4 min), 154 (from 6.4 to 6.8 min), 166 (from 6.8 to 7.5 min), 178 (from 7.5 to 8.5 min), 202 (from 8.5 to 10.2 min) and 228 (from 10.2 to 12.3 min) at 5 scan s^{-1}. Total ion current chromatograms were acquired and processes using MS Workstation (Varian) on a AMD SemproTM Processor computer which also controlled the whole system.

2.3. Preparation of the dispersion of single–walled carbon nanohorns

The procedure was as follows. First, an amount of 10 mg of pure SWNHs was accurately weighted in a glass vial. Subsequently, the nanomaterial was subjected to a functionalization using microwave energy (800 W, 10 min) [14]. According to the authors, this process generated oxygenated functional groups on the nanoparticle surface, which could facilitate their dispersion in polar media. Then, the functionalized solid was dispersed in 50 mL of Milli–Q water and then stirred for 60 min in an ultrasonic bath. The dispersion was centrifuged at 10,000 rpm for 15 min to remove potential non–functionalized material, thus improving the reproducibility between dispersions. The dispersions obtained are stable for ca. 24 h being possible their re–suspension by the application of ultrasounds for 1 h. Experiments carried out using several o–SWNHs dispersions prepared in different days showed a high batch–to–batch reproducibility.

2.4. Dispersive micro solid–phase extraction procedure

The proposed miniaturized extraction method was as follows: An aliquot of 10 mL of sample or an aqueous standard containing the eight PAHs at concentrations within the linear range was placed in a 20 mL glass vial. Next, 1 mL of the dispersed o–SWNHs at a final concentration of 0.2 g L^{-1} was added. Subsequently, the vial was sealed and stirred at 1600 rpm for 2 min. Homogenization was immediately achieved, which promotes the interaction between the analytes and carbon nanoparticles. Once the extraction was completed, the whole volume was passed through a 0.45 µm disposable Nylon filter (13 mm i.d.) previously conditioned passing 5 mL of hexane and 5 mL of Milli–Q water. The o–SWNHs enriched with the analytes were retained on the filter while the aqueous phase was discarded. Then the elution of the PAHs was accomplished by passing 100 µL of hexane through the filter. The eluate was collected in a vial and 2 µL injected in the gas chromatograph – mass spectrometer for further qualitative and quantitative analysis.

2.5. Safety considerations

The organic solvents and analytes used in this work are relatively volatile and slightly toxic and they should be handled using protective gloves and face mask. All the wastes were collected in special bottles for a proper management. The nanoparticles were handled under the above described safety considerations.

3. Results and discussion

3.1. Preliminary experiments

In previous research of our group, it was established the usefulness of single–walled carbon nanohorns as stationary phase in capillary electrochromatography [15]. However, no references have been published up to date dealing with the use of single–walled carbon nanohorns in solid phase extraction. Therefore, our first interest was to evaluate their sorbent capacity, using PAHs as model analytes taking into account their hydrophobic and aromatic characteristics. For this purpose, 10 mg of SWNHs were packed in a conventional SPE cartridge and 10 mL of an aqueous standard containing the PAHs at a concentration of 500 µg L^{-1} were passed through the sorbent. The aqueous phase was collected in a glass vial, extracted with 1 mL of hexane and injected in the GC–MS for analysis. As a result, no signal for the target compounds was observed in the chromatogram at the corresponding retention times. The elution of the retained PAHs was accomplished by using 1 mL of hexane, the analytes being identified at their characteristics retention times although the signal was low. It was attributed to the technical problems in the packing of the sorbent as it was very difficult to maintain the nanoparticles in the low part of the cartridge as they tend to be deposited on the walls. However, the absence of the analytes in the aqueous phase corroborates the potential sorption capacity of the nanoparticles. In order to overcome the problems associated with the packing of the SWNHs and to reduce the aggregation of the nanoparticles, which results in a lower sorption capacity, the dispersion of the SWNHs in water was accomplished in order to use the final dispersed nanoparticles as extractants of the PAHs.

3.2. Initial conditions for the dispersive micro solid–phase extraction

The dispersion of the carbon nanohorns in an aqueous medium can be achieved by following two main approaches: (a) the addition of a surfactant to the raw material; or (b) the functionalization via oxidation to increase the solubility in the aqueous phase. Both alternatives were evaluated.

Surfactant coated single–walled carbon nanohorns were prepared by adding 25 mg of the nanoparticles to 25 mL of a 17 mM sodium dodecyl sulphate (SDS) aqueous solution. Then, the mixture was sonicated for 60 min. Functionalized single–walled carbon nanohorns were obtained by microwave irradiation of the raw solid (25 mg) for 10 min at 800 W. Then, the oxidated nanoparticles were dispersed in 25 mL of water using ultrasounds radiation for

60 min. Both dispersions were evaluated for the extraction of PAHs from waters, being acenaphthene selected as model analyte.

For this purpose, 5 mL of a standard solution of acenaphthene, prepared at a concentration of 500 μg L^{-1}, were placed in a vial and 1 mL of the dispersed SWNHs (surfactant coated or oxidized) was added. The mixture was mixed using a vortex for 2 min. Then, the dispersion was passed through a 0.45 μm Nylon filter where the nanoparticles enriched with the analytes were retained while the aqueous phase was discarded. The elution was accomplished by passing 1 mL of hexane through the filter and aliquots of 2 μL of the organic extract were injected into the GC–MS for analysis. In order to determine the potential contribution of the SDS micelles in the extraction, and extraction of the acenaphthene aqueous standard using 1 mL of 17 mM SDS solution was also carried out. From this experiment, it was concluded that the highest recovery (ca. 35% better) was achieved when the oxidized SWNHs were used. Moreover, it was also observed that the extraction with SDS and surfactant coated SWHNs was very similar. It can be attributed to the fact that the reduced dimensions of the SWNHs included in the core of the SDS micelles, did not present a synergic contribution to the extraction process. Therefore, o–SWNHs were used as dispersed phase for subsequent experiments.

3.3. Selection of the o–SWNHs dispersive micro solid–phase extraction conditions

The initial values for the different variables involved in the extraction process were fixed as follows: Sample, 2.5 mL of an aqueous standard containing the PAHs at a concentration of 50 μg L^{-1}; extractant: 2.5 mL of o–

SWNHs (1 g L^{-1}) dispersed in water; vortex agitation for 5 min and filtration of the aqueous phase through a 0.45 µm disposable Nylon filter (25 mm internal diameter); eluent: methanol, 500 µL.

The first variable studied was the eluent. For this purpose, organic solvents compatible with the instrumental technique (GC–MS) were evaluated, namely: methanol, hexane, acetone and acetonitrile. In all cases, the volume was fixed at 500 µL. Hexane provided the best results in terms of chromatographic peak areas. Moreover, the chromatograms were cleaner in comparison with those obtained with the other solvents and therefore, it was chosen for further studies.

The concentration of o–SWHNs in the aqueous dispersion (extractant) affects to the sensitivity of the determination with two opposite effects. On the one hand, higher concentration of nanoparticles will lead to higher enrichment factors. On the other hand, it also increases the aggregation of the o–SWNHs which effectively reduces the nanoparticles surface available to interact with the analytes. This variable was studied in the interval 1–0.1 g L^{-1}. The best results were obtained when a concentration of 0.2 g L^{-1} was used, which corroborates the negative influence of the aggregation in the extraction capacity of the carbon nanoparticles, in general, and o–SWNHs in particular.

Next, the extractant to sample ratio was evaluated. For this study, a final volume of 10 mL and a concentration of 50 µg L^{-1} for all the analytes were fixed in all the experiments. The results obtained are depicted in Figure 1, where the variations of the peak areas versus the amount of dispersed o–SWNHs are represented.

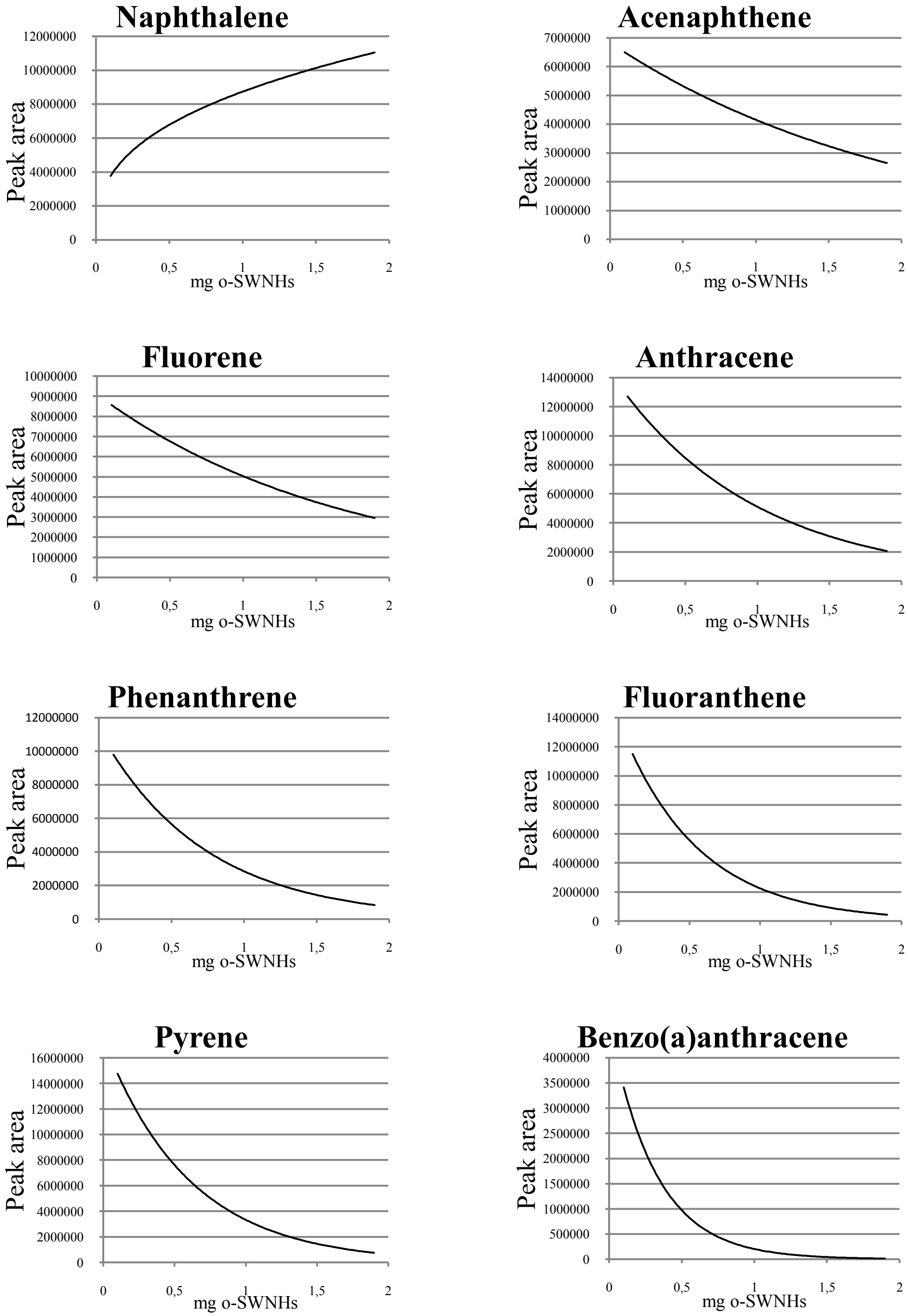

Fig. 1. Influence of the amount of o–SWNHs dispersed in the extractant on the chromatographic peak area of the selected PAHs.

As it can be seen, for all of the PAHs, there was a decrease in the peak area when the volume of dispersed extractant increases, except for naphthalene, whose signal remained almost constant within interval. Therefore, a 1:10 extractant:sample volumes ratio was selected as optimal.

The eluent volume influences the sensitivity of the method as it determines the maximum preconcentration factor that can be achieved for the target analytes. Ideally, it should be as low as possible but providing a quantitative and reproducible elution of the compounds. The hexane volume was studied between 100 and 1000 µL, the results being summarized in Figure 2. For values higher than 500 µL, 25 mm i.d. filters were used while smaller ones (13 mm i.d.) were used for lower volumes. The equivalence of these filters was corroborated as the same signal was obtained for 500 µL in both cases. As it can be seen, the best results were obtained for 100 µL due to the highest analytes preconcentration. Moreover, the elution was quantitative as when a fresh volume of hexane was passed through the filter, no analytical signal was obtained. Therefore, 100 µL of hexane was selected as optimum.

The last variable studied was the extraction time in the vortex. It was evaluated in the interval 30 s to 10 min. The peak areas for all PAHs increased with the extraction time up to 2 min, remaining almost constant over this value, being thus selected as optimum.

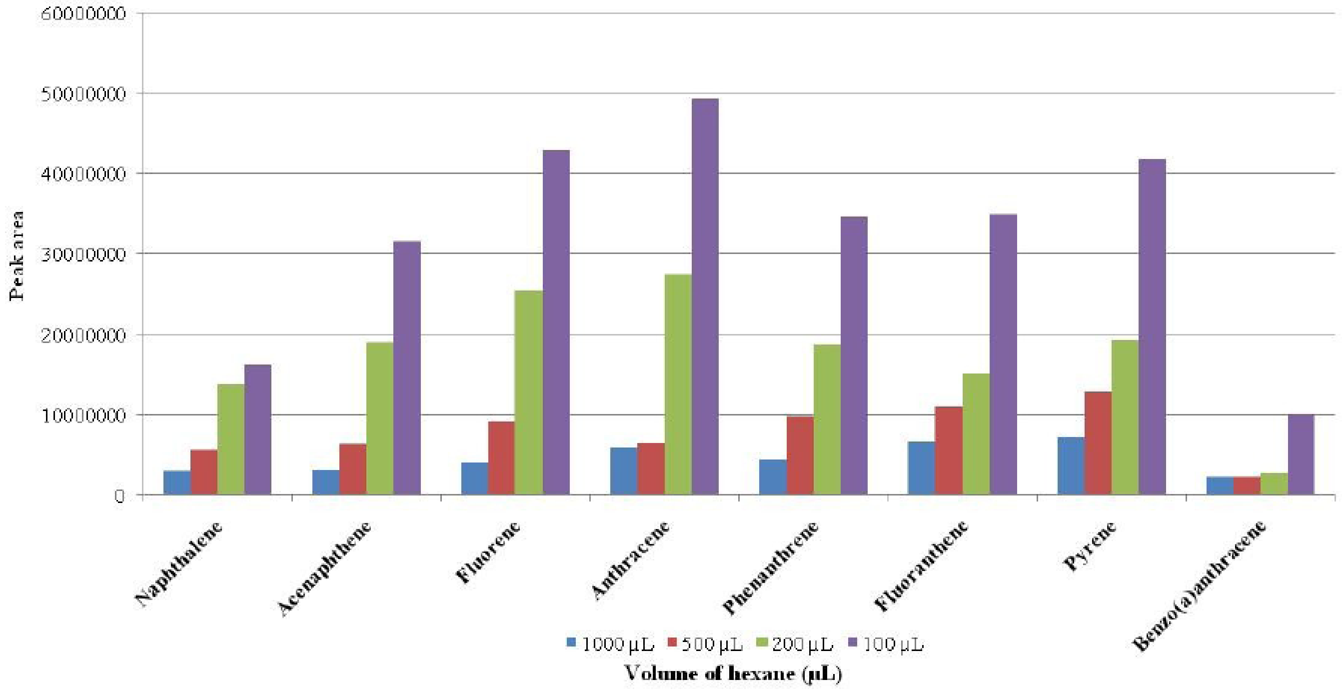

Fig. 2. Effect of the volume of hexane on the chromatographic peak area of the selected PAHs.

3.4. Analytical figures of merit of the SWNHs dispersive micro solid–phase extraction

The optimized miniaturized dispersive extraction method was characterized in terms of sensitivity, linearity and precision. The calibration graphs were constructed for the eight PAHs selected using aqueous standards extracted under the optimized conditions. The limits of detection were calculated as the minimum concentration providing a chromatographic peak area three times higher than the background noise. The values are listed in Table 1 and they varied between 30 and 60 ng L^{-1}.

The linearity was between 100 ng L^{-1} and 500 μg L^{-1}. The precision of the method (expressed as relative standard deviation) was calculated for eight individual standards prepared at 500 ng L^{-1} and ranged between 5.3% (acenaphthene) and 12.5% (pyrene).

Also, the absolute extraction recoveries were evaluated for each PAH. This parameter is defined as the percentage of total analyte that can be efficiently extracted by the o–SWNHs and further eluted with hexane. They are presented in Table 1 and ranged from 21 to 96%, the lower values being obtained for the heavier PAHs. The absolute extraction recoveries obtained demonstrated the efficiency of the dispersed nanoparticles for the extraction of the PAHs as these values are higher than those provided by other micro solid–phase extraction techniques which do not use the dispersion of the sorbent material.

Table 1. Analytical figures of merit of the proposed dispersive micro solid–phase extraction method using o–SWNHs as sorbent material.

Analyte	m/z	Limit of detection (ng L^{-1})	Limit of quantification (ng L^{-1})	Relative standard deviation (%, n=5)	Absolute extraction recoveries (%)
Naphthalene	128	50	150	11.9	96
Acenaphthene	154	40	120	5.3	69
Fluorene	166	30	100	8.1	83
Anthracene	178	30	100	7.7	80
Phenanthrene	178	40	120	10.9	89
Fluoranthene	202	40	120	7.1	86
Pyrene	202	40	120	12.5	37
Benzo(a)anthracene	228	60	200	7.2	21

Recovery studies were carried out by spiking the selected PAHs to tap, river and bottled mineral water samples at two concentration levels, 5 and 100 µg L^{-1}. In order to evaluate the applicability of the proposed method to the analysis of water samples, a recovery study was carried out in tap, river and bottled water. The samples were analyzed after collection and no signal for the target analytes were obtained. Then, the samples were spiked with the selected PAHs at two concentration levels, 5 and 100 µg L^{-1} and left stand for 76 h after enrichment in order to allow potential interactions between the PAHs and the sample matrix. The results obtained are listed in Table 2. The average recoveries for each matrix were 74% (tap water), 83% (river water) and 78% (bottled mineral water).

By way of example, Figure 3 shows the chromatogram obtained for a tap water sample spiked with the target PAHs at a concentration of 100 µg L^{-1}.

Table 2. Recovery study of the selected PAHs from three different water samples using the proposed dispersive micro solid–phase extraction method.

Analyte	**Concentration added** ($\mu g\ L^{-1}$)	**Tap water** (%, n=3)	**River water** (%, n=3)	**Bottled mineral water** (%, n=3)
Naphthalene	5	57 ± 6	59 ± 6	60 ± 6
	100	96 ± 12	97 ±12	96 ± 11
Acenaphthene	5	47 ± 2	56 ± 2	72 ± 5
	100	68 ± 4	110 ± 6	103 ± 5
Fluorene	5	52 ± 4	71 ± 6	61 ± 5
	100	93 ± 8	100 ± 8	83 ± 7
Anthracene	5	52 ± 4	92 ± 8	64 ± 4
	100	88 ± 7	94 ± 8	99 ± 8
Phenanthrene	5	25 ± 2	93 ± 10	57 ± 5
	100	78 ± 8	84 ± 9	101 ± 11
Fluoranthene	5	66 ± 5	73 ± 5	69 ± 5
	100	90 ± 7	74 ± 5	92 ± 7
Pyrene	5	60 ± 7	92 ± 11	69 ± 8
	100	88 ± 10	78 ± 9	85 ± 10
Benzo(a)anthracene	5	90 ± 6	88 ± 7	50 ± 4
	100	80 ± 6	61 ± 4	88 ± 7

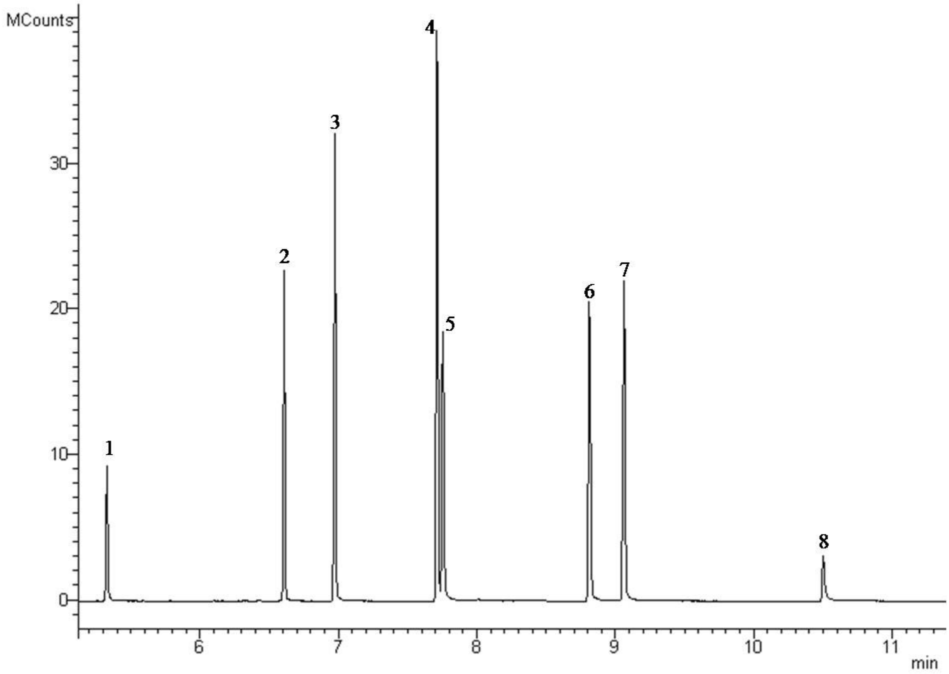

Fig. 3. Selected ion monitoring gas chromatogram obtained for a tap water sample spiked with the PAHs at a concentration of 100 µg L^{-1}.
Peaks: 1, Naphthalene; 2, Acenaphthene; 3, Fluorene; 4, Anthracene; 5, Phenanthrene; 6, Fluoranthene; 7, Pyrene; 8, Benzo(a)anthracene.

3.5. Comparison with carbon nanotubes and carbon nanoconos

As it was stated in a previous section of this article, the potential of carbon nanoparticles in solid–phase extraction is well–established [7,16]. Therefore, the efficiency of o–SWNHs in dispersive µ–SPE was compared with that provided by single–walled carbon nanotubes and carbon nanocones.

For this purpose, thermally treated carbon nanocones [17], single–walled carbon nanotubes functionalized by microwave radiation and carboxylated single–walled carbon nanotubes were dispersed under the optimized conditions of the method. Stable dispersions were obtained for the thermally treated carbon nanocones and the carboxylated single–walled carbon nanotubes, while the carbon nanotubes treated with microwave radiation presented a certain degree of aggregation. In all cases, the concentration of the carbon nanoparticles was 0.2 g L^{-1} and the optimized experimental conditions were used in this comparison. The results are illustrated in Figure 4. As a general conclusion, it can be said that the best extraction was achieved with the conical nanoparticles as the sorption on the carbon nanotubes was, in comparison, very low. In general, o–SWNHs were the best sorbent in dispersive μ–SPE for all the PAHs, being only slightly better carbon nanocones for the isolation of benzo(a)anthracene. However, it should be noted that the efficiency of the dispersive μ–SPE using carbon nanocones increased when increasing the number of aromatic rings in the PAH. This behaviour can be explained taken into consideration the dimensions of carbon nanocones, which are ca. 20 times larger than the SWNHs. The lower size of the o–SWNHs favours the interaction on the nanoparticle surface. In addition, the base diameter of carbon nanocones varied between 1 and 2 μm. This will allow the interactions of the PAHs in the inner part of the nanoparticle, being more difficult their release in the elution step. It also causes a poor reproducibility in the extractions.

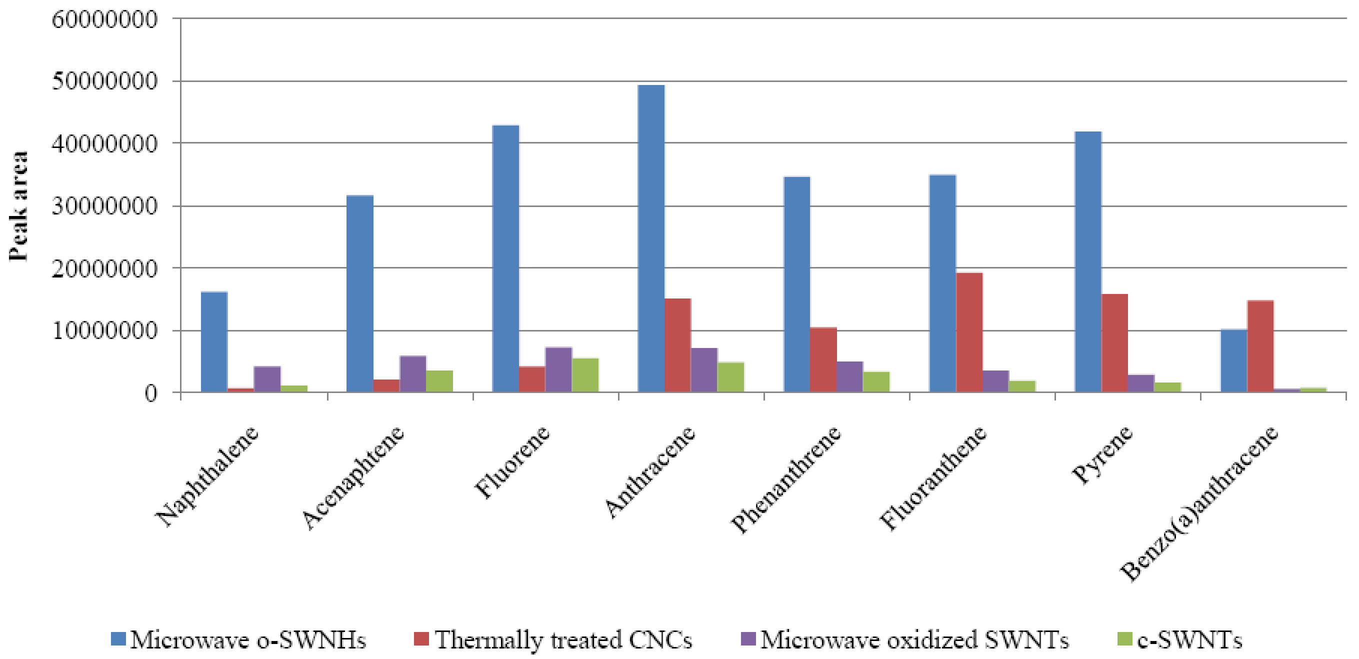

Fig. 4. Comparison of the performance of single walled carbon nanohorns, single walled carbon nanotubes and carbon nanocones in dispersive micro solid–phase extraction.

4. Conclusions

The research presented in this article demonstrated the potential of o–SWNHs as sorbent in miniaturized solid phase extraction techniques. The dispersive approach focused on sensitivity enhancement overcomes the main limitation of the use of carbon nanoparticles in solid phase extraction techniques as it is their aggregation. Different methodologies for the dispersion were evaluated, being the functionalization with microwave energy the most suitable for this aim. The dispersed nanoparticles are stable more than 24 h and the reproducibility of the extraction acceptable for the analytical problem studied. Moreover, the comparison with typical carbon nanoparticles such as carbon nanotubes and carbon nanocones, which are more similar in shape and electronic distribution, pointed out the better performance of conical carbon nanoparticles in dispersive μ–SPE. The differences in size between carbon nanohorns and carbon nanocones also condition their affinity for a specific group of PAHs, which are also retain in a different way on the two carbon nanoparticles. The carbon nanocones are by far the cheapest among the carbon nanoparticles (ca 33 $/g) although its purity is the lowest (20% cones and 70% disks). However, they can be easily purified by thermal treatment. The carbon nanohorns are cheaper (ca. 200 $/g) than the single walled carbon nanotubes (ca. 950 $/g) and of higher purity. In addition, it is also inexpensive their functionalization and almost all the solid is functionalized while the carboxylated nanotubes are very expensive (ca. 2300 $/g); also the laboratory functionalization is rather tedious and with a low percentage of functionalization. In our opinion, the SWNHs are the best alternative among carbon nanoparticles in terms of efficiency and cost. Concerning other conventional solid sorbents, the highest retention capacity of o–SWNHs, which allows the reduction of the dimensions of the extraction procedure, can be highlighted as the capital advantage. It also reduces the eluent (organic solvent) volume required, which makes the cost and environmental aspects of the method more favourable.

Further studies will be aimed at evaluating the behaviour of carbon nanohorns in dispersive µ–SPE for the isolation of different families of compounds of variable polarity.

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Capítulo 7

Dispersive micro solid–phase extraction of triazines from waters using oxidized single–walled carbon nanohorns as sorbent

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ABSTRACT

This article evaluates the usefulness of dispersed single–walled carbon nanohorns as sorbent for the isolation and preconcentration of triazines from waters. For this purpose, the carbon nanoparticles were oxidized to increase their solubility in aqueous media in order to obtain a stable dispersion that can be used as extractant of the selected pollutants. Then, 1 mL of the dispersion containing the oxidized single–walled carbon nanohorns at a concentration of 0.2 g/L was added to 10 mL of sample and stirred for 2 min using a vortex. Then, the whole volume was passed through a disposable 0.45 μm Nylon filter which retained the nanoparticles enriched with the triazines. Further elution with methanol permitted the gas chromatographic analysis of the analytes and subsequent identification and quantification by mass spectrometry working under the selected ion monitoring mode (SIM). The limits of detection (LODs) were in low nanogram per liter level which allowed the detection of the selected triazines at the concentration stated by legislation. The precision of the method, calculated as relative standard deviation, was acceptable in all instances. Finally, the recovery study carried out in different water samples provided average values between 87% and 94%. The results obtained revealed the applicability of oxidized single–walled carbon nanohorns for the proposed analytical problem.

1. Introduction

Triazines were introduced in the market about 50 years ago and since then, they have been widely used as herbicides for the control and elimination of weeds in a wide range of crops. The triazines are in solid state at room temperature and they have a low vapor pressure. Moreover, they are characterized by having a wide range of solubility in aqueous media according to their structure [1], which allows their easy distribution in different environmental compartments through the surface and underground waters [2]. Atrazine and simazine are the two triazines most commonly detected in natural waters. These herbicides present a high toxicity, high persistence and accumulation in the environmental compartments. For example, atrazine is classified as a compound which causes problems in the cardiovascular and reproductive systems in humans. For this reason, these herbicides have received particular attention in environmental and health monitoring [3]. Both the Environmental Protection Agency of the U.S. (EPA) and the EU, have developed standards for the control and monitoring of these herbicides, setting out the maximum level permitted in water for human consumption. In the case of the EPA, the maximum allowable level of atrazine is 3 μg/L [4]. The existing EU legislation sets a maximum level for each individual herbicide at 0.1 μg/L and 0.5 μg/L for mixtures of triazines [5].

Taking into account the low concentration levels established by legislation, the availability of the analytical strategies capable to efficiently preconcentrate the triazines from environmental matrices is desirable. These methodologies should also be simple and compatible with analytical instruments of the highest analytical rank, such as chromatographic separation coupled with mass spectrometric detection. Concerning sample treatment, most official methods for analysis of pesticides in water, including triazines, still use liquid-liquid extraction (LLE) technique [6]. Taking into account its negative connotations, several alternatives have been proposed in this context, such as semipermeable membrane device [7], supercritical fluid extraction [8], solid–phase extraction (SPE) [9] and solid–phase microextraction (SPME) [10]. In SPE it is of great importance the selection of the most suitable sorbent material. For the extraction of triazines from water, non–polar sorbents such as RP–C_8 and RP–C_{18} [11], carbon black [12] and polymeric resins [13] are the widely employed. Also, polyacrilate [10] and carbowax–divinylbenzene [14] have been used for the extraction of triazines by SPME.

Currently there is a trend to miniaturize all the steps of the analytical process. The miniaturization of the extraction techniques requires the use of highly efficient extractant media in order to maintain or even improve the preconcentration of the analytes using only a few milligrams or microliters of extractant. Concerning micro solid–phase extraction, the use of nanomaterials, such as magnetic or carbonaceous nanoparticles, produce a synergistic effect to achieve high efficiency in the extraction processes [15]. It provides not only an improvement of the analytical properties (sensitivity, selectivity and precision), but also helps to reduce the use of organic solvents, thus obtaining an extraction technique environmentally friendly, with lower risk for the analyst and with a considerable reduction of cost per analysis [16].

This article evaluates the applicability of dispersive micro solid–phase extraction using single–walled carbon nanohorns (SWNHs) as sorbent material for the extraction of triazines from waters. The usefulness of SWNHs in this miniaturized extraction technique has been recently evaluated by our research group using polycyclic aromatic hydrocarbons (PAHs) as family of target analytes with high aromaticity [17]. The dispersive microextraction methodology was successfully applied to the determination of the target compounds in waters. Also, the performance of the single-walled carbon nanohorns was compared to that obtained using carbon nanotubes and nanocones, being the selected nanoparticles more efficient in the dispersive solid-phase microextraction approach. In order to extent the applicability of the methodology, a family of analytes with lower aromaticity and also of environmental relevance has been selected. Taking into account the different nature of the analytes, all the variables involved in the process has been systematically studied and evaluated. The optimized procedure was applied to the determination of triazines in different water samples.

2. Experimental

2.1. Reagents and samples.

The reagents used were of analytical grade or better. The eleven triazines selected (atrazine, desmetryn, prometon, prometryn, propazine, secbumeton, simazine, simetryn, terbumeton, terbuthylazine desethyl and terbutryn) were purchased from Sigma–Aldrich (Madrid, Spain). Stock standard solutions of individual analytes were prepared in acetonitrile (HPLC gradient grade with a purity ≥ 99.9%, supplied by Sigma-Aldrich) at a concentration of 1 g/L and stored at 4 ºC. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in Milli–Q water (Millipore Corp., Madrid, Spain).

Single–walled carbon nanohorns (SWNHs) were purchased from Carbonium S.r.l. (Padua, Italy). The production of SWNHs was carried out by direct graphite evaporation in Ar flow, and the purity obtained was above 90 wt.%. SWNHs form stable dahlia–shaped aggregates. According to the information reported by the manufacturer, these aggregates have an average diameter of 60 – 80 nm. Individually, the lengths of these SWNHs are in a range 40 – 50 nm, and the diameter in the cylindrical structure varies between 4 and 5 nm.

Methanol (HPLC gradient grade with a purity ≥ 99.9%, supplied by Sigma-Aldrich) was used for triazines elution.

250 mL amber glass bottles (Albus, Córdoba, Spain) were employed for water samples collection. Before use, they were cleaned with acetone and sonicated in an ultrasonic bath for 30 min. Then, they were dried in an oven for 1 h at 80 °C.

River, tap and bottled waters were selected for the determination of the target triazines following the proposed dispersive micro solid–phase extraction approach. Bottled water was purchased in local markets and it was packed in 500 mL plastic containers. The individual bottles were maintained refrigerated in their original packing until analysis. Water samples for the Genil River were collected in amber glass bottles without headspace and stored at 4 ºC until analysis. The sample pH was 6.9 and all the aliquots were filtered through a 0.45 µm disposable Nylon filter prior to analysis.

2.2. Apparatus.

A domestic microwave oven equipped with a magnetron of 2450 MHz and a nominal maximum power of 800 W was used for SWNHs oxidation.

Vortex stirrer Reax Top Model (Heidolph, Merida, Spain) with a speed range between 0 and 2500 rpm was used for sample agitation. To complete the preparation of the extractant phase of o–SWNHs, a centrifuge Centronic–BL II model (Selecta, Barcelona, Spain) and an ultrasonic bath model 3510 from Branson (Connecticut, USA) were also used.

Chromatographic analyses were carried out on a gas chromatograph (Varian CP–3800) – mass spectrometer (Varian 1200 MS/MS) in single quadrupole mode and with an electron multiplier detector. The gas chromatograph was equipped with a fused silica capillary column VF–5ms, (30 m x 0.25 mm i.d.) coated with 5% phenyl – 95% dimethylpolysiloxane (film thickness 0.25 µm) (Supelco, Madrid, Spain) to separate the eleven triazines. The GC oven was programmed as follows: the initial temperature, 40 °C, was maintained for 2 min and then raised to 170 °C at 40 °C/min and further to 200 °C at 2 °C/min. The final temperature, 260 °C, was reached with a ramp of 10 °C/min and maintained for 2 min. The injector temperature was 280 °C and it was used in splitless mode. The injection volume, 2 µL, was measured with a 5 µL microsyringe (Hamilton Co., Nevada, USA). The carrier gas used was helium (6.0 grade, Air Liquide, Seville, Spain) at a flow rate of 1.0 mL/min, and it was regulated by a digital pressure and flow controller. The transfer line and ionization source were maintained at 280 ºC and 250 ºC, respectively. The ionization mode employed in the mass spectrometer was electron impact (EI) with ionization energy of 70 eV. Mass spectra were acquired using the selected ion monitoring mode (SIM), dividing the analysis time in different time windows, each of them containing the characteristic ion fragments of each triazine. The m/z selected were 186 (from 8.00 to 10.09 min); 200, 201, 210 and 214 (from 10.09 to 11.54 min); 196 (from 11.54 to

12.71 min); 213 (from 12.71 to 14.30 min); 241 (from 14.30 to 14.80 min); and 226 (from 14.80 to 16.00 min) at 1 scan/s. Chromatograms were acquired and processes using MS Workstation (Varian) on a AMD Sempro™ Processor computer which also controlled the whole system.

2.3. Oxidation and preparation of the single–walled carbon nanohorns dispersion.

The microwave assisted functionalization of SWNHs consisted of the introduction of oxygen–containing functional groups on the surface of these nanostructures as described by Yoshida and Sano [18]. These authors calculated the addition of oxygen on the carbon nanohorns surface in a percentage of ca. 5%. They also found that, depending on the irradiation power and the temperature during the process, the percentage can be lower as consequence of the decomposition of the carboxylic groups. However, the ether or quinone groups formed after degradation maintain the polarity of the nanomaterial, thus allowing its dispersibility in polar media. The functionalization step followed in this paper has been described elsewhere [17]. Briefly, an accurately weighed amount of 10 mg of pristine SWNHs was placed in a glass vial and it was irradiated with microwave energy (800 W, 10 min) using a household microwave oven. After cooling at room temperature, 50 mL of Milli–Q water were added. The dispersion was achieved by immersion of the mixture in an ultrasonic bath for 60 min. Then, the oxidized SWNHs (>95 wt.%) were separated from the non–oxidized ones (<5 wt.%) by centrifuging the dispersion at 10,000 rpm for 15 min. The supernatant containing the dispersed o–SWNHs, was transferred to a glass bottle while the non–functionalized SWNHs, deposited in the bottom of the tube, were discarded. Following this procedure, a stable dispersion of o–SWNHs was obtained with a high batch–to–batch reproducibility.

2.4. Analytical procedure of dispersive micro solid–phase extraction.

The determination and quantification of eleven triazines in water samples was performed by dispersive micro solid–phase extraction using o–SWNHs as sorbent material. For this purpose, aliquots of 10 mL of the aqueous standard or the water samples containing the eleven triazines at a concentration within the quantification linear range were placed in a 20 mL glass vial. Subsequently, 1 mL of the o–SWNHs dispersion at a final concentration of 0.2 g/L was added and the mixture was stirred at 1600 rpm for 2 min using a vortex. During this step, interaction between the analytes and the o–SWNHs occurred, being isolated and preconcentrated on the sorbent material. Then, the o–SWNHs enriched with the analytes were recovered by filtration on a 0.45 μm disposable Nylon filter, which was previously conditioned with 5 mL of methanol and 5 mL of Milli–Q water. Next, the analytes were eluted by passing 200 μL of methanol through the Nylon filter. Finally, the methanol containing the eleven triazines was collected in a vial and 2 μL of the organic solvent was injected into the gas chromatograph – mass spectrometer for identification and quantification of the analytes.

2.5. Safety considerations.

The organic solvents and analytes used in this work are relatively volatile and slightly toxic and they should be handled using protective gloves and face mask. All the wastes were collected in special bottles for a proper management. The nanoparticles were handled under the above described safety considerations.

3. Results and discussion

Dispersive solid–phase extraction was proposed in 2003 by Anastassiades et al. [19]. It allows the direct contact between the analytes/interferents with the sorbent thank to the homogeneous dispersion of the solid in the liquid matrix sample or its extract. Although it was initially proposed to increase the method selectivity, it can also be used to increase the sensitivity by retaining the target analytes on the appropriate sorbent material [20]. Another advantage of this technique is its easy miniaturization, which permits the reduction of the sample volume (a few mL) and the sorbent (in the order of several mg or less). In addition, it is highly compatible with a wide variety of analytical techniques, since the elution step of analytes can be carried out by using of organic solvents [17] or thermal desorption for volatile analytes [21]. It is even possible to make in–sorbent measurements, using spectroscopic techniques [20].

The use of nanometric sorbent materials in dispersive micro solid–phase extraction is advantageous taking into account their higher surface area, which increases the potential interaction with the analytes. The excellent sorbent capacity of the SWNHs is well established, especially for analytes containing aromatic rings in their structures [17,22,23]. Indeed, our research group has demonstrated the applicability of these nanoparticles in dispersive micro solid–phase extraction for the isolation and preconcentration of PAHs from water samples [17]. In this article, the usefulness of o–SWNHs for the extraction of less aromatic compound, such as triazines, is evaluated. All the variables affecting the microextraction procedure have been evaluated following an univariant approach and using three replicates for each value. The initial conditions fixed were as follows: sample: 10 mL of an aqueous standard containing the eleven triazines at a concentration of 0.1 mg/L; extractant: 1 mL of o–SWNHs dispersion 0.1 g/L; eluent: acetonitrile, 500 µL; extraction time: 5 min under magnetic stirring.

3.1. Optimization of variables involved in the dispersive micro solid–phase extraction.

The first variable studied was the eluent. For this purpose, four different organic solvents: acetonitrile, ethyl acetate, hexane and methanol were evaluated as eluent, being the volume fixed at 500 µL in all instances. Methanol and hexane offered the best elution values, although methanol provided better results in chromatographic terms. For this reason, it was selected as eluent.

The concentration of o–SWNHs in the extractant phase was evaluated in the range 0.1–0.5 g/L. The results obtained are shown in Fig. 1. As it can be seen, a minimum concentration of 0.2 g/L is required to reach the maximum extraction. The decrease observed for higher concentrations can be attributed to a potential aggregation of o–SWNHs, which results in a reduced surface available for analytes interaction. Therefore, a dispersion containing the o–SWNHs at a concentration of 0.2 g/L was selected as optimal. The extractant volume was then studied at the optimum o–SWNHs concentration and fixing 10 mL of aqueous standard of triazines as sample. This variable was studied between 0.25 and 2 mL. As expected, at lower extractant volumes, the chromatographic peak areas decreased as the likely result of the reduced amount of sorbent available for analytes interactions. The signals remained constant over 1 mL and therefore it was fixed for subsequent studies.

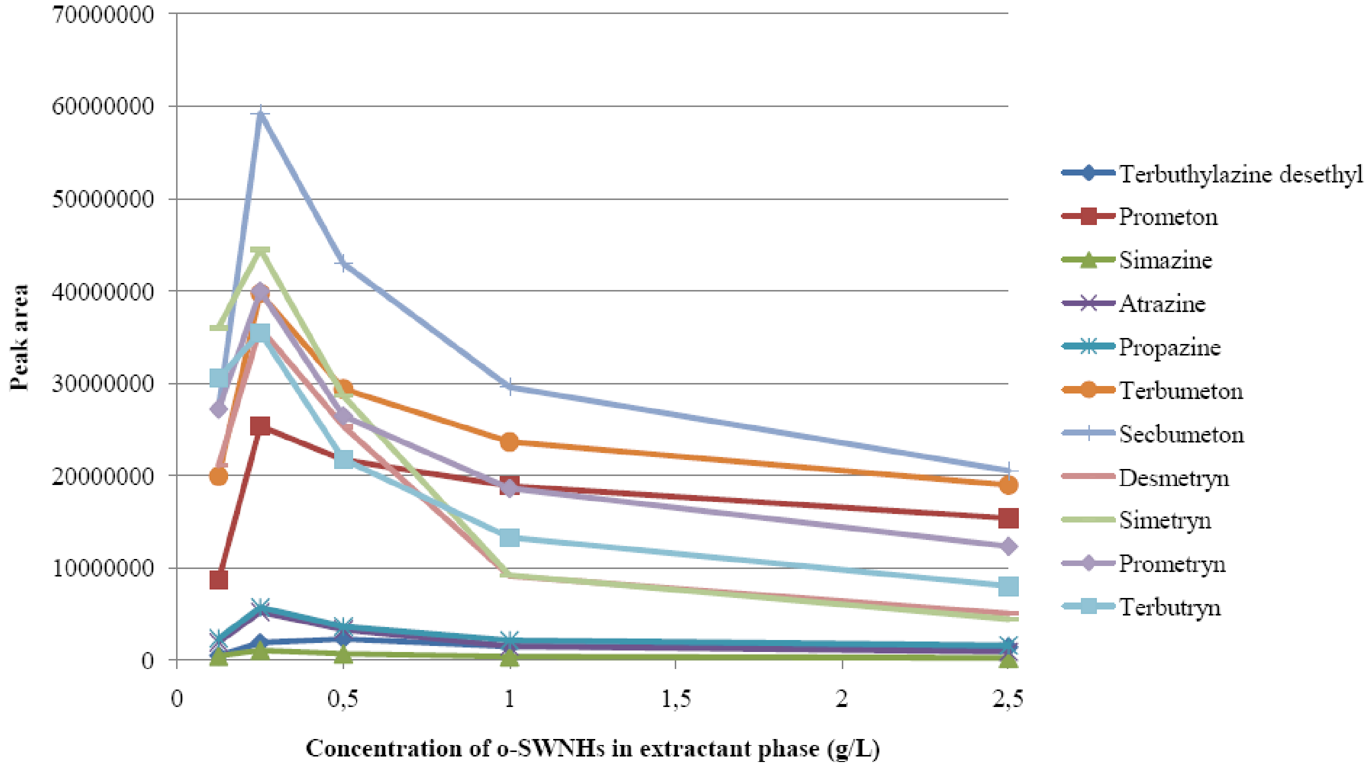

Fig. 1. Influence of the amount of o-SWNHs dispersed on the chromatographic peak area of the selected triazines.

The volume of methanol needed to obtain quantitative elution of the triazines was studied between 0.1 and 1 mL. As it can be seen in Fig. 2, a slight increase in the peak areas for almost all the analytes was observed in the interval 0.1 – 0.2 mL. Larger volumes result in lower peak areas owing to the dilution of the analytes in the eluent. Therefore, 0.2 mL of methanol was selected to optimize the next variables.

The potential benefits derived from increasing the ionic strength of the sample during the extraction (salting out effect) was evaluated using NaCl as model electrolyte, being its concentration studied within the interval 0 – 375 g/L. As it can be seen in Fig. 3a, the presence of the NaCl even at low concentration negatively affected to the dispersive (micro)extraction as it unstabilized the o–SWNHs dispersion, generating nanoparticles aggregation. Therefore, no salt was added during the dispersive micro solid–phase extraction. A similar situation was observed with the pH of the samples. It was studied between 2 and 12 using dilute HCl or NaOH for adjustment. As it can be seen in Fig. 3b, the extreme pH values reduced the solubility of the o–SWNHs which resulted in reduced extraction efficiency. The chromatographic signals remained almost constant within the interval 5 – 8 and therefore a pH of 6 was selected as the best option. Under these conditions, the pH adjustment of the water samples would not be required.

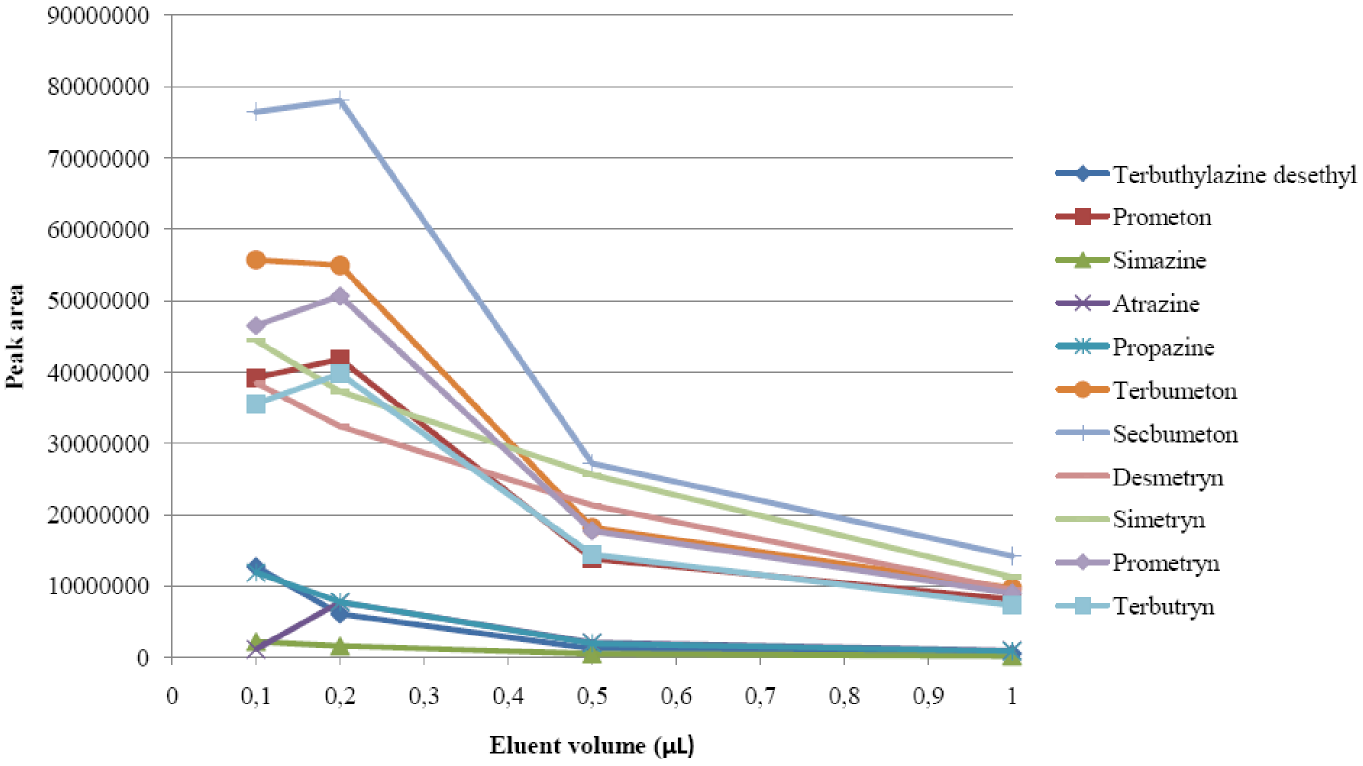

Fig. 2. Influence of the volume of elution using methanol.

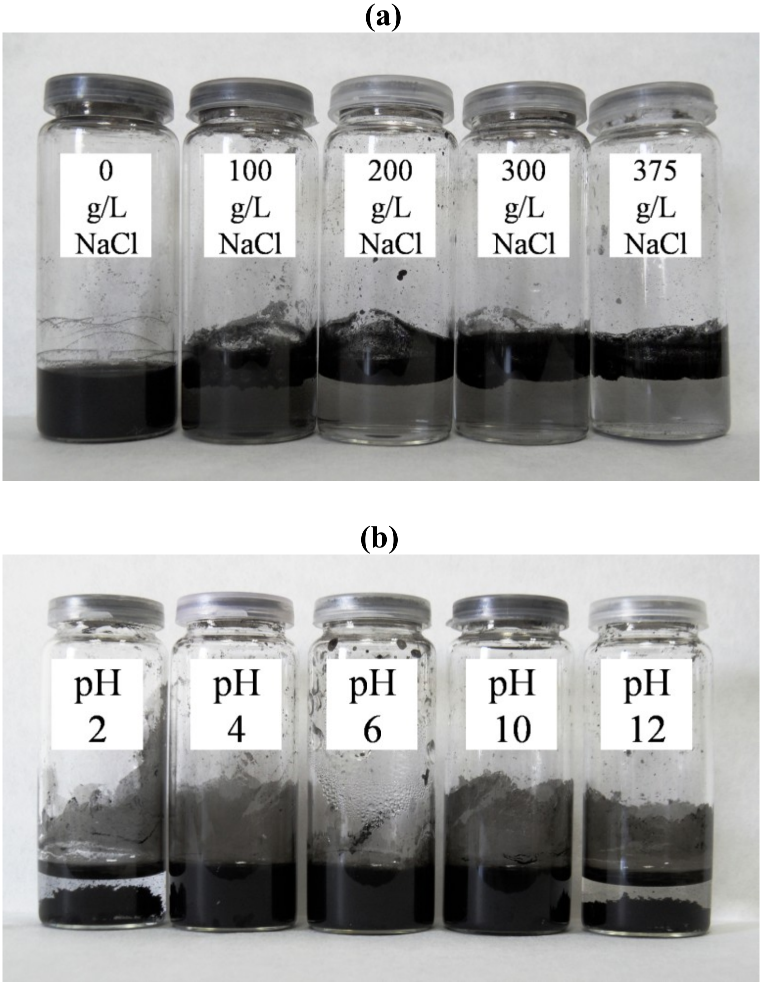

Fig. 3. (a) Images of a dispersion of extractant phase containing different concentration of NaCl. (**b)** Images of a dispersion of extractant phase containing o-SWNHs (at a concentration of 0.05 g/L) at different pH values.

Several alternatives for sample agitation during the extraction step were evaluated. Initially, magnetic stirring was discarded because the o–SWNHs were eventually deposited on the magnetic bar, which reduced the extraction capacity of the dispersion. The ultrasound were not enough to facilitate the homogeneous interactions of the nanoparticles and analytes, and therefore, the vortex was selected as the best option as it provided a vigorous stirring of the sample and extractant, favoring the reproducibility of the measurements. Concerning the extraction time, it was evaluated within the interval 1 – 10 min, being necessary only 2 min to reach a steady state for the peak areas of the eleven triazines.

Next, the influence of the geometry of the disposable Nylon filters used to retain the o–SWNHs and subsequent elution of target analytes was evaluated. For this purpose, disposable Nylon filters of 0.45 µm pore size, with internal diameters of 13 and 25 mm were used. It was found with 25 mm i.d. filters, the methanol could not be quantitatively recovered, since a small portion remained on the filter which made the elution less reproducible. Therefore, the 13 mm i.d. disposable Nylon filters were selected as the best alternative.

3.2. Analytical features of merit of the proposed dispersive micro solid–phase extraction method.

Table 1 summarizes the features of merit of the dispersive micro solid–phase extraction method proposed in this article. The corresponding calibration graphs were constructed by using ten aqueous standard solutions (pH 6.0) containing the eleven triazines in the range of 0.05 – 200 µg/L.

Table 1. Analytical figures of merit of the proposed dispersive micro solid phase extraction method.

Analyte	**m/z**	**LOD (ng/L)**	**LOQ (ng/L)**	**RSD (%, n=7)**
Terbuthylazine desethyl	186	50	120	9.2
Prometon	210	45	140	8.0
Simazine	201	100	300	9.0
Atrazine	200	60	190	9.3
Propazine	214	60	200	6.5
Terbumeton	210	40	125	10.7
Secbumeton	196	15	50	7.0
Desmetryn	213	25	75	4.9
Simetryn	213	15	50	6.2
Prometryn	241	15	50	9.3
Terbutryn	226	25	80	9.0

Detection and quantification limits and precision (calculated as relative standard deviation) were evaluated for each target analyte. The detection limits were calculated as the concentration providing an analytical signal three times higher than the background noise. They were in the range 15 – 100 ng/L and the quantification limits varied between 45 and 300 ng/L. The precision of the method, expressed as relative standard deviation (RSD) was calculated from seven individual standards prepared at concentration of 50 µg/L, and it was lower than 10% for all triazines (terbumeton excepted).

The proposed method was applied to the identification and quantification of eleven triazines in the different water samples such as: river, tap and bottled mineral waters. Aliquots of 50 mL of each water sample were separated from the raw water sample and the pH adjusted if required. The river water was previously filtered through a 0.45 µm Nylon filter to remove the potential suspended particulated matter. None of the samples provided positive results for the selected analytes. Therefore, new aliquots of 50 mL of each sample were spiked with the eleven triazines at two concentration levels, 5 and 100 µg/L, and they were maintained in amber glass bottles without headspace in the dark for four days before analysis in order to allow potential interaction between analytes and the sample matrix occur. Using the proposed method, three independents aliquots of 10 mL were analyzed. The recovery values obtained are shown in Table 2. These recoveries were calculated by interpolating the analytical signals obtained for the fortified water samples in the corresponding calibration curve constructed with aqueous standards processed following the same analytical procedure. The average recovery for each matrix was 87±12% for river water, 88±8% for tap water and 94±10% for bottled mineral water. From the individual data obtained for each analyte in the different samples evaluated, it was observed that the highest variability was for river water samples. The particulate matter potentially present in this matrix can be behind the variability as a sorption process can occur and, taking into account that this material was removed by filtration prior to analysis, a lower recovery was obtained as result.

Table 2. Recovery values from the study of water samples from different sources (river, tap and bottled mineral water) by the method of dispersive micro solid-phase extraction.

Analyte	Concentration added (µg/L)	River water (%, n=3)	Tap water (%, n=3)	Bottled mineral water (%, n=3)
Terbuthylazine desethyl	5	97 ± 9	68 ± 6	102 ± 10
	100	64 ± 6	83 ± 8	101 ± 10
Prometon	5	63 ± 5	89 ± 7	102 ± 8
	100	98 ± 8	80 ± 7	97 ± 8
Simazine	5	80 ± 7	80 ± 7	74 ± 7
	100	79 ± 7	91 ± 8	88 ± 8
Atrazine	5	105 ± 10	90 ± 8	101 ± 9
	100	79 ± 7	72 ± 7	110 ± 10
Propazine	5	86 ± 6	95 ± 6	109 ± 7
	100	87 ± 6	89 ± 6	95 ± 6
Terbumeton	5	74 ± 7	90 ± 9	104 ± 10
	100	101 ± 10	100 ± 10	96 ± 10
Secbumeton	5	77 ± 5	98 ± 7	105 ± 7
	100	104 ± 7	90 ± 7	96 ± 7
Desmetryn	5	99 ± 5	100 ± 5	67 ± 3
	100	79 ± 4	91 ± 5	94 ± 5
Simetryn	5	90 ± 6	89 ± 6	108 ± 7
	100	87 ± 6	93 ± 6	95 ± 6
Prometryn	5	104 ± 9	83 ± 8	56 ± 6
	100	74 ± 7	86 ± 8	87 ± 9
Terbutryn	5	97 ± 9	97 ± 9	88 ± 8
	100	84 ± 8	87 ± 8	86 ± 8

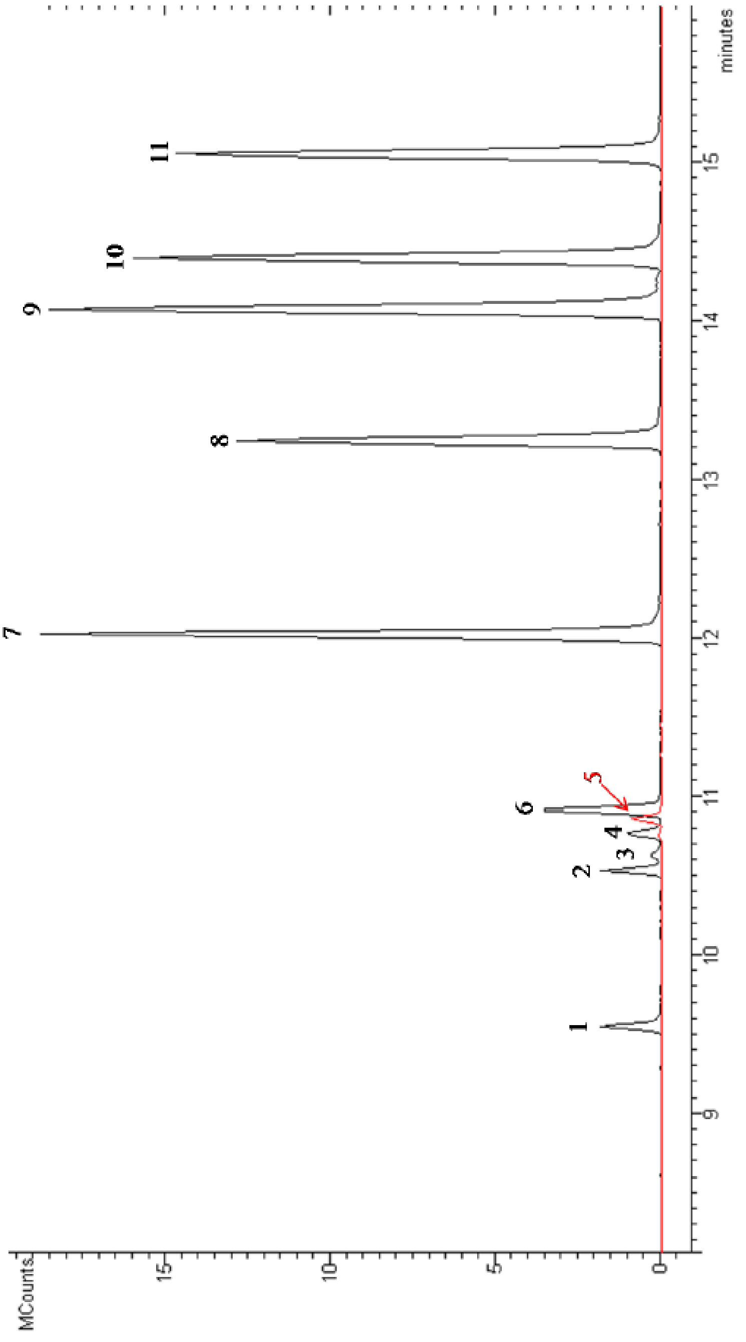

Fig. 4. Typical chromatogram obtained for a tap water sample spiked with eleven triazines at a concentration of 100 μg/L. Peak: 1, terbuthylazine desethyl; 2, prometon; 3, simazine; 4, atrazine; 5, propazine; 6, terbumeton; 7, secbumeton; 8, desmetryn; 9, simetryn; 10, prometryn; 11, terbutryn.

The variability of the results was acceptable taking into account the reproducibility of the method and, therefore, it can be concluded that it is appropriate for the determination of triazines in different water samples being negligible the influence of the sample matrix for the majority of the pollutants. By way of example, Fig. 4 shows a typical chromatogram obtained after the analysis of a tap water sample spiked with the eleven triazines at a concentration of 100 µg/L.

4. Conclusions

This article has evaluated the sorbent capacity of o–SWNHs toward triazines in different water samples. Among the different solid–phase microextraction techniques available, the modality of dispersive micro solid–phase extraction was selected to minimize or avoid the aggregation that presents the pristine SWNHs. By using a previous functionalization step by microwave energy, an aqueous dispersion of o–SWNHs, which was completely homogeneous and stable with the time, was obtained. This fact reduced the nanoparticles aggregation and allowed its use as extractant phase in a dispersive extraction mode. The eleven triazines selected as target analytes present a central aromatic ring with a nitrogen atom, thus being less aromatic than the polycyclic aromatic hydrocarbons (PAHs), previously studied [17]. After a careful optimization of each of the variables affecting to the analytical procedure, the sorbent capacity of the o–SWNHs was tested in water samples from different sources (river water, tap and bottled mineral water), obtaining satisfactory results both in recovery values and in terms of sensitivity, linearity and precision. The proposed dispersive microextraction method can be compared with other miniaturized approaches proposed in the literature for the determination of triazines in waters using gas chromatography-mas spectrometry as instrumental technique. Table 3 compiles the comparison of the analytical features of the selected references, including also the main characteristics of the extraction step.

As it can be seen, the analytical performance of the proposed dispersive micro solid-phase extraction system is similar to or better than the miniaturized extraction methods described in the literature for the same analytical problem, taking into account the sample volume used, the reduced amount of sorbent used and the short time required for the extraction. Two exceptions should be made, however, as solid-phase extraction on a multiwalled carbon nanotubes disk [24] and the use of stir bar sorptive extraction [25] provide better sensitivity. These improved analytical features can be justified taking into account the higher sample volumes and sorbent amount employed. In the case of the sorptive bar, the thermal desorption was accomplished in an automated thermal desorber device, being the triazines further trapped at –30 °C and then desorbed again through a heated transfer line directly connected to the capillary column.

Taking into account the excellent sorptive performance of o-SWNHs, further work should be aimed at evaluating their usefulness in other microextraction techniques to extend the application field of these carbon nanoparticles.

Table 3. Comparison of selected analytical methods proposed for the determination of triazines in waters using different miniaturized extraction techniques and GC-MS separation and detection.

Extraction technique	Sample vol. (mL)	Extraction time (min)	Elution step	LOD (µg/L)	Precision (% RSD)	Relative recovery (%)	Ref.
Dispersive micro solid phase extraction (DµSPE)	10	2	Methanol (200 µL)	0.015–0.1	6.2–10.7	63–110	Proposed method
Solid-phase extraction (SPE) with multiwalled carbon nanotubes (MWCNTs) disk	200	-	Acetone (5 mL)	2.5–5*	3.0–6.9	87–110	[24]
Stir bar sorptive extraction (SBSSE)	20	60	Thermal desorption (275 °C - 5min)	0.2–3.4*	2.4–6.3	94.4–106.0	[25]
Solid-phase extraction (SPE) with packed multiwalled carbon nanotubes (MWCNTs)	100	25	Ethyl acetate (4 mL)	0.02–0.05	2.3–5.3	85.7–109.7	[26]
Solid phase microextraction (DI-SPME)	3	25	Thermal desorption (250 °C - 1min)	20–88	6.5–11.6	96.3–99.6	[27]
Dispersive liquid-liquid microextraction (DLLME)	5	3	Chlorobenzene (12.0 µL)	0.021–0.12	1.4–8.7	85.2–119.4	[28]
Immersed solvent microextraction (SME)	1	15	Butyl acetate (3 µL)	0.02–0.4	5.6–10.9	18–90	[29]
Dual-resin solid-phase extraction (SPE)	125	-	Methanol (5 mL) and methylene (25 mL)	0.01–0.02	-	85–109	[30]
Membrane-assisted solvent extraction	15	30	Hexane (500 µL)	0.01–0.1	2.1–14.3	60–100	[31]

(*) ng/L

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Capítulo 8

Evaluation of the performance of single–walled carbon nanohorns in capillary electrophoresis

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WILEY ELECTROPHORESIS

Evaluation of the performance of single–walled carbon nanohorns in capillary electrophoresis

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ABSTRACT

This paper describes for the first time the use of single–walled carbon nanohorns (SWNHs) as pseudostationary and stationary phases for EKC and CEC, respectively, taking advantage of their characteristic features, such as conical–end termination, formation of spherical assemblies dahlia–flower like superstructure and easy functionalization. The use of SWNHs as pseudostationary phase for EKC required the study of their dispersion in different surfactants as well as their compatibility with the electrophoretic system. The carboxylation and subsequent immobilization of carboxylated SWNHs in fused–silica capillary to obtain useful, reproducible and stable stationary phases for CEC has also been investigated, with promising results. The electrophoretic separations obtained for water–soluble vitamins in both modalities (EKC and CEC) have been systematically compared with those obtained with single–walled carbon nanotubes.

1. Introduction

Analytical nanotechnology is undergoing an incredible development in the last years owing to the recent discoveries of new nano–materials with extraordinary physical and chemical properties. Carbon nanostructures (CNs) are one of the clearest examples in the last years and among them, single–walled carbon nanotubes (SWNTs) and multiwalled carbon nanotubes are by far the most used in analytical sciences. Their sorbent capacity has been exploited in SPE [1,2], solid–phase microextraction [3], stationary phases in gas chromatography [4,5], liquid chromatography [6] and CEC [7–9] as well as pseudostationary phases for CE [10–14].

Carbon nanohorns (CNHs) were firstly discovered by Iijima et al. in 1999 [15]. Concretely, single–walled carbon nanohorns (SWNHs) are a new class of material related to SWNTs but with a unique structural feature composed by a typical diameter of 2 nm, a length between 30 and 50 nm and the end of these tubes are closed by a conical cap with an averaged diameter cone angle of 20º. SWNHs easily form stable aggregates which have been identified as dahlia flower–like structures [15] in which the tubule walls are attracted to each other by van der Waals forces. This SWNHs structure provides high porosity and large surface area and therefore it presents high affinity for organic compounds showing a large adsorbing capacity [16]. In fact, they have been described as sorbent for ethanol [17], oxygen [18], nitrogen [19] and water vapor [20]. Additionally, other advantages of SWNHs compared with CNTs are the absence of catalyst in their synthesis [21] and their non–toxicity, being potential synthetic vehicles for programmed drugs delivery [22].

Despite of the potential of SWNHs, they have not been yet described for analytical purposes, probably because of their scarce commercialization. Knowing the precedents of other CNs as powerful analytical tools in EKC and CEC as pseudostationary phase and stationary phase [8, 10, 11], respectively, and taking into account the experience of our research group in this area, the aim of this paper was to study the possibilities of their application in CE. We investigated if their characteristic features can be used to improve EKC and CEC separations.

2. Materials and methods

2.1. Reagents

SWNTs with diameters between 0.7 and 1.2 nm and lengths of 2–20 µm were obtained from Sigma Aldrich (Madrid, Spain). SWNHs were a gift from Mer Corporation (Tucson, AZ, USA). Figure 1 shows the major differences schematically in size and shape between SWNTs and SWNHs.

3–aminopropyl triethoxy silane (APTS), 1,3–dicyclohexylcarbodiimide, glutaraldehyde and sodium hydroxide (Sigma Aldrich, Madrid, Spain) were used for the immobilization of the CNs on the fused–silica capillary. Sulfuric acid (98%), nitric acid (60%) and hydrochloric acid (35%) (Panreac, Barcelona, Spain) were used for the carboxylation of the CNTs. SDS and sodium dodecylbenzene sulfonate (NaDDBs) (Panreac) were employed for the dispersion of the CNTs and nanohorns. Boric acid, DMF, ethanol and anhydrous acetone (Panreac) were also used in this work.

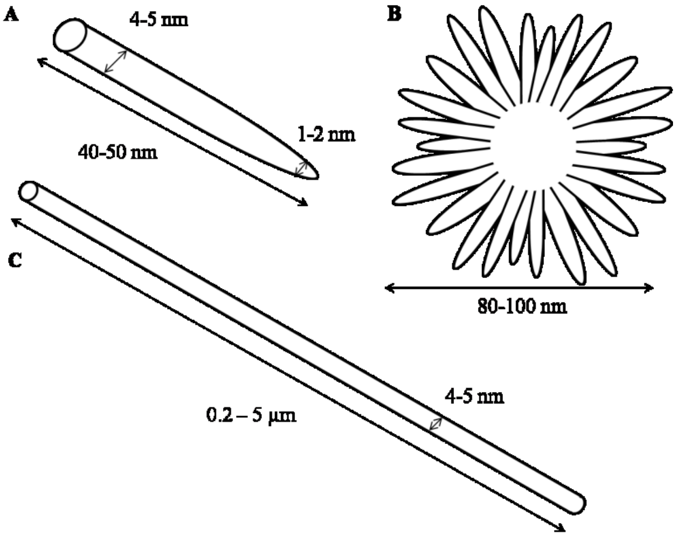

Fig. 1. Schematic representation of the relative dimensions of (A) single walled carbon nanohorn; (B) dahlia flower–like aggregate of single walled carbon nanohorns; and (C) single walled carbon nanotube.

Water–soluble vitamins: thiamine hydrochloride, ascorbic acid, riboflavin, pyridoxine hydrochloride and vitamin B_{12} were selected as target analytes. Stock standard solutions of the individual compounds were prepared at a concentration of 1000 mg L^{-1} by dissolving the appropriate amount in Milli–Q water. Working standard solutions were prepared on a daily basis by rigorous dilution of the stocks in Milli–Q water.

2.2. Safety considerations

The reagents used in this work are relatively toxic and irritant if exposed to skin and they should be handled using protective gloves and face mask. Concerning the CNs, as their impact in human health is not well established [23], they were handled as potential toxic. All the wasted were collected in plastics containers and properly managed to prevent environmental damage.

2.3. Apparatus

A Beckman Coulter (Palo Alto, CA, USA) P/ACE 5500 CE system equipped with a diode array detector was used to separate and quantify the analytes. Fused–silica capillaries (75 mm id) with an effective length between inlet and detector of 50 cm (total length of 57 cm) obtained from Beckman Coulter (Palo Alto, CA, USA) were used for the electrophoretic separation. The background electrophoretic buffer used was 50 mM boric acid adjusted at pH 8 with 1 M NaOH. Samples were injected hydrodynamically at 0.5 psi for 10 s. In all cases, the temperature of the capillary was set at 20 ºC and the applied voltage for the electrophoretic separation was 15 kV. The wavelength was fixed at 254 nm. When the nanoparticles were used as pseudostationary phase, the fused–silica capillary was initially conditioned by flushing Milli–Q water for 5 min, and then with running buffer (50 mM boric acid, pH 8) containing the CNTs or CNHs at their optimum concentration 3.2 mg L^{-1} and 3.0 mg L^{-1}, respectively, for 15 min. In the case of the carboxylated SWNHs (c–SWNHs) immobilized capillary, the conditioning was with Milli–Q water for 5 min, and then with running buffer for 20 min. Between runs, the capillary was flushed sequentially at 20 psi with Milli–Q water (2 min) and the electrophoretic buffer (5 min) in both cases.

2.4. Dispersion of single–walled carbon nanostructures (SWNSs)

SWNTs and SWNHs were dispersed following the optimized procedure developed by our research group in a previous study [10]: 1 mg of SWNTs or SWNHs was mixed with 25 mL of an aqueous solution containing

SDS 17.3 mM and sonicated (50 W, 60 Hz) for 20 min to obtain a final SWNS (CNTs or CNHs) concentration of 40 mg L^{-1}. These stocks solution were properly diluted in the running buffer to obtain the optimum conditions for the electrokinetic separation. The mixture was sonicated to ensure the homogeneity of the solution.

2.5. Preparation of carboxylic SWNSs

c–SWNTs were prepared following the procedure described by Suárez et al. [24]. The oxidation of SWNHs was carried out in a household microwave oven. For this purpose, 3.0 mg of SWNHs were placed in a glass vial which was further located inside the oven. Then, the microwave radiation (80 W) was applied during 4 min.

2.6. Immobilization of carboxylic SWNSs on fused–silica capillaries

Carboxylated SWNTs (c–SWNTs) and c–SWNHs were immobilized in the electrophoretic capillary following a previously described procedure [8]. First, the capillary was rinsed with NaOH 1 M for 30 min, followed by Milli–Q water (5 min). The second step was the introduction of an amino group by using a solution of 2% v/v APTS prepared in anhydrous acetone (15 min), followed by flushing with water (5 min) and methanol (5 min) to eliminate the excess of APTS. In the next step, a 10% v/v glutaraldehyde dissolved in 50 mM borate buffer at pH 9.0 was applied to the capillary for 1 h. Finally, a c–SWNTs and c–SWNHs solution (5 mg mL^{-1}) dissolved in 4.5 mL of DMF containing 0.5 mg of 1,3–dicyclohexylcarbodiimide was passed through the capillary for 1 h. Finally, the capillary was rinsed with water to remove the unimmobilized c–SWNTs and c–SWNHs.

2.7. Sample preparation

A pharmaceutical formulation containing ascorbic acid (tablets, 60 mg) was obtained from a local drugstore. Five tablets were weighted, grounded in a mortar and an amount of solid equivalent to 60 mg of the pure compound was placed in a glass vessel mixed with 50 mL of water, dissolved by sonication for 20 min and filtered through a filter paper. The filtrate was diluted with water to a final volume of 100 mL (final concentration 600 mg L^{-1}). For CE analysis, the sample was diluted in Milli–Q water.

3. Results and discussion

To achieve the electrophoretic separation of water–soluble vitamins by using of carbon nanoparticle based on pseudostationary or stationary phases, reversible interactions between them have to occur. In this article, we have tested two different electrophoretic operational modes, including the employment of immobilized CNs and the use of soluble surfactant–coated carbon nanoparticles. Depending on the electrophoretic modality used, various interactions can be established between the analytes and CNs. When the carbon nanoparticles are immobilized in the inner surface of the capillary, the analytes can be retained through π– π interactions or van der Waals forces interacting with SWNTs or SWNHs pores or even with polar groups resulting from the functionalization of CNs. Considering the particular electronic distribution of the CNHs, these interaction will be favored in the conical end for the more polar compounds while the wider end preferably retains less polar analytes. The separation of compounds using PSP based on nanoparticles has been previously described [25]. Notwithstanding this, the CNHs cannot be dispersed in single structures as they form very stable aggregates. They have the advantage of large surface available for retention. This effect is not observed for nanotubes aggregates.

Such interactions have been used for separating analytes mixtures of different nature such as caffeine and theobromine [26], chlorophenols, anti–inflammatory drugs and penicillins [9], ephedrine enantiomers [10], PAHs [27] β–lactam antibiotics, anti–inflammatory drugs and amphenicols [28].

3.1. Study of the dispersion of SWNSs

The dispersion of SWNTs with SDS to obtained surfactant coated carbon nanotubes (SC–SWNTs) has been fully established by our research group [10,11].

The solubilization of SWNHs in aqueous media was afforded in this work. SWNHs form stable aggregates of a total diameter ranging around 80–120 nm. Each aggregate is composed of approximately 2000 units, grouped by strong hydrophobic interactions and van der Waal forces. Unlike SWNTs, CNHs are more difficult to disperse in aqueous media, although the aggregates can be solubilized by means of surfactants. The exact mechanism by which surfactant molecules organize on SWNSs surfaces remains unclear, however. These surfactant–coated SWNHs (SC–SWNHs) can be further used as pseudostationary phase in EKC. Recently, the use of NaDDBs for the dispersion of SWNHs has been reported [29]. Therefore, the electrophoretic compatibility of the SC–SWNHs using SDS and NaDDBs was evaluated by adding an appropriated amount of both dispersions of SWNHs to the electrophoretic buffer (50 mM boric acid, pH 8). In both cases, 1 mg of the nanoparticles and a surfactant concentration of 17.3 mM were used. Moreover, a sonication step was used in order to break the agglomerates of CNHs. The results showed that the use of SDS in combination with sonication resulted in a better suspension of the dahlia nanoparticles and thus better compatibility with the electrophoretic system. Finally, the maximum concentration of SC–SWNHs that can be used as pseudostationary phase with SDS in the electrophoretic buffer was studied. This variable is of great importance as it will provide the maximum analyte interaction with the nanoparticles. However, as it was stated before, the amount of SWNHs is limited by the compatibility of the pseudostationary phase with the optical detector used. Figure 2 shows the baselines obtained at different concentrations, being 3 mg mL^{-1} finally selected as a compromise between the stability of the baseline and the separation capability of the pseudostationary phase.

3.2. Carboxylation and immobilization on fused–silica capillaries of c–SWNSs

Carboxylation of SWNTs was carried out following a previously described procedure [24], while for SWNHs, microwave [30] and a light–assisted oxidation [31] have been reported in the literature. Basically, those procedures rapidly open holes in the SWNHs walls and creates carboxylic groups at the hole edges. In this work, microwave assisted oxidation was selected on account of its simplicity and the lowest power, 80 W, was fixed to facilitate process control. The time required to complete the oxidation process was 4 min which is in good agreement with the literature [30]. The simplicity of the procedure in comparison with that described for CNTs is a clear advantage for SWNHs and points out the different reactivity between these nanostructures. The immobilization was carried out as described in the experimental section.

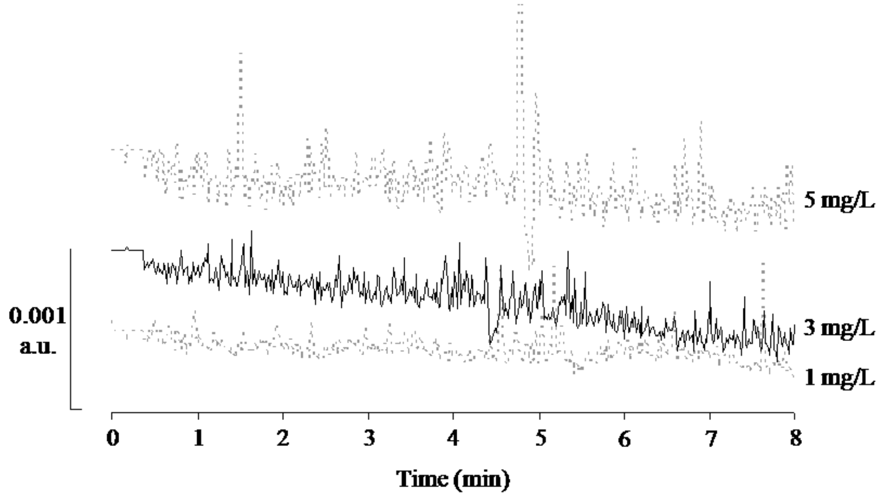

Fig. 2. Influence of the concentration of SC–SWNHs on the electrophoretic baseline a) 1 mg L^{-1}, b) 3 mg L^{-1} and c) 5 mg L^{-1}.

Figure 3 shows the SEM micrographs for a given section of the c–SWNHs immobilized fused–silica capillary at different magnifications. The treatment with c–SWNHs creates a layer of this nanostructured material on the surface of the fused–silica capillary, in contrast with the network generated by the SWNTs [8]. The differences in length between CNs as well as the different distribution for the functional groups through the individual CNTs and the nanohorns aggregates can be the reason for this behavior.

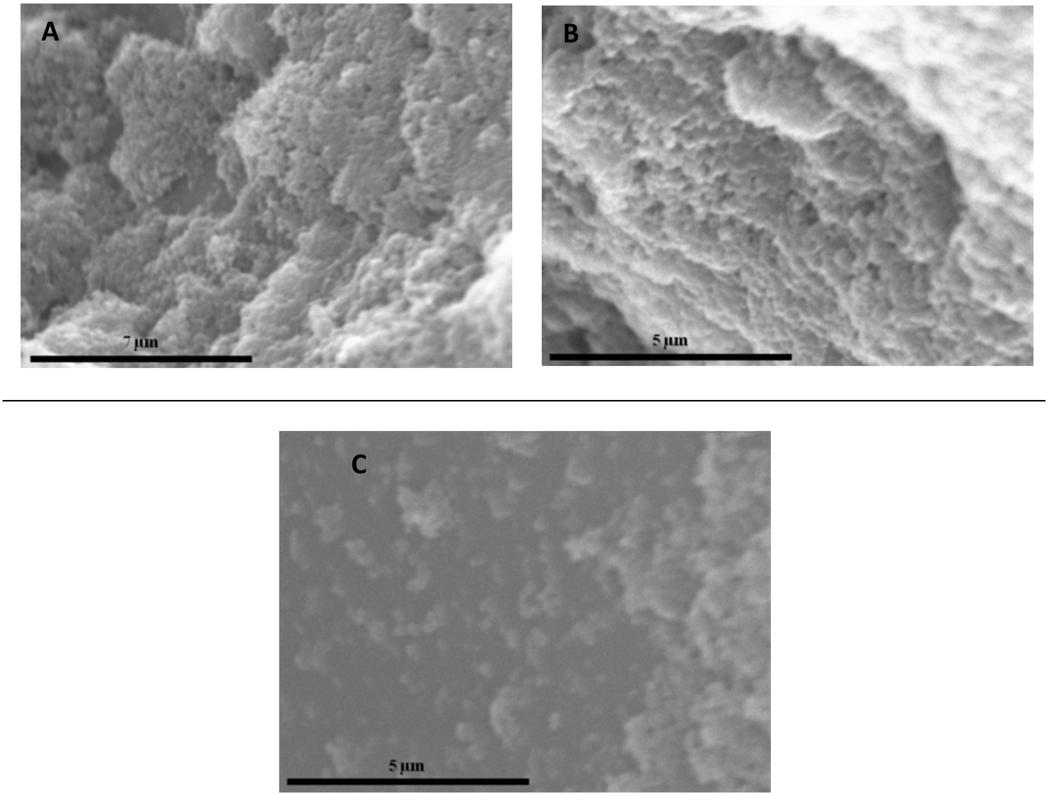

Fig. 3. SEM images obtained for the inner surface of the SWNHs immobilized fused silica capillary at different magnifications A) 6500, B) 10000 and C) 20000.

The electroosmotic mobility (μ_{eof}) of the SWNTs and SWNHs immobilized capillaries were $(40.4\pm0.9)\cdot10^{-5}$ and $(38.8\pm0.8)\cdot10^{-5}$ $cm^2\ V^{-1}s^{-1}$, respectively. The similarity obtained between these values revealed that the inner surfaces of the immobilized capillaries were negatively charged in both cases. The immobilization of these nanostructures in a fused capillary lead to a decrease in the negative charge of the inner capillary surface (μ_{eof} for a bared fused–silica capillary $(70.9\pm0.3)\cdot10^{-5}$ $cm^2\ V^{-1}s^{-1}$). This fact can be attributed to the charge balance between unreacted amino groups and carboxylic/hydroxylic groups on the CN surface.

A study of variation electroosmotic flow with respect to pH was carried out and the results are depicted in Figure 4. Through this test, it was found that at pH lower than 8.0, the electroosmotic flow of the bare capillary, c–SWNTs and c–SWNHs decreases, whereas at pH values above 8.0, this parameter is increased. This is because an increase in pH means an increase in the number of ionized carboxyl groups, thus, the overall negative charge within the capillary is greater.

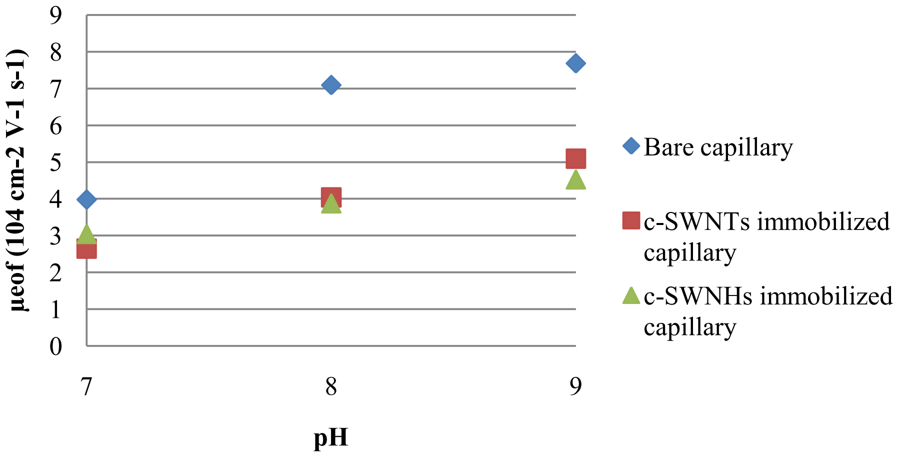

Fig. 4. Variation of electroosmotic flow with respect to pH of: bare capillary, c–SWNTs and SWNHs immobilized capillary.

3.3. Electrokinetic separation of water–soluble vitamins using surfactant–coated CNs as pseudostationary phase

Water–soluble vitamins, thiamine hydrochloride, ascorbic acid, riboflavin, pyridoxine hydrochloride and vitamin B_{12} were selected as target analytes for the comparison of SC–SWNTs (3.2 mg L^{-1}) and SC–SWNHs (3.0 mg L^{-1}) as pseudostationary phase in EKC. Under these electrophoretic conditions, the analytes are separated according to their effective charge and also by their particular interactions with the surfactant–coated CN (viz. pseudostationary phase). This additional partitioning effect increases the number of separation degrees of freedom and enables the separation of charged and uncharged analytes, thanks to the residual charge of the nanoparticles [25].

Table 1 shows the effective electrophoretic mobilities (μ_{effec}) and the resolution values obtained for each compound with both pseudostationary phases. The μ_{effec} were similar for both carbon nanoparticles. However, improved resolution was obtained with the SC–SWNTs as the mixture of the analytes was completely separated, while in the case of SC–SWNHs, vitamin B_{12} and riboflavin migrated at the same time. This result is also showed in Figures 5A and B which depicts the electrochromatograms obtained for a mixture of vitamins with SC–SWNTs and SC–SWNHs based pseudostationary phases. It is important to remark that in absence of surfactant–coated nanoparticles (BGE: 50 mM borate buffer, pH 8.0), vitamin B_{12} and riboflavin migrated with the EOF which hindered their determination under these electrophoretic conditions.

Table 1. Comparison of the effective electrophoretic mobilities and the resolution calculated for the vitamins with both PSPs tested.

	SC–SWNTs		**SC–SWNHs**	
	Resolution *R*	**Electrophoretic mobilities·10^{-4} ($cm^2\ s^{-1}\cdot V^{-1}$)**	**Resolution *R***	**Electrophoretic mobilities ·10^{-5} ($cm^2\ s^{-1}\cdot V^{-1}$)**
Thiamine		–3.10 ± 0.04		2.30 ± 0.30
$R_{thiam–B12}$	10.5		8.1	
Vitamin B_{12}		–4.65 ± 0.07		–6.04 ± 0.10
$R_{B12–Rib}$	7.5		0	
Riboflavin		–5.40 ± 0.10		–6.04 ± 0.10
$R_{Rib–Pyrx}$	8.6		4.8	
Pyridoxine		–6.50 ± 0.20		–6.53 ± 0.07
$R_{Pyrx–Asc}$	6.25		3.2	
Ascorbic acid		–7.57 ± 0.05		–7.04 ± 0.05

a) thiam: thiamine, B_{12}: vitamin B_{12}, Pyrx: pyridoxine, Asc: ascorbic acid.
Experiments conditions: BGE: 50 mM boric acid, pH 8.0. Applied voltage: 15 kV. Temperature: 20 ºC.

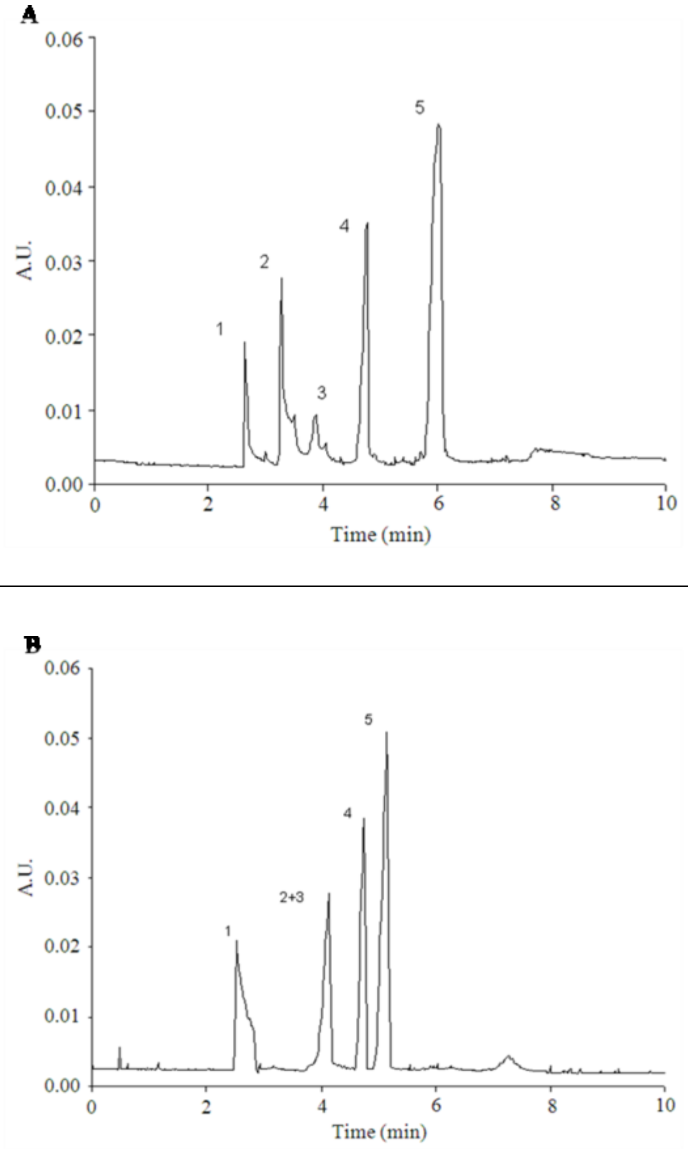

Fig. 5. Electropherograms obtained for a mixture of the target analytes: 1, thiamine; 2, vitamin B_{12}; 3, riboflavin; 4, pyridoxine; and 5, ascorbic acid at 100, 300, 300, 200 and 100 mg L^{-1}, respectively) using both carbon nanostructures as PSP A) 3.2 mg L^{-1} SC–SWNTs and B) 3.0 mg L^{-1} SC–SWNHs. The background electrolyte was 50 mM boric acid pH 8.0.

3.4. Electrochromatographic separation of watersoluble vitamins using CNs as stationary phase

Table 2 compares the electrochromatographic features of c–SWNTs and c–SWNHs immobilized capillaries for the separation of the five target vitamins.

The use of the c–SWNTs immobilized capillary resulted in good separation efficiencies. The retention factors and resolution that could be calculated were higher than those obtained for bared fused capillary. The same conclusion can be stated for the electrophoretic mobilities. However, for the analytes selected, the use of electrophoretic capillary immobilized with c–SWNTs did not improve the analyte separations and vitamin B_{12} migrates with the electroosmotic flow. Also, as it can be seen in Figure 6A, a high background noise in the baseline was obtained.

The electrochromatogram obtained for a mixture of the vitamins with the c–SWNHs immobilized capillary is shown in Figure 6B. This stationary phase allowed the complete separation of the analytes by means of afore mentioned interactions, which were also the responsible for the peak front tailing that can be observed in the electrochromatogram, being this fact more marked for pyridoxine and ascorbic acid.

Table 2. Electrophoretic parameters obtained for the SWNTs– and SWNTs immobilized fused silica capillaries.

	c–SWNTs immobilized fused silica capillary				c–SWNHs immobilized fused silica capillary			
	Number of theoretical plates, N (plates m^{-1})	**Retention factor k´**	**Resolution R**	**Electrophoretic mobility·10^{-5} ($cm^2\ s^{-1}\ v^{-1}$)**	**Number of theoretical plates, N (plates m^{-1})**	**Retention factor k´**	**Resolution R**	**Electrophoretic mobility·10^{-5} ($cm^2\ s^{-1}\ v^{-1}$)**
Thiamine	14400	−0.30		1.8 ± 0.9	8860	−0.33		1.9 ± 0.9
$R_{thiam–B12}$			–				3.03	
Vitamin B_{12}	–	–		–	17105	−0.22		8.0 ± 1.0
$R_{B12–Rib}$			–				5.60	
Riboflavin	24402	0.36		−1.0 ± 0.9	4570	0.27		−7.8 ± 0.5
$R_{Rib–Pyrx}$			6.66				5.15	
Pyridoxine	8294	1.07		−2.0 ± 0.7	10660	0.36		−1.8 ± 0.5
$R_{Pyrx–Asc}$			5.90				6.23	
Ascorbic acid	15430	2.01		−2.7 ± 0.5	8406	2.02		−2.6 ± 0.4

a) thiam: thiamine, B_{12}: vitamin B_{12}, Pyrx: pyridoxine, Asc: ascorbic acid

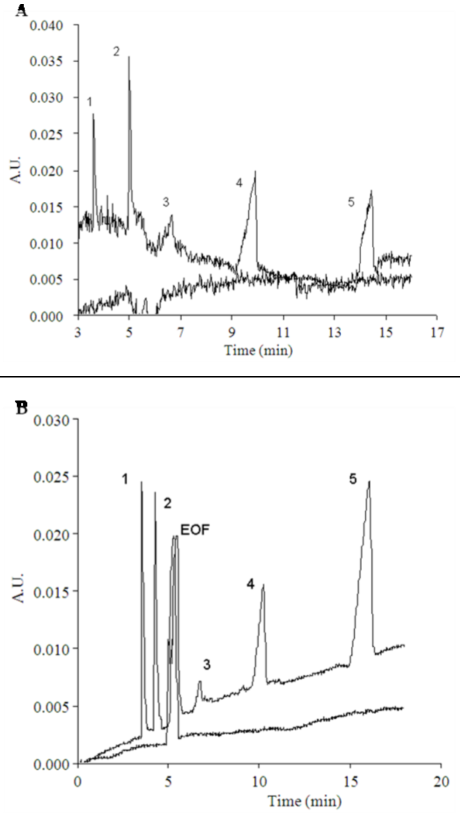

Fig. 6. Electropherograms obtained for the mixture of target analytes: 1, thiamine; 2, vitamin B_{12}; 3, riboflavin; 4, pyridoxine; and 5, ascorbic acid at 50, 150, 150, 100 and 50 mg L^{-1}, respectively) using A) c–SWNTs immobilized fused silica capillary and B) c–SWNHs immobilized fused silica capillary. The BEG was 50 mM boric acid pH 8.0.

The electrochromatographic features obtained for the c–SWNHs immobilized capillary showed good separation efficiencies and higher retention factors than those obtained with a bared fused–silica capillary and comparable with those obtained with the c–SWNTs immobilized capillary. As far as resolution is concerned, the c–SWNHs immobilized capillary showed an improvement on this parameter as the five analytes were completely separated and none of them migrated with the EOF. The advantages derived from the presence of c–SWNHs in the inner surface of the capillary can be explained owing to two types of interactions. On the one hand, the electrostatic interactions that occurred between the analytes and the carboxylic groups of the oxidized SWNHs, on the other hand, the π–π interactions that took place between the analytes and the dahlia flower–like superstructure owing to their large surface area. Considering the good results obtained, the analytical performance of the c–SWNHs as stationary phase in CEC was further evaluated.

3.4.1. Analytical figures of merit for the c–SWNHs immobilized capillary

Table 3 summarizes the main analytical features of the c–SWNHs immobilized capillary. The limits of detection and precision (calculated as relative standard deviation) of the electrochromatographic method were evaluated for the five water–soluble vitamins. The instrumental limits of detection were calculated by the direct injection of a standard solution of the analytes at variable concentrations. The concentration providing a signed–to–noise ratio equal to 3 was selected as the detection limit for the given analytes. The values obtained were ranged between 5 mg L^{-1} (thiamine) and 15 mg L^{-1} (vitamin B_{12}).

The reproducibility of the method was calculated using an aqueous standard (50 mg L^{-1}) between runs, days and capillaries, as shown in Table 3. In all cases, run–to–run (n=5) and day–to–day (n=10), the RSD values obtained for the retention times were lower than 1.0 and 1.9%, respectively. If the peak areas were considered as analytical parameter, the RSD values were lower than 2.5%. The capillary–to–capillary RSD values for the migration times varied between 2.0 and 3.0% (2.8–4.0% in terms of peak areas). Even though they are higher than the 1.0% allowed for this parameter, they were considered acceptable as the mixture of the five compounds was always resolved.

Finally, a regeneration step using a fresh solution of c–SWNHs every 50 injections allowed the use of the immobilized capillaries during 5 months without resolution losses.

3.4.2. Prospective application

Finally, the c–SWNHs immobilized capillary was applied to the determination of ascorbic in a pharmaceutical sample (tables). The sample was prepared as described in Section 2.5. The regression equation for ascorbic acid was $y=(0.049\pm0.002)x+(0.3\pm0.4)$, $R^2=0.991$ (where y = analytical signal, and x = analyte concentration in mg L^{-1}). The concentration of ascorbic acid found in the sample was 57±2 mg. That value was in accordance with the labeled content (60 mg) with a relative error lower than 10%. The recoveries obtained for a spiked sample with a mixture of the five vitamins are shown in Table 3. Those recoveries were satisfactory bearing in mind the tolerances allowed in pharmaceutical analysis.

Table 3. Reproducibility of c–SWNHs immobilized capillary modality, between runs and between days.

	LOD (mg L^{-1})	**Reproducibility (%RSD)**						**Concentr. spiked (mg L^{-1})**	**Recov. (%)**
		Migration time (min)			**Peak area**				
		Run–to run (n=5)	Day–to day (n=10)	Capillary–to–capillary (n=10)	Run–to run (n=5)	Day–to day (n=10)	Capillary–to–capillary (n=10)		
Thiamine	5	0.5	1.3	2.0	1.7	2.0	2.8	50	105 ± 3
Vitamin B_{12}	15	0.8	1.9	3.0	1.9	2.2	3.5	150	103 ± 1
Riboflavin	10	0.7	1.9	1.5	2.0	2.5	3.5	150	90 ± 5
Pyridoxine	8	0.8	1.5	2.9	1.8	2.3	3.7	100	98 ± 2
Ascorbic acid	8	0.7	1.5	3.1	1.6	2.4	4.0	50	97 ± 2

4. Concluding remarks

This paper shows for the first time the potential of SWNHs as pseudostationary and stationary phase in CE. The electrophoretic parameters have been systematically compared with those obtained with analogous CNs such as SWNTs. As a result, SC–SWNTs seem to be more adequate for their use as pseudostationary phase in EKC, as the interactions between the target analytes and SC–SWNHs did not improve the electrophoretic parameters. On the other hand, the electrochromatographic behavior of c–SWNHs immobilized on a fused–silica capillary was better than that of c–SWNTs as complete separation of the target analytes was obtained with the former.

Further research should be aimed at expanding the applicability of SWNHs to improve the electrophoretic separations, by including different functionalization of the nanoparticles and different agents capable of breaking the very stable dahlia aggregates. Interactions with analytes of different polarity and structure should be afforded in the near future.

It should be also be said that the analytical method proposed in this article can be extended to other samples by including the appropriate pretreatment steps in order to eliminate the potential interferences of the sample matrix for the quantitative analysis of thiamine, vitamin B_{12}, riboflavin, pyridoxine and ascorbic acid. The electrophoretic separation of other families of compounds such as non steroidal anti–inflammatory drugs will be afforded in the near future.

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Resultados y discusión

En este apartado de la memoria de Tesis Doctoral se resumen los resultados más relevantes obtenidos a lo largo del desarrollo experimental de la misma. En este sentido, se han mantenido los dos bloques en los que se ha estructurado la investigación llevada a cabo: nanoconos y nanocuernos de carbono como objetos y como herramientas analíticas en técnicas de (micro)extracción. En el primer bloque se va a hacer especial hincapié en las diferencias observadas en términos de purificación, caracterización y funcionalización. En el segundo apartado se compararán las propiedades sorbentes de las nanopartículas cónicas de carbono en relación con otros sólidos sorbentes convencionales como son el RP-C_{18} y el LiChrolut-EN; con nanotubos de carbono; y finalmente se compararán los nanoconos y nanocuernos de carbono entre sí.

1. Nanoconos y nanocuernos de carbono como objeto de análisis

El inicio de una línea de trabajo con un nuevo nanomaterial supone siempre un reto, cuyo elemento más crítico es poder adquirirlo en el mercado perfectamente caracterizado y con un nivel de pureza elevado, de manera que se pueda asegurar la reproducibilidad de los resultados obtenidos y que estos no dependan del lote o casa comercial que los suministre. En el campo de la Nanociencia y Nanotecnología, esto ha sido un cuello de botella para su desarrollo. Ocurrió en la década de los 90 con los fullerenos y en la primera década de este siglo con los nanotubos de carbono. Otra circunstancia a tener en cuenta es el elevado precio del material al inicio de su descubrimiento debido a las dificultades inherentes a los procesos de síntesis a gran escala.

Estos inconvenientes estuvieron presentes al inicio del trabajo experimental de esta Tesis Doctoral ya que, si bien se disponía de referencias sobre la utilidad de los nanocuernos de carbono para distintos fines ya comentados en capítulos anteriores de la Memoria, no existía en ese momento ninguna casa comercial que los distribuyera. De hecho, como se ha recogido en el capítulo dedicado a las herramientas analíticas, los primeros nanocuernos de carbono de pared simple, 200 mg, se obtuvieron por cortesía de Mer Corporation, cuya tradición en el suministro de nanopartículas de carbono es ampliamente reconocida. No obstante, la información facilitada por este proveedor se limitaba a las dimensiones del nanomaterial y una aproximación a la pureza del mismo.

De forma paralela, se evaluó también la posibilidad de incluir en la investigación otras nanopartículas cónicas, los nanoconos de carbono de los cuales ya existían algunas referencias en la bibliografía. En este caso sí fue posible encontrar una casa comercial que los suministraba con una buena caracterización del producto. Como se ha puesto de manifiesto en capítulos anteriores, el sólido comercial estaba formado por un 70% de discos de carbono, un 20% de nanoconos de carbono y un 10% de carbón amorfo (porcentajes en peso).

A la vista de esta situación, se hizo patente la necesidad de realizar varios estudios relacionados con la caracterización del material que se adquiría y de llevar a cabo, en su caso, procesos de purificación para poder eliminar posibles impurezas que interfieran en la utilidad analítica del material nanoestructurado. Este hecho era especialmente importante en el caso de los nanoconos de carbono, ya que la presencia del carbón amorfo introducía no sólo irreproducibilidad en las medidas, sino que además daría lugar a una pérdida de sensibilidad debido a la interacción irreversible entre los analitos y el material sorbente.

1.1. Caracterización de los nanocuernos de carbono de pared simple

La investigación correspondiente a este bloque de la Memoria se llevó a cabo en el Dipartimento di Scienze Chimiche e Farmaceutiche de la Università degli Studi di Trieste (Italia), en el grupo de investigación del Prof. Prato, especialista en la caracterización de nanopartículas de carbono. Se utilizó para ello la técnica de FlFFF acoplada a los detectores de UV-Visible y *multi-angle light scattering* (MALS) dispuestos en línea. El objetivo del trabajo realizado fue distinto dependiendo del nanomaterial analizado. En el caso de los nanocuernos de carbono de pared simple, la investigación estuvo centrada en encontrar en primer lugar una distribución de tamaños del material comercial con vistas a un posible fraccionamiento del mismo en base a este parámetro. En el caso de los nanoconos de carbono, el objetivo fue la separación del material nanoestructurado del carbón amorfo.

La primera dificultad que hubo que superar fue el poder disponer de una dispersión de las nanopartículas que fuese estable y que no presentase agregados que obturaran el sistema. Para ello fue necesario funcionalizar los nanocuernos de carbono de manera que, al generar grupos carboxilo e hidroxilo en la superficie de dichas nanopartículas, se consiguiera la solubilización de las mismas. De los distintos procedimientos descritos en la bibliografía, se optó por el empleo de la energía de microondas debido a la mayor rapidez del mismo frente al empleo de la radiación UV. Conviene resaltar que la facilidad de oxidación de los SWNHs contrasta notablemente con la dificultad de llevar a cabo el mismo proceso con los nanotubos de carbono, ya que requiere el tratamiento con ácidos fuertes y subsecuentes etapas de filtración y lavado. La complejidad del proceso dificulta la obtención de nanotubos oxidados con un porcentaje de grupos funcionales reproducibles.

La oxidación de los SWNHs se llevó a cabo utilizando un microondas doméstico y un equipo de microondas focalizado. En el primer caso se tomaron las condiciones descritas por Yoshida y Sano [1] y que implicaba una irradiación del sólido durante 10 min a 800 W de potencia. En el caso del equipo de microondas focalizado se llevó a cabo una optimización del tratamiento. Para ello, se realizaron análisis termogravimétricos y por espectroscopía Raman del material obtenido en cada caso de manera que se pudiera correlacionar la pérdida de peso con la generación o aparición de grupos funcionales en los SWNHs. Los resultados obtenidos se muestran la Tabla 1.

Como se puede comprobar en la tabla, las condiciones óptimas de funcionalización mediante el empleo de radiación microondas focalizada se corresponden a una potencia de 60 W y un tiempo de irradiación de 4 min, ya que bajo dichas condiciones es cuando se produce una mayor pérdida de peso al someter dichas muestras a un análisis termogravimétrico (TGA).

Tabla 1. Estudio de la influencia de las condiciones de irradiación focalizada de energía microondas en la oxidación de los SWNHs.

Condiciones irradiación microondas		Peso inicial	Peso recuperado	Pérdida de peso
Potencia (W)	**Tiempo (min)**	**(mg)**	**(mg)**	**(%)**
40	4	1.60	1.10	2.907
50	4	1.62	0.88	2.723
60	4	1.60	1.50	6.145
60	6	1.70	0.65	3.568
70	4	1.66	0.94	5.108
80	4	1.69	0.96	4.488
800[a]	10[a]	5.11	5.10	0.397

[a] Oxidación realizada empleando un microondas doméstico.

Para comprobar el grado de funcionalización se realizaron pruebas de espectroscopía Raman utilizando para ello un láser con una longitud de onda de 532 nm. Los resultados se muestran en la Figura 1.

A través de los espectros Raman obtenidos se calculó la relación existente entre las intensidades de la banda D (1300-1400 cm^{-1}) y la banda G (en torno a los 1600 cm^{-1}). Dicha relación indica el grado de funcionalización alcanzado, de tal modo que, cuanto mayor es la relación I_D/I_G, mayor es el grado de funcionalización de la nanopartícula. Como se puede ver en la gráfica, y en consonancia con los resultados obtenidos del análisis termogravimétrico, la funcionalización llevada a cabo mediante el uso de un microondas focalizado realizada a 60 W durante 4 min se consideró óptima.

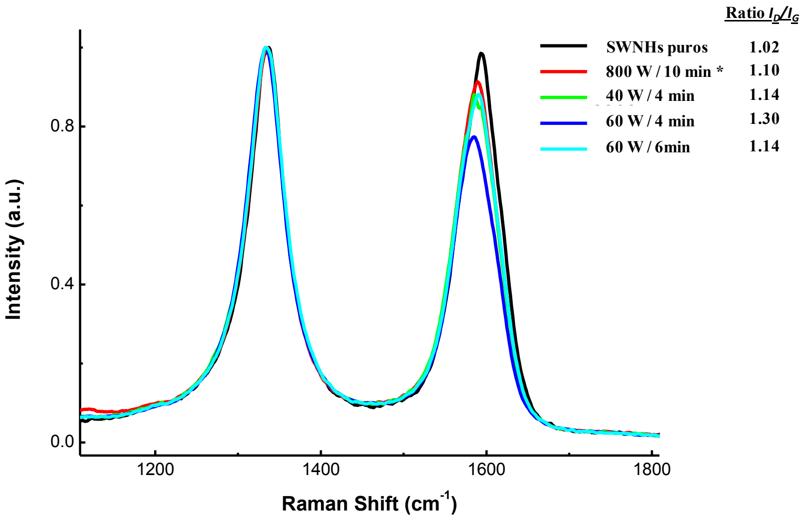

Figura 1. Espectros Raman obtenidos para SWNHs sometidos a distintos tratamientos con radiación de microondas.

Posteriormente, se utilizaron tanto los nanocuernos de carbono de pared simple oxidados mediante energía microondas (o-SWNHs) como nanocuernos de carbono funcionalizados mediante la técnica de cicloadición 1,3-dipolar (f-SWNHs) [2] para llevar a cabo su caracterización y separación por tamaños a través de la técnica de FlFFF. Para ello, se dispersaron los o-SWNHs y f-SWNHs en fase móvil acuosa (pH=9-10) que contenía SDS a una concentración de 0.086 mg/mL, inferior a la concentración micelar crítica, para de esta manera favorecer la estabilidad de la dispersión de las nanopartículas de carbono. A continuación, se llevó a cabo la optimización del procedimiento de separación evaluando los parámetros que afectan a la separación de las nanopartículas como son: a) la composición de la fase móvil, b) concentración de o-SWNHs y f-SWNHs (entre 0.01 y 0.1 mg/mL); y c) parámetros relacionados con el flujo obtenido en la cámara de separación (flujo de elución, flujo de inyección, flujo del detector y flujo cruzado). En la Tabla 2 se muestran las condiciones óptimas utilizadas en el fraccionamiento de los nanocuernos de carbono.

Tabla 2. Condiciones óptimas del fraccionamiento.

Parámetros de FlFFF	**Condiciones óptimas**
Fase móvil	NH_4OH (pH = 9-10)
Flujo de elución	2.0 mL/min
Flujo cruzado	1.0 mL/min o 1.5 mL/min
Flujo de inyección	0.3 mL/min
Volumen de inyección	100 µL
Concentración de la muestra	0.1 mg/mL
Tipo de membrana	Celulosa regenerada
Porosidad de la membrana	10 KDa
Espaciador	350 µm

Bajo estas condiciones se llevaron a cabo los siguientes estudios:

a) Separación de los o-SWNHs mediante un flujo cruzado de 1.0 mL/min y fraccionamiento del eluido en 5 fracciones.

b) Separación de los o-SWNHs mediante un flujo cruzado de 1.5 mL/min y fraccionamiento del eluido en 6 fracciones.

c) Separación de los f-SWNHs mediante un flujo cruzado de 1.0 mL/min y fraccionamiento del eluido en 5 fracciones.

Tras realizar dicho fraccionamiento, las muestras individuales obtenidas a la salida del equipo de FlFFF se evaporaron hasta reducir su volumen a 1 mL aproximadamente, con el objetivo de tener suficiente cantidad de nanopartículas dispersadas como para ser cuantificadas y poder medir sus diámetros mediante TEM. Después de realizar un análisis microscópico detallado, se compararon los resultados con los datos obtenidos mediante el análisis por UV-Visible y MALS. Dichos datos se pueden tratar mediante diferentes algoritmos (Zimm, Debye o Berry) en función de las características físico-químicas de las nanopartículas analizadas. Tras un estudio sistemático con los distintos algoritmos, se encontró que el modelo de cálculo Berry es el que mejor se ajusta a los tamaños determinados previamente mediante TEM. Esta selección estuvo en consonancia con las recomendaciones del fabricante del equipo de FlFFF ya que el sistema de análisis Berry se aconseja cuando se trabaja con moléculas con un diámetro superior a 50 nm.

Los resultados obtenidos que se han recogido en el Capítulo 1 de la Memoria, demuestran que la técnica de FlFFF se puede emplear para la caracterización, separación y purificación de nanocuernos de carbono de pared simple dispersados en matrices acuosas, y permite fraccionar las muestras para su posterior uso en otras aplicaciones sin que estas se vean física o químicamente alteradas.

1.2. Purificación de nanoconos/discos de carbono

Como se ha comentado en el apartado introductorio de la Memoria, la síntesis de nanoconos de carbono permite obtener un producto cuya pureza máxima esta en torno al 20% en peso. El 80% restante se distribuye entre discos de carbono (70%) y carbón amorfo (10%). Esta pureza puede aumentar hasta el 90% en peso ya que algunos autores consideran los discos de carbono como nanoconos de carbono que no presentan ningún pentágono en su estructura, lo que hace que no adquieran la forma cónica característica de los nanoconos.

Uno de los objetivos iniciales fue la separación de los distintos tipos de nanoconos (en función del ángulo de apertura del cono) de los discos y del carbón amorfo para poder obtener un material suficientemente homogéneo para emplearlo como herramienta analítica en el campo del tratamiento de muestra. En este sentido, se desarrollaron dos metodologías de purificación de nanoconos de carbono.

En primer lugar se optó por someter la mezcla comercial de nanoconos/discos de carbono y carbón amorfo a un tratamiento térmico en atmósfera de aire a distintas temperaturas y tiempos. Mediante este tratamiento se consiguió reducir la cantidad de carbón amorfo presente en el producto comercial, aunque, bien es cierto que un aumento de la temperatura (550 ºC) produjo un deterioro en las paredes de los nanoconos y los discos de carbono, como se muestra en la Figura 2. Las condiciones óptimas para eliminar el carbón amorfo sin deteriorar los nanoconos/discos y manteniendo unas condiciones óptimas de adsorción implican el calentamiento a 450 ºC durante 20 min bajo atmósfera de aire.

La otra metodología de purificación y separación de nanoconos/discos y carbón amorfo consistió en el empleo de la técnica de fraccionamiento en flujo por campo de flujo (FlFFF). Aunque no se han incluido datos experimentales en el Bloque I de la presente Memoria, cabe destacar que, tras llevar a cabo la optimización de las variables que afectan al proceso de separación de nanoconos/discos y carbón amorfo, se recogieron diferentes fracciones y se analizaron mediante TEM, comprobándose que mediante esta técnica de separación es posible retirar el carbón amorfo de la mezcla comercial que contiene nanoconos/discos de carbono. Sin embargo, la problemática derivada de la configuración instrumental impidió obtener datos que pudieran correlacionar los tamaños medidos por MALS y los obtenidos mediante TEM.

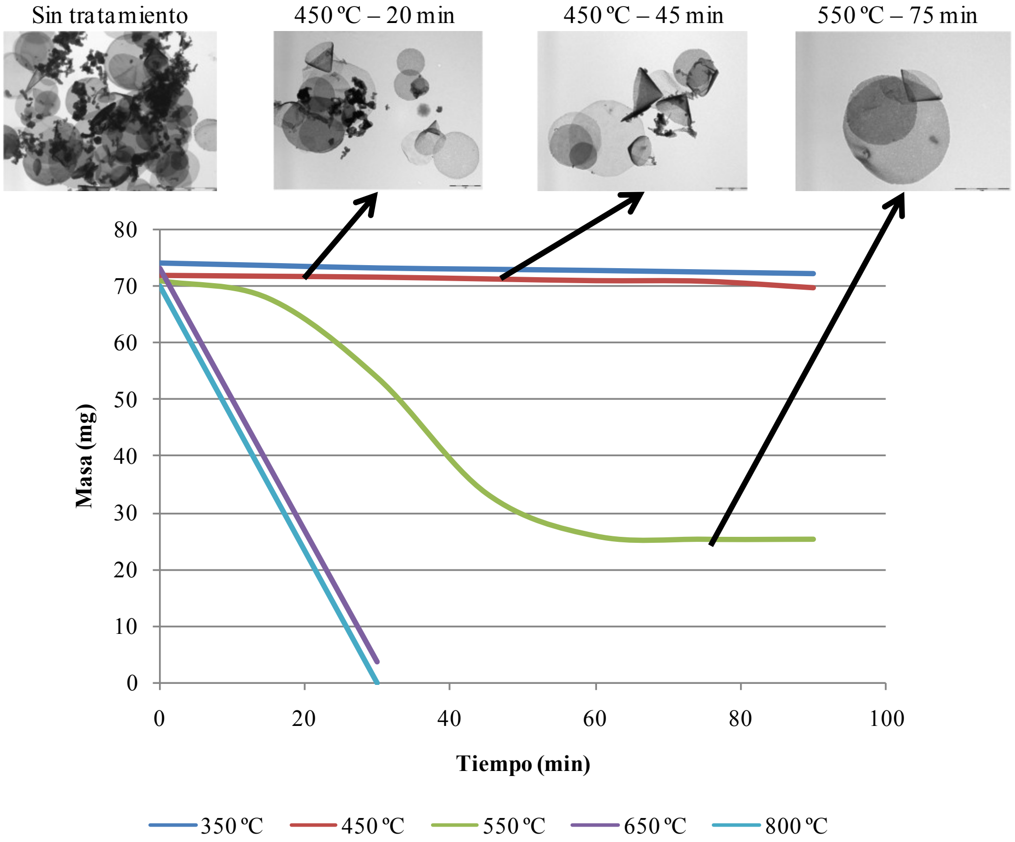

Figura 2. Pérdida de peso de los nanoconos/discos de carbono y carbón amorfo a diferentes temperaturas ensayadas. Las fotografías se tomaron mediante TEM y se corresponden con el tratamiento térmico aplicado a diferentes temperaturas y tiempos.

En resumen, resulta evidente que el tratamiento térmico es una alternativa más económica que el FlFFF si bien puede conllevar un deterioro de las nanoestructuras cuando la temperatura se eleva por encima del valor óptimo. Por otro lado, la técnica de FlFFF se puede utilizar para la caracterización y separación sin deterioro de las nanopartículas. No obstante, con vistas a obtener una elevada cantidad de material, el tratamiento térmico resulta más adecuado en términos de tiempo y coste instrumental.

2. Nanopartículas cónicas de carbono como herramientas en el proceso de medida

La miniaturización es una tendencia de la Química Analítica que ha ido adquiriendo importancia en los últimos años, implantándose en todas las etapas del proceso de medida. Sin duda, es la etapa de las operaciones previas la que más esfuerzos investigadores demanda a la hora de llevar a cabo una reducción de dimensiones en el tratamiento de las muestras. La miniaturización de las operaciones previas es de especial relevancia cuando se necesita trabajar con muestras que son valiosas, o de las que se pueden disponer de cantidades pequeñas. Esta miniaturización reduce también el coste del análisis por el menor consumo de reactivos y disolventes y la reducción del volumen de residuos que deben ser gestionados.

Dentro de las distintas subetapas que engloban las etapas previas del proceso de medida, se suelen incluir técnicas de separación no cromatográficas con vistas a aumentar la selectividad (eliminación de interferencias) y/o sensibilidad (preconcentración de los analitos) de la medida. Dentro de ellas, las técnicas de extracción, fundamentalmente líquido-líquido y en fase sólida, son las más habituales. La evolución de estas técnicas de extracción no ha sido ajena a la miniaturización, siendo inherente en algunos casos la automatización de las mismas. No obstante, para poder trabajar a esa escala reducida, resulta imprescindible el disponer de materiales (sólidos sorbentes y líquidos extractantes) cuya capacidad de extracción supere en eficiencia a los materiales convencionales, ya que de otro modo resulta inviable obtener factores de enriquecimiento elevados utilizando cantidades de sólidos o líquidos en el entorno de los micro-nanogramos o micro-nanolitros.

La Nanociencia y la Nanotecnología puede aplicarse al ámbito químico-analítico desde varias perspectivas, todas ellas complementarias y buscando dos objetivos básicos: miniaturizar los sistemas analíticos, en virtud del tamaño nanométrico del material y explotar las excepcionales propiedades de la materia. Esta última característica sólo se manifiesta si se mantiene el tamaño nanométrico. La combinación de ambos factores da lugar a los nanosistemas analíticos.

En el caso concreto de la incorporación de nanopartículas de carbono al proceso de medida, su papel es muy variado y se usan fundamentalmente en el tratamiento de muestra, separaciones cromatográficas y electroforéticas, y detección electroquímica.

En general, las propiedades químicas de las nanopartículas, con independencia de su naturaleza, son las más explotadas. Las propiedades eléctricas de las nanopartículas de carbono se han utilizado extensamente para el desarrollo de electrodos más competitivos. En el caso de las nanopartículas metálicas, (de oro, óxidos metálicos y puntos de luz), las propiedades eléctricas, ópticas y magnéticas compiten en utilidad con las químicas.

En el contexto de esta Tesis Doctoral, las aplicaciones desarrolladas se han centrado exclusivamente en evaluar las propiedades sorbentes de las nanopartículas cónicas de carbono (nanoconos y nanocuernos), en el ámbito de la (micro)extracción en fase sólida y la separación electroforética. La discusión de los resultados más relevantes alcanzados se realizará a través de una comparación de las tres nanopartículas de carbono a tres niveles: con materiales sorbentes convencionales; con otras nanopartículas de carbono (nanotubos fundamentalmente); y finalmente, de las dos nanopartículas cónicas entre sí.

2.1. Nanopartículas de carbono como sorbentes frente a materiales convencionales

Resulta difícil establecer una comparación directa sobre la capacidad sorbente de las nanopartículas de carbono respecto a los materiales convencionales dada la diferencia en el tamaño de partícula de los sólidos. Están ampliamente descritas en la bibliografía las ventajas que proporcionan los materiales nanométricos en el campo de la separación y la preconcentración de analitos, si bien se resaltan como principales inconvenientes la pureza a la que estos se pueden encontrar en el mercado y la irreproducibilidad del material disponible, consecuencia en muchos casos de los procesos de síntesis empleados. En ocasiones, es la poca homogeneidad del material, entendida como la diferencia en el tamaño de las nanopartículas, la que puede generar problemas de irreproducibilidad en sus resultados cuando son empleadas como sorbentes. No obstante, conviene destacar que estos factores se han ido minimizando con el paso del tiempo y el material disponible comercialmente está lo suficientemente caracterizado como para poder ser usado con garantía desde el punto de vista analítico.

No obstante, existe una limitación para el empleo de las nanopartículas de carbono en técnicas de extracción en fase sólida (SPE) que es la tendencia a la agregación que presentan éstas y que conlleva la pérdida de la dimensión nanométrica y por tanto la mayor capacidad sorbente frente a otros sólidos convencionales. Por ello, su uso empaquetadas en cartuchos de extracción en fase sólida no resulta especialmente adecuado. Este efecto es especialmente relevante para los nanotubos de carbono. La agregación del sorbente es un inconveniente para la retención de los analitos ya que disminuye la superficie activa disponible para interaccionar con los compuestos de interés.

Sin embargo, las nanopartículas de carbono resultan especialmente competitivas en las técnicas de extracción miniaturizadas. La microextracción en fase sólida emplea pequeñas cantidades de sorbente, en el orden de los microgramos, para aislar los analitos de interés de la muestra. Para mantener su eficacia, el material sorbente debe tener una gran capacidad de adsorción, de manera que se puedan alcanzar elevados factores de enriquecimiento y por tanto alcanzar valores de sensibilidad suficientemente elevados para poder detectar los analitos a bajos niveles de concentración, en la mayoría de los casos, fijados por la legislación.

Las nanopartículas de carbono presentan unas excepcionales propiedades sorbentes y una elevada área superficial, por lo que son una alternativa válida a los sólidos sorbentes convencionales, que por su menor eficacia no pueden utilizarse con resultados satisfactorios en las técnicas de microextracción en fase sólida.

En el trabajo desarrollado a lo largo de esta Tesis Doctoral no se ha llevado a cabo la comparación experimental del comportamiento del material sorbente convencional respecto a las nanopartículas de carbono debido a que la mayor parte de las herramientas desarrolladas se basan en técnicas de microextracción. Por lo tanto, la comparación más interesante es entre las nanopartículas entre sí. Esto se desarrollará en las secciones siguientes de este apartado de la Memoria.

2.2. Nanopartículas cónicas de carbono como sorbente frente a nanotubos de carbono

Los nanotubos de carbono y los fullerenos han sido las nanopartículas de carbono más empleadas en técnicas de extracción en fase sólida. Los primeros han demostrado ser más eficientes para la separación de compuestos orgánicos, mientras que las nanopartículas esféricas resultan más adecuadas para la deteminación de metales. Por tanto, en el contexto de esta Tesis Doctoral se han seleccionado los nanotubos de carbono a efectos de comparación con los nanoconos y nanocuernos de carbono como materiales sorbentes.

2.2.1. Nanoconos de carbono en SPE en cartuchos

En primer lugar, se seleccionaron los nanotubos de carbono de pared múltiple (MWNTs) para llevar a cabo una comparación de la eficacia de extracción de seis clorofenoles en muestras de agua mediante extracción en fase sólida en cartucho, frente a la extracción realizada con nanoconos/discos de carbono en el mismo dispositivo. Para ello, se empaquetaron 20 mg de ambas nanoestructuras (nanoconos/discos de carbono y nanotubos de pared múltiple) en diferentes cartuchos de SPE de 3 mL y se llevó a cabo el procedimiento analítico de extracción en fase sólida optimizado anteriormente mediante el uso de nanoconos/discos de carbono (2 mL de muestra acuosa que contenía a los seis clorofenoles, 200 µL de hexano para la elución de los clorofenoles e inyección de 2 µL en el GC/MS).

En términos de área de pico se pudo comprobar que, cuando se usaban nanoconos/discos de carbono como material sorbente, los resultados obtenidos fueron aproximadamente tres veces superiores a los proporcionados por los MWNTs, de tal modo que, para llevar a cabo una elución cuantitativa de los clorofenoles empleando los MWNTs fue necesario realizar 3 eluciones secuenciales con 200 µL, como se puede comprobar en la Figura 3. De esta comparación se puede concluir que, cuando la extracción en fase sólida se realiza con los MWNTs, los factores de preconcentración disminuyen y los límites de detección aumentan con respecto a los obtenidos mediante el uso de los nanoconos/discos de carbono como material sorbente.

Además, se consultó la bibliografía para corroborar estos hechos experimentales. Cai y colaboradores llevaron a cabo estudios para la extracción de cinco clorofenoles presentes en muestras de agua mediante el procedimiento de extracción en fase sólida empleando nanotubos de carbono de pared múltiple como material sorbente [3]. En este caso, los autores necesitaron 300 mg de MWNTs y 6 mL de metanol para llevar a cabo la extracción de los clorofenoles, utilizando 200 mL de muestra.

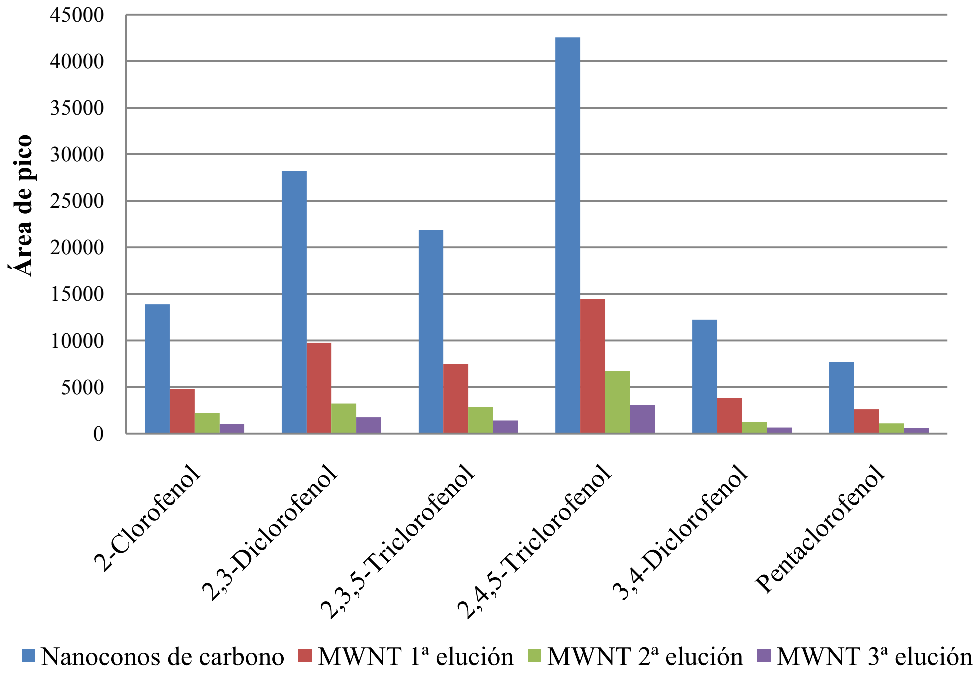

Figura 3. Comparación de los resultados obtenidos con nanoconos/discos de carbono y MWNTs (20 mg empaquetados en cartuchos SPE de 3 mL) como sorbentes para la preconcentración de seis clorofenoles.

Estos resultados ponen de manifiesto que es necesario utilizar mayores volúmenes de disolvente orgánico (en este caso metanol) para completar una elución cuantitativa de los clorofenoles cuando se emplean los MWNTs como material sorbente en SPE. Una reflexión sobre estos datos puede llevar a la conclusión de que tanto el tamaño y forma como la tendencia a la agregación del material sorbente empleado juega un importante papel en el proceso de extracción. De esta manera, la estructura cerrada y estrecha de los nanotubos de carbono, así como su alta tendencia a formar agregados, puede dificultar la interacción de las moléculas de analito con las paredes externas del nanotubo (e interior de la nanopartícula en caso de que se abran por procesos de oxidación), debido a la menor superficie efectiva disponible. Sin embargo, la baja tendencia a la agregación y la estructura abierta de los nanoconos/discos de carbono, facilitan en gran medida que los analitos interaccionen a través de interacciones π-π entre la parte aromática del analito y la superficie de las nanopartículas. Por otra parte, la mayor densidad electrónica que puedan presentar los nanotubos de carbono hace que la interacción con los analitos sea más fuerte y se requiera un mayor volumen de disolvente orgánico para conseguir la elución cuantitativa de los mismos.

2.2.2. Nanoconos/discos de carbono en SPME

También se evaluó la capacidad sorbente de los nanoconos/discos de carbono en la técnica de microextracción en fase sólida sobre fibra en su modalidad de espacio de cabeza (HS-SPME). Para ello, el primer reto abordado fue la selección del tipo de inmovilización de los nanoconos/discos de carbono sobre el soporte de microextracción. En este sentido, se consideraron dos técnicas de inmovilización: por un lado la técnica de sol-gel y por otro, el empleo de un pegamento orgánico. En esta Tesis Doctoral se seleccionó la segunda opción para inmovilizar los nanoconos/discos de carbono sobre una fibra de acero inoxidable de 3.5 cm de longitud. Para ello, se fabricó una pasta que contenía, además del material sorbente, una mezcla de compuestos orgánicos que actuaron como pegamento. Una vez inmovilizados, la fibra se sometió a un proceso de acondicionamiento a alta temperatura y en una corriente de gas inerte para eliminar todos aquellos compuestos volátiles que pudieran haber quedado como resultado del proceso y que podrían interferir en el análisis cromatográfico. La fibra preparada según este procedimiento se empleó en la modalidad de espacio de cabeza para la determinación del tolueno, etilbenceno, isómeros del xileno y estireno en muestras de agua.

En este caso no se comparó experimentalmente la eficacia de los nanoconos/discos de carbono y los nanotubos de carbono de pared múltiple (MWNTs) en HS-SPME, pero sí se recurrió a una comparación exhaustiva entre dichos sorbentes mediante los trabajos descritos en la bibliografía donde se usaban los mismos analitos (compuestos volátiles monoaromáticos) y la misma técnica de análisis (cromatografía de gases – espectrometría de masas).

Cabe destacar que en el momento de la publicación del artículo objeto de comparación (Capítulo 4), tan sólo existía un trabajo donde llevaba a cabo una microextracción en fase sólida de BTEX mediante nanotubos de carbono inmovilizados en una fibra [4]. Sin embargo, en ese artículo únicamente se evaluaba la metodología de inmovilización de los nanotubos de carbono sobre la fibra, y no se entraba en detalles analíticos ni en la realización de ningún tipo de aplicación a muestras reales. Es por ello por lo que en esta sección de Resultados y Discusión se ha optado por incluir una comparación más detallada incluyendo los tres nuevos trabajos aparecidos con posterioridad en la bibliografía. Los aspectos más relevantes se han incluido en la Tabla 3 donde se recogen diferentes conceptos como el tipo de inmovilización, el procedimiento de extracción y elución de SPME, las diferentes características analíticas de los métodos (limites de detección y precisión) y el análisis de muestras de agua (intervalos de recuperación).

Tabla 3. Comparación de distintas fibras de SPME preparadas con nanopartículas de carbono como material sorbente para la determinación de compuestos volátiles monoaromáticos en muestras de agua en la modalidad de espacio de cabeza.

Sorbente	Tipo de inmovilización	Reusabilidad (veces)	Volumen de muestra	Tiempo de extracción (min)	Detección	LOD (ng/mL)	Precisión (% RSD)	Recup. (%)	Ref.
Nanoconos y discos de carbono	Pegamento orgánico	100	10 mL	15	GC–MS	0.15 – 0.60	4.2 – 10.7	52 – 104	Método propuesto
Nanotubos de carbono funcionaliz.	Sol-gel	–	25 mL	20	GC–MS	–	1.8 – 2.5	–	[4]
o- SWNTs	Deposición electroforética	120	10 mL	20	GC–FID	0.005 – 0.026	1.5 – 5.6	75 – 105	[5]
o-MWNTs	Sol-gel	200	15 mL	30	GC–FID	0.6 – 3.0 pg/mL	4.4 – 5.8	90 – 102	[6]
o-MWNTs	Sol-gel	100	10 mL	30	GC–FID	0.001 – 0.8	4.7 – 7.1	–	[7]

GC: Cromatografía de gases; FID: Detección por ionización en llama; MS: Espectrometría de masas

En relación al artículo publicado por Jiang y colaboradores en 2009 [4], los nanotubos de carbono (no especifican si son de pared simple o de pared múltiple) se inmovilizaron mediante la técnica de sol-gel sobre una fibra de acero inoxidable previamente acondicionada durante 14.5 h. Posteriormente, los autores evaluaron la eficacia del proceso de inmovilización mediante el empleo de la técnica de SPME usando como analitos diferentes BTEX. Para poder llevar a cabo la inmovilización, fue necesario funcionalizar los nanotubos para hacerlos solubles en los disolventes orgánicos utilizados en el proceso. En nuestro caso, la buena capacidad de dispersión de nanoconos/discos de carbono en disolventes orgánicos (como el 1,2-dicloroetano) evitó esta etapa, reduciendo las fuentes de irreproducibilidad y prescindiendo del empleo de ácidos fuertes concentrados necesarios para la funcionalización de nanotubos, con el consiguiente riesgo para el operador. Para la obtención de la fibra, los autores idearon un dispositivo mediante el cual el soporte de acero inoxidable se introducía en la disolución sol-gel a una velocidad constante de 20 cm/min, controlando de esta manera el espesor del recubrimiento de la fibra. A continuación, procedieron a cortarla en trozos de 2 cm y a fijarla sobre un ensamblaje de SPME mediante un pegamento epoxi. En nuestro caso, se sumergió el soporte en la dispersión de nanoconos/discos dos veces hasta obtener un espesor de película de 50 µm. La uniformidad del mismo se comprobó mediante microscopía. No obstante, no fue suficientemente robusto como para traspasar el septum del inyector del cromatógrafo de gases, así que se optó por la desorción térmica de los analitos retenidos para poder reutilizar la fibra. La longitud óptima de la misma fue de 35 mm, lo que permitió disponer de una mayor cantidad de sólido y por tanto, mejorar la sensibilidad del método propuesto.

En el año 2010 se publicaron 3 artículos que empleaban nanotubos de carbono, de pared simple y pared múltiple, oxidados como material sorbente en SPME [5-7]. Las características más relevantes de los mismos se han resumido en la Tabla 3. En los tres casos se empleó la modalidad de HS-SPME.

En cuanto al método de inmovilización del nanomaterial sorbente, Li y col. optaron por una deposición electroforética de o-SWNTs sobre una fibra de platino empleada como ánodo; y, tanto Amiri y col. como Sarafraz-Yazdi y col. hicieron lo propio mediante la técnica sol-gel empleando MWNTs oxidados y posteriormente modificados con polietilenglicol. El tipo de inmovilización está directamente relacionado con la robustez de las fibras obtenidas. En este sentido, la de nanoconos/discos se pudo reutilizar 100 veces, Li y col., Amiri y col. y Sarafraz-Yazdi y col. aseguraron que sus fibras pueden ser utilizadas 120, 200 y 100 veces respectivamente sin que se vea afectada su capacidad sorbente.

En lo referente al proceso de extracción, cabe resaltar que en todos los casos se utilizaron 10 mL de muestra (a excepción de Amiri y col. que usaron un volumen de muestra de 15 mL) lo cual indica que no es necesario emplear elevados volúmenes de muestra para alcanzar unos buenos niveles de sensibilidad gracias a la elevada capacidad sorbente de los nanomateriales. En cuanto al tiempo de extracción requerido para completar la extracción, cabe resaltar que en nuestro caso fueron necesarios 15 min, mientras que Li y col., Amiri y col., y Sarafraz-Yazdi y col. requirieron tiempos de extracción de 20, 30 y 30 min respectivamente.

En relación con las características analíticas del método, tan solo Amiri y col. consiguieron alcanzar límites de detección del orden de los pg/mL, mientras que en el resto de metodologías estos se situaron en torno a los ng/mL. Teniendo en cuenta que de todos los compuestos volátiles monoaromáticos analizados, el benceno es el compuesto que tiene un nivel regulado por la legislación en aguas más bajo, 0.005 µg/mL según la Agencia de Protección Medioambiental (EPA) de Estados Unidos, todas las metodologías de extracción permiten la detección de estos compuestos en muestras de agua de interés medioambiental.

En los referente a la precisión de los métodos, expresada mediante la desviación estándar relativa utilizando una única fibra y llevando a cabo varias repeticiones del procedimiento de SPME, en el caso de la fibra de nanoconos/discos de carbono se obtuvieron valores de desviación estándar relativa inferiores al 6% (exceptuando el tolueno, con valores de 10.7%), lo cual estuvo en concordancia con los valores máximos de precisión obtenidos por el resto de autores, que se situaron en torno a 6.5%.

Por último, en relación con la recuperación de analitos de muestras de agua de diferente precedencia fortificadas, con la fibra de nanoconos/discos de carbono, exceptuando el tolueno en la muestra de agua de río fortificada a 20 ng/mL (valor de recuperación del 52%), el resto de valores estuvieron en un intervalo de 76 a 104%. Estos valores fueron similares a los obtenidos por Li y col. (75–105%). Amiri y col. consiguieron mejorar los valores de recuperación (90–102%).

De esta comparación se puede concluir que el empleo de nanoconos/discos de carbono en técnicas de microextracción en fase sólida puede ser una buena alternativa a los nanomateriales de carbono más empleados para este fin.

2.2.3. Nanocuernos de carbono inmovilizados en fibra hueca

En el desarrollo de esta Tesis Doctoral también se ha evaluado la capacidad sorbente de los SWNHs inmovilizados en los poros de una fibra hueca de polipropileno. El problema analítico modelo seleccionado fue la determinación de triazinas en aguas. Si bien en el artículo científico se lleva a cabo una comparación con otras técnicas de microextracción, en esta sección se van a comparar los resultados obtenidos con los publicados por Wu y col. [8], basados en la combinación de la microextracción líquido-líquido (LLME) y la técnica de microextracción en fase sólida con nanotubos de carbono inmovilizados sobre una fibra hueca. Estos artículos tienen algunos puntos de coincidencia, como pueden ser:

a) la familia de analitos empleada,
b) la funcionalización tanto de los SWNHs como de los MWNTs para mejorar la solubilidad. Esta etapa es más sencilla en el caso de los SWNHs ya que sólo requiere energía microondas y en el caso de los MWNTs se llevó a cabo a través de un tratamiento con ácidos fuertes,
c) el eluyente (metanol) y,
d) el tipo de muestras analizadas, las cuales fueron muestras de agua de diferente procedencia.

Las principales diferencias entre ambos métodos se comentan a continuación. La primera divergencia encontrada es la metodología de inmovilización de los nanosorbentes sobre la fibra hueca. Wu y col. dispersaron los o-MWNTs en 1-octanol y procedieron a inmovilizar el sorbente inyectando 15 µL de dicha dispersión dentro de una fibra hueca de 30 mm de longitud y sonicando posteriormente hasta que la mezcla quedó dispersada dentro de los poros de la fibra hueca. Los o-SWNHs se dispersaron en metanol y a continuación se sumergió una fibra hueca de 25 mm de longitud en dicha dispersión y se sometió a ultrasonidos para que los o-SWNHs quedaran retenidos dentro de los poros de la fibra hueca. Posteriormente, se retiró la fibra y se secó a 80 ºC durante 8 h. Puesto que esta etapa no se realizó en la fibra reforzada con o-MWNTs, tanto el material sólido como el 1-octanol actuaron como fase extractante.

Otra diferencia es el procedimiento de extracción. Wu. y col. partieron de una muestra acuosa (15 mL) a la cual añadieron NaCl y C_6H_5Cl (30 µL). A continuación, insertaron la fibra y la pusieron en contacto con el C_6H_5Cl mediante agitación durante 20 min. Inicialmente, las triazinas se extrajeron en el C_6H_5Cl y posteriormente se difundieron por los poros de la fibra hueca hasta quedar adsorbidas sobre los o-MWNTs. Por último, la elución se realizó introduciendo la fibra hueca en un vial que contenía 50 µL de metanol y sin necesidad de agitación. En el caso de los o-SWNHs, la metodología seguida fue la correspondiente a una microextracción en fase sólida mediante inmersión directa (DI-SPME). Para ello, se sumergió la fibra hueca con los o-SWNHs inmovilizados en una muestra de agua (50 mL) que contenía las once triazinas. Dicha fibra se mantuvo en la parte superior del vaso de precipitados fijando el fino alambre de acero inoxidable que se había introducido dentro de la fibra a una cinta adhesiva. A continuación las triazinas quedaron retenidas sobre los o-SWNHs mediante agitación de la muestra durante 15 min.

Posteriormente, se retiró la fibra de la muestra acuosa y se introdujo en un inserto de vidrio que contenía 150 µL de metanol para llevar a cabo la elución de las triazinas mediante agitación en ultrasonidos durante 5 min. Finalmente, se evaporó el disolvente orgánico y se reconstituyó el residuo con 10 µL de metanol para mejorar la sensibilidad.

Una vez optimizadas, ambas metodologías de microextracción se caracterizaron en términos de sensibilidad y precisión, obteniéndose límites de detección muy parecidos en ambos casos (ver Tabla 4) los cuales fueron suficientes para alcanzar los límites máximos permitidos por la Agencia de Protección Medioambiental norteamericana y la Unión Europea (UE) para estos compuestos en matrices acuosas, que son de 3 µg/L y 0.1 µg/L, respectivamente. Tan sólo un analito (simazina, con un límite de detección de 0.15 µg/L en la propuesta de Wu y col.) estaría fuera del límite establecido por la UE. La precisión de los métodos se evaluó en términos de repetitividad, llevando a cabo 5 repeticiones del procedimiento utilizando la misma fibra hueca en todos los casos. En el caso de los o-MWNTs, el intervalo de valores obtenidos en términos de desviación estándar relativa (RSD, %) fue de 4.6–6.9% mientras que con o-SWNHs oscilaron entre 5.1 y 10.2%.

Tabla 4. Comparación de la capacidad sorbente de o-SWNHs y o-MWNTs inmovilizados sobre fibra hueca para la determinación de triazinas en agua por inmersión directa.

Metodología de extracción	**Técnica instrumental**	**Tipo de muestra**	**Longitud de la fibra (mm)**	**Disolvente orgánico / Nanosorbente (membrana)**	**Elución**	**LD (µg/L)**	**Precisión (RSD, %)(n=5)**	**Recup. media (%)**	**Ref**
SPME de fibra hueca con o–SWNHs	GC/MS	Agua de rio, grifo y mineral embotellada	25	o-SWNHs	Metanol	0.05-0.10	5.1-10.2	90	Método propuesto
LLME (C_6H_5Cl) y HF-MMSPE simultánea	HPLC-diodos en fila	Agua de grifo, de lago y de escorrentía (5 triazinas)	30	o-MWNTs en 1-octanol	Metanol	0.08-0.15	4.6-6.9	92	[8]

LD: Límites de detección

RSD: Desviación estándar relativa

En relación con los valores de recuperación obtenidos para muestras fortificadas, la fibra con o-MWNTs proporcionó porcentajes entre 87 y 107% mientras que para o-SWNHs este rango estuvo entre 72 y 108%, si bien es cierto que los valores promedio de desviación estándar relativa fueron de 92 y 90%, respectivamente.

De esta comparación se constata la capacidad sorbente de los o-SWNHs inmovilizados en los poros de una fibra hueca, así como su uso en microextracción en fase sólida por inmersión directa para determinar y cuantificar once triazinas en muestras de agua de diferente procedencia.

2.2.4. Nanocuernos de carbono en microextracción en fase dispersiva

Finalmente, en el ámbito de las técnicas de microextracción se puede comparar la eficacia de los nanocuernos de carbono y los nanotubos de carbono cuando estos se utilizan como material sorbente en microextracción en fase sólida dispersiva. Cabe resaltar que en este caso, el objetivo principal del desarrollo de esta metodología fue el de preconcentrar analitos, y no tanto el de eliminar interferencias de la muestra, que es el origen de la técnica de extracción en fase sólida dispersiva [9].

Dicha comparación se realizó experimentalmente (Capítulo 6) empleando (a) nanocuernos de carbono de pared simple previamente funcionalizados mediante energía microondas; (b) nanotubos de carbono de pared simple oxidados mediante energía microondas (o-SWNTs), siguiendo el mismo procedimiento de funcionalización que el desarrollado para los nanocuernos; y (c) nanotubos de carbono de pared simple carboxilados (c-

SWNTs) comerciales. Como problema analítico modelo se seleccionó la extracción de ocho hidrocarburos policíclicos aromáticos mediante la técnica de microextracción en fase sólida dispersiva con las condiciones previamente optimizadas para el uso con nanocuernos de carbono.

En primer lugar, se llevó a cabo la dispersión en medio acuoso de las nanopartículas de carbono funcionalizadas. Mientras que los o-SWNHs y c-SWNTs se dispersaron sin dificultad en un volumen de agua establecido, este proceso no fue tan eficaz para o-SWNTs. Los resultados obtenidos en los tres casos se muestran en la Figura 4. Como puede observarse, los o-SWNHs presentan una mayor capacidad de adsorción que los o-SWNTs y los c-SWNTs. En realidad, en la dispersión obtenida de o-SWNHs se mantienen los agregados estables de estas nanopartículas comentadas en secciones anteriores de esta Memoria. Estos agregados que presentan una forma típica de dalia tienen mayor capacidad de adsorción que las nanopartículas individuales (por ejemplo, nanoconos) ya que presentan sitios adicionales de interacción. Así, además de la superficie de los SWNHs, dichas interacciones se pueden producir en el espacio interno del agregado y en las regiones intersticiales entre agregados. Además, como el proceso de oxidación puede abrir nanoventanas en los SWNHs, también se pueden generar sitios de interacción preferentes en los extremos cónicos y curvados de los mismos.

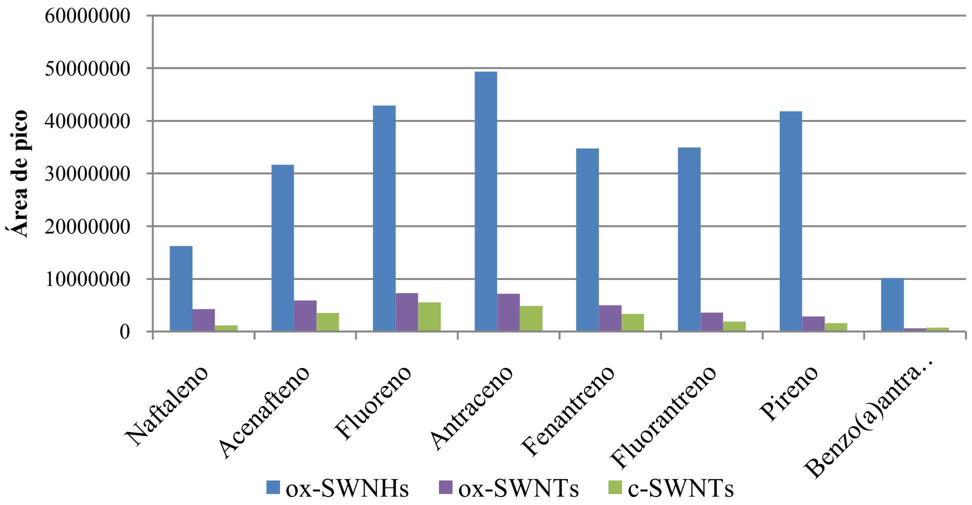

Figura 4. Comparación del comportamiento de los nanocuernos de carbono de pared simple oxidados, nanotubos de carbono de pared simple oxidados y nanotubos de carbono de pared simple carboxilados como sorbentes en microextracción en fase sólida dispersiva

2.2.5. Nanocuernos de carbono en electroforesis capilar.

Fuera del ámbito de las técnicas de (micro)extracción, también se ha considerado en esta Memoria la aplicabilidad de los nanocuernos de carbono monocapa en electroforesis capilar. En este contexto, se ha descrito la utilidad de las nanopartículas como aditivos del buffer electroforético (fase pseudoestacionaria) en la modalidad de cromatografía electrocinética micelar [10], así como inmovilizadas sobre la pared del capilar en electrocromatografía capilar. En el Capítulo 8, se ha comparado el comportamiento de SWNHs y SWNTs en ambas modalidades para llevar a cabo la separación de cinco vitaminas hidrosolubles (tiamina, vitamina B_{12}, riboflavina, piridoxina y ácido ascórbico). Así, se aprovecharon las capacidades de interacción de nanotubos y nanocuernos de carbono con moléculas que presentan cierta aromaticidad en su estructura y que además pueden presentar carga para hacerlas compatibles con el equipo de electroforesis capilar.

Puesto que en el artículo publicado se incluye una comparativa detallada entre la eficacia de separación llevada a cabo con ambas nanopartículas mediante las dos modalidades electroforéticas mencionadas, sólo se destacará a modo de resumen que los nanotubos de carbono de pared simple recubiertos de tensioactivo (dodecil sulfato sódico, SDS) permitieron llevar a cabo una completa separación de las cinco vitaminas hidrosolubles presentes en muestras de agua, mientras que, siguiendo la misma metodología y bajo las mismas condiciones experimentales, los nanocuernos de carbono de pared simple recubiertos con SDS no consiguieron separar entre sí dos vitaminas (vitamina B_{12} y riboflavina) de la mezcla de cinco analizadas, como se muestra en los electroferogramas de la Figura 5.

Además del SDS, se evaluó la eficacia de separación de los SWNHs cuando estos se dispersaron en otro tensioactivo como el dodecil bencenosulfonato sódico (NaDDBS). En la Figura 6 se muestra una imagen comparativa de dos dispersiones obtenidas con SWNHs a los que se les ha añadido SDS y NaDDBS a una concentración de 17.3 mM. El aspecto es diferente puesto que se ha descrito en la bibliografía que NaDDBS es capaz de romper los agregados de SWNHs y obtenerse las nanopartículas individuales recubiertas de tensioactivo [11]. Ambas dispersiones se ensayaron como fase pseudoestacionaria proporcionando el SDS mejores resultados. Esto puede explicarse en base a que una rotura de los agregados con NaDDBS resulta en un menor efecto sinérgico nanopartícula-tensioactivo, lo que conlleva menor capacidad de separación en cromatografía electrocinética micelar.

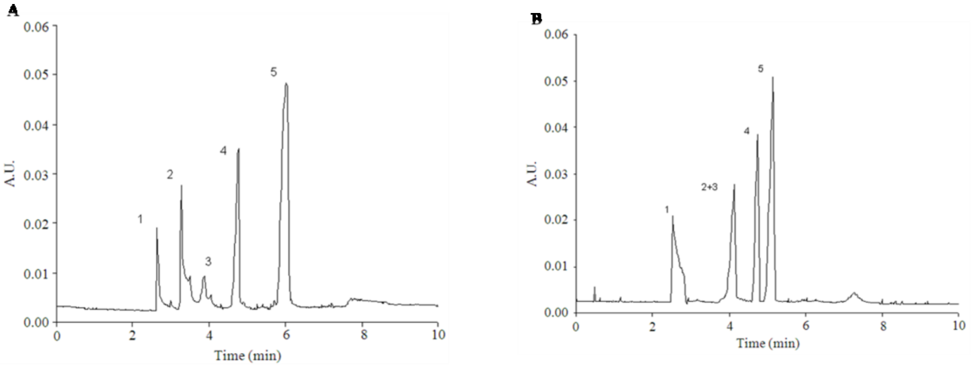

Figura 5. Electroferogramas obtenidos para una mezcla de los analitos diana: 1, tiamina; 2, vitamina B_{12}; 3, riboflavina; 4, piridoxina; y 5, ácido ascórbico a 100, 300, 300, 200 y 100 mg/L, respectivamente) usando ambas nanoestructuras de carbono como fase pseudoestacionaria A) 3.2 mg/L SC–SWNTs y B) 3.0 mg/L SC–SWNHs. El electrolito empleado fue ácido bórico a 50 mM y pH 8.0.

Sin embargo, cuando se utilizan los SWNHs previamente carboxilados mediante energía microondas e inmovilizados en el interior de un capilar de sílice fundida para la separación de las cinco vitaminas hidrosolubles mediante CEC, se obtuvo una mejora tanto en términos de capacidad de resolución como en la estabilidad de la línea de base, con respecto a los SWNTs carboxilados mediante un tratamiento con ácidos e inmovilizados a través del mismo procedimiento (Figura 7). En estas condiciones electroforéticas, los o-SWNHs resultaron ser más eficaces para la separación de los analitos de interés, lo que corrobora su excelente capacidad sorbente y su potencial en esta técnica electroforética.

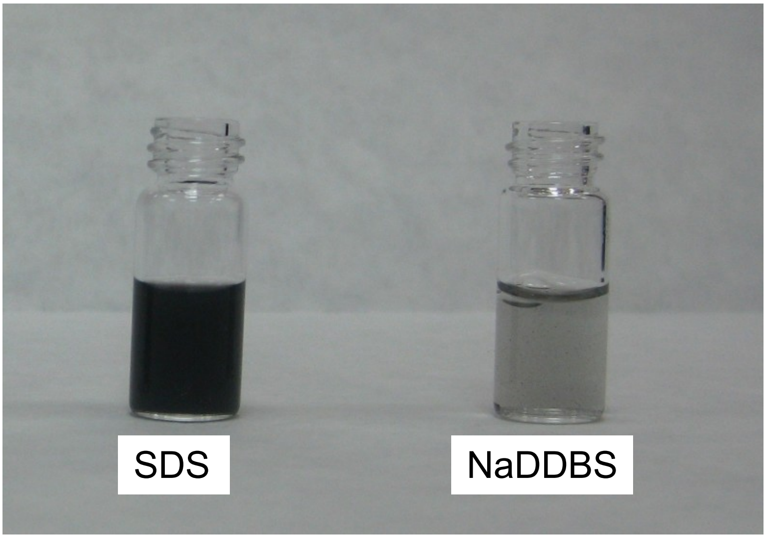

Figura 6. Fotografías de las dispersiones de SWNHs en SDS (17.3 mM) y en NaDDBS (17.3 mM)

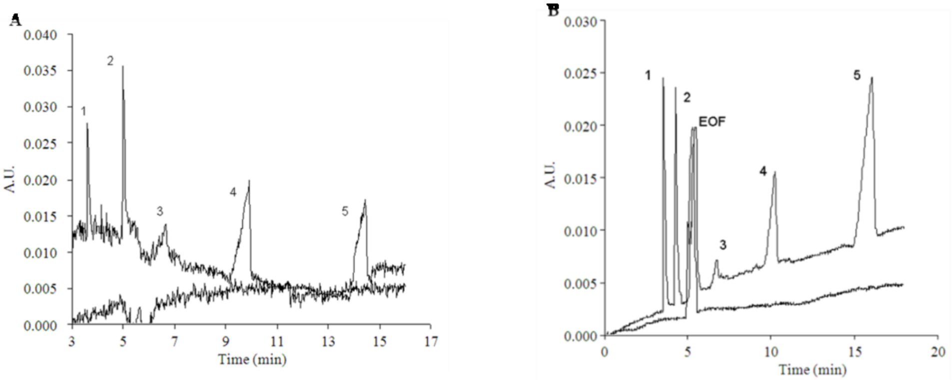

Figura 7. Electroferogramas obtenidos para una mezcla de los analitos diana: 1, tiamina; 2, vitamina B_{12}; 3, riboflavina; 4, piridoxina; y 5, ácido ascórbico a 50, 150, 150, 100 and 50 mg/L, respectivamente usando A) c–SWNTs inmovilizados sobre un capilar de sílice fundida y B) c–SWNHs inmovilizados sobre un capilar de sílice fundida. El electrolito empleado fue ácido bórico a 50 mM y pH 8.0.

2.3. Comparación entre nanopartículas cónicas de carbono: nanocuernos y nanoconos/discos de carbono

En esta última sección se abordará la comparación entre la capacidad sorbente de los nanocuernos y los nanoconos/discos de carbono en el marco de las diferentes técnicas de (micro)extracción en fase sólida desarrolladas en esta Tesis Doctoral.

En este sentido cabe destacar que, al comienzo del desarrollo del trabajo experimental no existía ninguna casa comercial que comercializara los nanocuernos de carbono. Es por ello por lo que se decidió realizar las primeras investigaciones empleando otro tipo de nanopartículas cónicas, los nanoconos de carbono. Por este motivo no se han obtenido resultados analíticos de la eficacia de extracción los nanocuernos de carbono en técnicas de extracción en fase sólida empaquetados en cartuchos (SPE), ni en microextracción en fase sólida inmovilizados sobre una fibra de acero inoxidable (SPME). No obstante, una vez que se dispuso de los SWNHs, y previamente al desarrollo de la investigación recogida en el Capítulo 6 de esta Memoria, se realizaron diferentes pruebas que consistieron en el empaquetamiento de 10 mg de SWNHs en cartuchos de extracción en fase sólida a través de los que se pasó un estándar acuoso con PAHs. A continuación, se recogió la fase acuosa, se extrajo con hexano y se inyectaron 2 μL en el GC-MS. Se obtuvieron señales muy bajas a los tiempos de retención característicos de los analitos, lo que corrobora la capacidad sorbente. Sin embargo, resultó técnicamente muy difícil mantener el empaquetamiento de los SWNHs en el cartucho, por lo que esta alternativa de extracción no parece la más adecuada.

En relación con el empleo de SWNHs en SPME, no se ha abordado en esta Memoria de Tesis y tampoco se han encontrado hasta la fecha referencias bibliográficas que hayan propuesto esta técnica utilizando SWNHs como fase extractiva.

Por otro lado, aunque no se ha recogido en el artículo correspondiente al Capítulo 5 de esta Memoria, sí que se pueden comparar algunos aspectos de la inmovilización de los nanoconos/discos y nanocuernos de carbono en la fibra hueca, ya que durante las pruebas preliminares de dicho trabajo se realizaron diferentes ensayos con ambas nanopartículas. En el caso de los nanoconos/discos de carbono, tras completar el proceso de inmovilización optimizado, se pudo apreciar que la fibra hueca resultante presentaba cierta heterogeneidad en su recubrimiento. Dicha irregularidad puede ser debida a la diferencia de tamaños existente entre ambas nanopartículas lo cual puede suponer un impedimento físico a la hora de la inmovilización de los nanoconos/discos de carbono dentro de los poros de la fibra ya que, según el suministrador de la fibra hueca, su tamaño de poro está en torno a los 0.2 μm, mientras que los nanoconos de carbono presentan unas longitudes que varían entre 0.3 y 0.8 μm y un diámetro máximo en la base del cono que oscila entre 1 y 2 μm, con lo cual, se puede suponer que los nanoconos/discos de carbono no penetren en los poros de la fibra hueca.

También se puede comparar la respuesta de ambas nanopartículas de carbono en la modalidad de microextracción en fase sólida dispersiva, tanto en capacidad de dispersión como en eficacia de extracción en la determinación de hidrocarburos policíclicos aromáticos (Capítulo 6 de la Memoria). Aunque no se ha recogido en el artículo, cabe resaltar la buena capacidad de dispersión en fase acuosa que presentaron tanto los nanoconos/discos tratados térmicamente, como los nanocuernos de carbono oxidados mediante energía microondas. Para completar dicha dispersión, las mezclas se agitaron mediante ultrasonidos durante 1 h, lo cual permitió que las dispersiones fueran estables durante al menos 24 h. Además, su redispersión fue posible mediante una agitación con ultrasonidos durante 1 h. De este modo se aseguró que dichas dispersiones fueran homogéneas y estables a lo largo del tiempo.

En cuanto a la comparación de la eficacia de extracción de ambas nanopartículas cónicas, previamente se hicieron dispersiones acuosas de cada sorbente a una concentración optimizada de 0.2 g/L. A continuación se añadió 1 mL de dicha dispersión a 10 mL de estándar acuoso que contenía ocho hidrocarburos policíclicos aromáticos a una concentración de 50 µg/L y la mezcla se mantuvo en agitación durante 2 min. Después, se separaron las nanopartículas sobre las que habían retenido los analitos mediante filtración sobre un filtro de Nylon de 0.45 µm de tamaño de poro. A continuación, se eluyeron con 100 µL de hexano, de los cuales se inyectaron 2 µL en el cromatógrafo de gases – espectrómetro de masas para su análisis cualitativo y cuantitativo. Evaluando las señales analíticas obtenidas en ambos casos (Figura 8), se puede concluir que los o-SWNHs proporcionaron mejores resultados en la microextracción en fase sólida dispersiva de hidrocarburos policíclicos aromáticos, siendo sólo ligeramente mejores los nanoconos/discos de carbono para la extracción del benzo(a)pireno. Sin embargo hay que señalar que la eficiencia de extracción de los nanoconos/discos de carbono se incrementó al aumentar el número de anillos aromáticos de los hidrocarburos policíclicos aromáticos. Este comportamiento puede explicarse teniendo en cuenta las dimensiones de los nanoconos/discos de carbono, que son aproximadamente 20 veces más grandes que los agregados de SWNHs. Esto favorece las interacciones de los hidrocarburos en la parte interna de los nanoconos de carbono. El menor tamaño de los o-SWNHs favorece la interacción de los analitos con la superficie de las nanopartículas.

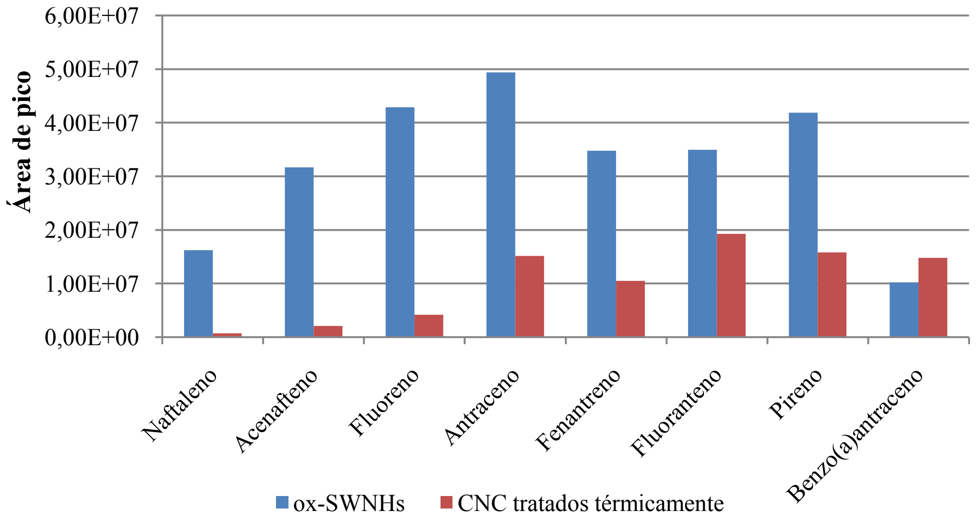

Figura 8. Comparación del comportamiento de los nanocuernos de carbono de pared simple oxidados frente a los nanoconos/discos de carbono tratados térmicamente en microextracción en fase sólida dispersiva.

Conviene además resaltar que el empleo de nanoconos/discos de carbono aumento la irreproducibilidad de los resultados, posiblemente debido a la heterogénea composición del producto comercial.

Como se ha comentado anteriormente, en el Capítulo 8 de la Memoria relativo al empleo de nanopartículas de carbono en la separación electroforética de vitaminas, se llevó a cabo una comparación entre los SWNHs y los SWNTs. Sin embargo, desde el inicio de esta tarea experimental se barajó la posibilidad de incluir en el estudio los nanoconos/discos de carbono como una nanopartícula más objeto de estudio. Sin embargo, se comprobó que su uso en los sistemas de separación electroforéticos está bastante limitado debido tanto a la dificultad que estos presentan a la hora de ser funcionalizados como a su mayor tamaño con respecto a SWNHs y SWNTs. Esta dificultad impide que se puedan usar como fase estacionaria en electrocromatografía capilar ya que no se pueden inmovilizar sobre las paredes internas del capilar de sílice fundida. Es por ello por lo que se descartó el uso de nanoconos/discos de carbono en ambas aplicaciones electroforéticas.

Con esto se cierra este apartado de Resultados y Discusión donde se han abordado de manera comparativa el trabajo experimental desarrollado en esta Memoria de Tesis Doctoral.

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Conclusiones

Conclusions

La investigación desarrollada a lo largo de estos cuatro años se ha centrado en profundizar en el conocimiento de las nanopartículas cónicas de carbono, en concreto los nanoconos/discos y nanocuernos de carbono de pared simple. La novedad de la temática y las dificultades encontradas al inicio se han recogido en los apartados previos de esta Memoria de Tesis Doctoral. La compilación de los resultados obtenidos, materializados en una serie de artículos científicos y la reflexión incluida en el apartado de Resultados y Discusión permiten realizar una evaluación crítica del trabajo realizado e identificar aquellos aspectos que aún quedan por abordar y que resultarán en el planteamiento de futuras líneas de investigación a desarrollar por el grupo.

Han sido dos las temáticas abordadas en esta Tesis Doctoral: profundizar en la caracterización de nanoconos/discos y nanocuernos de carbono, y explorar su potencial analítico en el campo de las técnicas de microextracción. En este apartado de la Memoria se resumirán las conclusiones de los resultados obtenidos para cada una de las nanopartículas cónicas de carbono estudiadas.

Los nanoconos/discos de carbono son productos comerciales que se pueden adquirir con una caracterización bastante detallada, siendo además la reproducibilidad del material suministrado bastante elevada, como se ha podido comprobar a lo largo de este tiempo.

El trabajo de caracterización se ha centrado sobre todo en la reducción del pequeño porcentaje (10%) de carbón amorfo que acompañaba a los nanoconos/discos, con vistas a mejorar su eficacia y reproducibilidad en su faceta aplicada. Esto se ha alcanzado satisfactoriamente tanto por calentamiento del sólido comercial en condiciones controladas de temperatura y tiempo, así como haciendo uso de la técnica de FlFFF. El tratamiento térmico resultó ser más adecuado para la obtención de elevadas cantidades de producto con fines analíticos. En este sentido, queda pendiente intentar la separación de los nanoconos de carbono de los discos de carbono. Asimismo se podría profundizar en procedimientos de funcionalización de este material para poder incrementar las aplicaciones de estas nanopartículas en técnicas de microextracción, electroforéticas y cromatográficas. Igualmente, sería también interesante incorporar (bio)moléculas sobre este nanomaterial para evaluar su potencial en el ámbito (bio)químico.

No existían en la bibliografía referencias relacionadas con el uso de los nanoconos/discos de carbono en técnicas de (micro)extracción en fase sólida, por lo que las aplicaciones desarrolladas en esta Memoria son las primeras hasta la fecha. Se han propuesto como sorbentes en técnicas de extracción en fase sólida en cartucho e inmovilizadas sobre una fibra metálica de microextracción en fase sólida. Su comportamiento empaquetadas en cartuchos convencionales de SPE fue mejor al de otras nanopartículas en base a su menor tendencia a la agregación. En cuanto a su empleo en SPME, la principal limitación es la poca resistencia mecánica de la fibra lo que imposibilita la introducción directa de la misma en el inyector del cromatógrafo para llevar a cabo la desorción de los analitos en el equipo. La necesidad de realizar una etapa de calentamiento en un vial y posterior inyección de un volumen del espacio de cabeza generado en el GC-MS conllevó una dilución final que repercutió negativamente en la sensibilidad del proceso analítico.

Los nanocuernos de carbono de pared simple se pueden adquirir recientemente en el mercado con una caracterización suficiente para garantizar la reproducibilidad de los resultados obtenidos. Partiendo de esta premisa, el mayor interés se centra en la caracterización de los agregados estables en forma de dalia que estos forman en disolución. Este estudio sistemático se ha abordado por primera vez en esta Memoria de Tesis Doctoral haciendo uso de la técnica de FlFFF, presentando los resultados obtenidos una buena correlación con las medidas realizadas por TEM de cada una de las fracciones separadas mediante la técnica instrumental.

En relación con la aplicación de estas nanopartículas en el ámbito de la Química Analítica, se han desarrollado herramientas novedosas en el contexto de las técnicas de microextracción en las modalidades:

- en fase sólida dispersiva con vistas a incrementar la sensibilidad de las medidas, aumentando el contacto entre la fase extractante y el analito mediante la dispersión de los nanocuernos funcionalizados en la muestra.
- soportadas sobre una fibra hueca porosa

resultando en ambos casos ser más eficientes que otras nanopartículas de carbono como los nanoconos/discos o nanotubos.

También se ha evaluado su potencial en dos condiciones electroforéticas, dispersadas con tensioactivo e inmovilizadas sobre la pared del capilar, resultando esta última alternativa la más eficiente para la separación de los analitos de interés en base al menor efecto sinérgico de los nanocuernos recubiertos de tensioactivo en relación con la presencia de tensioactivo en el *buffer* electroforético.

Finalmente, a lo largo de la Memoria se ha abordado el análisis de muestras relativamente simples, como muestras de agua para la determinación de analitos de naturaleza generalmente hidrofóbica (excepto las vitaminas hidrosolubles) y con un elevado grado de aromaticidad. Queda pendiente pues, extender la aplicación de las herramientas desarrolladas a matrices más complejas, así como evaluar la capacidad de extracción de las nanopartículas cónicas de carbono con otras familias de analitos con menor grado de aromaticidad e hidrofobicidad. Para ello podrían estudiarse distintas funcionalizaciones de los sorbentes para favorecer la interacción con dichos analitos. Por último, se podría evaluar si esta modificación superficial resulta adecuada para la extracción de especies inorgánicas, aspecto no contemplado en la Memoria.

The research conducted in this doctoral work was aimed at obtaining a deeper knowledge about carbon conical nanoparticles (specifically, nanocones/disks and single-walled nanohorns). The novelty of this topic and the initial difficulties encountered are described in previous sections. The body of results obtained, which have materialized in a series of scientific papers, and the reflections made in the Results and Discussion section, have allowed us to critically assess the work done and identify existing gaps to be addressed in future research to be conducted by our group.

This doctoral thesis was aimed to advance the existing knowledge about carbon nanocones/disks and nanohorns with a view to assessing their analytical potential in microextraction techniques. This section provides a brief, overview of the results obtained with each type of nanoparticle.

Carbon nanocones/disks are today commercially available in well-defined compositions that facilitate reproducible use as confirmed by their use in this work. Their characterization focused on the reduction of a small amount of amorphous carbon (10%) with an aim to improve their efficiency. This objective was fulfilled both by heating the commercially available solid under controlled temperature and time conditions, as well as by using the FlFFF technique. The thermal treatment proved more effective to obtain large amounts of product for analytical purposes. Two objectives remaining to be fulfilled in this respect are to accomplish the separation of carbon nanocones from disks and to advance existing functionalization procedures for this material in order to expand its use in microextraction, electrophoretic and chromatographic techniques, in addition to incorporate (bio)molecules into the nanoparticles and assessing their potential for (bio)chemical applications.

There was no single reference to the use of carbon nanocones/disks in solid-phase microextraction at the time this work was started, so those described here are the first ever to be reported. We have used them as sorbents for solid-phase extraction in cartridges, and also, immobilized on metal fibres, in solid-phase microextraction. Carbon nanocones/disks packed in conventional SPE cartridges were found to outperform other types of nanoparticles by virtue of their lesser tendency to aggregate. Their use in SPME is limited mainly by the low mechanical strength of the immobilizing fibre, which precludes its direct insertion into the chromatograph injector for desorption of the analytes. The need for heating in a vial prior to injection into a headspace volume of the GC–MS equipment introduces a diluting effect with an adverse impact on the analytical sensitivity.

<u>Single-walled carbon nanohorns</u> were recently made commercially available in reproducible compositions. Our greatest interest in this respect was to characterize the stable, dahlia-shaped aggregates they form in solution. This was for the first time systematically studied in this doctoral work, where the FIFFF technique provided quite good results consistent with TEM measurements of each fraction obtained with the instrumental technique. We have developed two interesting analytical applications for these nanoparticles in two different microextraction modes, namely: (a) in a dispersive solid phase used to enhance sensitivity of measurement, as a result of closer contact between the extractant phase and the analyte by means of the presence of functionalized nanohorns dispersed in the sample; and (b) in a hollow porous fibre. Both proved more efficient than other types or carbon nanoparticles such as nanocones/disks and nanotubes. Single-walled nanohorns were also assessed for use in electrophoretic applications, both

dispersed in a surfactant and also immobilized on the capillary walls. The latter choice proved the more efficient for separating the target analytes by virtue of the reduced synergistic effect of surfactant-coated nanotubes relative to the presence of the surfactant in the electrophoretic buffer.

We also addressed the analysis of relatively simple samples such as water containing mostly hydrophobic analytes (water-soluble vitamins excluded) of high aromaticity. In future work, the tools developed here will be applied to more complex matrices. Also, the extraction capacity of carbon conical nanoparticles for other, less hydrophobic and aromatic analyte families, will be assessed. For this purpose, sorbents functionalization methods intended to enhance interactions with the analytes will be studied. Finally, whether surface functionalization of these nanoparticles can facilitate extraction of inorganic species (a subject not addressed in this work) will possibly be tested and confirmed by further research.

Producción científica derivada de la Tesis Doctoral

ARTÍCULOS CIENTÍFICOS

Tipo de Publicación	Artículo Científico.
Autores	Juan Manuel Jiménez-Soto, Soledad Cárdenas, Miguel Valcárcel.
Título	**Evaluation of carbon nanocones/disks as sorbent material for solid-phase extraction.**
Revista	Journal of Chromatography A. Volumen 1216, Páginas 5626-5633.
ISSN	0021-9673.
Índice de impacto	3.756. (Sexta posición en la sección de Química Analítica del *Journal Citation Report* de 2008)

Tipo de Publicación	Artículo Científico.
Autores	Juan Manuel Jiménez-Soto, Yolanda Moliner-Martínez, Soledad Cárdenas, Miguel Valcárcel.
Título	**Evaluation of the performance of single-walled carbon nanohorns in capillary electrophoresis.**
Revista	Electrophoresis. Volumen 31 (2010), Páginas 1681-1688.
ISSN	0173-0835.
Índice de impacto	3.509. (Séptima posición en la sección de Química Analítica del *Journal Citation Report* de 2008)

Tipo de Publicación	Artículo Científico.
Autores	Juan-Manuel Jiménez Soto, Soledad Cárdenas, Miguel Valcárcel.
Título	**Carbon nanocones/disks as new coating for solid-phase microextraction.**
Revista	Journal of Chromatography A. Volumen 1217 (2010). Páginas 3341-3347.
ISSN	0021-9673.
Índice de impacto	3.756. (Sexta posición en la sección de Química Analítica del *Journal Citation Report* de 2008)

Tipo de Publicación	Artículo Científico.
Autores	Jiménez Soto, Juan-Manuel; Cárdenas, Soledad; Valcárcel, Miguel.
Título	**Evaluation of single-walled carbon nanohorns as sorbent in dispersive micro solid-phase extraction.**
Revista	Analytica Chimica Acta. Volumen 714 (2012) Páginas 76-81.
ISSN	0003-2670.
Índice de impacto	4.311. (Sexta posición en la sección de Química Analítica del *Journal Citation Report* de 2010)

Tipo de Publicación	Artículo Científico.
Autores	Juan-Manuel Jiménez Soto, Soledad Cárdenas, Miguel Valcárcel.
Título	**Dispersive micro solid-phase extraction of triazines from waters using oxidized single-walled carbon nanohorns as sorbent.**
Revista	Journal of Chromatography A. Volumen 1245 (2012), Páginas 17-23
ISSN	0021-9673.
Fecha de Publicación	4.194. (Séptima posición en la sección de Química Analítica del *Journal Citation Report* de 2010)

Tipo de Publicación	Artículo Científico.
Autores	J. M. Jiménez-Soto, C. Hadad, A. Micoli, F. Giacalone, F. M. Tomas, S. Cárdenas, M. Valcárcel, M. Prato.
Título	**Purification, separation and diameter determination of modified single-walled carbon nanohorns using the flow field flow fractionation technique.**
Revista	Enviado a Carbon
ISSN	0008-6223.

Tipo de Publicación	Artículo Científico.
Autores	Jiménez-Soto, Juan Manuel; Cárdenas, Soledad; Valcárcel, Miguel.
Título	**Oxidized–single walled carbon nanohorns as sorbent for in–porous hollow–fiber solid phase microextraction**
Revista	Enviado a Analytica Chimica Acta.
ISSN	0003-2670.

CAPÍTULOS DE LIBRO/ENCICLOPEDIA

Tipo de Publicación	Capítulo de Libro.
Autores	Juan Manuel Jiménez-Soto, Rafael Lucena, Soledad Cárdenas, Miguel Valcárcel.
Título	**Solid-Phase (Micro)Extraction tools based on Carbon Nanotubes and Related Nanostructures.**
Libro	Carbon Nanotubes (IN-TECH, 2010). 766 páginas.
ISBN	978-953-307-054-4.
Fecha de Publicación	Enero de 2010.

Tipo de Publicación	Capítulo de Encyclopedia of Analytical Chemistry, por invitación.
Autores	Jiménez-Soto, Juan Manuel; Cárdenas, Soledad.
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Libro	Encyclopedia of Analytical Chemistry
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Título	**Evaluación de los Nanoconos de Carbono como material sorbente en Extracción en Fase Sólida.**
Tipo de participación	Póster.
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Autores	Jiménez-Soto, Juan Manuel.
Título	**Los Nanocuernos y Nanoconos de Carbono como Herramientas en Nanociencia y Nanotecnología Analíticas.**
Tipo de participación	Póster. ***Premio al Mejor Cartel Ilustrado.***
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Autores	Jiménez-Soto, Juan Manuel; Cárdenas, Soledad; Valcárcel, Miguel.
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Tipo de participación	Oral.
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Título	**Evaluation of the performance of single-walled carbon nanohorns in capillary electrophoresis.**
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Lugar y fecha de celebración	Córdoba, 14 y 15 de Enero de 2010.

Autores	Jiménez-Soto, Juan Manuel; Moliner-Martínez, Yolanda; Cárdenas, Soledad; Valcárcel, Miguel.
Título	**Evaluación del empleo de nanocuernos de carbono de pared simple en electroforesis capilar**
Tipo de participación	Oral y Póster. ***Premio al mejor Póster.***
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Lugar y fecha de celebración	Córdoba, 10 y 11 de Junio de 2010.

Autores	Jiménez Soto, Juan Manuel, Soledad Cárdenas, Miguel Valcárcel.
Título	**Sorbent properties of carbon nanoparticles: Nanotubes, Nanocones & Nanohorns.**
Tipo de participación	Oral y Póster.
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Autores	Jiménez-Soto, Juan Manuel; Cárdenas, Soledad; Valcárcel, Miguel.
Título	**Propiedades sorbentes de nanopartículas de carbono: Nanotubos, Nanoconos y Nanocuernos.**
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Autores	Caroline Hadad, Juan Manuel Jiménez-Soto, Alessandra Micoli, Chiara Fabbro, Soledad Cárdenas, Miguel Valcárcel and Maurizio Prato.
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Tipo de participación	Póster.
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Título	**Evaluación de los nanocuernos de carbono de pared simple como material sorbente en microextracción en fase sólida dispersiva**
Tipo de participación	Oral y Póster.
Congreso	XIII Reunión del Grupo Regional Andaluz de la Sociedad Española de Química Analítica.
Lugar y fecha de celebración	Málaga, 7 y 8 de Junio de 2012.