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Octadecyl functionalized core–shell magnetic silica nanoparticle as a powerful nanocomposite sorbent to extract urinary volatile organic metabolites

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ABSTRACT

In this present study, magnetic Fe_3O_4@SiO_2 nanoparticles (MNPs) functionalized with octadecyl groups (Fe_3O_4@SiO_2-C_{18} NPs) were synthesized, characterized and employed, for the first time, as powerful nanosorbent to extract endogenous volatile organic metabolites (EVOMs) namely, hexanal, heptanal, decanal, benzaldehyde, 4-heptanone, 5-methyl-2-furfural and phenol, described as potential biomarkers of cancer, from human urine. By using co-precipitation, surface modification methods, the carbon-ferromagnetic nanocomposite was synthesized and characterized by infrared spectrum (IR) and transmission electron microscopy (TEM). By coupling with gas chromatography–mass spectrometry (GC–qMS), a reliable, sensitive and cost-effective method was validated. To test the extraction efficiency of the carbon-ferromagnetic nanocomposite toward urinary EVOMs experimental variables affecting the extraction performance, including nanosorbent amount, adsorption time, elution time, and nature of elution solvent, were investigated in detail. The extraction process was performed by dispersing Fe_3O_4@SiO_2-C18 NPs into working solution containing targeted VOMs, and into urine samples, and then eluted with an adequate organic solvent. The eluate was collected, concentrated and analyzed by GC–qMS. Under the optimized conditions, the LODs and LOQs achieved were in the range of 9.7–57.3 and 32.4–190.9 ng/mL, respectively. Calibration curves were linear ($r^2 \geq 0.988$) over the concentration ranges from 0.25 to 250 ng/mL. In addition, a satisfying reproducibility was achieved by evaluating the intra- and inter-day precisions with relative standard deviations (RSDs) less than 3 and 11%, respectively. The method also afforded satisfactory results in terms of the matrix effect (72.8–96.1%) and recoveries (accuracy) higher than 75.1% for most of the studied EVOMs. The Fe_3O_4@SiO_2-C_{18} NPs-based sorbent extraction combined with GC–qMS revealed that the new nanosorbent had a strong ability to retain the target metabolites providing a new, reliable and high throughput strategy for isolation of targeted EVOMs in human urine, suggesting their potential to be applied in other EVOMs.

1. Introduction

Cancer is a leading cause of death worldwide accounted for about 8 million deaths. According to World Health Organization (WHO; www.who.int/en) the most widespread cancers in the developed world are lung, stomach, liver, colorectal and breast, accounting for half of cancer death. In European Union (EU) countries, breast, prostate, large bowel and lung cancers present the highest incidence rates in the 27 countries of the EU while lung, large bowel, breast and prostate cancers, present the highest ratio of mortality. Besides deaths due to cancer have decrease in recent years, due in part to improved early detection and better treatment, their incidence and mortality rates are still unacceptably high. The global burden of cancer continues to increase because of the aging of population through all the world alongside increasing cancer-causing behavior, such as smoking and unhealthy lifestyle in many

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countries [1]. Deaths from cancer worldwide are projected to continue to rise to over 13.1 million in 2030. This sobering fact in itself is a strong argument to pursue research both basic and clinical.

Early detection of cancer in the general population is highly necessary in a society where cancer is a leading cause of death, which minimizes therapeutic intervention and reduces morality [2]. Currently, different methods for diagnosis and staging are used in clinical practice including those based on diagnostic imaging such as X-ray, computed tomography (CT) [3], magnetic resonance imaging (MRI) [4], positron emission tomography (PET) [5], ultrasonography [6] and endoscopy [7]. However, these methods are both expensive and invasive, and in addition susceptible to false positive and false negative results, had associated risks and are very expensive. The ability to combine imaging techniques with other methodologies such as protein and gene profiling are new promising techniques with the potential for determining cancer risk or to enhance cancer detection. Although various biomarkers from blood, saliva and urine have been detected, including proteins, tumor antigens, anti-tumor antibodies, cell type-specific peptides, and epigenetic phenomena such as hyper-methylated DNA, RNA, and the expression of specific genes [5], to date none of these biomarkers has the adequate sensitivity, specificity and reproducibility to be considered for its clinical use. For this reason, the development of a novel diagnostic test, which can facilitate the early detection of cancer, could contribute to vastly reduce cancer mortality rates.

Since cancer is a disease which is known to alter cellular metabolism, recent investigations indicated that some volatile metabolites are related with the presence of cancer [8]. In this context a complementary, emerging and powerful approach that overcomes many constraints of the conventional techniques relies on the establishment of patterns of EVOMs that are linked to cancer and can be detected in biological fluids, namely exhaled breath, urine and saliva, from cancer patients. EVOMs emerged as a new frontier in medical diagnosis and as an attractive and exciting tool in disease prevention, monitoring therapy, disease recurrence and disease state monitoring. Using sensitive instruments, researchers have been able to measure and identify different patterns of EVOCs in urine samples from cancer patients compared to those who are cancer-free [9–13]. Many research groups reported some EVOMs which can be divided into four compound families, for instance, alkanes (e.g. undecane) [14], aldehydes (e.g. hexanal, heptanal, decanal, and benzaldehyde) [14–17], ketones (e.g. 4-heptanone) [17], and aromatic compounds (e.g. 5-methyl-2-furural and phenol) [17,18]. Thus, plentiful efforts have been made toward the development of EVOMs testing for cancer diagnosis.

Urinary EVOMs are recognized as a useful medical approach since they are the end-products of metabolic processes and non-invasively sampling [19]. Normally, a majority of methods developed for EVOMs monitoring in urine is sorbent-based extraction [20]. Briefly, the extraction process could be achieved due to the affinity of EVOMs existing in urine to solid sorbent. The sorbent therefore plays vital role in the extraction process. Among all kinds of sorbent, nanomaterials (NMs) are considered as good candidates for extracting urinary EVOMs of cancer patients because of their large surface area and fascinating dispersibility in usually aqueous solution [21], but high-speed centrifugation is usually inevitable in the extraction process [22]. Intensive centrifugation at high speed may lead to co-precipitation of undesirable interferents and even loss of some target analytes, which, in large part, restricts the application of these advanced materials [23]. A simple, rapid, gentle and efficient method is therefore urgently needed. In the past decades, separation technology based on magnetic NMs (MNMs) has become a powerful complement to sorbent-based extraction [24–26].

MNMs have been widely applied in various research fields, for example, chemosensors [27], catalysis [28], drug delivery [29], MRI [30] and sample preparation [27,31–33]. Although a number of magnetic materials are available now (e.g. iron, cobalt, nickel, magnetite, maghemite and alloys), Fe_3O_4 NPs are the most frequently used magnetic materials in sample preparation due to their easy preparation, surface modification and good recoverability [34]. Although bare Fe_3O_4 NPs can be directly used for isolation and pre-concentration of some target analytes, they are prone to the formation of large aggregates resulting in changes of their magnetic properties. In addition, their lack of selectivity makes them unsuitable for samples with complex matrices [35]. Normally, coating with silica is viewed to improve the stability and prevent oxidation of the Fe_3O_4 NPs, moreover, modification of Fe_3O_4@SiO_2 NPs can be achieved by silanation using silane coupling agents (e.g. C_{18}). In this study, core@shell Fe_3O_4@SiO_2 functionalized with octadecyl groups (C_{18}) were successfully synthesized, though this NPs have been widely used as sorbents for the adsorption and separation of methylprednisolone (MP) [36], lidocaine [37], and puerarin [38] from the complex matrix of rat plasma, and for removal of sudan dyes [39], pesticide residues [40], and polycyclic aromatic hydrocarbons (PAHs) [41] from water samples. Additionally, the adsorption of ergosterol [42] from cigarette could be achieved by employing Fe_3O_4@SiO_2-C_{18} NPs.

To our best knowledge, the extraction of EVOMs from the exhaled biological fluids of cancer patients, particularly urine, by Fe_3O_4@SiO_2-C_{18} has not been reported. Therefore in this paper we reported, for the first time, the use of MNPs Fe_3O_4@SiO_2-C_{18}-based as powerful nanosorbent to isolate urinary EVOMs. GC–qMS was used for qualitative and quantitative analysis. Explored the optimized combination of experimental variables with the help of an extract urinary EVOMs from cancer patients. To optimize the performance of extraction procedure, several experimental variables such as sorbent amount, adsorption time, elution time, and nature of elution solvent, were evaluated and compared. The newly Fe_3O_4@SiO_2-C_{18}/GC–qMS methodology was validated for selectivity, linearity, LODs, LOQs, accuracy, precision and matrix effect.

2. Experimental

2.1. Chemicals and reagents

All the reagents, purchased from commercially sources, were of analytical grade and used without further purification. Sigma-Aldrich (Madrid, Spain) provided the analytes: benzaldehyde (99%), phenol (99%), 4-heptanone (96%), toluene (99.8%) while decanal (95%), hexanal (96%), heptanal (95%), undecane (99%) and 5-methyl-2-furfural (98%) was obtained from Acros Organics (Geel, Belgium). The chemical structures of EVOMs investigated in this study are shown in Fig. 1.

For the synthesis of the Fe_3O_4@SiO_2-C_{18} NPs, required reagents were also obtained from Sigma-Aldrich. $FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$ and ammonia were used for the synthesis of the Fe_3O_4 NPs. Tetraethyl orthosilicate (TEOS) was employed for endowing a protective silica-based coating onto the surface of Fe_3O_4 NPs. Finally, octadecyltriethoxysilane was used to introduce hydrophobic groups on the Fe_3O_4@SiO_2 surface. Methanol (99.99%) and ethanol (99.99%) used as solvent were purchased from Fisher Scientific (Loughborough, UK).

The stock standard solution of each EVOMs were prepared in ethanol with a concentration of 100 mg/L and stored at −20 °C in darkness. Working solutions were prepared daily by appropriate dilutions of their stock solutions in the synthetic urine whose formula was described by Uppuluri et al. [43]. The ranges of concentrations (Table 1) were selected according to the sensitivity of the GC–qMS toward each EVOMs (as the physical–chemical