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Cork-based activated carbons as supported adsorbent materials for trace level analysis of ibuprofen and clofibric acid in environmental and biological matrices

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ABSTRACT

In this contribution, powdered activated carbons (ACs) from cork waste were supported for bar adsorptive micro-extraction (BAμE), as novel adsorbent phases for the analysis of polar compounds. By combining this approach with liquid desorption followed by high performance liquid chromatography with diode array detection (BAμE(AC)-LD/HPLC-DAD), good analytical performance was achieved using clofibric acid (CLOF) and ibuprofen (IBU) model compounds in environmental and biological matrices. Assays performed on 30 mL water samples spiked at the 25.0 μg L^{-1} level yielded recoveries around 80% for CLOF and 95% for IBU, under optimized experimental conditions. The ACs textural and surface chemistry properties were correlated with the results obtained. The analytical performance showed good precision (<15%), suitable detection limits (0.24 and 0.78 μg L^{-1} for CLOF and IBU, respectively) and good linear dynamic ranges ($r^2 > 0.9922$) from 1.0 to 600.0 μg L^{-1}. By using the standard addition methodology, the application of the present approach to environmental water and urine matrices allowed remarkable performance at the trace level. The proposed methodology proved to be a viable alternative for acidic pharmaceuticals analysis, showing to be easy to implement, reliable, sensitive and requiring low sample volume to monitor these priority compounds in environmental and biological matrices.

1. Introduction

The growing worldwide consumption of pharmaceuticals and their proved occurrence in the environment has become an important issue in recent years [1–4]. In this sense, the number of studies focused in the determination and/or removal of these contaminants has strongly increased. Although these pollutants are not considered in the water quality legislation they may be introduced in future, depending on the results concerning their occurrence, toxicity and potential harmful effects in the environment and on human health [5,6]. Pharmaceuticals in their original form or as metabolites, are continuously being introduced into sewage waters, mainly indirectly by excreta, and through disposal of unused or expired drugs, or directly in discharges from pharmaceutical manufacturing plants, influencing the quality of water resources [7,8]. Moreover, some of these pharmaceuticals are non-prescription drugs, such as the class of non-steroidal acidic anti-inflammatory drugs, which aspirin, ibuprofen, and diclofenac are good examples [9,10]. Considering that these substances are present in the environment at trace levels, suitable sample preparation approaches need to be applied to isolate and pre-concentrate the targets from complex matrices prior to chromatographic analysis [6], which represents a challenge since these compounds have very distinct structures and chemical properties.

Common enrichment techniques, like solid phase extraction [11,12], solid phase micro-extraction [13,14] and stir bar sorptive extraction [10,15], have been proposed to monitor many classes of priority pollutants. However, these approaches present low effectiveness, in particular, to recover trace levels of polar compounds. Therefore, novel sample preparation approaches must be developed using efficient extractive sorbents, such as polyurethane [16] or activated carbons (ACs) [17,18] to retain analytes with higher polarity. Recently, our group introduced a novel enrichment technique, bar adsorptive micro-extraction (BAμE), where supported materials such as commercial ACs showed to present convenient properties to retain polar compounds for trace analysis [19,20].

In the present study lab-made ACs prepared from cork powder waste were assayed as novel enrichment supported materials for BAμE, since their textural and surface chemistry properties compare favourably with those of commercial ACs [21,22]. Furthermore, cork-based ACs has showed to be suitable as supported adsorbents for the removal of pollutants in aqueous [21–24] or gas phase [25,26]. To test the performance of these novel ACs, ibuprofen (IBU), an over the counter anti-inflammatory drug and clofibric acid (CLOF), an active metabolite of blood lipid regulators, where selected as model compounds since both have been detected in the environment water worldwide [7,27,28]. The optimization of

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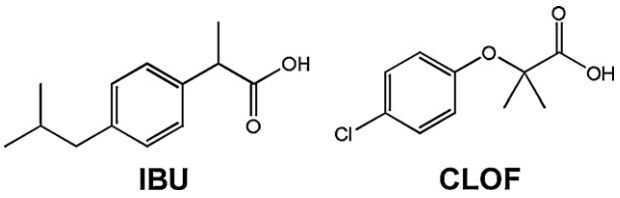

Fig. 1. Chemical structure of IBU and CLOF used in the present work.

the analytical process using liquid desorption (LD) prior to high performance liquid chromatography with diode array detection (BAμE(AC)-LD/HPLC-DAD), the influence of several experimental parameters, as well as, the cork-based ACs properties will be discussed in detail. The developed methodology was also validated and applied for the determination of traces of IBU and CLOF in environmental and biological matrices.

2. Materials and methods

2.1. Chemicals and samples

All reagents and solvents were of analytical grade and used with no further purification. HPLC-grade methanol (MeOH, 99.9%) and acetonitrile (ACN, 99.9%) were purchased from Merck (Germany). Sodium chloride (NaCl, 99.9%) and sodium hydroxide (NaOH, 98.0%) were purchased from AnalaR (England). *o*-Phosphoric acid (85.0%) was purchased from Aldrich (Germany). Hydrochloride acid (HCl, 37.0%) and sodium carbonate (99.5%) were purchased from Riedel-de Haën (Germany). Ultra-pure water was obtained from Milli-Q water purification systems (Millipore, USA). IBU was synthesized by Shasun Chemicals and Drugs Ltd. (lot IBU0307598) and CLOF was purchased from Alfa Aesar GmbH & Co KG (lot G1266B). The dimensions of both compounds were estimated by molecular modelization [29] and are (in nm) 1.03 (length) × 0.52 (width) × 0.43 (thickness) for IBU [23] and 1.90 (length) × 0.58 (width) × 0.50 (thickness) for CLOF [22]. Fig. 1 depicts the chemical structure of these two molecules. Tap, surface and groundwater samples were collected in the metropolitan area of Lisbon (Portugal). Estuary and sea water samples were collected in Tagus river and in Costa da Caparica (Portugal), respectively. Three wastewater samples were obtained from the urban treatment plant located in east side of Lisbon (Beirolas, Portugal). The water samples were collected before and after primary decantation and after the final treatment (ultra-violet disinfection). Two urine samples were collected in the morning from a healthy 27 years old woman after consumption of Brufen® 600 (2 doses, one per night) and from a healthy 29 years old man that, for control purpose, did not consume any kind of pharmaceuticals. All samples were previously filtered (Whatman No 1 filters) and stored refrigerated at 4 °C until analysis.

2.2. Experimental set-up

2.2.1. Cork-based ACs preparation and characterization

The ACs (C1 and C2) were prepared using cork powder waste (fraction < 0.297 mm) as raw material, following the experimental procedure described before [23]. Briefly, carbon C1 was obtained by chemical activation of cork with K_2CO_3 at 700 °C for 1 h (burn off degree 88%). Carbon C2 was prepared by further physical activation of Carbon C1 with steam at 750 °C for 1 h up to a burn-off degree in the physical activation of 66%. The textural properties of the carbon materials were characterized by N_2 and CO_2 adsorption at −196 and 0 °C, respectively. The nitrogen isotherms were obtained in an automatic volumetric apparatus ASAP 2010 from Micromeritics while the carbon dioxide isotherms were determined in a manual volumetric pyrex-made apparatus equipped with a pressure transducer from MKS-Baratron (310BHS-1000). Before the isotherms acquisition the samples (~50 mg) were outgassed for 2 h at 300 °C, under vacuum better than 10^{-2} Pa. Apparent surface areas, A_{BET}, were calculated from BET equation ($0.05 < p/p^0 < 0.15$) [30], microporous volume, V_{DR}, from the Dubinin–Radushkevich (DR) equation [30]. The mesoporous volume, V_{meso}, was obtained from the difference between the amount adsorbed at 0.95, V_{total}, and V_{DR}. Mean micropore half width, L_0, was evaluated from the characteristic adsorption energy, E_0 (obtained from the slope of the DR plots), and using the empirical equation $L_0 = (13.028 - 1.53 \times 10^{-5} E_0^{3.5})/E_0$ [31]. The microporosity characterization was complemented by applying α_S method, taking as reference the isotherm reported in Rodríguez-Reinoso et al. [32]. Applying this method the total microporous volume ($V_{\alpha\ total}$) and the ultra and supermicroporous volume ($V_{\alpha\ ultra}$ and $V_{\alpha\ super}$, respectively) were obtained. Additionally, the volume of ultramicropores was assessed from CO_2 adsorption isotherms at 0 °C with DR formulism. The ash content of the ACs was estimated by the mass residue left after the combustion of the samples in air, according to the procedure described elsewhere [23]. The samples were further characterized by the determination of the pH at the point of zero charge (pH_{PZC}), using the mass titration procedure [23,33].

2.2.2. BAμE(AC)-LD assays and method validation

The bar devices were prepared in the laboratory by supported polyethylene bars with adhesive films where powdered AC (approximately 4.0 mg for C1 and 3.0 mg for C2) was supported. The extractive bars were cleaned by treatment with ultra-pure water before use. In a typical assay, 30 mL of ultra-pure water spiked with CLOF and IBU working standards at desired concentrations and bar devices were introduced into glass flasks (50 mL, Macherey-Nagel, Düren). For the optimization of BAμE efficiency, assays were performed in a 15 stirring point plate (Variomag H + P Labortechnik AG Multipoint 15, Germany) at room temperature. Parameters such as stirring speed (750 and 990 rpm), extraction time (1, 2, 3, 4 and 16 h), pH (1, 2, 3, 5, 8 and 11), organic modifier (MeOH; 5, 10 and 15%, v/v) and ionic strength (NaCl; 5, 10 and 15%, w/v) were systematically studied in triplicate. The pH was adjusted with 5% (v/v) HCl or 0.1 M NaOH, and checked periodically using a Metrohm 744 pH Meter (Switzerland). To evaluate the best LD conditions, several assays using MeOH, ACN and equal volumes of both as back-extraction solvents and different desorption times (30, 45 and 60 min) were assayed in triplicate. For back-extraction, the bar devices were removed from the samples with clean tweezes, dried with a lint-free tissue, placed into a 2 mL vial (VWR, Portugal) containing 1.5 mL of the stripping solvent, ensuring their total immersion during the ultrasonic treatment at a constant temperature (25 °C). After back-extraction, the bar devices were removed by clean tweezes, the stripping solvent was evaporated to dryness under a gentle stream of nitrogen (>99.5%) followed by reconstitution with 200 μL of mobile phase. The vials were then sealed and placed on the auto-sampler for HPLC-DAD analysis. For the method validation experiments, 30 mL of ultra-pure water were spiked with 200 μL of both working standards at desired concentrations (1.0–600.0 $\mu g\,L^{-1}$), and the extraction and back-extraction assays were performed in triplicate as described above under optimized conditions. For real sample assays, 30 mL of water samples were used, spiked with both working standards at the desired concentrations (5.0–20.0 $\mu g\,L^{-1}$), and for biological matrices, 1 mL of urine was diluted to 30 mL with ultra-pure water, spiked with IBU working standard (20.0–600 $\mu g\,L^{-1}$). To suppress matrix effects the standard addition methodology (SAM) was used under optimized experimental conditions. Blank assays were also performed using the procedure described above, without spiking.

Table 1
Nanotextural characterization, ash content and pH_{PZC} of the two cork-based ACs.

ACs	A_{BET} ($m\,g^{-1}$)	V_{total}[a] ($cm^3\,g^{-1}$)	V_{meso}[b] ($cm^3\,g^{-1}$)	DR equation			α_s method			$W_{0\,CO_2}$ ($cm^3\,g^{-1}$)	Ash (%)	pH_{PZC}
				V_{DR} ($cm^3\,g^{-1}$)	E_0 ($kJ\,mol^{-1}$)	L_0 (nm)	$V_{\alpha\,total}$ ($cm^3\,g^{-1}$)	$V_{\alpha\,ultra}$ ($cm^3\,g^{-1}$)	$V_{\alpha\,super}$ ($cm^3\,g^{-1}$)			
C1	891	0.42	0.03	0.39	25.8	0.45	0.37	0.25	0.12	0.38	4.1	7.5
C2	1060	0.57	0.13	0.44	22.2	0.55	0.44	0.26	0.28	0.27	12.8	9.9

[a] Volume adsorbed at $p/p^0 = 0.95$.
[b] Difference between V_{total} and V_{DR}.

2.3. Instrumental settings

HPLC-DAD analyses were carried out on an Agilent 1100 Series LC system (Agilent Technologies, Germany), constituted by the following modules: vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), thermostated column compartment (G1316A) and the diode array detector (G1315B). The data acquisition and instrumental control were performed by the software LC3D ChemStation (version Rev.A.10.02[1757], Agilent Technologies). Analyses were performed on a Tracer excel 120 ODS-A column, 150 mm × 4.0 mm, 5 μm particle size (Teknokroma, Spain). The mobile phase consisted on a mixture of MeOH and 0.1% phosphoric acid with ratio of 40/60% (25 °C) and a flow of 1.0 mL min^{-1}. The injection volume was 20 μL with a draw speed of 200 μL min^{-1}. The detector was set at 220 nm. For identification purposes, standard addition was used by spiking the samples with both pure standards, as well as by comparing the retention parameters and peak purity with the UV–vis spectral reference data. For recovery calculations, peak areas obtained from each assay were compared with the peak areas of standard controls used for spiking. For quantification purposes on real matrices calibration plots using the SAM was also performed.

3. Results and discussion

3.1. Characterization of the cork-based ACs

The nitrogen adsorption isotherms (data not shown) of the two cork-based ACs have different shapes. Carbon C1 exhibits a type I isotherm characteristic of a microporous carbon, and carbon C2 presents a type I allied with type IV character [34], indicating that this is a micro + mesoporous carbon. Carbon C2 presents higher adsorption capacity and the adsorption isotherm has a rounder off knee for low relative pressures, than C1, revealing that the physical activation step led to a more pronounced development of porosity. Physical activation promoted the widening of the microporous size distribution due to the formation of larger micropores (supermicropores – pores with dimensions between 0.7 and 2.0 nm) and the development of mesoporosity, having the same dimensional order of magnitude of both target analytes estimated by molecular modelization (see Section 2.2). The N_2 isotherm of carbon C2 also shows a H4-type hysteresis loop, characteristic of slit-shaped pores where the adsorption and desorption branches are parallel. This analysis is in good agreement with the results of the nanotextural characterization quoted in Table 1. Carbon C2 has the highest micro, meso and total pore volumes and the main textural difference between the two samples is related with the supermicroporous volume, which almost triple after the physical activation with steam. The data obtained from CO_2 adsorption isotherms agrees with the data from the N_2 isotherms, since the micropore volume obtained by CO_2 for carbon C1 is larger than the corresponding volume of micropores assessed by N_2 confirming that the narrow microporosity (ultramicropores – width less than 0.7 nm) has a very important contribution to the overall porosity of this carbon. Additionally, the mesopore volume accounts for 7 and 23% of the total pore volume for carbon C1 and C2, respectively, confirming the essential microporous nature of carbon C1 and the micro and mesoporous structure of the carbon C2. From the data quoted in Table 1, it is clear that the preparation procedures led to ACs materials with different surface chemistry properties. The ash content of the carbon C2 is three times higher than that of carbon C1 revealing the higher inorganic content of the material activated with steam. Considering the pH_{PZC} values we can conclude that carbon C1 has a less basic surface than C2, since it presents a lower pH at the point of zero charge.

3.2. Optimization of the BAμE(AC)-LD assays

By combining a conventional reversed phase column with a mobile phase constituted by MeOH and 0.1% phosphoric acid aqueous solution, very good response was attained for both compounds through HPLC-DAD, showing enough resolution within convenient analytical time (<16 min). The instrumental limits of detection (LODs) and quantification (LOQs) for CLOF and IBU were calculated with signal-to-noise ratios (S/N) of 3/1 and 10/1, where values ranging from 40.0 to 50.0 μg L^{-1} for LODs, and from 120.0 to 140.0 μg L^{-1} for LOQs, were measured, respectively. Subsequently, the instrumental calibration was performed with eight standard solutions having concentrations between 0.15 and 90.0 mg L^{-1}, for which good linear dynamic responses were observed for both targets with correlation coefficients higher than 0.9998. The precision was also evaluated through injection repeatability studies, resulting in relative standard deviations (RSD) below 4.0% and no carry-over was observed by series of replicate injections (90.0 mg L^{-1}), since the background was always below the LODs achieved.

Throughout the present work, several parameters affecting BAμE efficiency yields were evaluated. Therefore, systematic assays were performed to optimize parameters that are known to influence the extraction process such as equilibrium time, agitation speed, matrix characteristics (pH, ionic strength and polarity) and back-extraction conditions using the two cork-based ACs as adsorbent phases.

In a first approach, we start to evaluate the best LD conditions that ensure complete back-extraction for both compounds from the supported adsorbent bar devices. Thereby, solvents such as MeOH, ACN and a mixture of both in equal volumes were assayed in order to survey the desorption performance followed by solvent switch, *i.e.* evaporation to dryness followed by reconstitution with mobile phase, which is the most convenient for HPLC-DAD analysis. From the data depicted in Fig. 2(a), obtained, under standard experimental conditions (extraction time: 3 h (990 rpm) and LD time: 45 min under sonification), the mixture of MeOH/ACN (50/50%) was chosen as LD solvent for further studies, due to the higher ability to desorb both targets from the cork-based ACs.

After the selection of the most effective solvent for back-extraction, LD times of 30, 45 and 60 min were also assayed. A slight increment on the recovery efficiency was observed from 30 to 45 min and no advantages were observed for longer periods of

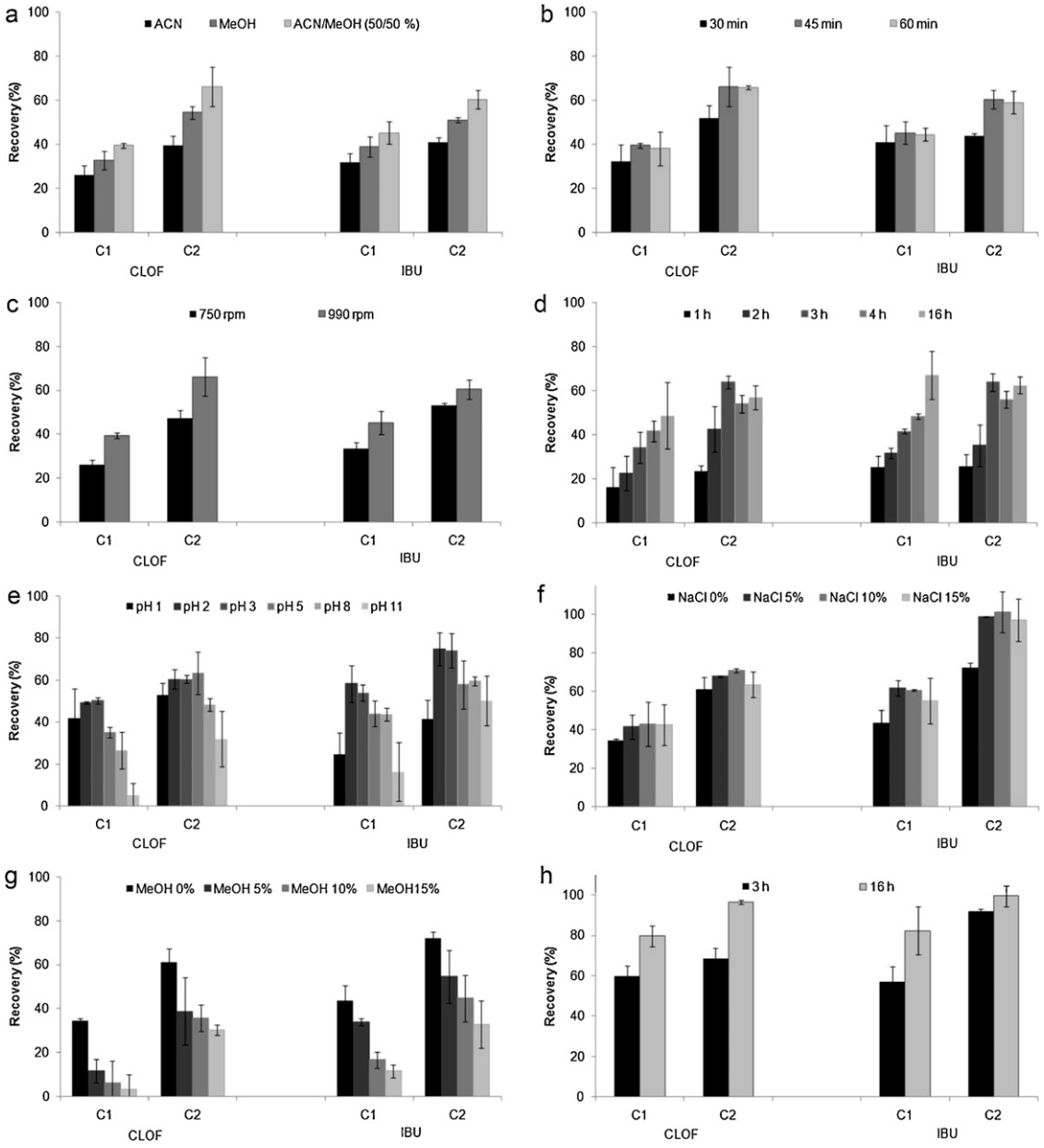

Fig. 2. Systematized effects on the recovery yields of CLOF and IBU from spiking ultra-pure water samples (25 $\mu g\,L^{-1}$) obtained by BAμE(AC)-LD/HPLC-DAD, using two (C1 and C2) cork-based ACs, For conditions see Section 2.2. Effect of solvent type (a) and time (b) on back-extraction. Effect of agitation speed (c), equilibrium time (d), matrix pH (e), ionic strength (f) and polarity (g) on extraction. Effect of equilibrium time under optimized conditions (h).

time (Fig. 2(b)). Consequently, 45 min under sonification treatment was established for the back-extraction process.

From the data obtained, carbon C2 presents always higher recoveries than carbon C1, for all the cases, showing that its textural and/or surface chemistry properties favour the overall process. Moreover, the adsorption capacity of carbons C1 and C2 for IBU and CLOF were already determined in previous studies [22,23] and are 145 and 378 mg g^{-1}, and 138 and 295 mg g^{-1}, respectively. Therefore, considering these values and the mass of ACs used in each bar device, it can be anticipated that, for the concentrations used in the present study, both carbons remove completely the two target compounds. Since the analytes are equally adsorbed by both ACs, the differences observed in the LD time effect must be only due to the back-extraction process. A possible explanation for the different behaviour of the ACs is related with their textural characteristics. In fact, carbon C1 has higher percentage of ultramicropores, pores where the adsorption energy is higher, and consequently, the back-extraction process is disfavoured when compared with carbon C2 that has lower ultramicropore volume. Moreover, carbon C1 has almost no mesopores (transport pores) and so, the analytes desorption from the porous structure will have more difusional constrictions than in the case of carbon C2. Another possible explanation could be related with the specific interactions carbon/analyte. However, considering that the working solution pH is 5.5, and both solids have neutral or positively charged surfaces ($pH < pH_{PZC}$) one could expect similar behaviours. On the other hand, at pH 5.5, IBU and CLOF molecules have different ionizations degrees (around 50 and 100% deprotonated, respectively), therefore if the specific interactions were the dominant factor of the process one could expect different recoveries achieve for each analyte [35,36]. The experimental data for each carbon are very similar pointing out that in these conditions the surface chemistry properties of both ACs are not responsible for their different performance.

Since the evaporation step is essential for solvent switch, it is necessary to check for possible analyte losses in this procedure. The results obtained shown that during the evaporation step only negligible losses of CLOF and IBU occur, since these compounds are non-volatile. Furthermore, no carry-over was observed by series of replicate desorptions, in which the background was always below the instrumental LODs achieved.

The evaluations of the equilibrium time and agitation speed effects are very important parameters to better control the enrichment conditions. Stirring rate can have a great influence in the mass transfer of the analytes towards the AC during the equilibrium process. Therefore, two levels (750 and 990 rpm) were assayed and the results (Fig. 2(c)) shown that the higher the stirring speed the greater will be the recovery yield for both targets, under the experimental conditions used. As a consequence, 990 rpm was selected for further assays. These results are in line with the fact that carbon C2 has a great open structure that lowers the difusional constrictions, and also with the fact that in this AC the target analytes are less strongly adsorbed.

Subsequently, the extraction time was also evaluated by performing experiments within 1 and 16 h, as illustrated in Fig. 2(d). From the data obtained, it is possible to notice that while for carbon C1, and both analytes, the higher the equilibrium time the higher is the recovery yield; for carbon C2 the recovery increases only up to an extraction time of 3 h, after what it remains almost constant. Once again, the behaviour of both ACs is most likely related with their textural properties. The adsorption of the probe molecules in the porous structure of carbon C1 has more diffusional limitations than in the case of carbon C2, as this last one has a higher percentage of mesopores and also wider micropores. From these results, carbon C2 seems to be the more suitable for this enrichment process, since it has equal or higher recoveries than carbon C1 for IBU and CLOF after just 3 h. To attain similar performance carbon C1 needs an extraction time of 16 h. Therefore, the extraction time was fixed at 3 h and 16 h for further assays with carbons C2 and C1, respectively.

In accordance with previous works [37], the characteristics of the aqueous medium, *i.e.* pH, ionic strength and polarity characteristics are also important factors with significant effects on the extraction efficiency, so these parameters were also studied individually. As expected, Fig. 2(e) shows that the pHs of the extraction step play an important role in the overall process. As it is well known, the ionization of the adsorbate and the carbon surface charge varies with the pH. On the first hand, pH changes affect the dissociation of IBU and CLOF that have pK_a 4.9 [35] and 3.6 [36], respectively. Since both molecules have a carboxylic acid group, if pH rises the deprotonation will occur leading to species negatively charged. Nevertheless for pH ≤ 2 both molecules are neutral. IBU and CLOF will have 50% of protonated and deprotonated species at pH around 4.5 and 3.5, respectively, and will be completely deprotonated (anionic form) for pH ≥ 7 and pH ≥ 5, respectively. On the other hand, pH changes affect the surface chemistry of the adsorbent due to the dissociation of the surface functional groups. The carbon surface may be either positively or negatively charged depending on the nature of the AC; therefore, at a given pH, the carbon surface and the adsorbate species may coexist in a complex system, in which the same or opposite charges may be present. However, as the cork-based ACs assayed has distinct pH_{PZC} values, the influence of the pH on the surface chemistry of both will be different.

Each of the ACs was assayed at six different pH values, chosen in order to study the overall process at different adsorbate–adsorbent systems, that is, systems with different charge interactions. The results show that for both ACs the best recoveries are obtained at acidic pH. In what concerns carbon C1 the best recovery yields, for both IBU and CLOF, are observed for pH 2 and 3, respectively. At these values, the surface of the AC is neutral or positively charged and the two adsorbates exist also as neutral species. For pH > 3, the recovery decreases, being more accentuated for CLOF, which is totally deprotonated at pH 5. Carbon C1 has the lower recoveries at pH 11, most probably due to repulsive electrostatic interactions, since at this value the surface of the carbon have a high negative charge and both molecules are in the anionic form. In the case of carbon C2 the best recoveries were also obtained at pH 2 and 3 for both molecules, decreasing for pH > 5 for CLOF and pH > 3 for IBU. At pH 11 the recoveries obtained with the carbon C2 are much higher than with carbon C1 since the pH_{PZC} of the former is 9.9 (Table 1), and so, at pH 11 the net positive surface charge is lower than that presented by the latter (pH_{PZC} (C1) = 7.5; Table 1). The overall data show that carbon C2 presents always better recoveries for all the pH studied, due to the fact that it has higher volume of micropores with suitable dimensions to adsorb the probe molecules and also because it has less acidic surface groups.

The ionic strength and polarity were modified through the addition of 5, 10 and 15% NaCl and MeOH onto matrix media,

Table 2
Recovery, correlation coefficients (r^2), LODs and LOQs achieved for IBU and CLOF in ultra-pure water samples by BAμE(AC)-LD/HPLC-DAD using the two cork-based ACs, under optimized experimental conditions.

ACs	Compounds	Recovery[a] (% ± RSD; n = 3)	r^2[b]	LOD (μg L^{-1})	LOQ (μg L^{-1})
C1	IBU	82.4 ± 11.9	0.9994	0.25	0.82
	CLOF	79.7 ± 5.2	0.9988	0.28	0.92
C2	IBU	99.6 ± 5.1	0.9922	0.21	0.70
	CLOF	96.5 ± 1.1	0.9972	0.24	0.78

[a] Method efficiency after extraction and back-extraction with AC in water sample spiked at the 25.0 μg L^{-1} level.
[b] Eight levels of concentration ranging from 1.0 to 600.0 μg L^{-1}.

Table 3
Regression parameters obtained from the SAM for both target analytes determined in environmental water and urine matrices by BAμE(AC)-LD/HPLC-DAD using the two cork-based ACs, under optimized experimental conditions.

Matrices	ACs	C1		C2	
	Compounds	IBU r^2[d]	CLOF	IBU r^2[d]	CLOF
Tap water		0.9920	0.9981	0.9976	0.9931
Surface water		0.9974	0.9933	0.9934	0.9962
Ground water		0.9962	0.9937	0.9964	0.9992
Estuary water		0.9934	0.9973	0.9911	0.9926
Sea water		0.9951	0.9969	0.9936	0.9961
Wastewater 1[a]		0.9914	0.9921	0.9936	0.9957
Wastewater 2[b]		0.9955	0.9900	0.9992	0.9970
Wastewater 3[c]		0.9925	0.9937	0.9971	0.9938
Urine		0.9986		0.9983	

[a] Before primary decantation.
[b] After primary decantation.
[c] After UV–vis treatment.
[d] Five levels of concentration.

Table 4
Content obtained from the SAM for IBU and CLOF determined in wastewater and urine samples by the proposed methodology, using the two cork-based ACs.

ACs	Compounds	Concentration (μg L^{-1})			
		Wastewater 1[a]	Wastewater 2[b]	Wastewater 3[c]	Urine
C1	IBU	13.6 ± 1.4	6.1 ± 0.7	<LOD	6.0 ± 0.4
	CLOF	17.8 ± 1.5	7.9 ± 1.2	<LOD	–
C2	IBU	15.0 ± 1.3	8.6 ± 0.3	<LOD	6.2 ± 0.4
	CLOF	18.9 ± 1.2	9.1 ± 0.7	<LOD	–

[a] Before primary decantation.
[b] After primary decantation.
[c] After UV–vis treatment.

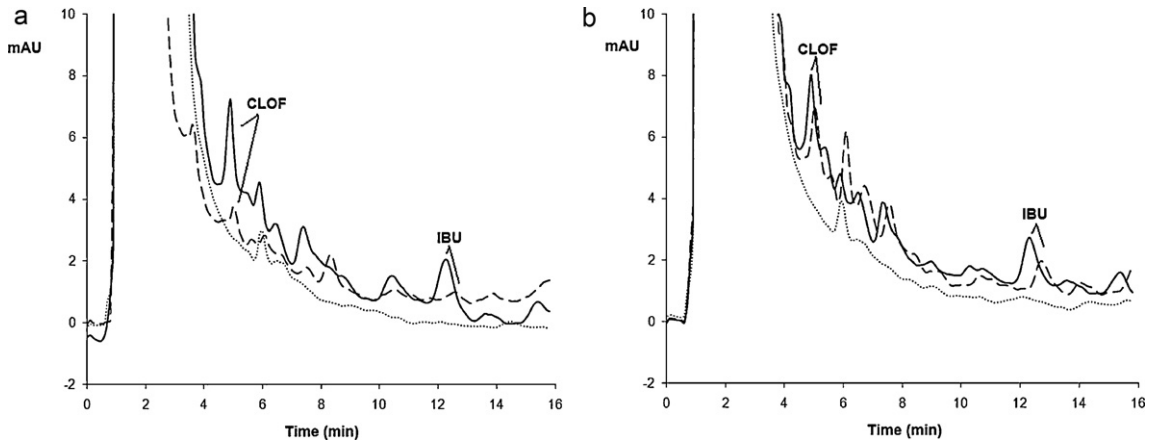

Fig. 3. Chromatograms profile from a wastewater sample without spiking, obtained by the proposed methodology using C1 (a) and C2 (b) cork-based ACs, under optimized experimental conditions (— before primary decantation; – – after primary decantation; ····· after UV–vis treatment).

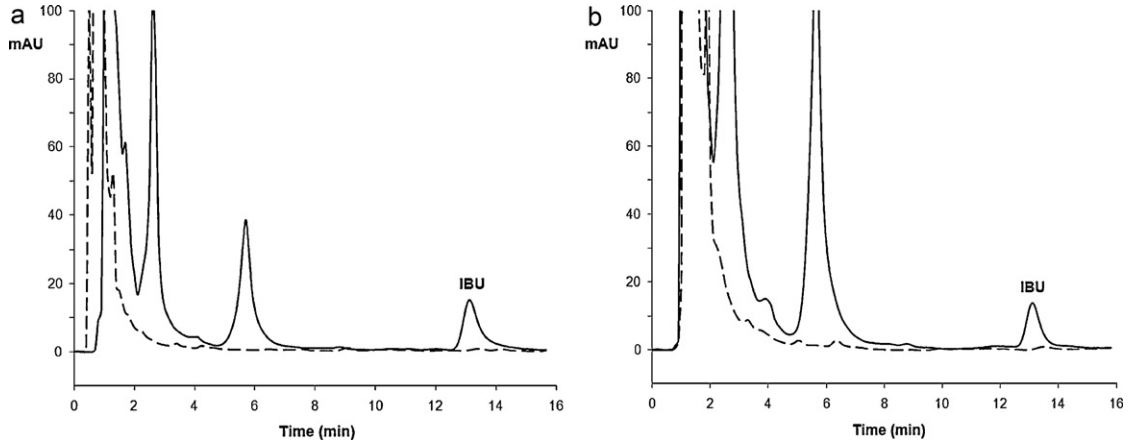

Fig. 4. Chromatograms profile from a urine sample without spiking, obtained by the proposed methodology using C1 (a) and C2 (b) using two cork-based ACs, under optimized experimental conditions (— urine with consumption of Brufen; – – urine without consumption of Brufen).

respectively. As depicted in Fig. 2(f), the salt addition produced a noticeable increment on the recovery of IBU for both ACs. On the other hand, the addition of MeOH has, in many cases, a positive influence on the recovery efficiency, eliminating the adsorption phenomena of the analytes onto the glass wall ("wall-effect") of the sampling flask [38]. However, as it is clearly observed, the progressive addition of MeOH reduces significantly the recovery yield of both targets, as depicting in Fig. 2(g). From the matrix modifications assays, it can be demonstrated that the addition of MeOH is disadvantageous, but the ionic strength increment promotes much higher yields. As a consequence, the further experiments on real matrices were performed with 10% of NaCl.

During the optimization process, the equilibrium time tested for carbons C1 and C2 were, respectively, 16 and 3 h. Meanwhile, as it was proved that the matrix pH and ionic strength have great influence on the recovery of both analytes, the effect of equilibrium time was repeated by using optimized experimental conditions. As depicted in Fig. 2(h), an extraction time of 16 h lead to recovery yields above 80 and 95% for both analytes with carbons C1 and C2, respectively (Table 2). Consequently, the optimized experimental parameters were set as, extraction: 16 h (990 rpm), pH 2 and 10% NaCl; back-extraction: 45 min, MeOH/ACN (50/50%) under sonification treatment.

3.3. Validation of the BAμE(AC)-LD/HPLC-DAD methodology

After optimizing the analytical process we proceed to the validation of the proposed methodology. From the data obtained (Table 2), recovery yields of $79.7 \pm 5.2\%$ and $96.5 \pm 1.1\%$ for CLOF, and $82.4 \pm 11.9\%$ and $99.6 \pm 5.1\%$ for IBU were obtained using C1 and C2 based-cork ACs, respectively, where the latter present higher efficiency, under optimized experimental conditions. It must be emphasized that the recoveries provided by the proposed methodology for both target analytes is much higher than other analytical approaches already reported [39,40]. Furthermore excellent linearity was obtained with good correlation coefficients (>0.9922) for concentrations ranging from 1.0 to 600.0 $\mu g\,L^{-1}$. It is also noteworthy that the precision accomplished for the present methodology, using within- and between-day repeatability assays, calculated as RSD on five replicates, gave rise to variations lower than 15.0%. According to the requirements of Directive 98/83/EC [41] for trace level analysis of organic compounds, the proposed methodology may be considered acceptable since it has a precision below 25%. The analytical limits of the actual methodology were also checked through the LOD (>0.21 $\mu g\,L^{-1}$) and LOQ (>0.70 $\mu g\,L^{-1}$) obtained for the target compounds. Table 2 summarizes the experimental recovery yields, the correlation coefficients, LODs and LOQs achieved for IBU and CLOF in ultra-pure water matrices by the present methodology using the two cork-based ACs, under optimized experimental conditions.

3.4. Application to real matrices

To evaluate the applicability of the proposed methodology, assays were performed on environmental matrices, namely tap, surface, ground, estuary and sea water samples. Additionally, urine

as well as wastewater samples, the latter collected from a water treatment plant, were also assayed. To account for intrinsic contamination and particular pronounced matrix effects, the SAM approach was also implemented. In a first approach, the matrix was fortified with four working standards to produce the corresponding spiking levels (5.0–20.0 $\mu g\,L^{-1}$ for water and 20.0–600.0 $\mu g\,L^{-1}$ for urine samples) for both targets under study, with the exception for urine samples, in which only IBU was studied. Blank assays ("zero-point") were also performed without spiking to assure maximum control of the analytical methodology. Table 3 summarizes the regression parameters obtained from the SAM for both analytes by the proposed methodology, under optimized experimental conditions. The data obtained from the assays performed shown very good linearity ($r^2 > 0.9900$; Table 3) and also demonstrated that in the tap, surface, groundwater, estuary and sea water samples analyzed both analytes content are below the LOD achieved for the present methodology. Fig. 3 depict chromatograms from a wastewater sample without spiking, obtained by the present methodology using both cork-based ACs, under optimized experimental conditions. The data obtained for these samples shown the presence of both analytes before (wastewater 1; 13.6–18.9 $\mu g\,L^{-1}$) and after (wastewater 2; 6.1–9.1 $\mu g\,L^{-1}$) the primary decantation. As expected, after the UV–vis treatment (wastewater 3), the analytes concentrations were below the LOD achieved for the proposed methodology. During the present work, physiological fluids were also evaluated and assays performed on urine matrices showed the occurrence of substantial contents of IBU ($\sim$6 $\mu g\,L^{-1}$), as depict in Fig. 4, since the sample was collected from a patient after Brufen consumption. Table 4 summarizes the contents detected of both compounds in the real water matrices by the SAM using the present methodology, under optimized experimental conditions.

The proposed methodology, besides being very sensitive, as demonstrated above, also showed a very high selectivity even for complex matrices such as biological or water matrices, which could have potential interferences (*e.g.* metabolites, humic acids, natural organic matter, etc.) that could block the porous available for the adsorption of the target compounds under study. Although it has proven to be a suitable analytical tool to monitor trace levels of acidic pharmaceuticals in real matrices, the performance can be further improved mainly by using LC coupled to mass spectrometry or tandem systems (LC–MS or LC–MS/MS), to achieve much better selectivity, sensitivity, and possible identity confirmation.

4. Conclusions

The activation of cork powder waste with K_2CO_3 and steam produces ACs (carbons C1 and C2) with textural and surface properties that permit their use as adsorbents materials for enrichment purposes prior to chromatographic analysis. By using these corked-based ACs as adsorbent phases in bar adsorptive micro-extraction, followed liquid desorption and high performance liquid chromatography with diode array detection (BAμE(AC)-LD/HPLC-DAD), very good performance was attained for the analysis of trace levels of IBU and CLOF in aqueous media. Experimental parameters affecting the extraction and back-extraction processes were optimized, where the influence of equilibrium time and matrix pH on the recovery yields was discussed and correlated with the ACs properties and the speciation of IBU and CLOF. Carbon C2, with the higher pH_{PZC} and the higher porous volume, showed the best recovery yields for both target molecules. Under optimized experimental conditions, the efficiencies for these two compounds provided recovery yields higher than 80% and 96% for carbons C1 and C2, respectively. Furthermore, good accuracy, suitable precision, low detection limits and excellent linear dynamic range were also achieved. The application of the proposed analytical approach through SAM, to real matrices, provided very good performance at the trace level. The method also demonstrated to be easy to work-up, sensitive and requires low sample volumes to monitor pharmaceutical compounds in environmental water or urine samples. The methodology employing ACs as enrichment materials has proved to be suitable to monitor polar compounds at the trace level, and presents the advantage of being designed to support ACs with different properties, allowing the study of their analytical performances, in the demand of searching for better enrichment techniques prior to chemical analysis.

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