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# Evaluation of graphitized carbon black as a selective adsorbent for extracting acidic organic compounds from water

Antonio Di Corcia\*, Stefano Marchese and Roberto Samperi

Dipartimento di Chimica, Università degli Studi di Roma "La Sapienza", Piazza Aldo Moro 5, 00185 Rome (Italy)

## ABSTRACT

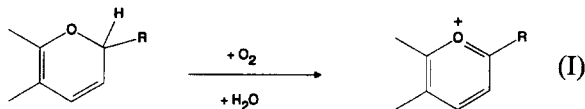
By a simple and rapid treatment, graphitized carbon black (GCB) can be made to act as both a reversed phase and an anion exchanger. The base–neutral/acid fractionation of organic species extracted from water with a GCB cartridge can be easily achieved by differential elution, provided that the  $pK_a$  values of the acidic species are not higher than about 7. A tetramethylammonium hydroxide-basified solvent mixture is the most effective eluent for the rapid elution from a GCB extraction cartridge of any kind of organic acid. The subfractionation of acidic species can be easily performed on the basis of their acidity strength by passing sequentially through a GCB cartridge various solvent mixtures containing suitable phase modifiers. The ability of a GCB extraction cartridge to retain organic acids was unaffected by the ionic strength of the water sample. The influence of fulvic acids dissolved in water on the capability of GCB to extract acidic compounds and isolate them from co-extracted base–neutral species was evaluated. The advantage of using a GCB cartridge over that containing a chemically bonded silica for accurately determining acidic compounds is demonstrated by two practical applications.

## INTRODUCTION

Graphitized carbon blacks (GCBs) are adsorbing media that are produced by heating carbon blacks at 2700–3000°C in an inert atmosphere. GCBs are essentially non-specific, non-porous sorbents with surface areas ranging about between 8 and 100 m<sup>2</sup>/g, depending on the type of starting material submitted to the graphitization process. GCBs have proved to be useful stationary phases for gas–liquid–solid chromatography [1–3]. In the last 10 years, GCB with the highest surface area, commercially referred to as Carboxpack B or Carbograph 1, has been successfully used for the liquid–solid extraction (LSE) of analytes of both clinical [4–6] and environmental [7–11] interest.

Some years ago, on studying the nature of the chemical heterogeneities contaminating the GCB

surface, we obtained experimental evidence [12] for the presence of an oxygen complex having a chromene-like structure, that is a burnt-off residue left over from the heating of carbon blacks [13] in producing graphitic carbons. In the presence of water this surface group is rearranged to form benzopyrylium salts, according to



The presence of these positively charged chemical impurities on the GCB surface enables it to act as both an anion exchanger and a non-specific sorbent. By using GCB extraction cartridges and differential elution, the singular feature has been empirically exploited for fractionating estrogens and their conjugated forms in body fluids [14,15] and for

\* Corresponding author.

isolating acidic pesticides from co-extracted base-neutral pesticides present in aqueous environmental samples [16,17]. In these reports, several solvent systems have been proposed for desorbing from the GCB surface particular target acidic compounds. Moreover, no systematic investigation on the efficiency and reliability of GCB in specifically adsorbing acidic species from various aqueous matrices has yet been performed.

This study was devoted to the systematic characterization of GCB as an anion exchanger for establishing whether GCB extraction cartridges can be advantageously included in analytical schemes elaborated for determining acidic organic compounds at trace levels in natural waters. The effects that the pH of the aqueous matrix, its ionic strength and the presence in it of fulvic acids and acidic surfactants can have on the capability of a GCB cartridge to extract quantitatively sixteen selected acidic model compounds and to isolate them from base-neutral species by selective desorption were studied. The possibility of subfractionating co-extracted acidic compounds on the basis of their acid strength by stepwise desorption was also investigated.

## EXPERIMENTAL

### Reagents

For this study, the model compounds used were selected with the criterion of choosing, among the acidic organic compounds of environmental interest, those having different  $pK_a$  values in order to cover a large range of acidity. The test compounds selected were as follows: 2,4-dichlorophenol ( $pK_a$  8.0) (2,4-DCPh); *p*-nitrophenol ( $pK_a$  7.2) (*p*-NPh); 2,4,6-trichlorophenol ( $pK_a$  7.0) (2,4,6-TCPH); 4-hydroxy-3-(3-oxo-1-phenylbutyl)coumarin ( $pK_a$  5.7) (warfarin); 4-(2,4-dichlorophenoxy)butyric acid ( $pK_a$  4.8) (2,4-DB); pentachlorophenol ( $pK_a$  4.7) (PCP); 2-*sec.*-butyl-4,6-dinitrophenol ( $pK_a$  4.6) (dinoseb); 2,4-dinitro-*o*-cresol ( $pK_a$  4.4) (DNOC); 2,4-dinitrophenol ( $pK_a$  4.1) (2,4-DNPh); 4-hydroxy-3,5-diiodobenzonitrile ( $pK_a$  3.9) (ioxynil); 4-chloro-2-methylpropanoic acid ( $pK_a$  3.7) (mecoprop); 3-isopropyl-1*H*-2,1,3-benzothiadiazin-4-one 2,2 dioxide ( $pK_a$  3.5) (bentazone); 2,4-dichlorophenoxyacetic acid ( $pK_a$  2.6) (2,4-D); 2,4,5-trichlorophenoxyacetic acid ( $pK_a$  2.2) (2,4,5 T); 3,6-dichloro-2-methoxybenzoic acid ( $pK_a$  1.9, (dicamba); and

octylbenzenesulphonic acid ( $pK_a$  0.8) ( $C_8$ -LAS). These compounds were supplied by Riedel-de Haën (Hannover, Germany) and Aldrich (Milwaukee, WI, USA). The  $pK_a$  values in water reported above were taken from various sources. Individual standard solutions were prepared by dissolving 100 mg of each compound in 100 ml of acetonitrile. A composite working standard solution was prepared by mixing 0.2–0.4 ml of each standard solution and diluting to 10 ml with acetonitrile.

Fulvic acids were kindly donated by Dr. A. Piccolo, who prepared them as described elsewhere [18]. A commercial  $C_{10}$ – $C_{13}$  linear alkylbenzenesulphonate (LAS) mixture (Marlon A) was supplied by Chemische Werke Hüls (Marl, Germany).

Trifluoroacetic acid (TFA), tetramethylammonium hydroxide (TMAOH), tetrapropylammonium bromide (TPABr) and formic acid were purchased from Aldrich. For HPLC, distilled water was further purified with an Elgastat UHQPS apparatus (Elga, Bucks, UK). Methanol and acetonitrile of gradient grade were obtained from Riedel-de Haën. All other solvents were of analytical reagent grade (Carlo Erba, Milan, Italy) and were used as received.

Graphitized carbon black (120–400 mesh size), commercially referred to as Carbograph 1, and the other materials used for preparing the extraction cartridges were kindly supplied by Alltech (Deerfield, IL, USA). GCB cartridges (300 mg) were prepared as reported previously [16,17]. The trap was fitted into a side-arm flask and liquids were forced to pass through the cartridge by vacuum from a water pump.

The measurements of the anion-exchange capacities of both GCB and its parent material were conducted by passing through the 300-mg sorbent cartridges 10 ml of a  $CH_2Cl_2$ – $CH_3OH$  mixture (50:50, v/v) containing 2 g/l of  $C_8$ -LAS. The fraction of this acid unspecifically adsorbed was removed by washing the cartridge with 12 ml of  $CH_2Cl_2$ – $CH_3OH$  (80:20, v/v). The  $C_8$ -LAS fraction specifically sorbed on the benzopyrylium ions were desorbed by passing 8 ml of  $CH_2Cl_2$ – $CH_3OH$  (80:20, v/v) basified with TMAOH (20 mmol/l). After solvent removal, the residue was reconstituted by a suitable acidified aqueous methanolic mixture and a portion of it was injected into the HPLC apparatus.

### Procedure

Recovery studies were performed by pretreating the GCB cartridge with 7 ml of water acidified with HCl (pH 1). After extracting the water samples, the cartridge was washed with an aqueous solution containing 20 mmol/l of  $\text{KHCO}_3$ . The major part of water was eliminated by room air drying for 30 s. Thereafter, residual water was eliminated by passing 1 ml of methanol. Before desorbing acids, the cartridge was washed with 7 ml of  $\text{CH}_2\text{Cl}_2$ – $\text{CH}_3\text{OH}$  (90:10, v/v). This solvent mixture is able to remove from the cartridge any non-acidic compounds. Unless indicated otherwise, acidic compounds were eluted with 6 ml of  $\text{CH}_2\text{Cl}_2$ – $\text{CH}_3\text{OH}$  (90:10, v/v) basified with 20 mmol/l of TMAOH.

When using TMAOH as an eluent modifier, the extract was acidified with 100  $\mu\text{l}$  of 10% TFA in methanol, before evaporating the eluate. This was done in order to avoid the decomposition of 2,4-DNPh and DNOC that occurs under alkaline conditions. In all instances, the extracts were evaporated to a volume of *ca.* 100  $\mu\text{l}$  using a stream of nitrogen at 30°C. To this, 150  $\mu\text{l}$  of acidified water (pH 1) were added and, after measuring the exact volume, 50  $\mu\text{l}$  of this solution were injected into the HPLC apparatus.

### HPLC analysis

Liquid chromatography was carried out with a Varian (Walnut, Creek, CA, USA) Model 5000 chromatograph equipped with a Rheodyne Model 7125 injector having a 50- $\mu\text{l}$  loop and with a Model 2550 UV detector (Varian). A 25 cm  $\times$  4.6 mm I.D. column filled with 5- $\mu\text{m}$  LC-18 packing (Supelco, Bellefonte, PA, USA) was used. For separating the acidic compounds selected, gradient elution was performed. Solvent A was water acidified with 0.05% TFA; solvent B was acetonitrile–methanol (80:20, v/v) acidified with 0.01% TFA. The initial mobile phase composition was 47% solvent B, linearly programmed to 85% after 25 min. The eluted compounds were monitored with the UV detector set at 220 nm. The flow-rate of the mobile phase was 1.5 ml/min.

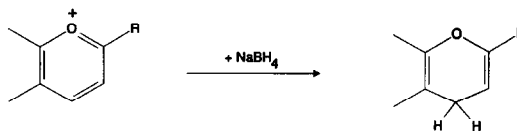
The concentrations of the acidic compounds in the final extract were calculated by measuring the peak heights for each compound and comparing them with those obtained from standard solutions. These were prepared by taking known and appro-

pritate volumes of the working standard solution, evaporating the solvent and reconstituting the residue with 100  $\mu\text{l}$  of methanol and 150  $\mu\text{l}$  of water acidified to pH 2.

### RESULTS AND DISCUSSION

The anion-exchange capacity of GCB was measured by packing 300 mg of it in a cartridge and pretreating the adsorbent with various solutions. The results are reported in Table I. According to the proposed reaction mechanism (I), conversion of chromene-like structures to benzopyrylium ions was much more readily achieved by washing the sorbent column with acidified water. Moreover, only a slight increase in the number of exchange sites was obtained by both increasing the volume of the acidified water and decreasing the pH of the water. This indicates that chromene groups are rapidly rearranged to give benzopyrylium salts in the presence of acidified water.

It has been reported [19] that benzopyrylium salts are promptly reduced to benzopyrans under mild conditions according to



In order to obtain additional evidence about the nature of the exchange sites populating the surface framework of GCB, this material was first washed

TABLE I  
THE EFFECT OF VARIOUS PRETREATMENTS ON THE ANION-EXCHANGE CAPACITY OF GCB

Pretreatment	Capacity, $\mu\text{equiv./g}^a$
None	0.89
TFA in $\text{CH}_2\text{Cl}_2$ –MeOH	3.5
14 ml Water (pH 6)	3.1
7 ml Water (pH 2)	7.5
14 ml Water (pH 2)	7.4
7 ml Water (pH 1)	7.8
7 ml Water (pH 0)	7.8
7 ml Water (pH 1) followed by $\text{NaBH}_4$	0.84

<sup>a</sup> Mean values from duplicate experiments.

with an acidic aqueous solution and then treated with sodium tetrahydroborate dissolved in acetonitrile. As can be seen in Table I, after this treatment the anion-exchange capacity of the GCB material decreased abruptly, confirming that benzpyrylium ions are responsible for binding organic anions via electrostatic forces. Interestingly, the exchange site concentration of the NaBH<sub>4</sub>-treated GCB was very similar to that measured for untreated GCB. This finding suggests that, in addition to chromene groups, the GCB surface might also be contaminated by a small number of chemical heterogeneities of different nature, even those capable of exchanging anions.

The ion-exchange capacity of non-graphitized carbon black was also measured and it was found to be about twice that of the GCB surface. This seems surprising considering that carbon blacks are graphitized at very high temperatures at which any surface organic chemical groups should be decomposed. Probably chemical heterogeneities are partially reformed during cooling of the carbonaceous material after the graphitization process.

When water contaminated by organic compounds passes through a GCB column, base-neutral compounds are adsorbed on the graphitic framework of the sorbent, whereas adsorption of acidic compounds takes place on benzpyrylium ions. In this situation, the base-neutral/acid fractionation can be easily achieved by first eluting base-neutral species with a neutral organic solvent mixture and then passing a basified or acidified solvent system to desorb acidic compounds. For conciseness, from this point onwards the solvent systems designed to elute non-acidic and acidic adsorbates will be called eluent A and eluent B, respectively.

This scheme succeeded in isolating acidic from base-neutral pesticides [16,17]. The acidic pesticides considered had pK<sub>a</sub> values ranging between about 2 and 5. We evaluated whether even weaker acidic compounds could still be isolated from base-neutral compounds by stepwise desorption. For this purpose, 1 l of distilled water was spiked with the sixteen acidic model compounds selected and with a neutral compound, such as linuron, which is a well known phenylurea herbicide. This artificially contaminated aqueous sample was then extracted with the 300-mg GCB cartridge. After extraction, the

cartridge was washed with 7 ml of an aqueous solution containing 20 mmol/l of KHCO<sub>3</sub> (pH 8.1).

Differential elution was carried out as described under Experimental. The results reported in Table II shows that both *p*-NPh and 2,4,6-TCPH, having pK<sub>a</sub> values of about 7, were found quantitatively in the acid-containing extract. When the step of washing the cartridge with water slightly basified with KHCO<sub>3</sub> was omitted, ca. 30 and 15% of *p*-Nph and 2,4,6-TCPH, respectively, were eluted by eluent A. It can be assumed that the undissociated fraction of an acidic compound present in water is, as such, unspecifically adsorbed on the GCB surface. This fraction is probably ionized on passing through the sorbent bed a basified aqueous solution and then it moves from unspecific adsorption sites to those ones bearing a positive charge.

Under the experimental conditions selected, 2,4-DChPh, having a pK<sub>a</sub> about ten times higher than those of the two phenols mentioned above, was al-

TABLE II

BASE-NEUTRAL/ACID FRACTIONATION AFTER EXTRACTION FROM WATER OF SELECTED COMPOUNDS WITH A GCB CARTRIDGE

Compound	Recovery (%) <sup>a</sup>	
	Eluent A <sup>b</sup>	Followed by eluent B <sup>c</sup>
Linuron	97	—
2,4 DCPH	80	12
<i>p</i> -NPh	—	98
2,4,6-TCPH	—	95
Warfarin	—	99
2,4-DB	—	101
PCPh	—	96
Dinoseb	—	99
DNOC	—	90
2,4-DNPh	—	98
Ioxynil	—	100
Mecoprop	—	100
Bentazone	—	101
2,4-D	—	99
2,4,5-T	—	98
Dicamba	—	100
C <sub>8</sub> -LAS	—	97

<sup>a</sup> Mean values obtained from triplicate measurements.

<sup>b</sup> 7 ml of CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH (90:10, v/v).

<sup>c</sup> 6 ml of CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH (90:10, v/v) basified with TMAOH (20 mmol/l).

most completely washed out by the neutral mobile phase. Increasing the pH of the aqueous washing phase decreased only to a slight extent the percentage of 2,4-DChPh found in the neutral extract. Probably methanol contained in eluent A is able to compete with very weakly acidic compounds for adsorption on benzopyrylium ions. On the other hand, it has been reported [16] that solvent systems not containing methanol are ineffective for rapidly eluting from GCB cartridges any kind of neutral adsorbate. In fact, when eluent A was composed of methylene chloride or methylene chloride–acetonitrile, more than 85% of linuron was found in the second extract containing the acidic species. Definitely, the goal of isolating an acidic compound from co-extracted base–neutral compounds can easily be achieved by differential elution, provided that the  $pK_a$  value is not higher than about 7.

In the past, several solvent mixtures composed of various amounts of methanol in chloroform or methylene chloride and containing various concentrations of different acid-displacing agents, such as tetrapropylammonium bromide (TPABr) [14], tet-

ramethylammonium hydroxide (TMAOH) [8,20], potassium hydroxide [16] and trifluoroacetic acid (TFA) [17], have been used empirically for eluting particular acidic analytes from a GCB cartridge. By varying both the nature of the displacing agent and the composition of the solvent mixture in which they were dissolved, the abilities of various eluent systems to re-extract rapidly acidic adsorbates from a 300-mg GCB extraction cartridge were compared. These experiments were conducted by passing through the cartridge 1 l of distilled water spiked with the acidic model compounds considered at individual concentrations of 0.5  $\mu\text{g/l}$ . The adsorbates were eluted by passing through the GCB bed eight 2-ml aliquots of the eluent system selected and analysing each individual aliquot. The concentration of any phase modifier considered was 20 mmol/l. The results are reported in Table III.

Both TPABr and KOH were effective phase modifiers for rapidly removing from the GCB surface organic anions, except for some phenol derivatives. In general, their effect was enhanced when dissolved in solvent systems rich in methylene chloride. TFA

TABLE III

VOLUMES OF VARIOUS ELUENT SYSTEMS NEEDED FOR RE-EXTRACTING ACIDIC COMPOUNDS FROM THE GCB CARTRIDGE

Compound	Eluent system volume (ml) <sup>a</sup>											
	TPABr			KOH			TFA			TMAOH		
	90:10 <sup>b</sup>	80:20	60:40	90:10	80:20	60:40	90:10	80:20	60:40	90:10	80:20	60:40
<i>p</i> -NPH	6	4	4	4	4	4	4	4	4	4	4	4
2,4-DNPh	6	12	>16	10	12	14	6	10	12	4	4	4
Dicamba	4	4	4	4	4	4	4	4	4	4	4	4
Bentazone	4	4	4	4	4	4	4	4	4	4	4	4
C <sub>8</sub> -LAS	4	4	4	4	4	4	>16	>16	>16	4	4	4
2,4-D	4	4	4	4	4	4	4	4	4	4	4	4
DNOC	14	16	>16	14	10	>16	12	16	>16	6	6	6
Ioxynil	8	8	12	8	6	10	4	4	4	4	4	4
Mecoprop	4	4	4	4	4	4	4	4	4	4	4	4
Warfarin	4	4	4	4	4	4	4	4	4	4	4	4
2,4,5-T	4	6	16	4	4	16	4	4	4	4	4	4
2,4-DB	4	4	6	4	4	6	4	4	4	4	4	4
TCPh	4	4	4	4	4	4	4	4	4	4	4	4
Dinoseb	4	6	16	8	4	10	4	6	6	4	4	4
PCP	12	12	>16	10	8	>16	12	16	>16	6	6	6

<sup>a</sup> Volume of the eluent system needed to elute at least 90% of the adsorbate. Mean values obtained from duplicate experiments.

<sup>b</sup> CH<sub>2</sub>Cl<sub>2</sub> to CH<sub>3</sub>OH volume ratio in the solution containing the acid-displacing agent.

was unable to elute C<sub>8</sub>-LAS quantitatively from the GCB cartridge. This can be accounted for by considering that the sulphonated compound has a higher acidity than TFA. Regardless of the composition of the solvent system in which TMAOH was dissolved, this organic base was capable of rapidly eluting from the GCB cartridge even those phenol derivatives which exhibited a particularly high affinity for binding with benzpyrylium ions. This ability is probably the result of a synergic action played by the OH<sup>-</sup> ion, which competes with organic anions for adsorption on the exchange sites, and the “naked” tetraalkylammonium cation able to form with organic anions stable ion pairs, which are readily soluble in organic solvents.

Recently, we reported [17] that 8 ml of CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH (80:20, v/v) acidified with TFA sufficed to elute quantitatively from a 300-mg cartridge eighteen acidic pesticides, including DNOC and PCP. This appears to be in contrast to data obtained in this study. For this reason, we evaluated the extent to which batch-to-batch variations in the physico-

chemical characteristics of the GCB surface could affect the re-extraction of acidic compounds from the GCB cartridge when eluting them with a TFA-containing solvent mixture. Two distilled water samples were spiked with the selected acidic compounds considered at different individual concentrations, namely 0.25 and 2.0 µg/l. Each artificially contaminated water sample was analysed six times by extracting 1-l aliquots with cartridges containing GCB from six distinct batches. The re-extraction of the acidic compounds was performed by using both TFA and TMAOH as eluent modifiers. The results are reported in Table IV.

With TFA as modifier, unsatisfactorily low mean recoveries of DNOC, 2,4-DNPh and PCPh with values scattered over a wide range were obtained when analysing the water sample containing the lowest concentration of acids. Under the same conditions, the recovery and precision for the three phenols mentioned above were also measured by extracting water with cartridges filled with GCB from one individual batch. The recovery and the

TABLE IV

RECOVERY OF SELECTED ACIDS FROM GCB CARTRIDGES PREPARED FROM SIX DISTINCT SORBENT BATCHES BY PASSING THROUGH TWO SELECTED ELUENT SYSTEMS

Compound	Recovery (%)							
	8 ml CH <sub>2</sub> Cl <sub>2</sub> -CH <sub>3</sub> OH (90:10, v/v)-20 mM TFA				6 ml CH <sub>2</sub> Cl <sub>2</sub> -CH <sub>3</sub> OH (90:10, v/v)-20 mM TMAOH			
	0.25 µg/l <sup>a</sup>		2.0 µg/l <sup>a</sup>		0.25 µg/l <sup>a</sup>		2.0 µg/l <sup>a</sup>	
	Mean <sup>b</sup>	Range	Mean <sup>b</sup>	Range	Mean <sup>b</sup>	Range	Mean <sup>b</sup>	Range
<i>p</i> -NPH	99	96–103	99	96–99	99	98–101	100	97–101
2,4-DNP	79	70–93	96	90–99	98	94–103	98	96–100
Dicamba	99	96–103	99	96–98	99	98–102	99	96–100
Bentazone	100	98–104	99	97–100	100	99–102	100	99–100
2,4-D	97	91–102	98	96–99	98	96–100	98	96–99
DNOC	62	50–85	91	85–95	95	91–98	95	90–99
Ioxynil	99	96–102	100	97–101	98	96–100	100	99–100
Mecoprop	97	95–101	99	98–100	101	98–102	98	97–100
Warfarin	99	97–102	98	96–100	100	99–103	100	99–100
2,4,5-T	98	96–103	98	95–100	99	98–102	99	99–100
2,4-DB	100	97–104	99	97–101	101	99–104	100	99–100
2,4,6-TCPh	99	98–103	98	98–100	100	98–102	100	99–100
Dinoseb	94	92–100	97	95–99	99	97–103	99	97–100
PCPh	83	70–91	90	86–95	98	96–105	99	98–100

<sup>a</sup> Concentration in water of the model compounds.

<sup>b</sup> Average recovery calculated from six determinations.

range were 84% (79–86%), 75% (72–79%) and 82% (78–84%) for 2,4-DNPh, DNOC and PCPh, respectively. By comparing these values with those ones reported in Table IV, it can be concluded that batch-to-batch variations of the GCB material were responsible for large variations in the recovery of these three phenols. Conversely, the re-extraction efficiency of the TMAOH-basified organic mixture was not dependent on the particular GCB batch.

This finding is not thoroughly understood. However, one could speculate that the mobilities along the GCB column of particular non-ionized phenol derivatives are influenced by additional interactions occurring between these eluates and unknown active sites present on the GCB surface. The surface concentration of these chemical heterogeneities is very low, as their action is evident only when a few hundred nanograms of the three phenol derivatives are adsorbed on the GCB surface. Variations in the surface concentration of these active sites could arise from insufficient control of all the factors affecting the quality of the graphitization process.

With respect to the past, the multi-component determination of organic contaminants in environmental waters is nowadays made more difficult as a much larger number of target compounds have to be monitored and the demand is for methods of greater sensitivity. Therefore, the use of a single sorbent cartridge able not only to extract quantitatively a large number of pollutants having a broad range of physico-chemical properties but also to fractionate them by differential elution is highly desirable. As shown above, base-neutral/acid fractionation can be easily accomplished by the use of a GCB extraction cartridge.

The possibility of subfractionating acids on the basis of differences in their acid strengths by a multi-stage desorption process was also investigated. For these experiments, a water sample spiked with the acidic compounds considered was passed through the GCB cartridge. After washing the cartridge with the solvent mixture designed to elute base-neutral compounds, stepwise desorption of acids was performed by passing sequentially three different, selected eluent systems. The results reported in Table V show that, except for a slight carryover between DNPh, DNOC, PCPh and C<sub>8</sub>-LAS, subfractionation of acidic compounds was accomplished on the basis of their pK<sub>a</sub> values by prop-

TABLE V

GROUP SEPARATION OF SELECTED ACIDIC COMPOUNDS BY DIFFERENTIAL ELUTION FROM A GCB CARTRIDGE

Compound	Recovery (%) <sup>a</sup>		
	Eluent B <sub>1</sub> <sup>b</sup>	Eluent B <sub>2</sub> <sup>c</sup>	Eluent B <sub>3</sub> <sup>d</sup>
<i>p</i> -NPH	97	—	—
2,4-DNPh	—	96	5
Dicamba	—	101	—
Bentazone	—	101	—
C <sub>8</sub> -LAS	—	4	95
2,4-D	—	98	—
DNOC	—	93	5
Ioxynil	—	100	—
Mecoprop	—	100	—
Warfarin	96	—	—
2,4,5-T	—	99	—
2,4-D B	98	—	—
2,4,6-TChPh	99	—	—
Dinoseb	—	98	—
PCPh	—	96	4

<sup>a</sup> Mean values obtained from triplicate measurements.

<sup>b</sup> 6 ml of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (90:10, v/v) containing acetic acid (4 mmol/l).

<sup>c</sup> 8 ml of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (90:10, v/v) containing formic acid (250 mmol/l).

<sup>d</sup> 6 ml of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (90:10, v/v) containing TMAOH (20 mmol/l).

erly selecting the eluotropic strength of the eluent system. In particular, a 5 mmol/l acetic acid-containing mobile phase was able to remove from the GCB surface only those acids having pK<sub>a</sub> values between 7 and 5. The addition of 0.25 mol/l of formic acid to the CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH mixture succeeded in eluting acids with pK<sub>a</sub> values between about 5 and 2. Finally, by passing TMAOH in CH<sub>2</sub>Cl<sub>2</sub>-CH<sub>3</sub>OH, even the compound bearing a sulphonic group was quantitatively recovered in the third fraction. Formic acid was preferred to TFA as a displacer of medium-strength acidic compounds because with TFA a certain, variable amount of C<sub>8</sub>-LAS was found in the second fraction. More interestingly, it was observed that by replacing TFA with formic acid the re-extraction efficiency of even traces of 2,4-DNPh, DNOC and PCPh was unaffected by batch-to-batch variations in the surface characteristics of GCB.

It is known that one serious drawback discourag-

ing the use of conventional anion exchangers for the selective determination of acidic compounds in environmental waters is that the extraction efficiency of these materials is dependent on the ionic strength of the aqueous matrix. The influence of inorganic anions present in water on the ability of a 300-mg GCB cartridge to extract organic acids and isolate them from co-extracted base-neutral compounds was evaluated and compared with that of a cartridge containing the same mass of a high-capacity resin-based strong anion exchanger, such as Amberlite CG-400-II. Aliquots of 1 l of distilled water were spiked with the acidic model compounds and 35 g of sodium chloride were added to simulate a sea-water specimen. Before extraction with the conventional anion exchanger, the pH of the water sample was adjusted to 9 to increase the dissociation of the weakest acids.

The recovery data reported in Table VI show that, in contrast to that of the resin-based anion exchanger, the extraction efficiency of GCB was unaffected by the presence in water of a large amount of inorganic anions. Also, no significant amount of any acidic compounds was lost by washing the GCB cartridge with eluent A designed to remove base-neutral compounds from the sorbent surface. With respect to inorganic anions, the higher affinity that organic ions have for specific adsorption on the benzpyrylium ions of the GCB surface can be explained by assuming that non-specific interactions taking place between the hydrophobic part of an ionized organic compound and the graphitic surface framework of the sorbent make a significant contribution to the overall heat of adsorption. An analogous situation takes place on conventional ion exchangers but, in this instance, the contribution mentioned above is far more reduced as the number of sites for the ion exchange greatly predominate over those for non-specific adsorption. From a practical point of view, the GCB cartridge appears to be suitable for determining acidic pollutants in sea water.

It has been reported [21] that about 80% of the dissolved organic content (DOC) in environmental waters is due to the presence of fulvic acids (FA). Owing to their nature, FA can compete with acidic analytes for adsorption on benzpyrylium ions present on the GCB surface. The effect of the presence of FA in water on the extraction and isolation of

TABLE VI

RECOVERY OF SELECTED ACIDIC COMPOUNDS FROM A WATER SAMPLE WITH A HIGH IONIC STRENGTH (0.6 mol/l) BY EXTRACTING WITH THE GCB CARTRIDGE AND WITH A CARTRIDGE FILLED WITH A CONVENTIONAL ANION EXCHANGER

Compound	Recovery (%) <sup>a</sup>	
	Amberlite CG-400-II	GCB
<i>p</i> -Nph	38	97
2,4 DNP	99	98
Dicamba	2	96
Bentazone	96	100
C <sub>8</sub> -LAS	98	99
2,4-D	12	98
DNOC	97	94
Ioxynil	98	97
Mecoprop	10	96
Warfarin	81	95
2,4,5-T	38	97
2,4-DB	30	100
2,4,6-TChPh	95	100
Dinoseb	97	96
PCPh	98	96

<sup>a</sup> Mean values obtained from duplicate determinations.

acidic compounds by a GCB cartridge was evaluated. For these experiments, simulated surface water samples were prepared by adding to distilled water samples various amounts of FA, from 2.5 to 20 mg/l, after adjusting the pH of the water to 8. Each of these samples was spiked with known amounts of the model compounds and 0.5-l aliquots were analysed. The re-extraction of the acidic compounds was performed by passing the neutral eluent A followed by the basified eluent B (see Experimental).

The results reported in Table VII show that there was an increasing tendency of the model compounds to be eluted from the GCB cartridge by the neutral eluent A as the FA content in water increased. This was probably due to the fact that the ion-exchange sites on the GCB surface were progressively saturated by FA with the result that the adsorption of the ionized model compounds partially occurred also on the predominant unspecific surface sites of the sorbent.

Another negative effect induced by the presence in water of large amounts of FA was that some of



TABLE VII

RECOVERY OF SELECTED ACIDIC COMPOUNDS FROM 0.5 l OF WATER SAMPLES CONTAINING VARIOUS AMOUNTS OF FULVIC ACIDS BY EXTRACTION WITH GCB CARTRIDGES

Compound	Recovery (%) <sup>a</sup>									
	2.5 mg/l FA		5 mg/l FA		10 mg/l FA		20 mg/l FA		10 mg/l FA <sup>b</sup> , B <sup>c</sup>	20 mg/l FA <sup>b</sup> , B <sup>c</sup>
	A <sup>c</sup>	B <sup>c</sup>	A <sup>c</sup>	B <sup>c</sup>	A <sup>c</sup>	B <sup>c</sup>	A <sup>c</sup>	B <sup>c</sup>		
<i>p</i> -NPh	–	98	16	80	18	65	23		90	84
DNPh	–	95	–	96	12	85	30	74	100	95
Dicamba	–	94	–	93	10	52	20	2	87	75
Bentazone	–	97	–	97	4	92	66	33	95	90
C <sub>8</sub> -LAS	–	98	–	98	–	97	8	90	101	96
2,4-D	–	103	–	97	5	88	16	80	100	101
DNOC	–	95	–	94	–	93	–	96	100	96
Ioxynil	–	100	–	97	7	88	8	85	98	98
Mecoprop	–	98	–	93	5	88	15	73	93	88
Warfarin	–	96	–	91	3	80	7	47	94	79
2,4,5-T	–	100	–	95	–	90	–	92	99	95
2,4-DB	–	96	–	92	5	88	5	68	95	99
TCPH	–	98	–	96	–	92	25	–	96	88
Dinoseb	–	96	–	94	–	94	6	86	97	96
PCP		98		97		96	4	90	96	96

<sup>a</sup> Mean values from duplicate measurements.<sup>b</sup> Water acidified to pH 2.2.<sup>c</sup> The compositions of mobile phases A and B are the same as reported in Table II.

the acids were partially lost in the water effluent. Two different causes may be responsible for this loss. One is that the graphitic framework of the GCB surface is scarcely able to adsorb from water very polar organic anions. Another source of loss might be traced to some kind of association occurring between FA and some of the acidic compounds considered. Binding of organic molecules with FA in water has already been observed [22,23]. If this occurs, a sorbent column is unable to retain solutes associated with that fraction of FA passing unrestrained through it. At concentrations of FA in water  $\geq 10$  mg/l, we observed that the water samples after extraction had a brownish colour, indicating the presence of FA in the effluent. In our experience, river waters contain FA at concentrations lower than 10 mg/l. Anyway, when acidic analytes have to be monitored in a river water sample suspected to contain an anomalously high content of FA (a brownish colour of the sample is a valid indication), in order to avoid loss of the analytes and as an alternative to the obvious expedient of sampling

smaller water volumes, the aqueous sample could be acidified before extraction. By doing so with water samples having high contents of FA, only a moderate loss of the water effluent of some of the acidic compounds was observed. By suppressing ionization, the adsorption of acids mainly occurs on the unspecific sites of the GCB surface, which are much larger in number than specific sites and are not readily saturated by FA. This result was obtained, however, at the expense of selectivity, because on passing through the sorbent bed the neutral eluent A designed to elute non-acidic compounds, variable fractions of most of the acidic model compounds considered were washed out from the cartridge.

Linear alkyl benzenesulphonates (LAS)<sub>1</sub> are anionic surfactants of major use in detergent formulations. Commercial LAS materials are mixtures of various alkyl homologues that vary from C<sub>10</sub> and C<sub>13</sub> and phenyl positional isomers. Because of their widespread use, they occur in many different environmental compartments. In our experience,

typical concentrations of LAS in surface water samples range from 5 to 50  $\mu\text{g/l}$  and only rarely do they occur in higher concentrations. When extracting with a GCB cartridge surface water samples contaminated by LAS, these compounds can interfere with the analysis or target acidic compounds in two ways. One is that, being strongly adsorbed on the benzopyrylium ions present on the GCB surface, they tend to saturate them. Moreover, LAS co-extracted with target acidic compounds may interfere with the subsequent HPLC analysis of the latter as the former produce large and broadened peaks. The effect of the presence in water of LAS on the quality of the analysis of acidic analytes compounds was evaluated by artificially contaminating 0.5 l of pure water samples with both the model acidic compounds and various amounts of a mixture composed of  $\text{C}_{10}$ – $\text{C}_{13}$  LAS and analysing. The recovery data showed that the quantitative extraction by the GCB cartridge of acidic compounds and their suc-

cessive isolation from base-neutral compounds by stepwise desorption were unaffected by the presence of LAS even when they were present in water at a total level of 300  $\mu\text{g/l}$ . In addition, using the formic acid-containing solvent mixture mentioned above, we were able to elute completely from the GCB cartridge the model compounds, except  $\text{C}_8$ -LAS, and to determine them by HPLC without any interference by LAS, as the latter compounds were not desorbed from the GCB surface by the selected eluent.

For monitoring acidic compounds in natural waters, the advantage of using a GCB extraction cartridge over the popular octadecyl-bonded silica ( $\text{C}_{18}$ ) cartridge is that the analysis can be made more selective by a suitable choice of both the washing and the eluent phases. Practical evidence for this statement was obtained by performing two experiments. In the first, a river water sample was spiked with 0.2  $\mu\text{g/l}$  of PCPh and 2.0  $\mu\text{g/l}$  of a polychlorinated biphenyl (PCB) mixture (Arochlor 1254). It is known

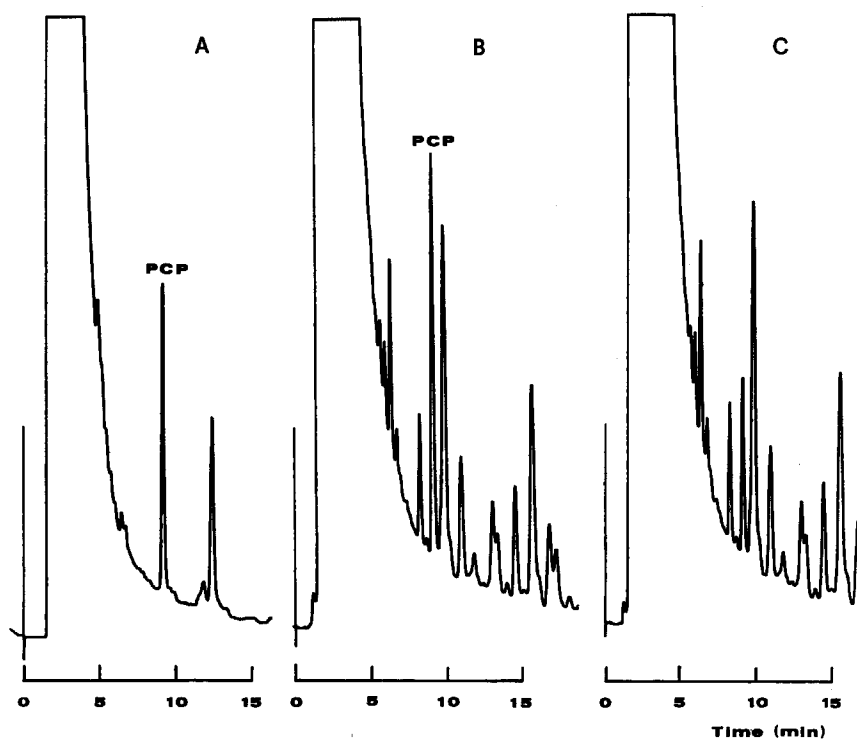


Fig. 1. Chromatograms obtained on analysing 1-l aliquots of river water (Tevere) spiked with 0.2  $\mu\text{g/l}$  of pentachlorophenol and 2.0  $\mu\text{g/l}$  of a mixture of polychlorobiphenyls (Arochlor 1254) by two procedures involving the use of (A) the GCB extraction cartridge and (B) a 0.5-g  $\text{C}_{18}$  extraction cartridge. Chromatogram C was obtained by the latter procedure on analysing 1 l of the same water sample spiked only with 2  $\mu\text{g/l}$  of Arochlor 1254.

that, as a consequence of their widespread use, PCBs are present in all compartments of the natural environment. A 1-l aliquot of the artificially contaminated water sample was analysed as such by extracting PCPh with the 300-mg GCB cartridge and, after washing PCBs from the GCB surface by passing 7 ml of  $\text{CH}_2\text{Cl}_2$ - $\text{CH}_3\text{OH}$  (90:10, v/v), PCPh was eluted with the formic acid-containing mobile phase. Before extracting with the 0.5-g  $\text{C}_{18}$  cartridge (Supelco), the pH of a 1-l aliquot of the same water sample was adjusted to about 2.5. After the water had passed through the  $\text{C}_{18}$  cartridge, PCPh was eluted by passing through it 6 ml of

methanol. The two eluates were dried and the residues reconstituted with 0.25 ml of an acidified aqueous methanol mixture. Volume of 50  $\mu\text{l}$  of each of two extracts were then injected into the HPLC system. The column was operated isocratically with a water-methanol mixture (20:80, v/v) acidified with TFA (0.05%, v/v). The resulting chromatograms are shown in Fig. 1, which also shows a third chromatogram obtained by analysis with the procedure involving the use of the  $\text{C}_{18}$  extraction cartridge the surface water sample spiked only with PCBs. From comparison of the chromatograms, it appears that the use of a non-selective analytical procedure, such

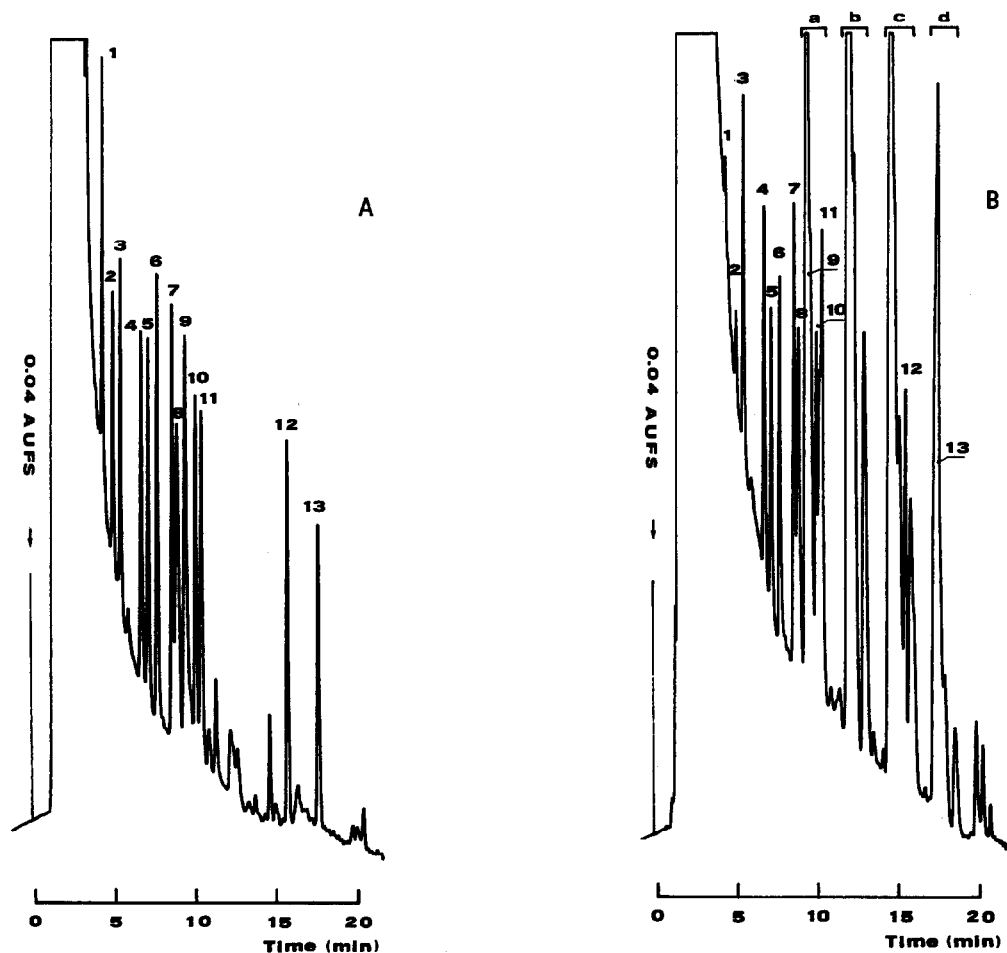


Fig. 2. Chromatograms obtained on analysing 0.5 l aliquots of river water (Teverce) spiked with 1-2  $\mu\text{g/l}$  of thirteen selected acidic compounds and with 100  $\mu\text{g/l}$  of a  $\text{C}_{10}$ - $\text{C}_{13}$  LAS mixture by two procedures involving the use of (A) the GCB extraction cartridge and (B) a 0.5-g  $\text{C}_{18}$  cartridge. 1 = 2,4-DNPh; 2 = dicamba; 3 = bentazone; 4 = 2,4-D; 5 = DNOC; 6 = ioxynil; 7 = mecoprop; 8 = warfarin; 9 = 2,4,5-T; 10 = 2,4-DB; 11 = 2,4,6-TCPh; 12 = dinoseb; 13 = PCPh; a =  $\text{C}_{10}$  LAS; b =  $\text{C}_{11}$  LAS; c =  $\text{C}_{12}$  LAS; d =  $\text{C}_{13}$  LAS.

as that involving the C<sub>18</sub> extraction cartridge, resulted in overestimation of PCPh, because one peak for PCBs overlapped that for the phenol derivative.

In the second experiment, the determination of thirteen of the acidic compounds considered in a surface water sample contaminated by LAS was simulated. This was done by artificially contaminating a surface water sample with both the thirteen acidic compounds at the individual level of 1–2 µg/l and with 100 µg/l of the C<sub>10</sub>–C<sub>13</sub> LAS mixture, and assaying 0.5-l aliquots of the sample by the same two procedures described above. On comparing the resulting two chromatograms shown in Fig. 2, it appears that only the procedure involving the use of the GCB cartridge was suitable for determining all of the thirteen target compounds present in a water sample containing LAS. This result was achieved by selecting as the phase modifier formic acid, which is able to elute only those acids having pK<sub>a</sub> values higher than that of sulphonated compounds. In fact, when using TMAOH as phase modifier for eluting acidic compounds from the GCB cartridge, a chromatogram similar to that obtained by the analytical procedure making use of the C<sub>18</sub> extraction cartridge was obtained. Finally, we observed that the GCB extraction cartridge was able to retain quantitatively both 2,4-DNPh and dicamba. These compounds were almost completely lost in the water effluent when using the C<sub>18</sub> extraction cartridge.

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