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Determination of phenolics from sediments of pulp mill origin by *in situ* supercritical carbon dioxide extraction and derivatization

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ABSTRACT

A method for the determination of extractable chlorinated phenolics in sediments collected downstream of chlorine-bleaching mills was developed by using a single-step *in situ* derivatization technique in conjunction with supercritical fluid extraction (SFE). Phenolics in air dried samples were extracted with carbon dioxide and simultaneously acetylated under static SFE conditions by acetic anhydride in the presence of triethylamine. The derivatives were then removed from the matrix in the dynamic extraction stage. Within an extraction chamber temperature range from 40 to 120°C, the best overall recovery for the phenolics was obtained at 110°C. A carbon dioxide density of 0.71 g/ml (pressure 37 MPa) was used for the extraction–derivatization experiments since lower CO_2 densities adversely affected the recovery of the catechols. Two extractions of the same sample were necessary for the quantitative recovery of extractable phenolics in weathered sediments. For sample size of 1 g, 120 μl of acetic anhydride and 30 μl of triethylamine were found to produce the optimal results. While the results obtained by this SFE–derivatization method were comparable to conventional technique such as Soxhlet extraction, the SFE approach required no solvent in the extraction steps and was extremely time-efficient (*ca.* 35 min).

INTRODUCTION

Of all the pulp and paper mills operating in Canada, 47 of them use chlorine for bleaching either entirely or in at least one of the multiple bleaching steps. In a 1991 report jointly published by Environment Canada and Health and Welfare Canada [1], it was estimated that Canadian mills used over $610 \cdot 10^6$ kg of chlorine annually to produce over $10 \cdot 10^9$ kg of bleached pulp and released over 10^9 kg of chlorinated organics to the aquatic environment. Hundreds of compounds were found in the final effluents of the bleached kraft mills, including the chlorinated dibenzofurans and dibenzo-*p*-dioxins, phenolics, resin and fatty acids, and a variety of low-molecular-mass aliphatic compounds [2,3]. Recent studies carried out by the Pulp and Paper Research Institute of Canada indicated that the undesirable production of the highly toxic furans and dioxins can be greatly minimized by the elimination of the non-chlorinated dibenzo-*p*-dioxin and dibenzofuran in defoamers used in chlorine bleaching mills [4]. Chlorinated phenolics such as catechols, guaiacols, vanillins and syringols in the bleachery effluents are derived from the degradation of lignin during the

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bleaching process. Although substituting chlorine dioxide for chlorine in the bleaching steps reduces the formation of the total chlorinated phenolics [5], complete elimination of these compounds would require the use of non-chlorine bleaching techniques. Installation of secondary (biological) waste treatment facilities by the pulp mills also removes many toxic substances including the phenolics from the effluents before they are discharged into the receiving waters.

Many chlorinated phenolics are acutely toxic to fish and their 96-h LC_{50} values (concentration which kills 50% of a test population over a 96-h exposure) range from 0.3 to 3 mg/l [6]. The octanol–water partition coefficients (K_{ow}) of chlorinated guaiacols and catechols are similar to those of chlorophenols with the same level of chlorine substitution [7], thus, accumulation of the toxic phenolics in the sediments is predicted and has actually been observed [8,9]. Therefore, there is a need to monitor the level of phenolic contamination in sediments created by the bleaching process from the paper mills.

Different approaches to the extraction of phenolics from sediments have been used [10]. Nearly all of them are either time-consuming or use a lot of solvent or both. We have previously developed a method for the extraction of resin and fatty acids from sediments collected downstream of pulp mills using supercritical carbon dioxide [11]. This supercritical fluid extraction (SFE) method not only provided recovery of the acids equal to or better than the Soxhlet technique, but was also extremely time-efficient and used practically no solvent. Recently, a technique involving the *in situ* extraction and chemical derivatization of polar compounds under SFE conditions has been demonstrated. Some successful examples include silyation of acidic components in coffee beans, tea and marine sediments [12], methylation of herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and dicamba in soil [13], methylation of fatty acids from whole cells of *Escherichia coli* [13], methylation and trifluoroethylation of phenols in coal gasification wastewater and wood soot leachate [13], pentafluorobenzylation of resin and fatty acid in river sediments downstream of pulp mills [11], as well as acetylation of chlorophenols in soil from a wood treatment plant [14]. This approach further reduced sample preparation time and simultaneously enhanced the extractability of polar organic compounds. In this paper, we shall describe an efficient SFE method for the determination of extractable chlorinated phenolics commonly found in sediments downstream of chlorobleaching mills.

EXPERIMENTAL

Reagents and chemicals

All chlorinated phenolics were obtained from Helix-Biotech Scientific (Vancouver, Canada) and used without further purification. These included 4,5- and 4,6-dichloroguaiacols (45G and 46G), 3,4,5- and 4,5,6-trichloroguaiacols (345G and 456G), 3,4,5,6-tetrachloroguaiacol (3456G), 3,5- and 4,5-dichlorocatechols (35C and 45C), 3,4,5-trichlorocatechol (345C), 3,4,5,6-tetrachlorocatechol (3456C), 6-chlorovanillin (6V), 5,6-dichlorovanillin (56V), and 3,4,5-trichlorosyringol (345S). Stock solutions of each individual compound were prepared in acetone at 1000 μg/ml and kept at −20°C in crimped top vials. A mixture of the above 11 phenolics at 10 μg/ml was also prepared in acetone for spiking and preparation of the acetylated standards.

Triethylamine and acetic anhydride were purchased from Aldrich (Milwaukee, WI, USA). The anhydride was triple distilled before use. SFC-grade carbon dioxide without helium head pressure was obtained from Scott Specialty Gases (Troy, MI, USA) and Praxair (Oakville, Canada). Silica gel (GC grade 950, 60–200 mesh, Fisher Scientific) was activated overnight at 200°C and the 5% deactivated silica gel was prepared by adding 5 ml of water to 95 g of the activated adsorbent.

Grab sediment samples were collected downstream of several Ontario pulp mills using chlorine bleaching. These samples were air dried at room temperature, crushed, ground and sieved through a 60-mesh screen before they were used in the extraction experiments.

SFE of sediment samples

All supercritical fluid extractions were carried

out with carbon dioxide using the Hewlett-Packard 7680A or 7680T extractor module. The two modules have similar capabilities except that, in the case of the 7680T, a series of up to eight thimbles can be prepared and loaded into the extractor for unattended sequential extraction. Prior to the extraction, two layers of Whatman GFC filter paper cut to internal diameter of the extraction thimble were placed at the bottom of the thimble before it was filled with 200 mg of Celite. The filter paper and Celite kept the sediment fines from plugging the fritted thimble cap and also prevented the modifier from leaking out of the thimble. The thimble was then filled with 1 g of sediment, followed by spiking 30 μl of triethylamine to the sample. The thimble contents were mixed for 30 s on a vortex mixer before the addition of another 200 mg of Celite. The derivatization reagent, 120 μl of acetic anhydride, was added to the top Celite layer. The thimble was then mixed again for 30 s. In a typical extraction, the extractor was set at a temperature of 110°C and a constant pressure of 37 MPa. Sample extraction and derivatization were first performed in the static mode for 10 min, followed by a 5-min dynamic extraction with a flow-rate of 2 ml/min to remove the analytes. During the dynamic extraction stage, the acetylated phenolics were collected on a built-in octadecylsilane (ODS) trap connected to a variable diameter restrictor nozzle which was responsible for the depressurization of supercritical carbon dioxide. The trap temperature was set at 15°C for the extraction stages and 40°C during the rising stage. Finally, the derivatized extract was removed from the trap by two 1-ml rinses of dichloromethane.

Column cleanup

The above dichloromethane rinses were combined and solvent exchanged into 1 ml of isooctane. The extract was then cleaned up on a 5-cm 5% deactivated silica gel column prepared with a 23-cm Pasteur pipet. After the extract was applied, the column was eluted with 5 ml of 5% dichloromethane in light petroleum (b.p. 30–60°C) and the eluate was discarded. The acetyl derivatives of the phenolics were eluted from the column by 10 ml of 1% methanol in dichloromethane. This fraction was subsequently solvent exchanged into 1 ml of isooctane for final analysis.

Chromatographic analysis

Gas chromatographic analysis was performed with both electron-capture detection (ECD) and mass-selective detection (MS). ECD was used for the routine analysis of sediment extracts for all phenolics and MS was used for the confirmation of peak identity. Splitless injection (1 μl) was made by a HP7673 autosampler onto a 25 m × 0.2 mm I.D. HP-5 fused silica column. The initial oven temperature was 70°C (0.75 min hold) and it was programmed to 120°C at 30°C/min and then to 180°C at 2°C/min. Splitless time was 0.75 min. Constant carrier (hydrogen) flow at 1.5 ml/min was maintained by an electronic pressure controller. In the case of MS analysis, selected ion monitoring (SIM) of the characteristic $[M-42]^+$ and $[M-42-15]^+$ ions for each compound was performed [15].

A mixture of the acetyl derivatives was prepared by an aqueous acetylation of known amounts of the phenolics [15] and appropriate dilutions of this mixture were used as external standards for the quantitation of the samples.

RESULTS AND DISCUSSION

Conventional extraction of phenolics from sediments

Organics from sediments are usually extracted by a solvent or a mixture or solvents at an elevated temperature (*e.g.* the Soxhlet procedure) or at ambient temperature (*e.g.* by an ultrasonic or high-speed mixing technique). In many cases, acidic compounds are better recovered from the sediment if a strong acid is present with the solvent system. However, in the cases of sediments with high contents of humic substances such as those samples collected from pulp and paper mills, extraction under acidic conditions produces a large amount of coextractives which may precipitate when the solvent is being evaporated. The precipitate not only changes the homogeneity of the extract if it is to be subsampled but can also adversely affect the derivatization reaction which is often required

for the gas chromatographic analysis of the acidic compounds.

Another approach that has been applied to the determination of pentachlorophenol (PCP) in sediment was steam distillation [16]. In our work, we found that some free phenols such as the less chlorinated catechols could not be fully recovered by this technique, presumably due to their higher water solubilities than other chlorophenols. We have also attempted to acetylate the phenolics in the sediment suspended in a potassium carbonate slurry and subsequently steam distilled the acetyl derivatives from the mixture. This method worked well with all chlorinated phenols, guaiacols and syringols but did not work with the chlorinated vanillins and catechols. The latter compounds were not recovered since their acetyl derivatives were completely decomposed during the steam distillation stage. Thus, before the advent of the SFE technique, solvent extraction was the only way to recover all the phenolics from a sediment sample.

TABLE I

% RECOVERY OF CHLORINATED PHENOLICS FROM SPIKED SEDIMENT SAMPLES USING THE *IN SITU* EXTRACTION AND DERIVATIZATION TECHNIQUE

All extractions were done at 80°C and 37 MPa with 1-g samples.

Spiking level (ng/g)	500	500	500	50
Amount of Et_3N (μl)	30	30	30	30
Amount of Ac_2O (μl)	30	30	120	120
Static time (min)	5	10	10	10
Dynamic time (min)	5	5	5	5
No. of replicates (n)	3	3	6	6
Recovery (%)				
45G	80	89	97 ± 5	94 ± 7
45C	67	92	92 ± 4	93 ± 6
345G	78	95	100 ± 7	98 ± 4
56V	54	81	98 ± 5	89 ± 6
345C	50	89	96 ± 8	92 ± 6
3456G	56	87	89 ± 4	96 ± 5
345S	73	90	91 ± 5	87 ± 6
3456C	16	44	84 ± 8	92 ± 7

Development of a SFE method for chlorinated phenolics in sediment

In the beginning, we were using the *in situ* extraction and derivatization method developed for the determination of PCP and other chlorophenols [14]. Using sediment spiked at 500 ng/g of the phenolics, a 1-g aliquot was extracted for 5 min statically and then dynamically with 37 MPa supercritical carbon dioxide at a temperature of 80°C in the presence of 30 μl each of triethylamine and acetic anhydride. Although the above *in situ* derivatization condition was also feasible for the extraction of the catechols and guaiacols from sediment samples, the results (column 2, Table I) indicated the recovery of the phenolics was far from complete, particularly for 3456C. An increase in static extraction time from five to 10 min produced a significant improvement on the recovery of all compounds (column 3, Table I), yet longer dynamic extraction did not help since the derivatization occurred during the static extraction stage. While chlorophenols and chloroguaiacols were easily converted into their acetyl derivatives under SFE conditions, our previous work on the aqueous acetylation of phenolics indicated that complete derivatization of the chlorocatechols required an excess of acetic anhydride [15]. This principle again applied to our present work, since an increase of the amount of anhydride used from 30 to 120 μl produced a recovery better than 85% for each phenolic compound from spiked sediment samples using the SFE technique (columns 4 and 5, Table I).

Once we had a method that worked reasonably well with spiked samples, the next phase of development was to optimize this procedure by applying it to naturally contaminated samples. In the following work, a bulk sediment collected approximately two km downstream of a bleached kraft mill was used as a reference sample. Analysis of effluent samples collected in the same area indicated the site was contaminated by resin and fatty acids as well as the chlorinated phenolics. By following the procedure developed for the spiked samples, all the common phenolics were detected in this reference sample. However, we were also able to recover an additional 30% or more of these phenolics from a second extraction of the same sample, indicating that the extraction–derivatization conditions were still not optimized for natural samples.

Factors affecting the SFE recovery of phenolics

Among the many factors that can affect the SFE results, the effect of extraction chamber temperature was the first one to be studied. The temperature dependence on the recovery of six major phenolic components in the reference sample, namely, 45G, 45C, 345G, 56V, 345C, 3456G and 3456C, was examined in 10°C increments from 40 to 120°C. In these experiments, 1-g aliquots of the sample were extracted for 10 minutes in the static mode and for a further 5 min in the dynamic mode at 37 MPa using 30 μl of triethylamine and 120 μl of acetic anhydride for the acetylation reaction. In order to have an easy comparison of the results, percent recovery obtained at various temperatures relative to that at 110°C was calculated for each compound. At an extraction chamber temperature of 40°C, less than 15% of the phenolics were extracted from the sediment and acetylated. Although the recovery of the catechols was vastly improved when the extraction and derivatization was carried out at 60°C, the guaiacols and 56V were still poorly recovered (≤40%) at this temperature. Continuous increase in recovery for all phenolics were observed when the extraction chamber temperature was increased to 100°C, where the recovery of catechols reached a maximum. While the recovery of the catechols began to drop at higher temperatures, highest recoveries for 56V and the guaiacols were obtained at 120°C. We were not able to study the recovery of these phenolics at even higher temperatures since 120°C is the maximum extraction chamber temperature that our extractor can reach. Since the optimal recovery of different phenolics were obtained at different temperatures, 110°C was chosen for the extraction and derivatization of sediments since it gave the best overall recovery of all compounds. A graphical summary of the temperature effect on the recovery of phenolics is depicted in Fig. 1.

The recovery of the chlorinated phenolics was also studied at four different extraction fluid densities, namely, 0.71, 0.64, 0.55, and 0.50 g/ml. No difference in the phenolics results was observed at the two highest fluid densities, suggesting that a further increase in density (or carbon dioxide pressure) would not result in better extraction efficiency. Although the chlorinated guaiacols and vanillins did not seem to be affected, the recovery of the catechols, particularly 3456C, dropped substantially at fluid densities of 0.55 and 0.50 g/ml and thus extraction with the lower-density fluid is not recommended. Extraction times of 10 (static) and 5 min (dynamic) were always used since shorter static time caused a reduction in the recovery while longer static and dynamic extractions did not improve the yield for the reference sample.

The amount of reagents used and the presence of solvents can also affect the derivatization and

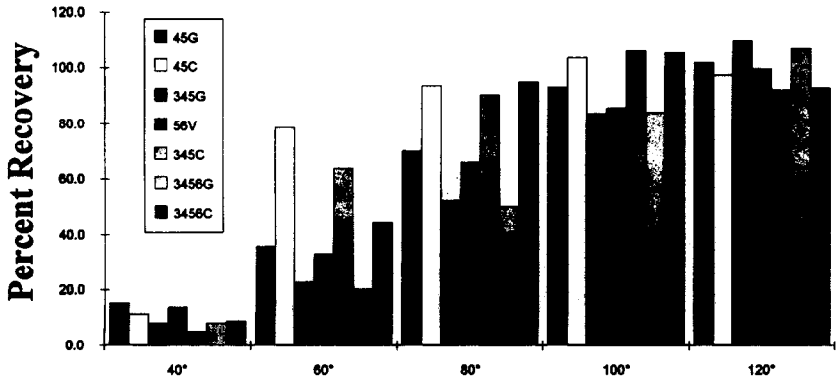

Fig. 1. SFE recovery of chlorinated phenolics from sediment at various extraction temperatures. All data are relative to those obtained at 110°C.

the recovery of the phenolics too. For example, the recoveries of guaiacols and catechols were *ca.* 60 and 15%, respectively, lower if triethylamine was not used in the derivatization. However, there was no significant change in the results when 60 instead of 30 μl of the base was used and there was a slight decrease in recovery when 240 instead of 120 μl of the anhydride was employed. We were also unable to improve the recovery of phenolics by the addition of a modifier such as dichloromethane to the sample. Yet, it was noted that the presence of either methanol or water was detrimental to the derivatization of all phenolics. Less than 25 or 50% of the phenolics could be recovered if 250 μl of methanol or water, respectively, were added to the sample prior to extraction. This result is not unexpected since both methanol and water react with the anhydride causing a deficiency in the reagent for derivatization. Therefore, the *in situ* SFE–acetylation technique should not be applied to a wet sediment sample.

Using the above optimized extraction and derivatization conditions, we were able to recover *ca.* 80% of the extractable phenolics from a natural sediment sample in the first extraction. An additional 10 to 20% of the phenolics could be recovered if a second extraction of the sample at 110°C with fresh reagents was performed. A third extraction, however, recovered less than 5% of the derivatized products. Therefore, two extractions of the same sample are required for the quantitative recovery of the extractable chlorinated phenolics from sediments.

Method evaluation and application

For further evaluation of this *in situ* extraction and acetylation technique, results for the reference sediment (sample A) obtained by SFE were compared with those acquired by conventional techniques such as steam distillation and Soxhlet extraction with acidified acetone (Table II). As mentioned earlier, only chloroguaiacols were recovered by our modified steam distillation procedure since the derivatives of chlorinated vanillins and catechols decomposed under such conditions. It is obvious from Table II that the SFE results, obtained by a single extraction, were very similar to the steam distillation results for chloroguaiacols and were slightly higher than all of the Soxhlet results. In the absence of a certified sediment reference material for total (free and bound) chlorinated phenolics, we were unable to ascertain how close were the SFE

TABLE II

LEVELS OF CHLORINATED PHENOLICS IN SEDIMENT SAMPLES DETERMINED BY VARIOUS TECHNIQUES AND FROM DIFFERENT LOCATIONS

All SFE results were based on a single extraction at 110°C and 37 MPa.

Compound	Concentration (ng/g)					
	Sample A, extraction by steam distillation[a]	Sample A, Soxhlet extraction[a]	Sample A, SFE[b]	Sample B, SFE	Sample C, SFE	Sample D, SFE
45G	410	381	396 ± 41	717	284	822
6V	N.D.	65	83 ± 6	505	222	303
45C	N.D.	305	325 ± 28	342	133	428
345G	123	126	131 ± 10	297	82	2258
56V	N.D.	40	44 ± 5	111	45	83
345C	N.D.	1205	1364 ± 75	1264	209	1416
3456G	13	11	15 ± 2	65	27	1502
3456C	N.D.	666	688 ± 42	982	113	2796

[a] Mean of two determinations. N.D. = None detected.

[b] Mean of six determinations and the uncertainty is 1 standard deviation.

results to the total phenolic contents in naturally contaminated sediments. However, our findings already indicated that the SFE technique was at least capable of producing precise and quantitative results for the free or extractable phenolics commonly found in sediments downstream of bleached kraft mill. Contrary to the procedures involving methanolic KOH hydrolysis [10], the SFE technique employed here will not convert catechols into guaiacols and produce biased results.

This SFE method has been applied to the determination of chlorinated phenolics in sediment samples of pulp mill origin and some of the results are tabulated in Table II. In all cases, the SFE extracts were subject to a silica gel column cleanup for the removal of polar coextractives such as acids and pigments. Failure to do so would cause interference in the subsequent GC–ECD analysis as well as a shortening of the life of the analytical column. Samples B and C were obtained from sites approximately 2 and 5 km, respectively, downstream of a chlorine-bleaching mill. A GC–ECD chromatogram of the acetylated SFE extract for sample B is shown in Fig. 2. Sample D came from the sedimentation basin of another bleached kraft mill and thus it is not surprising to find that its phenolic levels are higher than those in the river sediments. The predominant phenolics in these samples are 45G, 345G, 3456G, 45C, 345C and 3456C and their presence is consistent with previous findings [8–10]. Based on a 1-g sample and a final volume of 1 ml, the detection limit for these phenolics is *ca.* 10 ng/g. ECD gives a linear response over a range from 10 to 1000 pg/μl for all 11 acetyl derivatives [15].

CONCLUSIONS

An *in situ* extraction and acteylation procedure has been optimized for the determination of the extractable chlorinated phenolics in sediment samples. For the best recovery of all compounds involved in this work, the sample should be air dried prior to supercritical carbon dioxide extraction at 37 MPa and a temperature of 110°C. For 1 g of sediment, 30 μl of triethylamine and 120

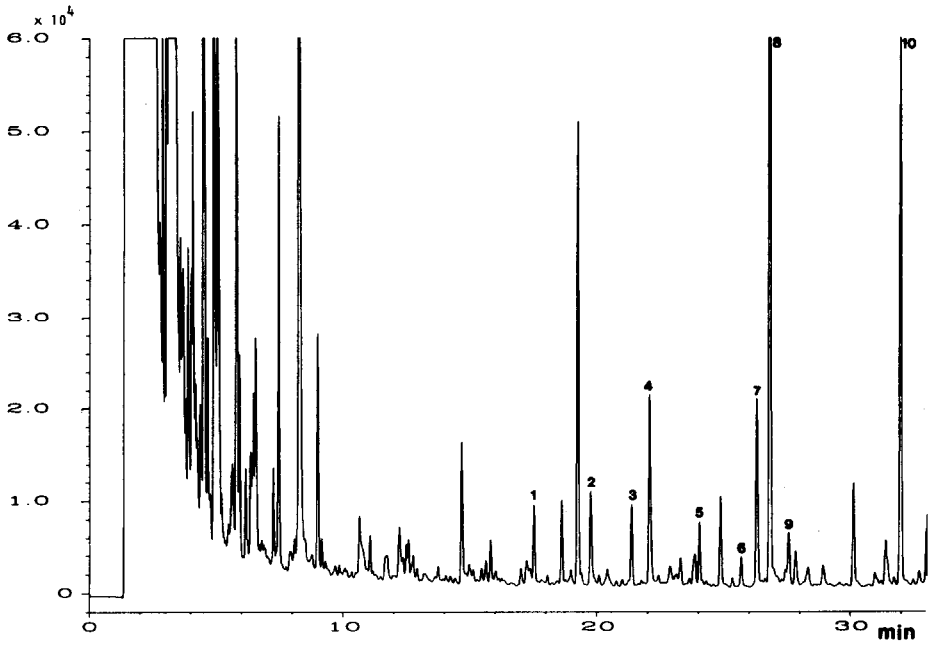

Fig. 2. A GC–ECD chromatogram of a SFE extract for a sediment (sample B) collected downstream of a chlorine bleaching mill. Peaks identified are acetyl derivatives of: 1 = 46G; 2 = 6V; 3 = 45C; 4 = 345G; 5 = 456G; 6 = 56V; 7 = PCP; 8 = 345C; 9 = 3456G; 10 = 3456C. y-Axis represents detector response.

μl of acetic anhydride were found to produce the best results for the acetylation of phenolics. A second extraction of the sample should be performed if quantitative recovery of the extractable phenolics in sediments is required. This procedure should not be applied to wet sediments since the presence of water in the sample is detrimental to the derivatization of the phenolics under SFE conditions.

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