Sorption Properties of Model Compounds on C₁₈ Adsorbents

Harold F. Walton

The bonded silica adsorbent, Bondapak-C₁₈® (Waters Associates) has been evaluated for removing organic matter from secondary sewage effluents and from solutions of pure organic compounds. The adsorbent is hydrophobic and will not function effectively in water unless it is first wet with methanol or another organic solvent; therefore, its behavior in water may be erratic. In water-methanol mixtures on the other hand it behaves reproducibly and gives linear absorption isotherms. Advantages of Bondapak-C₁₈®, compared with microparticulate adsorbents, include greater chemical robustness and tolerance to regeneration, lower flow resistance and lower cost. It has been used to remove organic compounds from water and as a support in low-resolution chromatographic analysis.

Part of this effort was to develop liquid-chromatographic methods of analysis for organic constituents of wastewater. For this purpose, microparticulate adsorbents were used along with a Sep-Pak® precolumn for rapid trace enrichment. Identification of the many chromatographic peaks was difficult, but the chromatograms were reproducible and permit different effluents to be compared and arbitrary treatment processes to be evaluated.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The research described herein is a continuation of that reported in March 1979: EPA Report No. 600/1-79-014; "Chemistry and Cytotoxicity of Renovated Wastewater." That report described a method of analysis of filtered secondary sewage effluents using selective adsorption and desorption of the less polar organic constituents and yielded a number of fractions of successively decreasing polarity. These fractions were tested for toxicity by a micro-scale cellular technique; some tests of mutagenic action were made, and attempts were made to resolve the fractions into individual, identifiable chemical compounds.

The primary purpose of the continued investigation, described in the final report, was to study in detail the adsorptive properties of Bondapak-C₁₈®. A secondary purpose was to determine how this material, and others like it, could be applied to the chromatographic analysis of the organic compounds in treated wastewater. The Bondapak-C₁₈® adsorbent was chosen because, when packed into a column, it retained an appreciable proportion of the organic material, about one-half of the total organic carbon, and released nearly all of it when water-methanol mixtures were passed through the column.

Bondapak-C₁₈® is a bonded-phase adsorbent designed for large-scale or preparative-scale liquid chromatography. It consists of irregular particles of superﬁcially porous silica, size range 37-75 micrometers, to whose surface is chemically bonded a hydrocarbon layer, equivalent to a coating of parafin one-molecule thick. The molecular fragments bound to silica are octadecyl groups, C₁₈H₃₇. For high-performance liquid chromatography (HPLC) very small and uniform particles, diameter 10 micrometers or less, are used, and they are
"totally porous," that is, they are penetrated by internal channels and have a large effective surface area. Ideally, every silicon atom on this surface carries a carbon chain, but not CH₂₃, then CH₃ groups used for "capping." The idea is to prevent free Si-OH groups that would otherwise exist at the surface of silica. It is impossible to avoid Si-OH groups entirely, however, and a part of the surface of Bondapak-C₁₈ may be considered to consist of Si-OH groups. They change the adsorptive properties, making the particles more prone to adsorb polar compounds, and even ionic compounds. Chemically, the coarse Bondapak-C₁₈ closely resembles fine-particle bonded-phase adsorbents such as Micro Bondapak-C₁₈ (Waters Associates), Partisil ODS® (Whatman), and Zorbax ODS® (DuPont)*.

**Experimental Procedures**

**Model Solutes**

**Breakthrough and Elution Studies**

Two kinds of tests were made: 1) breakthrough, in which solutions of the organic compounds were pumped through the column until the compounds "broke through" the column and emerged at the same concentration level as they went in, and 2) elution, in which small portions (0.1 ml or less) of solution were injected into a flowing stream of solvent, and the compounds were detected as a peak in ultraviolet absorbance when they emerged. "Elution" is the normal mode used in chromatographic analysis, but "breakthrough" and its reverse, "stripping," provides a better evaluation of the behavior of the adsorbent in retaining trace organic compounds from water. Further, "breakthrough" permits study of the effect of concentration and column loading.

Benzophenone was tested utilizing a Bondapak-C₁₈ column (0.45 cm ID x 10-15 cm long). Methanol-water mixtures were pumped through them under pressure, using a Waters pump, model 6000-A or 45; an ultraviolet absorbance detector operated at 254 nm was connected to the column exit. Methanol-water mixtures were used, rather than pure water, because benzophenone is almost insoluble in water; moreover, retention times in pure water are extremely large. Curves for breakthrough, stripping (desorption) and elution of benzophenone in 50% methanol are shown in Figure 1.

Caffeine was also chosen for detailed study because, as a common contaminant in wastewater, it is sufficiently hydrophobic to be adsorbed by Bondapak-C₁₈, yet is freely soluble in water. Adsorption-desorption curves (Figure 2) were obtained for caffeine at 10 ppm, in simulated city water (distilled water containing anhydrous CaSO₄, 120 ppm; NaHCO₃, 70 ppm, and CaCl₂·2H₂O, 47 ppm), on a Bondapak column (0.45 cm ID x 5.5 cm long) that had been previously conditioned with methanol.

Other compounds selected for evaluation were dibutylphthalate, diocetylphthalate, 2,4'-dichlorobiphenyl, anthraquinone, pyrene, atrazine, 4,4'-dichlorobiphenyl, 2,4-dichlorophenol, furfural, and pentachlorophenol. The majority of these compounds were selected from a list of Consensus Voluntary Reference Compounds (Keith et al., Environ. Sci. Tech., 13, 1469, 1979). These substances were passed separately through the column of Bondapak-C₁₈ (0.45 cm ID x 10.5 cm long) and elution volumes, (Vₑ), measured. Most of these substances are sparingly soluble in water and are strongly retained; retention volumes were therefore measured in water-methanol mixtures. Each measurement was made in triplicate. The concentration of solutes ranged between 1 and 50 ppm in order to permit direct UV measurement. The breakthrough volume (Vₑ) was determined to be independent of concentration in this range. For acidic compounds the addition of 0.1% acetic acid was required to repress ionization and to assure reproducible behavior. Plots of log Vₑ vs. methanol concentration are shown in Figures 3 and 4. By extrapolating the straight-line graphs to zero methanol concentration an estimate of breakthrough volume in pure water may be made.

**Liquid Chromatographic Analysis of Wastewater**

**Large-Scale Trace Enrichment and Gradient Elution**

This technique was described in the 1979 Report (EPA Report No. 600/1-79-104). Portions of treated wastewater containing 10-20 ppm of dissolved organic carbon were filtered under suction through glass fiber filter papers (Whatman GF-A and GFF, porosities 2.0 and 0.7 microns, respectively) before use. The pH after filtration was about 8; however, for some tests the pH was lowered to 4 by the addition of nitric acid. Columns consisted of stainless steel

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*Mention of trade names or commercial products does not constitute endorsement or recommendation for use

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**Figure 1.** Adsorption and desorption of benzophenone on Bondapak-C₁₈ from 50% methanol. These are schematic curves drawn from many strip-chart records. Note symmetrical shape. Theoretical-plate numbers calculated from the curve profiles were 115 for flow rate 1.0 ml./min, 165 for flow rate 0.5 ml./min.
Figure 2. Adsorption and desorption of caffeine on Bondapak-C<sub>18</sub> from water. These curves are copied from strip-chart records. Caffeine concentration was 10 mg/L; flow rate, curve (a), 2.5 mL/min, 0.275 cm/sec; (b), 5.0 mL/min, 0.55 cm/sec; (c), 7.5 mL/min, 0.825 cm/sec. Column dimensions, 0.45 cm x 5.5 cm. Absorbance measured at 280 nm, 1.0 absorbance units full scale. Note the small dependence on flow rate and the unsymmetrical shape, a nonlinear adsorption isotherm is indicated.

The Adsorbent

Regarding chemical selectivity, the partition of pure organic compounds between the adsorbent and a solvent (water or methanol-water mixture) and the order in which a series of compounds is adsorbed and released are the same for the 37-75 micron Bondapak-C<sub>18</sub> as the fine particle (< 10 micron) C<sub>18</sub> bonded phases used in HPLC. The main advantages of Bondapak-C<sub>18</sub> for wastewater analysis and the preparation of fractions for toxicity testing are that it is more robust and less prone to fouling by humic substances, and back pressures are much less than those observed with 10-micron particles, and the material is considerably less expensive.

When using Bondapak-C<sub>18</sub> to adsorb organic compounds from water, it must be recognized that it is hydrophobic and not wetted by water. It must first be thoroughly wetted with at least 50% methanol or another water-miscible solvent; then, when the greater part of this solvent has been washed out, the absorbent can be used to take up organic compounds from water. Its behavior towards purely aqueous solutions depends strongly on the small amounts of methanol sticking to it, and inevitable the behavior is unrepeatable, making it impractical to try to characterize it by exact physical tests. In mixed water-methanol solvents, however, its behavior is reproducible and similar to that of microparticulate C<sub>18</sub> bonded packings, except for the lower plate numbers.

Model Solutes

Experimental values of V<sub>des</sub>, volume of desorption, and V<sub>el</sub> for benzophenone; listed in Table 1, indicate that the adsorption of benzophenone on Bondapak-C<sub>18</sub> follows a linear isotherm. Assuming the isotherm to be linear, even at lowest concentrations one can predict that the breakthrough volume, V<sub>des</sub>, is the same at the very low concentrations prevailing in treated sewage effluents and in polluted water as it is using the concentrations in this work.

Figure 2 shows the adsorption-desorption curves for caffeine. A marked contrast exists between these curves and...
In Figure 2, there is a rapid concentration rise during breakthrough and a slower concentration fall during stripping. This behavior is due to a nonlinear adsorption isotherm. When nonlinear adsorption occurs, the adsorption becomes less strong as the concentration rises; this is a common phenomenon in chromatography. Measurements of $V_{oa}$ at different concentrations confirms this interpretation; as shown in Table 2.

Breakthrough volumes were measured for furfural at methanol concentrations ranging from 50% to 20% and in pure water. Figure 3 shows the relationship. At low methanol concentrations, the linear relationship breaks down. Adsorption on hydrophobic bonded-phase adsorbents is stronger in pure water and at very low methanol concentrations than the straightline plots predict. It has been suggested that in solvents rich in methanol, the long bonded hydrocarbon chains stretch out into the solvent and move freely about their anchor points on the silica surface, but in pure water they stick to each other to form a compact, hydrophobic, matted layer.

Data for the other model solutes (Figure 4) suggests a linear isotherm; however, the limited number of points makes it impossible to determine if a linear relationship holds at low methanol concentrations or whether an effect similar to that observed for furfural occurs.

**Wastewater Studies**

**Large Scale Trace Enrichment with Gradient Elution**

Figure 5 shows the results of tests with two Bondapak-C<sub>18</sub> columns. The erratic rise and fall of the dissolved TOC was mainly attributed to the inability to maintain a consistent flow rate. The effect of pH is clearly seen. Sorption was more effective at lower pH as expected since undissociated acids (mainly humic acids) are more strongly retained than the acid anions.

With the larger column, the TOC falls as more liquid is passed. At the very beginning of a run, the TOC is abnormally high due to methanol that is washed out of the adsorbent, but this effect only lasts for 100 mL at most. The gradual fall in TOC seen in Figure 5 might be due to another effect. Perhaps the humic acid adsorbed on the Bondapak-C<sub>18</sub> enhances the adsorption of other organic compounds. With the small column, however, the TOC rises slowly, that is, sorption becomes less efficient as time goes on. This is a more understandable effect, for the column must eventually become saturated with adsorbed material. Curve (d), Figure 5, shows that the column had adsorbed 6 mg of organic carbon during the run. The dry weight of Bondapak-C<sub>18</sub> in this column was 0.7 gram.

**Small-Scale Trace Enrichment**

A serious difficulty in analyzing the potentially toxic trace organic compounds in treated sewage isolated by the above procedure is the presence of humic material. Humic material is not a single chemical substance but an ill-defined high molecular weight polyelectrolyte with a variety of functional groups. By utilizing a C<sub>18</sub> Sep-Pak® that has been conditioned with methanol, the humic material is held back as a brown ring at the entrance to the Sep-Pak®. Elution with methylene chloride leaves nearly all the humic material behind. Despite this separation, the identification of individual peaks is nearly impossible because of their number and peak overlap. However, portions of the same filtered waste water passed through different Sep-Paks® and analyzed separately produced essentially identical chromatograms. Figure 6 shows the fluorescence record for a Denver and a Boulder wastewater. Note the prominent peaks in the Boulder sample.

**Conclusions**

Bondapak-C<sub>18</sub> adsorbs relatively hydrophobic compounds, including caffeine, phenols and chlorophenols, most pesticides, plasticizers and polycyclic aromatic hydrocarbons.

The main advantages of Bondapak-C<sub>18</sub> (37-75 microns) over micropropitulate Bondapak-C<sub>18</sub> (less than 10 microns) for wastewater analysis and the preparation of fractions for toxicity testing are: its lower cost and the fact that it is more robust and less prone to fouling by the humic substances.

Adsorption and retention volumes were determined for a variety of model solutes. These values were not affected appreciably by levels of salts and humic substances normally encountered in drinking water.

Preliminary data are given on a method of HPLC that uses a Sep-Pak® to adsorb and separate the less polar organic
Figure 4. Plots of capacity factors versus methanol concentration Bondapak-C<sub>18</sub>. Column (0.45 cm I.D x 10.5 cm long; void volume 0.8 mL; to find the adsorption volumes in mL, add (log 0.6 = -0.22) to ordinates.

<table>
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<th>Benzophenone concentration, ppm:</th>
<th>0.5</th>
<th>2.5</th>
<th>5.0</th>
<th>6.5</th>
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<tr>
<td>Flow rate, mL/min:</td>
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<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>F&lt;sub&gt;ads&lt;/sub&gt; (mL):</td>
<td>9.6</td>
<td>9.9</td>
<td>9.9</td>
<td>9.9</td>
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<tr>
<td>F&lt;sub&gt;ads&lt;/sub&gt; (mL)</td>
<td>10.2</td>
<td>9.0</td>
<td>9.9</td>
<td>9.8</td>
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</tbody>
</table>

*Table 1. Adsorption and Desorption Volumes of Benzophenone on Bondapak-C<sub>18</sub> from Solutions in 50% Methanol (V/V)*

**NOTES:**
(a) column dimensions, 0.45 cm x 10.5 cm.
(b) theoretical-plate numbers, from adsorption and desorption profiles, 115 (1 mL/min), 165 (0.5 mL/min).
(c) elution volumes, V<sub>elut</sub> for injections of benzophenone varying between 0.2 and 5.0 micrograms, were 10.0 ± 0.05 mL (mean of 14 measurements).

V<sub>elut</sub> = stripping or desorption volume.

**Recommendations**

Two practical purposes remain to be fully realized: one is the separation of the dissolved organic material in wastewater and treated sewage into well-characterized fractions, with a view to toxicity testing, and the other is the chemical analysis and recognition of specific organic compounds, including nonvolatile compounds, by high-resolution liquid chromatography. This work advanced significantly toward this goal through Sep-Pack® trace enrichment and high resolution liquid chromatography, as described in the latter part of the final report. These efforts should be continued, with emphasis on selective methods of chromatographic detection, particularly electrochemical, which distinguish oxidizable compounds like phenols and benzodiazines. Electrochemical techniques are now possible that can be used with gradient elution, which the older techniques could not.

A few tests were made with other adsorbents, notably anion-exchange resins, Duolite A-7 and Bio-Rad MP-1. Those resins removed organic compounds from water that were not removed by Bondapak-C<sub>18</sub>. Stripping each sorbent in turn using pH and solvent gradients would permit further selective fractionation. Additional work is needed in this area.
Table 2. Adsorption Volumes of Caffeine on Bondapak-C_{18} from Water

<table>
<thead>
<tr>
<th>Caffeine concentration, ppm:</th>
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<tr>
<td>Adsorption volume, mL:</td>
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<td></td>
<td></td>
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<tr>
<td>at flow rates:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mL/min</td>
<td>70</td>
<td>71</td>
<td>48</td>
</tr>
<tr>
<td>5.0 mL/min</td>
<td>65</td>
<td>62</td>
<td>46</td>
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<tr>
<td>9.9 mL/min</td>
<td>63</td>
<td>56</td>
<td>42</td>
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</tbody>
</table>

Column (0.45 cm ID x 10 cm long), freshly packed.

Figure 5. Dissolved organic carbon in effluent from Bondapak-C_{18}. Curves (a) and (c), column 1 cm x 10 cm; influent, 19.5 ppm carbon; (a), pH 7.2; (c), pH 3.5. Curve (b), column 1 cm x 10 cm; influent, 17 ppm carbon; pH 7.5. Curve (b) was taken a month after (a) and (c) but with the same packed column. Flow rate was about 5 mL/min but could not be controlled accurately. Points (d), column 0.45 cm x 10 cm; influent, 14 ppm carbon, pH 7.5; flow rate, 1.5 mL/min.

Figure 6. Fluorescence Chromatograms of two secondary sewage effluents: (a) Boulder, (b) Denver. Each injection corresponds to 10 mL of secondary effluent. Solvent gradient went from 25% to 75% methanol in 40 minutes.
Harold F. Walton is with the University of Colorado, Boulder, CO 80301. H. P. Ringhand is the EPA Project Officer (see below). The complete report, entitled “Sorption Properties of Model Compounds on C₁₈ Adsorbents,” (Order No. PB 85-125 839; Cost: $8.50, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
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