ABSTRACT. The breakthrough volume is the most important characteristic parameter to determine the suitability of a sampling device for isolating the analytes of interest. The home-made solid phase extraction-ultraviolet (SPE-UV) detection system has been developed for the determination of breakthrough volumes of adsorbents. The system consists of an HPLC pump that delivers the analyte, a stainless steel precolumn that contains the adsorbent, a selection valve, and a UV detector. This system was used to study the capacity and affinity analytes trapped in the modified PS-DVB adsorbents synthesized in the laboratory (PS-DVB heptadecyl ketone, PS-DVB chloromethyl, PS-DVB octadecoxy methyl) and commercial PS-DVB and ODS-silica adsorbents using nitrobenzene and 2-chlorophenol as the test compounds. The highest breakthrough volumes among the modified adsorbents were achieved for PS-DVB heptadecyl ketone i.e. 36.75 mL at 4 ppm of nitrobenzene and 4.68 mL at 4 ppm of 2-chlorophenol. PS-DVB heptadecyl ketone shows very good recovery values i.e 110 ± 2.01% for nitrobenzene and 68 ± 2.18% for 2-chlorophenol. Suggesting that PS-DVB heptadecyl ketone has greater capacity and affinity in trapping the analytes.

KEYWORDS. Breakthrough volume, polystyrene-divinylbenzene, Chromatography adsorbent.

INTRODUCTION

The ability of solid surfaces to bind molecules of organic compounds via different affinity mechanisms has been known for many decades. The analytical possibilities offered by this phenomenon were gradually recognized during long-term development of chromatographic techniques first introduced by Tswett at the turn of the twentieth century (Liska, 2000). Since then various hydrophilic functional groups were chemically attached to the benzene rings of porous, crosslinked polystyrene resins as stationary phase for SPE.

In SPE, the analytes to be extracted are partitioned between a solid and a liquid rather than between two immiscible liquids as in LLE and these analytes must have a greater affinity for the solid phase than for the sample matrix (retention or adsorption step) (Puig et al., 2007). The choice of adsorbent is therefore a key point in SPE because it can control parameters such as selectivity, affinity and capacity (Fontanals, 2005).

SPE is usually performed using a SPE tube containing appropriate packing. Modified ODS-silica reversed-phase adsorbent is one of the most common and widely used packing materials for SPE because of its greater capacity compared to other bonded silicas, such as the C₈ and CN types (Tang et al., 2008). The mechanism of retention is based on hydrophobic interactions between the solutes and the stationary phase (Van der Waals forces) (Leon-Gonzalez et al., 2000).
Nevertheless, the main drawback of such adsorbents is their narrow pH stability range. Consequently, when SPE has to be carried out in extremely acidic or basic media, reversed-phase polymeric adsorbents (generally based on PS-DVB) are used (Gülbakhan et al., 2008). In addition to their broader pH stability range that increase the flexibility of the method, these kind of adsorbents have a greater surface area per gram and they show relative selectivity for analytes with aromatic rings because of their π-π interactions (Fontanals et al., 2008). Owing to their hydrophobicity, they show a poor surface contact with predominantly aqueous solutions during SPE and cause a low breakthrough volume and adsorbent capacity.

The target of creating new types of chemically bonded resins is to overcome these drawbacks. For an improvement it has been shown that introduction of polar groups into a PS-DVB resin greatly increases the retention of polar organic compounds. Sanagi et al., (2005) modified PS-DVB with alcohol and acetyl functional groups which exhibited excellent hydrophilicity and a reduced dependence on wetting prior to extraction. In general, modified polymer phases have the advantage over bonded silicas that they can be used over the entire pH range.

One important parameter to control in the development of SPE method is the breakthrough volume, which is the sample volume where the analyte starts to elute from the exit of the column. The sample volume indicates the amount of analyte that can be preconcentrated and that is available for detection. The value of breakthrough volume is a function of the chromatographic retention of analyte on the particular sorbent in the SPE column and can only be altered by a change of sorbent (Hennion & Coquart, 1993).

The breakthrough volume can be determined by pumping a dilute solution of the analytes through the stainless steel precolumn connected to a detector through a selection valve (Hyötyläinen, 2007). The breakthrough volume and adsorbent capacity for a given packed bed are valuable information to determine SPE parameters such as adsorbent amounts and bed thickness (Liu et al., 2006). A home-made set-up for breakthrough volume and adsorbent capacity measurement can be utilized for characterization different adsorbents with ease and simplicity, time and cost effective.

The aim of this paper is to determine of breakthrough volume and percentage recovery of two different analytes namely, nitrobenzene and 2-chlorophenol on three different home-made adsorbents PS-DVB heptadecyl ketone, PS-DVB chloromethyl and PS-DVB octadecoxy chloromethyl. The SPE-UV detection system was developed in order to measure the breakthrough volume and percentage recovery of the analytes.

**EXPERIMENTAL**

**Instrumentation**

SPE tube in the form of stainless steel precolumn (Supelco, USA) (2.0 mm length and 0.6 mm I.D) was used to pack the sorbents, with an approximate weight of 0.150 g. A Rheodyne six port injection valve was used as switching valve (Cotati, USA) and the stainless steel precolumn being placed in the sample-loop position of the switching valve by using the set-up as shown in Figure 1. The analyte solution was passed through the precolumn by using HPLC pump, JASCO Waters-515 (Tokyo, Japan). Sample solution was introduced to precolumn at 0.1mL/min. UV detector from JASCO (Tokyo, Japan) was use to detect the analyte at 254 nm for nitrobenzene and 280 nm for 2-chlorophenol.
Breakthrough volume curves were acquired with a JASCO Waters-515 HPLC pump (Tokyo, Japan) and a JASCO Intelligent UV 2075 plus UV/Vis detector (Tokyo, Japan). All measurements were performed at 254 nm for nitrobenzene solution, and 280 nm for 2-chlorophenol solution. Data acquisitions were made using a Hewlett-Packard HP3396A integrator (USA). The percentage recovery of each analyte with different sorbents were carried out by using GC-FID; the analytes eluted from SPE tube were collected and then analysed using a Hewlett Packard Model 6890GC gas chromatography (GC) equipped with a flame ionisation detector (FID) and a data processor (USA). The gas chromatographic column used was Ultra-1 932530, a non-polar, fused-silica capillary column (30 m length × 250 μm inner diameter × 0.20 μm film thickness) (USA). Helium was used as the carrier gas at a flow rate of 1.1 mL/min at a pressure of 75 kPa. The injector temperature was set at 250°C and the detector temperature at 310°C. The gas chromatography oven was operated under programmed temperature with an initial temperature of 100°C, which was held for 2 minutes and ramped up to 140°C at a rate of 5°C/min (Puig, 2007). Each sample (1 μL) was injected into the gas chromatograph by using a 10 μL syringe obtained from Agilent (Little Fall, USA). Three injections were carried out for each sample to obtain a good accuracy.

Solid Phase Extraction (SPE) Precolumn Packing

In this study, a new set-up was developed to obtain the breakthrough volumes and recoveries data. Guard column / precolumn was used in place of traditional SPE polypropylene tubes to pack the sorbent. The original content of the guard column was removed and the column was thoroughly cleaned. For packing purpose the top plastic cap was removed and the sorbent was slowly added (which was assisted by or with the help of syringe barrel that acted as funnel) into the stainless steel precolumn. A small amount of sorbent was added at a time and each time the stainless steel precolumn will be given a gentle tap – to have homogenous/compact packing (Schmidt & Fritz, 1993). This was done until the whole stainless steel precolumn cavity was

Figure 1. (a) The experimental set up of the SPE-UV detection system. (b) The stainless steel precolumn and the housing.
filled with sorbent (150 mg). The plastic cap that was removed earlier for packing purpose will be fixed to the same position – top end of the stainless steel precolumn. The SPE stainless steel precolumn packing processes is illustrated in Figure 2.

![Figure 2. SPE precolumn packing process: (a) Construction of the SPE stainless steel precolumn (b) Cap removed for sorbent filling. (c) SPE stainless steel precolumn fully capped (d) SPE stainless steel precolumn placed inside the housing](image)

**Conditioning of Precolumn**

The SPE sorbent was activated / cleaned by pumping 2.0 mL of methanol through the stainless steel precolumn using HPLC pump at a flow rate of 0.5 mL/min. Displacement of methanol was done by using 2.0 mL of deionised water at the same flow rate as above. Upon completing the above steps, the sorbent is ready to receive sample solution. Test solution of 4 ppm 2-chlorophenol and nitrobenzene will be passed through the precolumn at a flow rate of 0.1 mL/min. Selection of the low flow rate is to maintain a low pressure which might interrupt the entrapment of analyte. Once the recording of the breakthrough volume is completed i.e the curve reached 10% of the initial UV absorbance, regeneration of the sorbent will be carried out where the sorbent will be cleaned with methanol and deionised water until it gives zero base line reading on UV detector. The equations used to calculate the breakthrough volumes are as follows:
Breakthrough volumes = Retention time x Flow rate \hspace{1cm} (1)
Retention time = Retention distance / Chart speed \hspace{1cm} (2)

The formula used to calculate the response factor for each of the analyte studied is

\[
\text{Response factor, } F_x = \frac{\text{Average peak area of test compound (pA)}}{\text{Concentration of test compound (ppm)}} \hspace{1cm} (3)
\]

**Breakthrough Volume Measurement Procedure**

Test stock solutions of 40,000 ppm were prepared by weighing 1.0 g of each nitrobenzene and 2-chlorophenol separately in two 25-mL volumetric flasks and diluted in methanol to 25 mL. The working test solution or sample aqueous solution for each analyte; nitrobenzene and 2-chlorophenol at 4 ppm were prepared by adding 0.2 mL of 40,000 ppm stock solution into two separate 2000 mL volumetric flasks and then diluted to the mark with deionized water. Conditioning of the precolumn was carried out. Solution containing 400 ppm of each test compound was passed through the precolumn at a flow rate of 0.1mL/min for 10 mins. The precolumn was purged with nitrogen gas to remove the water for a duration of 30 mins at 80 psi (Lefebvre, 2007). The precolumn was eluted with 1.0 mL of methanol and the eluate was collected in a centrifuge tube. The internal standard (0.1 mL) was added to the eluate, capped and agitated. The eluate was stored in a refrigerator while waiting for analysis. 1.0 uL aliquot was injected into GC to obtain the chromatogram. The working test solution or sample aqueous solution for each analyte; nitrobenzene and 2-chlorophenol at 400 ppm were prepared by adding 1.0 mL of 40,000 ppm stock solution into two separate 100 mL volumetric flasks and then diluted to the mark with deionized water.

**RESULTS AND DISCUSSION**

**Breakthrough measurements**

The retention efficiency of modified PS-DVB and unmodified PS-DVB were determined by measuring the breakthrough volume of the adsorbents. The equations used to calculate the breakthrough volumes are as in equation 1 and 2. The concentration of the analyte used in determining the breakthrough volumes was 4 ppm. Table 1 shows the breakthrough volume of ODS-silica, unmodified and modified PS-DVB sorbents using nitrobenzene and 2-chlorophenol as analytes.

Table 1. Breakthrough volumes for ODS-silica, unmodified PS-DVB and modified PS-DVB sorbents using nitrobenzene and 2-chlorophenol as analytes.
From Table 1, it is apparent that, the PS-DVB heptadecyl ketone has the highest breakthrough volumes for both analytes compared to those for commercial PS-DVB and modified sorbents used in this study. PS-DVB heptadecyl ketone having the highest capacity in trapping the analytes i.e 0.15 mg of nitrobenzene and 0.02 mg of 2-chlorophenol per 150 mg of sorbent. PS-DVB octadecoxy methyl sorbent capacity was lowest compared to other modified sorbents (Table 2). This may be due to the presence of the polar carbonyl groups on PS-DVB heptadecyl ketone surface, which improved the efficiency of the adsorbent by increasing the ability to undergo polar interactions with the polar analytes. In addition, the presence of polar groups also caused the sorbent to be wetted easily and have an intimate contact with aqueous solution, therefore the analyte can easily be extracted from the solution. Another reason could be that better interactions occurred between solutes (polar and non-polar) and PS-DVB heptadecyl ketone sorbent owing to higher surface area of the sorbent (the home-made PS-DVB have been synthesized using 8% of crosslinker whereas the commercial PS-DVB only 4%).

Table 2. Sorbent capacity of ODS-silica, unmodified PS-DVB and modified PS-DVB sorbents using nitrobenzene and 2-chlorophenol as analytes.
<table>
<thead>
<tr>
<th>Sorbent (150mg)</th>
<th>Nitrobenzene 4 ppm</th>
<th>2-Chlorophenol 4 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sorbent capacity (mg)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>ODS-silica</td>
<td>0.17</td>
<td>1.31</td>
</tr>
<tr>
<td>PS-DVB commercial</td>
<td>1.24x10^{-3}</td>
<td>8.89</td>
</tr>
<tr>
<td>PS-DVB heptadecyl ketone</td>
<td>0.15</td>
<td>2.69</td>
</tr>
<tr>
<td>PS-DVB Chloromethyl</td>
<td>1.24x10^{-3}</td>
<td>5.10</td>
</tr>
<tr>
<td>PS-DVB Octadecoxy methyl</td>
<td>0.48x10^{-3}</td>
<td>17.68</td>
</tr>
</tbody>
</table>

The breakthrough volume for nitrobenzene and 2-chlorophenol were 0.30 mL and 0.10 mL respectively. Figure 3 shows the breakthrough curve of nitrobenzene and 2-chlorophenol using commercial PS-DVB. Based on the results obtained, the analytes were poorly retained on the said sorbent. The nature of analytes plays an important role in retention mechanisms on the PS-DVB which involves the \( \pi-\pi \) interactions between the analyte and sorbent. PS-DVB possesses exceptionally strong \( \pi \)-electron donating-accepting ability, which causes retention of compounds that contain aromatic \( \pi \)-systems or functional groups with lone electron pairs such as carbonyl and nitro groups. Even though the mentioned factors are favorable for the retentions of analytes, the observed breakthrough volumes were small which could be caused by the small capacity of the sorbent.
Figure 3. Breakthrough volume curve of (a) nitrobenzene and (b) 2-chlorophenol using PS-DVB as the adsorbent.

Figure 4 shows the breakthrough curve of nitrobenzene and 2-chlorophenol using PS-DVB heptadecyl ketone. Higher breakthrough volume was observed for both analytes compared to other modified PS-DVB. The breakthrough volumes for nitrobenzene was higher compared to 2-chlorophenol on PS-DVB heptadecyl ketone sorbent.
Figure 4. Breakthrough volume curves of (a) nitrobenzene and (b) 2-chlorophenol using PS-DVB heptadecyl ketone.

The presence of electron-withdrawing or positive electron resonant capacity substituents on analyte caused the polymer to donate electron to the analyte. Higher retention of 2-chlorophenol on PS-DVB heptadecyl ketone was contributed by the hydrogen bonding between the hydrogen from hydroxyl groups of the analyte and the oxygen from the carbonyl groups on the adsorbent. According to the Lewis acid-base Theory, the benzene rings on PS-DVB and the carbonyl groups on PS-DVB heptadecyl ketone can be considered as Lewis base while the phenolic compounds can act as a Lewis acid. However, the oxygen on the carbonyl group on PS-DVB heptadecyl ketone exhibited larger dipole moment and resulted in better Lewis base
property in relative to the benzene ring on PS-DVB. Consequently, the interaction of phenolic compounds was found to be much better by using PS-DVB heptadecyl ketone instead of PS-DVB as the adsorbent. The breakthrough volume for nitrobenzene and 2-chlorophenol using PS-DVB chloromethyl were 0.31 mL and 0.16 mL respectively, meanwhile for PS-DVB octadecoxy methyl were 0.12 mL and 0.10 mL respectively. The small amount of breakthrough volumes observed for the PS-DVB chloromethyl and PS-DVB octadecoxy methyl mostly is attributed to the small capacity owned by the respective sorbent, incompatibility of wetting solvent used and the homemade sorbents were unsatisfactorily synthesized.

**Measurement of Percentage Recovery**

The chromatogram of test compounds and internal standard obtained is given in Figure 5. All the test compounds and butyrophenone were eluted within ten minutes. The retention times for the test compounds as well as internal standard are listed in Table 3.

![Image of chromatogram](image)

**Figure 5.** Separation of test compounds and butyrophenone (internal standard) using gas chromatography. Peaks: 1 – chlorophenol, 2 – nitrobenzene, 3 – butyrophenone

The response factor is usually utilized in GC accurate quantitative calculation. In this study, 1 µL of each test compounds and the internal standard stock solutions (400 ppm) were injected into the gas chromatograph to determine the response factor ($F_X$) for each compounds. Three injections were carried out to obtain a measure of accuracy. The response factors for each compound were calculated as per equation 3.

**Table 3.** Retention time of test compounds and butyrophenone in GC chromatogram.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time, (t&lt;sub&gt;R&lt;/sub&gt;/minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>4.244</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>5.153</td>
</tr>
<tr>
<td>Butyrophenone (Internal Standard)</td>
<td>7.931</td>
</tr>
</tbody>
</table>

The peak areas, response factors, concentrations, percentage recovery and relative standard deviations of each test compound is summarized and listed in Table 4. It was found that the recovery of the less polar compounds, nitrobenzene was higher (44%) compared to the more polar compound, 2-chlorophenol (35%).

**Table 4. The average peak areas, average concentration values, percentages recovery, and relative standard deviation from the test compounds with ODS-silica SPE precolumn**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Average Area</th>
<th>Average Concentration (ppm)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>4.2</td>
<td>139</td>
<td>35</td>
<td>7.92</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>7.6</td>
<td>175</td>
<td>44</td>
<td>2.17</td>
</tr>
</tbody>
</table>

The efficiency of PS-DVB heptadecyl ketone as SPE adsorbent was evaluated by determining the analytes recovery percentage. Based on the results obtained in Table 5 it was observed that the overall results demonstrated high recoveries in the range of 68%-110% with RSD values of 2.07% to 2.18% using the PS-DVB heptadecyl ketone as an adsorbent. This adsorbent shows a better affinity compared to ODS-silica with large difference between the percentage recovery of the analyte. A flow rate of 0.1 mL/min was used to ensure sufficient interaction between the analytes and elution solvent, which aids the mass transfer of analytes from sorbent to elution solvent. The PS-DVB heptadecyl ketone exhibited strong hydrophobic characteristics due to the polymer backbone and hydrophilic properties through the polar carbonyl functional group on its surface.

**Table 5. Average peak areas, average concentration values, percentage of recovery, and relative standard deviation for test compounds desorbed from PS-DVB heptadecyl ketone.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Average Area</th>
<th>Average Concentration (ppm)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>7.45</td>
<td>273</td>
<td>68</td>
<td>2.18</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>17.8</td>
<td>441</td>
<td>110</td>
<td>2.07</td>
</tr>
</tbody>
</table>

The analytes were not detected during the recoveries study using commercial PS-DVB and other modified PS-DVB. This might be due to the small adsorbent capacity to trap the analytes. The analytes desorbed from the sorbent was probably too low to be detected by GC.
FID. The capacity of the adsorbent can be increased by increasing the sorbent mass, but in this study, a fix volume of precolumn was used to pack the sorbents.

The efficiency of ODS-silica and PS-DVB heptadecyl ketone in SPE stainless steel precolumn was compared by means of percentage recovery of test compounds. Table 6 shows the comparison of recovery percentage and RSD values obtained using ODS-silica and PS-DVB heptadecyl ketone as adsorbents.

The higher recoveries of the test compounds on PS-DVB heptadecyl ketone were due to the hydrophilic character of the introduced functional groups which increases its surface polarity and improved the sorbent wetting property. The ability of polar surface to reduce the surface tension of the water thus enabled the aqueous sample to interact with the resin surface and enhanced the mass transfer of the analytes from the water solution to the sorbents. Although PS-DVB heptadecyl ketone has a hydrophobic surface, it also contains relatively large number of active aromatic sites, which allow π-π interactions between aromatic analytes and the sorbents.

### Table 6. Comparison of percentages of recovery (%R) and relative standard deviation for the extraction of test compounds using ODS-silica and PS-DVB heptadecyl ketone.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ODS-silica</th>
<th>PS-DVB heptadecyl ketone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% R</td>
<td>RSD %</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>35</td>
<td>7.92</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>44</td>
<td>2.17</td>
</tr>
</tbody>
</table>

Even though nitrobenzene is less polar compared to 2-chlorophenol, greater amount of nitrobenzene was eluted by methanol due to amount of nitrobenzene retained on the sorbent was very high. The low recoveries of ODS-silica might be due to the hydrophobic surface of the sorbent. The consequence is poor surface contact with predominantly aqueous solutions. Less analyte is retained on the sorbent and therefore less analyte desorbed when eluting with methanol.

In this experiment, methanol was chosen as the elution solvent because its volatile characteristic was compatible to the subsequent gas chromatography analysis. Methanol was found to be a good elution solvent for the extraction of polar analytes using reversed phase adsorbents. The hydroxyl group on methanol that contributed to its polarity enables the solubility of analytes that were retained on the adsorbent.

**CONCLUSIONS**
Three adsorbents were studied and ODS-silica was used as comparison. Based on this study, PS-DVB heptadecyl ketone adsorbent exhibited higher breakthrough volume of 36.75 mL and 4.68 mL for nitrobenzene and 2-chlorophenol respectively with % RSD at 2.69% and 21.52% compared to the other modified PS-DVB.

Thus, it was established that the PS-DVB heptadecyl ketone has a very good adsorbent capacity compared to other modified PS-DVB and comparable to that of commercial ODS-silica. As for percentage recovery, the PS-DVB heptadecyl ketone, shows high recoveries, 110% for nitrobenzene and 68% for 2-chlorophenol as well as good reproducibility with relative standard deviation between 2.07% and 2.18%.

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