Interpretation on Band-broadening in Chromatography with Spatial Peak Profiles Obtained Using Whole-column Detection

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Fifteen liquid chromatographic experiments were investigated using a whole-column detection (WCD) system and a conventional post-column UV/Vis detector. The peak widths obtained from chromatograms were found dependent on the retention factor; the larger the retention factor was the greater the peak width. However, the on-column spatial peak widths were dependent on the locations where they were measured in the column. The peak widths monitored at 17 cm from the column inlet were found essentially the same no matter what their retention factors were. In addition, a linear relationship was found between the chromatographic peak width and the reciprocal of the average linear rate of the solute migration. The peak widths on chromatograms did not reflect how they appeared in the column; instead, the widths were determined by the solute speed passing the detector.

Keywords: Liquid chromatography; Chromatography; Peak broadening; Whole-column detection.

INTRODUCTION

Chromatography is a powerful separation method that finds applications in all branches of science. Historically, chromatography can be dated back to the Russian botanist, Mikhail Tswett,\textsuperscript{1} who gave a lecture in 1903 on the separation of the pigments in green leaves on a chalk column. He employed the technique\textsuperscript{2} to separate various plant pigments such as chlorophylls and xanthophylls by passing solutions of these compounds through a glass column packed with finely divided calcium carbonate. The separated species appeared as colored bands on the column. At that time, chromatographers had to observe the separated bands with the naked eye because on-line detection techniques were not available. Scientists then realized that column efficiency could be improved by decreasing the particle size of the packing material. It was not until the late 1960s, however, that the technology for producing and using packings with particle diameters as small as 3 to 10 \(\mu\)m was achieved.\textsuperscript{3-5} Equipment had to be manufactured to accommodate columns packed with the fine particles without loss of performance and to withstand high back pressures. Accordingly, glass columns of classic gravity-flow liquid chromatography (LC) were gradually replaced by the stainless steel columns used as in the present form.\textsuperscript{6,7} In the meantime, on-line detectors were developed to monitor the flowing signals, for example, UV/Vis absorption detector for LC and flame ionization detector for gas chromatography (GC). Since either packed or capillary GC columns are housed in a temperature-controlled oven, just like the situation with the stainless steel LC columns, the inside of the column is no longer visible as it was before. After being equipped with on-line detectors, chromatograms of solute concentration profiles recorded as a function of time (on the temporal coordinate) became standard. All studies on column efficiency, i.e. band-broadening during the elution, were then analyzed through chromatograms. Since then, most chromatographers paid less or no direct attention to what really happened inside the column during the separation.

In a linear chromatographic elution, the compound with the lower retention time appears faster with a comparatively narrower and sharper peak shape compared to the wider and flatter shape of the later peak with the higher retention time on the chromatogram. Some would probably think that the explanation of band-broadening for the later peak is that the breadth of the band increases on the time coordinate for the higher retained solute with the higher retention time on the chromatogram. Some would probably think that the explanation of band-broadening for the later peak is that the breadth of the band increases on the time coordinate for the higher retained solute because more time is allowed for spreading to occur on the spatial coordinate. However, we intend to point out that the analyte peaks obtained temporally (as a function of time, such as in conven-
tional chromatograms) are by no means the same as those obtained spatially (the true analyte distribution in the column). Rowlen et al.\textsuperscript{8} pointed out that on-column peak widths depend only on the position in the column, but not on the retention factor ($k$). In other words, the peak width of a slow-moving solute with a larger $k$ value should be close to that of a fast-moving solute with a smaller $k$ value. Using a whole-column detection (WCD) measurement, Tamura et al.\textsuperscript{9} also found the same results. In their study, the solute bandwidth broadened more significantly from inlet to outlet due to the large packing material (30 µm). However, the solutes of different $k$'s still gave almost the same bandwidth as long as they were monitored at the same column positions.

We employed a laboratory-made WCD system and a high-pressure glass column to monitor the on-column chromatographic peaks. Since the whole peak profile of the moving analyte was scrutinized during the entire elution, it provided direct on-column observations and thus was able to show the true peak-broadening behavior.

**EXPERIMENTAL SECTION**

The schematic diagram of the experimental set-up is illustrated in Fig. 1. A detailed description of the system can be found elsewhere.\textsuperscript{10} A LC pump (Series 4, Lab Alliance, PA, USA) provided different isocratic elutions for a laboratory-made HPLC glass column.\textsuperscript{10} This column (25 cm long × 3 mm I.D.) was packed with 10 µm octadecylsilane stationary phase. A UV/Vis detector (Model 486, Waters, MA, USA) was installed at the column outlet to acquire chromatograms. A cold cathode fluorescence lamp was employed as the light source. A 21 cm × 2 cm interference filter (band center = 435 nm, bandwidth = 50 nm, transmittance = 90%) was placed between the source and the column in order to provide a narrower wavelength bandwidth for absorbance measurements. A linear charge-coupled device (CCD) transducer was installed in an optical box on the other side of the column. Solute absorbance profiles were condensed in the optical box to form images onto

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**Table 1. Mobile phase compositions and the experimental results**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mobile phase Methanol : Water</th>
<th>$k$</th>
<th>$\bar{u}$ (cm min$^{-1}$)</th>
<th>$W_{1/2}$ (min)</th>
<th>$W_{1/4}$ (cm) at 17 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99 : 1</td>
<td>0.54</td>
<td>9.92</td>
<td>0.14</td>
<td>2.13</td>
</tr>
<tr>
<td>2</td>
<td>97 : 3</td>
<td>0.65</td>
<td>9.26</td>
<td>0.16</td>
<td>2.24</td>
</tr>
<tr>
<td>3</td>
<td>95 : 5</td>
<td>0.77</td>
<td>8.64</td>
<td>0.18</td>
<td>2.24</td>
</tr>
<tr>
<td>4</td>
<td>93 : 7</td>
<td>0.93</td>
<td>7.93</td>
<td>0.19</td>
<td>2.28</td>
</tr>
<tr>
<td>5</td>
<td>91 : 3</td>
<td>1.10</td>
<td>7.29</td>
<td>0.21</td>
<td>2.21</td>
</tr>
<tr>
<td>6</td>
<td>89 : 11</td>
<td>1.35</td>
<td>6.52</td>
<td>0.23</td>
<td>2.21</td>
</tr>
<tr>
<td>7</td>
<td>87 : 13</td>
<td>1.63</td>
<td>5.82</td>
<td>0.25</td>
<td>2.13</td>
</tr>
<tr>
<td>8</td>
<td>85 : 15</td>
<td>1.97</td>
<td>5.17</td>
<td>0.28</td>
<td>2.06</td>
</tr>
<tr>
<td>9</td>
<td>83 : 17</td>
<td>2.35</td>
<td>4.57</td>
<td>0.31</td>
<td>2.02</td>
</tr>
<tr>
<td>10</td>
<td>81 : 19</td>
<td>2.86</td>
<td>3.97</td>
<td>0.34</td>
<td>1.99</td>
</tr>
<tr>
<td>11</td>
<td>79 : 21</td>
<td>3.46</td>
<td>3.43</td>
<td>0.39</td>
<td>1.91</td>
</tr>
<tr>
<td>12</td>
<td>77 : 23</td>
<td>4.19</td>
<td>2.95</td>
<td>0.44</td>
<td>1.88</td>
</tr>
<tr>
<td>13</td>
<td>75 : 25</td>
<td>5.09</td>
<td>2.51</td>
<td>0.51</td>
<td>1.80</td>
</tr>
<tr>
<td>14</td>
<td>73 : 27</td>
<td>6.06</td>
<td>2.17</td>
<td>0.57</td>
<td>1.76</td>
</tr>
<tr>
<td>15</td>
<td>71 : 29</td>
<td>7.27</td>
<td>1.85</td>
<td>0.66</td>
<td>1.73</td>
</tr>
</tbody>
</table>
the CCD. The measurement window on the column ranged from 5 to 20 cm, counted from the inlet.

Anthrarufin, obtained from Aldrich (WI, USA), was dissolved in acetone to make a concentration of 0.62 mmol L\(^{-1}\). Methanol and de-ionized water in various proportions were compounded into mobile phases (listed in Table 1). The flow rate was 1 mL min\(^{-1}\) for all experiments. Both organic solvents were also obtained from Aldrich.

![Fig. 2. (a) Chromatograms of 15 different retention factors. (b) On-column spatial peak profiles of 6 selected retention factors monitored at 9, 11, 13, 15, 17 cm from column inlet.](image-url)
RESULTS AND DISCUSSION

At the injection of the sample, both the WCD and the UV/Vis detector started to collect absorbance data of the on-column peak profiles and the post-column chromatograms. The experimental results, including the retention capacity \(k\), the average linear rate of solute migration \(\overline{u}\), the width of the chromatographic peak at half its maximum height, \(w_\frac{1}{2}\), and the width of the on-column spatial peak at half its maximum height \(w_\frac{1}{2}(s)\) obtained at 17 cm on the column for the 15 runs, are listed in Table 1. The chromatograms for 15 runs are shown in Fig. 2(a). As expected, elutions with smaller \(k\) values gave narrower and sharper peak profiles and those with greater \(k\) values gave wider and flatter profiles. The propagations of the spatial peaks for six selected runs (\(k = 0.54, 0.77, 1.10, 2.35, 4.19,\) and \(7.27\), respectively) are shown in Fig. 2(b). Five on-column peak profiles were recorded at column positions 9, 11, 13, 15, and 17 cm, respectively. As can be seen, the bandwidth increment within any one experiment from 9 cm to 17 cm was limited to ~10%.

The major purpose of this study is to explain that the peak widths shown on the chromatograms do not reflect directly what they are in the column. Accordingly, comparison of the peak widths between the post-column chromatograms and the on-column spatial profiles would reveal the fact. Since they were recorded using different units – min and cm, direct comparison was impossible. The ratio of peak width as a function of \(k\) is shown in Fig. 3. Assuming the width of the peak under \(k = 0.54\) is unity, the rest of the 14 peaks were calculated by taking the ratio against unity. Under the same operation, the width of the spatial peak at 17 cm under \(k = 0.54\) is unity, and the rest of the peaks were calculated. The ratio shows a linear increment with a significant slope as the \(k\) increases for the 15 chromatograms. The width of the run with the highest \(k\) (7.27) can be as large as ~5 times of the run with the lowest \(k\) (0.54). However, the ratio for the spatial peaks remains very close to 1 no matter what the \(k\) is. Not only did the ratio not increase with the \(k\) values, it became even lower than 1 when \(k\) increased. The chromatographic peak widths are then plotted against the reciprocal of \(\overline{u}\) in Fig. 4. The linear relationship gives positive evidence for the argument that peak width depends on the average linear rate of solute migration. In other words, a highly retained solute (larger \(k\)) results in slower analyte migration speed, thus a broader peak width.

CONCLUSION

The spatial peak profiles broadened during the elution depending on their on-column locations, but not their retention factors. The on-column spatial peak widths were very close to one another no matter what their retention factors were. However, the peak width recorded on the temporal coordinate using a conventional post-column detector was related to the solute migration speed. Peaks with smaller \(k\) values gave narrower widths while those of greater \(k\) values gave broader widths. This is why peaks coming out of the column later usually exhibit broader in isocratic LC and isothermal GC elutions. Both gradient elution in LC and temperature programming in GC are used...
to improve separation efficiency on a daily basis. Sometimes, peaks with larger retention times exhibit narrower widths than those with a smaller retention time in non-linear chromatography.\textsuperscript{11-14} This can be explained by the fact that the higher solute migration speeds occur during the latter part of the elution at which the greater solvent strengths in LC or the higher temperatures in GC are encountered. Thus, the peak widths obtained on the chromatogram do not reflect how they may look on the spatial coordinate in the column.

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