Waters
Lab Highlights
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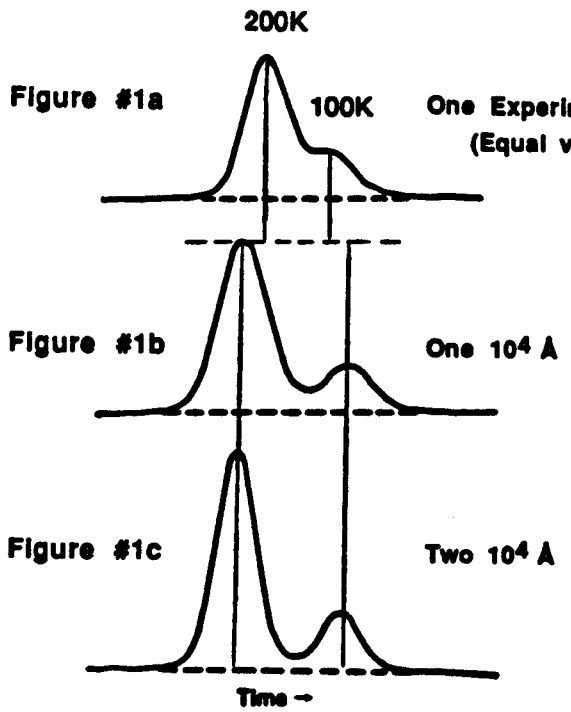
Resolution of Partially Overlapping Molecular Weight Distributions by GPC

The resolution of partially overlapping molecular weight distributions (MWDs) by gel permeation chromatography (GPC) can be improved by:

- selecting columns with more pores in the region (size) of interest.
- using more of the same pore size columns to improve the resolution with existing pore sizes.
- using higher efficiency columns to improve the resolving power of existing pores.

The first two principles are demonstrated below with a mixture of polystyrene standards. The use of higher efficiency columns to improve resolution in GPC has already been discussed.[1]

The GPC separation of two narrow distribution polystyrene standards with peak molecular weights of 200,000 (200K) and 100,000 (100K) is shown in Figure #1a. This separation was performed with a single experimental[2] Ultrastyragel® mixed bed column packed with equal volumes of 10^3, 10^4, and 10^5Å column packing material. The broad range of pore sizes in this column is ideal for separation over a very wide range of molecular weights but is clearly not optimized for resolution in the region of 100K to 200K. This mixed bed column has many pores that are too large as well as too small to provide resolving power in the desired range of molecular weight.



COLUMNS: ULTRASTYRAGEL® (as indicated)

SAMPLE: Polystyrene Blend (0.1% in THF)

INJECTION VOLUME: 50 µl per column

MOBILE PHASE: THF

FLOW RATE: 1 ml/min

DETECTOR: RI, 8X

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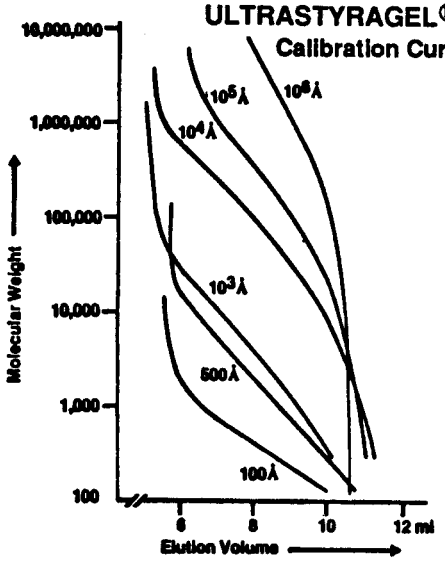
If we examine the calibration curves of the family of six individual pore size Ultrastyragel columns (Figure #2), we can see that polystyrene standards of 100K and 200K molecular weight would be totally excluded on a 10^3Å packing material, are in the center of the resolving range for 10^4Å packing, and fall on a more vertical portion (less resolution) of the 10^5Å calibration curve than for the 10^4Å column. Substitution of a single 10^4Å Ultrastyragel column for the experimental

Figure #2



COLUMNS:	As Indicated
SAMPLES:	Polystyrene Standards and n-Hydrocarbons
INJECTION VOLUME:	50µl
FLOW RATE:	1 ml/min
MOBILE PHASE:	Toluene
DETECTOR:	RI, 4X
TEMPERATURE:	Ambient

mixed bed column used in Figure #1a afforded the improved resolution of Figure #1b. Each column had the same total plate count (efficiency) but the calibration curve for the 10^4Å column is less steep (more resolving power) than that of the mixed bed column which is designed for separation of a wider range of molecular weights. The pore size distribution and resulting calibration curve for the 10^4Å column are optimum for these polystyrene standards. Notice that the improved resolution has been achieved by increasing the difference in elution volumes of the two standards while the plate count has remained constant. In this way, we can see that selecting a column with more pores in the region of interest can afford better resolution of partially overlapping molecular weight distributions.

When we use two 10^4Å Ultrastyragel columns and decrease the chart speed by a factor of two to "normalize" the chromatogram, we generate even better resolution of these polystyrene standards because we now have twice as many pores available (Figure #1c).

In some cases, optimization of pore sizes, addition of more of the same columns, or use of higher efficiency columns might afford no additional improvement in resolution of partially overlapping MWDs. This would indicate that the true MWD of the total sample had already been resolved as well as possible and that baseline resolution was not possible because there was true overlap of the individual molecular weight distributions. For example, if addition of the second 10^4Å column had not improved the resolution of the two polystyrene standards with a deeper valley between "peaks", it would indicate that the two MWDs of the standards actually overlap, i.e. the smallest molecules in the 200K standard are lower molecular weight than the largest molecules in the 100K standard. GPC is a technique that separates based on size of molecules in solution and it cannot completely resolve two polymers which have molecular size distributions that actually overlap. To the extent that the best separation of partially overlapping MWDs has not yet been achieved, intelligent selection of GPC columns can afford significant improvement in resolution. These same principles also apply, of course, to optimization of any GPC separation.

1). Ekmanis, J.L., Waters Lab Highlight # 0264 (1985)

2). This was an experimental column prepared during the development of the Ultrastyragel Linear (mixed bed) column and the recipe of pore sizes is different from that of the final product that was introduced for sale.

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