GPCSIM : an instrument simulator of polymer analysis by size exclusion chromatography for demonstration and training purposes

Educational paper

GPCSIM – an Instrument Simulator of Polymer Analysis by Size Exclusion Chromatography for Demonstration and Training Purposes

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Abstract

A computer simulation has been developed with the purpose of demonstrating and visualizing a multitude of effects in the molecular characterization of synthetic polymer mixtures by size exclusion (gel permeation) chromatography. The chromatographic results and their interpretation are influenced by numerous parameters originating from sample, column and instrumentation used (injection, detection etc). The target audience for the software tool consists of polymer scientists, teachers of separation science and students. Especially for the latter audience it is important to stress that the software *enables intentional creation of mistakes and learning from these mistakes*. What the user can do ranges from visualization (quantitatively) all retention and dispersion effects, validation of experimental setup, checking sensitivity for certain operating conditions, extrapolation of current instrument specifications, and in general performing hypothetical experiments. Several examples, such as column choice, band broadening, detection comparison and possible artifacts in the calculation of distributions are presented. This simulator is part of a family of similar tools for gas chromatography, high performance liquid chromatography, micellar electrokinetic chromatography and capillary electrophoresis. They have proved their effectiveness in education of separation science topics at several European universities.

Keywords: polymer analysis, size exclusion, gel permeation, simulation, visualization

1. Introduction

John Amos Comenius (Jan Amos Komenský, Figure 1) has been called the Father of Modern Education. He was born to Slovak parents on March 28, 1592 in Nivnice, Moravia (now in the Czech Republic) and he died on November 15, 1670 in Naarden, the Netherlands where his remains have found a resting place in a dedicated mausoleum. He advocated teaching in the common or vernacular language of students rather than in Latin, and the establishment of a universal system of education with opportunities that included women and peoples of all nations. Throughout his life, John Amos Comenius worked for educational, scientific, and cultural cooperation, enlightenment and understanding. He was a philosopher and cartographer, but most importantly the first modern educationalist. His book 'Orbis Pictus' in 1658 was the first picture book[1] for teaching children and remained a standard text in Europe and in America for over 200 years.[2]

Figure 1. Jan Amos Komenský (http://sk.wikipedia.org).

The use of illustrations in textbooks at the time of Comenius certainly was not self-evident. At present we

know, just as the proverb goes "a picture tells more than a thousand words", that in general, the use of graphics or a pictorial representation of theoretical concepts yields a higher retention rate than a textual or numerical representation alone. This of course is even more important as science education progresses into deeper level of abstraction, e.g. on a molecular level. Quantitatively this has been shown in Edgar Dale's Learning Pyramid:[3] visualization doubles the retention rate of traditional lecture plus reading text from 10 to 20%. Demonstrating increases the retention rate to 30%, whereas hands-on experience by students (practice by doing) yields a retention rate of 75%. Thus, visualization, demonstration and practice by doing have been precisely the reason why over the years we have developed and applied a number of simulation programs. The focus has been on analytical separation techniques, with applications in both academic education and research. The present simulator allows for molecular characterization of synthetic polymers and polymer mixtures with size exclusion (gel permeation) chromatography. It is part of a family of similar tools for gas chromatography,[4] high performance liquid chromatography,[5] micellar electrokinetic chromatography[6] and capillary electrophoresis.[7,8] An overview of the above is given on the Internet.[9]

Whereas the simulators mentioned above were mainly targeted for educational and training purposes, the general purpose of the present size exclusion chromatography (SEC) simulator is twofold. On the one hand it is equally intended as a training tool for students and those academics involved in education. For those it is important in using the simulator to be able to make mistakes, and learn from these mistakes. One the other hand, it gives polymer scientists and chromatographers insight into a large number of both instrumental parameters and fundamental aspects, in relation to the correct interpretation of experimental chromatograms. As such, it can even play a role in discussions about the influence of e.g. chromatographic band broadening in determining polydispersity of real polymers.

2. Program Setup

The difference between an experimental setup of instrumental analysis equipment and a simulation thereof for demonstration and educational purposes is quite simple. On the one hand, real experiments are intended to draw conclusions about an unknown sample component/mixture. In the case of chromatographic methods, comparing chromatograms of unknowns with chromatograms of known standards does this. In simulation on the other hand, we can only obtain chromatograms of standards for reason that their behavior in the chromatographic system must be known and well defined in order to be able to generate a realistic chromatogram.

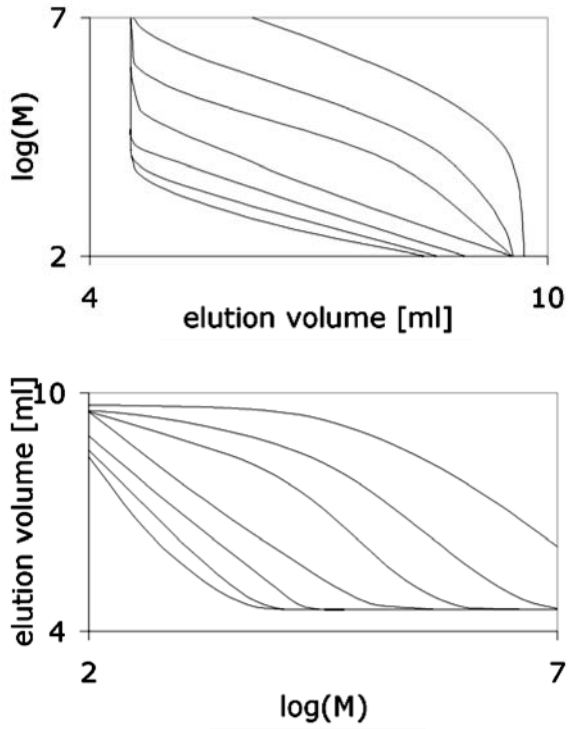

Figure 2. Different ways of depicting SEC calibration curves for 300 × 7.5 mm columns.

This can also be illustrated with the depiction of calibration graphs. Experimentally, we usually calculate the unknown molar mass M from the retention (elution) volume V_e, using a calibration plot of log(M) vs. V_e (Figure 2, left), because the calibration equation for commercially available columns[10] is usually written as log(M) as a polynomial expression of log M vs. V_e. In the simulation, for the purpose of generating a chromatogram, an essentially different approach is taken. V_e is calculated from log(M), (Figure 2, right), where M is chosen by the user. Here V_e is expressed as a five parameter polynomial equation of log(M).

A block diagram of the program is given in Figure 3. Upon startup, the program imports data from two data bases:

- Columns data base containing fitting coefficients for retention volumes of poly (styrene) standards for all 13 columns (normalized dimensions)
- Polymers data base containing molar mass of repeating unit, as well as K and a constants from viscosity law for 21 different polymers

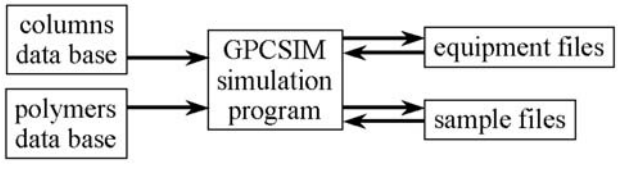

Figure 3. Block diagram of the simulation software.

The program then loads default versions of two additional files:

- Equipment file, in which the column settings (type, size) and instrument parameters (flow, detection, injection etc) are stored,
- Samples file, containing type(s) of polymer(s), concentration, and molar mass. If only one polymer is chosen, the molar mass distribution width and the type of distribution are also stored.

A chromatogram is immediately calculated and displayed on the screen output. The user can then change any parameter related to the column, the equipment or the sample, after which a new chromatogram is calculated and displayed. There is the option to display both the current and the previous chromatogram(s) together. Different equipment settings can be saved in different equipment files. The same applies to sample settings into sample files. The main screen, as depicted in Figure 4 shows a chromatogram, auto-scaled on a time axis, on which two dashed lines correspond to the volume of total exclusion and to that of total permeation respectively. The main menu line is on top of the screen.

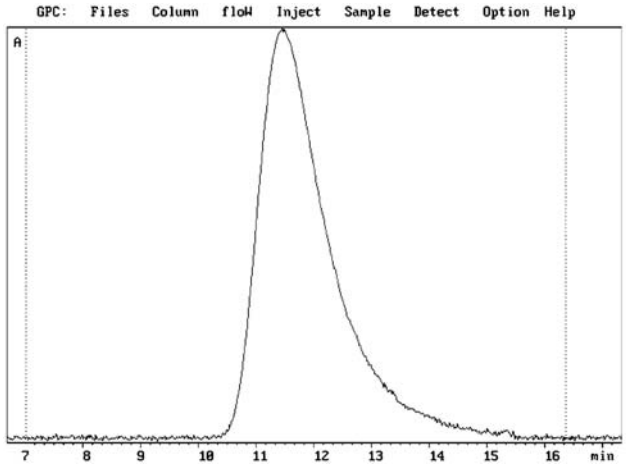

Figure 4. Typical screenshot of the simulator, RI detection, default column size, 0.7 mL min^{-1} THF, 15 µl injection of 100 mg L^{-1} poly(styrene) sample, average molar mass 10000 g mol^{-1}.

The user interface is simple. Settings are changed either though the items on the main menu (e.g. press F for File, then S for Sample, then L for Load), or through the use of single function key commands, such as F3 (switch detector) or F4 (switch column). A new chromatogram is immediately calculated and displayed.

2.1. Sample Parameters

A number of different, mainly linear polymers for which constants *K* and *a* from the Kuhn-Mark-Houwink-Sakurada viscosity law, taken from literature,[11] are collected in the sample database, see Table I. All linear polymers are assumed to behave like a random coil. In addition to that there is a branched polymer such as dextran. Three hypothetical polymers are finally added: a rigid rod (RR), a compact sphere (CS) and a hypothetical one (Hyp) of which *K* and *a* values can be freely chosen by the user.

Table 1. List of all polymer samples in the database. Incremental *dM* mass unit is in g/mol; *K* value is in 10^{-5}dL g^{-1}. (Solvent THF unless indicated otherwise, 40 °C).

Abbrev.	Polymer name (solvent)	*dM*	*K*	*a*	ref
PS	Poly(styrene)	104	11.4	0.716	12
PMMA	Poly(methyl methacrylate)	84	9.44	0.719	11
PEMA	Poly(ethyl methacrylate)	98	9.7	0.714	13
PBMA	Poly(butyl methacrylate)	112	14.8	0.664	13
PiBMA	Poly(iso-butyl methacrylate)	112	9.7	0.705	13
P2EHMA	Poly(2-ethylhexyl methacrylate)	210	6.3	0.707	13
PC10MA	Poly(isodecyl methacrylate)	238	5.18	0.72	13
PMA	Poly(methyl acrylate)	86	19.5	0.66	14
PEA	Poly(ethyl acrylate)	100	8.9	0.75	15
PBA	Poly(butyl acrylate)	114	7.4	0.75	15
P2EHA	Poly(2-ethylhexyl acrylate)	210	11	0.68	15
PDDA	Poly(dodecyl acrylate)	226	27.3	0.58	13
PiBoMA	Poly(isobornyl methacrylate)	223	13.5	0.56	16
PVAc	Poly(vinyl acetate)	86	16	0.7	18
PCL	Poly(ε-caprolactone)	114	13.95	0.786	11
PC	Poly(carbonate)	238	23.9	0.766	11
Dex	Dextran (water)	100	380	0.38	17
Pul	Pullulan (water)	504	16.6	0.696	17
RR	Rigid Rods	100	10	1.8	–
SS	Solid Spheres	100	10	0	–
Hyp	Hypothetical polymer	100	Var.	Var.	–

In case the sample solution consists of a single linear polymer, not only the molar mass can be varied but also the width of the distribution and the distribution function (Poisson, Lognormal or Flory). In case of a mixture of chemically different polymers and/or different molar masses of the same polymer, then each of them is assumed to have an uniform individual distribution. Concentration of each in the sample can be freely chosen.

2.2. Column and Instrument Parameters

The simulator enables us to choose among 12 different column materials with both broad and narrow molar mass separation range. Columns are calibrated with polystyrene PS. The columns are stored in the column database, using calibration curves for polystyrene (such as one depicted in Figure 2).

The data were obtained from typical commercially available columns,[10] with a default dimension of 300 × 7.5 mm. In the simulator, however, length and inner diameter of each column, as well as particle diameter and flow rate can be freely changed. In this way, chromatographic behavior can be additionally manipulated. The resulting teachware creates the base for a great variability of separation conditions enabling its extensive use in education of SEC or more generally, analytical separation methods. Table 2 lists these and other instrumental parameters, their range and default value.

Table 2. Column and Instrument parameters, their range and default values in parentheses. Default particle diameter* depends on column choice.

Parameter	Range (default)
Column length	50–1200 mm (300)
Column ID	2–20 mm (7.5)
Column particle diameter	1–25 μm (*)
Injection volume	1–1000 μL (50)
Eluent Flow rate	0.1–10 mL min^{-1}
Temperature	25–220 °C (40)
Connections volume	0–1000 μL (0)
Detector volume	1–1000 μL (50)
Detector path length	1–25 mm (10)
Detector time constant	0.01–5 s (1)

2.3. Retention

Often, SEC chromatograms are interpreted as follows. The column calibration graph is constructed using narrow molar mass distribution polystyrene "standards". From the retention times of an unknown polymer sample, retention volumes are calculated using flow rate. With the calibration graph mentioned, "polystyrene-equivalent molar masses" and distributions are calculated. If K and a constants are available for the polymer concerned (and for polystyrene), true molar masses can finally be obtained, using universal calibration.

In the case of the retention algorithm in the simulator, exactly the opposite procedure is applied. For polymers other than polystyrene, first the PS-equivalent molar mass M_{PS} is calculated from the polymer's molar mass M_i, using Kuhn-Mark-Houwink-Sakurada coefficients K_i and a_i and those of PS as follows:

$$\mathrm{Log}(M_{PS}) = (1/(a_{PS} + 1))\log(K_i/K_{PS}) + \\ + ((a_i + 1)/(a_{PS} + 1))\log(M_i). \quad (1)$$

Here it is assumed that equal hydrodynamic volumes (given by $HV_i = K_i \cdot M_i^{(ai+1)}$, in practice mean equal retention volumes. The retention volume V_e is subsequently calculated from M_{PS} obtained above, using the column calibration polynomial equation described earlier.

2.4. Band Broadening

Principally all components in the chromatographic system can lead to band broadening in the ultimate detector signal. Several earlier texts already indicate that chromatographic band broadening in SEC configurations is much less straightforward,[19,20] compared to lower-molar mass GC and HPLC systems. The dispersion model incorporated in the present algorithm is an extended chromatographic plate height model, comprising both in-column and extra-column effects. All effects are assumed to act mutually independent. The overall equation is given by:

$$H = H_{inj} + H_{con} + H_{det} + H_{tau} + \\ + d_p \cdot (A + B/Pe + C \cdot Pe) \quad (2)$$

The first four contributions on the right-hand side of Eq.2 represent peak broadening due to injection, connections, and detector volume and time constant respectively. The equations for these individual effects can be found in any chromatography textbook. The last term of the equation describes the band broadening due to in-column effect, written in a dimensionless form with the Peclet number $Pe = d_p \cdot v / D$. The A, B and C coefficients are specific for the type of chromatography used, the type of column and also the type of solute. Several approaches dealing with column band broadening have been published in recent years.[21–24] What we have used is the approach of Potschka[23,24] for the A, B and C coefficients. In these coefficients, there are a number of terms and factors that are still open for discussion in the polymer analysis community: these have been made a changeable parameter in the simulator. The terms will be discussed in more detail in the next section.

The diffusion coefficient for each solute was calculated as follows. Diffusion coefficient as a function of molar mass was modeled according to $D = 1.25.10^{-4} \cdot M^{-0.55}$ which fits well for PEG.[25] It was used for all polymers.

The temperature dependence of diffusion coefficient was modeled as $D = D_{25} \cdot (1.006)^{(T-25)}$. Possible temperature dependence of constants K and a is not considered.

Calculating a full chromatogram of a polymer distribution was done in the manner described below. For each polymer species in the distribution, the following is calculated:

1. The relative concentration;
2. The PS-equivalent molar mass, using Eq.1;
3. The retention volume, using the polynomial column calibration curve;
4. The retention time, using column dimensions and flow;
5. The total plate height, using Eq.2 and from this the time-based signal distribution due to the presence of the individual polymer species;
6. The signal due to each individual polymer species in the sample is then added to the signal baseline (noise depending on detector parameters), taking into account relative concentration, injection volume and detector response.

2.5. Detection

Several detection systems are available. Their response differs in two aspects: noise level and specific response. In spite of fundamental constructional differences, each detector can be assigned a detector volume V_{det} (defined as the product of flow rate and residence time) and path length (defined as the product of linear velocity and residence time). In line with the above, the cross sectional area is defined as the ratio of detector volume and detector path length.

The response of all detectors is proportional to the sample concentration c and injection volume V_{inj}; the proportionality constant depends on the detector type. The response of the refractive index detector is furthermore proportional to the path length and to the refractive index increment dn/dc. The response of the density detector is proportional to the differential density $d\rho/dc$ and the path length. The response of the viscosity detector is proportional to the molar mass M to the power a, the flow and the path length and inversely proportional to the cross sectional area. Finally, the response of the light scattering detector is proportional to the molar mass and to dn/dc (which in turn depends on the wavelength chosen).

The noise level, was made proportional to the inverse value of the cross sectional area, and also proportional to the temperature. Like in experimental setups, the noise level could be decreased using a moving average filter.

3. Results and Discussion

The number of different sample and column parameters and their range lead to a virtually infinite number of different combinations, many of them falling in the category "stupid mistakes". As a consequence, using the simulator for the trial and error optimization approach is not advised. In this section a number of properly chosen examples are given to highlight the main features of the program.

In modeling detection, not only signal amplitude but also the noise level of all four detectors is taken into account, with the result that at lower concentrations, the signal of the density detector soon drowns in the noise. An example of refractive index and viscosity detection is shown in Figure 5. Under these sample conditions, the use of a density detector (not shown in the figure) would lead to a noisy baseline on which hardly any peaks are recognized.

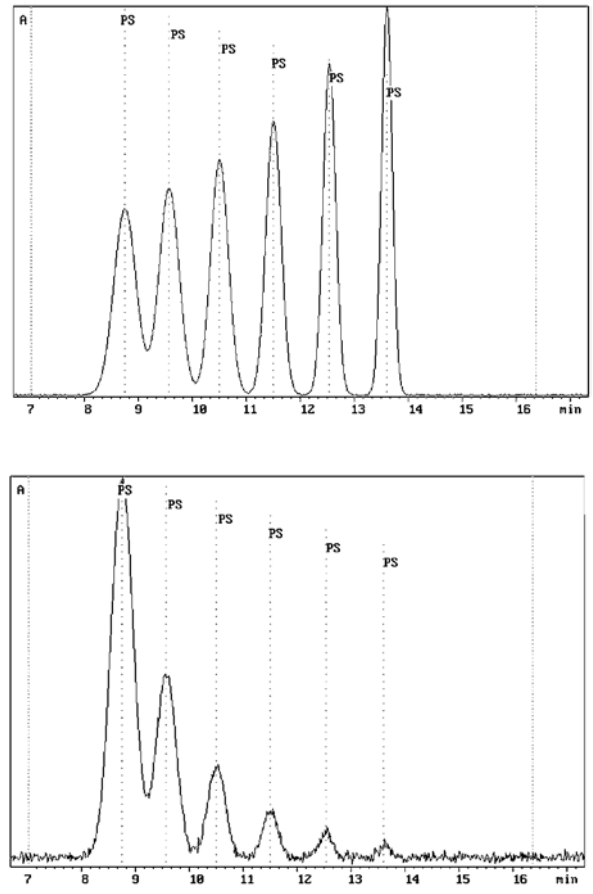

Figure 5. Separation of a mixture of uniform polystyrene standards (molar mass 1700, 5700, 18000, 57000, 180000, 560000 g mol^{-1}, other conditions as in Figure 4), detected with refractive index detector (top) and viscosity detector (bottom).

The separation of the same sample of uniform polystyrene standards is now simulated on two different columns (Figure 6). The column selection takes place on the basis of the calibration graphs as in Figure 2, depicted on the screen.

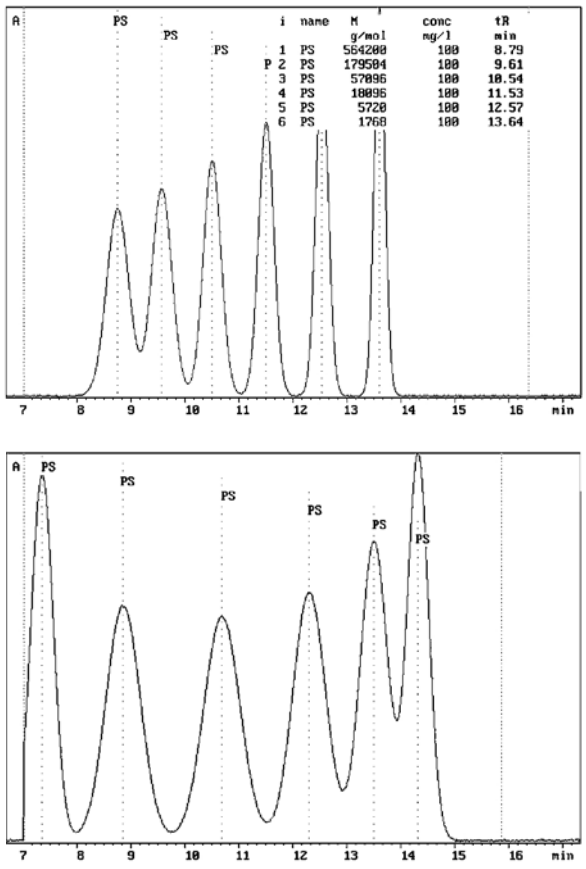

Figure 6. Screen shot of the simulator depicting separation of a number of uniform standards analyzed on a broad molar mass range column (top) and a narrow molar mass range column (bottom). For other conditions see Figure 5.

The difference in broad vs. narrow range columns (Figure 6) can be explained as follows: As the molar masses of the narrow standards are arranged in logarithmically equidistant manner, the peaks from the broad range column are equidistant in time. Those from the narrow range column are not; in addition, the peak capacity of the narrow range column is lower, but as a result molar masses of individual peaks can be determined more accurately.

The need for universal calibration in size exclusion chromatography can be illustrated by simulating the analysis of a mixture of all polymer components in the database, each having a molar mass of approximately 100000 g mol^{-1} and with a uniform molar mass distribution. As seen in the resulting simulation (Figure 7), one can observe that several linear polymers in fact co-elute with polystyrene, indicating that reliable results could have been obtained after calibrating the column with PS standards. Many others on the other hand show significantly different retention behavior. In order to obtain reliable data, universal calibration should have been used instead, using constants K and a from Kuhn-Mark-Houwink-Sakurada viscosity law, taken from literature.

Several components in the chromatographic system (other than the column packing itself) can lead to systematic retention time shifts. These can be due to the volumes of injection V_{inj}, connection V_{con} and detection V_{det} respectively. This time-shift δt is simply, for all peaks:

$$\delta t = (V_{inj}/2 + V_{con} + V_{det}/2) / F, \quad (4)$$

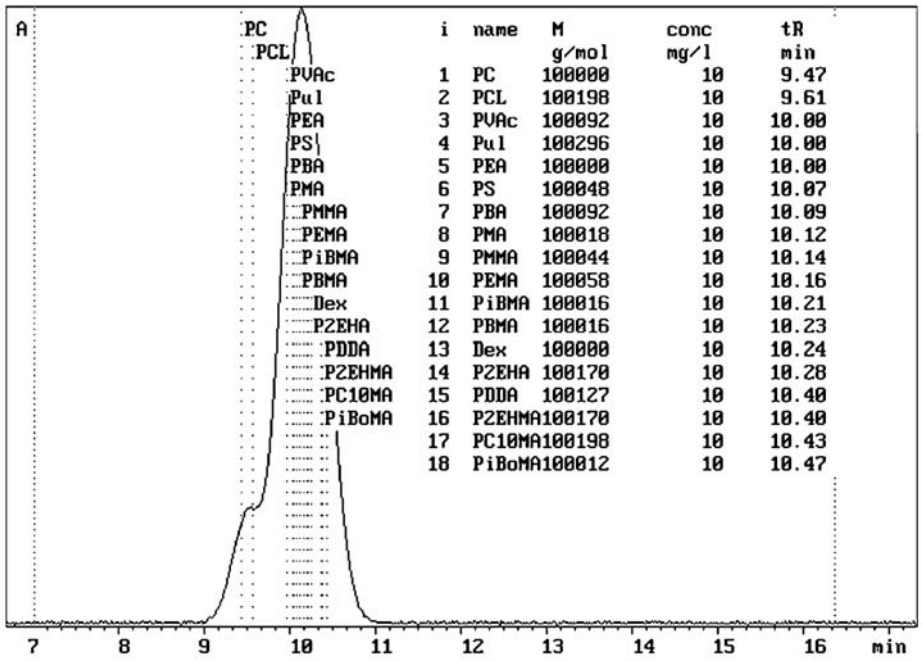

Figure 7. Separation of a mixture of all polymers in the database, each having a uniform molar mass distribution.

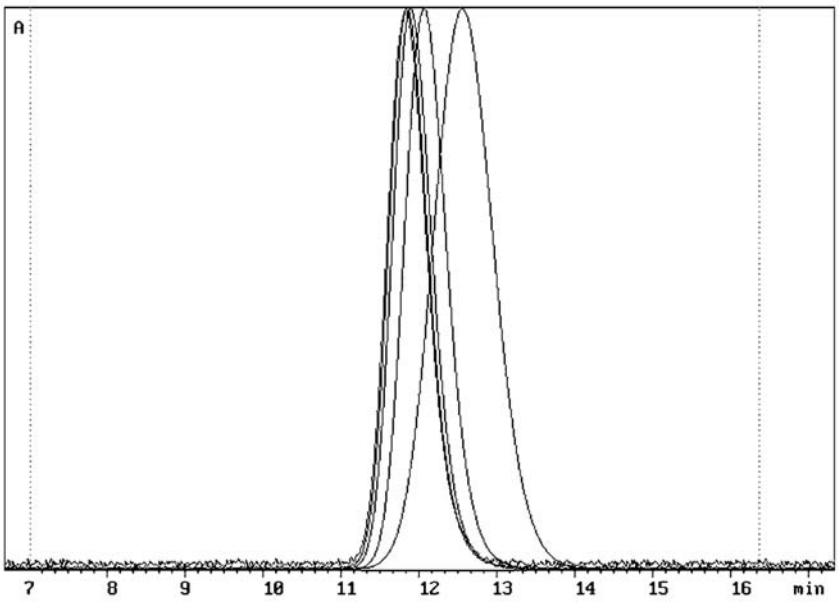

Figure 8. Superimposed (auto scaled) chromatograms of different injection volumes of the same poly(styrene) sample: 10, 30, 100, 300 and 1000 μL (see Figure 4 for other conditions).

in which F is the eluent flow rate. All of the above volumes can be changed within a broad range, in order for the user to become familiar with their sensitivity. If the column is calibrated with a certain value of V_{inj}, a systematic deviation of δt can be expected if V_{inj} is different for a subsequent sample. Also, when making a calibration graph, the injection volume should remain constant.

As an example of extra-column band broadening, consider analysing a uniform molar mass distribution, and injecting different volumes of the same sample. Peak areas would of course change proportionally. Two other effects are observed (Figure 8.). The peak is shifting due to the equation mentioned above. A large injection volume leads to additional band broadening, which under circumstances of narrow peaks can be directly noticeable. The last two effects may lead to systematic deviations of average molar mass and polydispersity, as calculated from the chromatogram.

Due to the absence of reliable modelling/data, the retention model inherently has some limitations. The effect of sample concentration for example has not been taken into account in the present version. Additionally, there is an uncertainty regarding the values of K and a constants: results from different sources scatter significantly. In addition, the Kuhn-Mark-Houwink-Sakurada viscosity law may lose its validity in case of very small or very large molar masses or high sample concentration. In a quantitative manner, the above uncorrected example will lead to misinterpretation of the chromatogram. The software is equipped with the following features to make this visible. Theoretical values (i.e. of the sample data input into the simulation algorithm) for number-average, mass-average, z-average, z +1-average and viscosity-average molar masses, respectively, are calculated as the first results. The same averages are calculated from the simulated chromatogram. Comparison of the two sets of averages illustrates e.g. a possible bias resulting from chromatographic band broadening.

The above statement summarizes the second purpose of the paper (the first comprising of the educational goal in teaching separation science): determining the role of band broadening in the possible misinterpretation of SEC results with respect to polydispersity. It should be emphasized that the present paper intends to provide a tool for investigating this issue qualitatively. It is not our intention to *a priori* settle the controversy whether the mentioned effect will take place in practice. For the second purpose of the paper we have to look into chromatographic band broadening phenomena in a more detailed way. As said, on a detailed level there is no consensus how to model the C term in the van Deemter equation, to mention only one thing. There already is quite some literature about correcting for chromatographic band broadening after separation, but only few approaches model the actual cause with van Deemter-like equations. Neither some of the older literature[19,20] nor most of the more recent approaches[21–24] seem to have settled the issue definitively.

As the dispersion models in this and our other simulators are plate height based, we have decided to model intra-column band broadening according to the equations proposed by Potschka.[23,24] Additional reason for this is that the approach chosen will be easily open to further refinement by the (advanced) user of the simulation. The last term in Eq.2, in dimensionless form, is rewritten as:

$$h = \mathrm{A}'(\varepsilon) + \mathrm{B}'/Pe + \xi_p\,[(\mathrm{C}'\,Pe)^{-1} + \mathrm{D}^{-1}]^{-1}. \qquad (4)$$

Here, ε accounts for boundary layer formation due to surface roughness and ξ_p is the tortuosity of the pores. For further details regarding A', B', C' and D we refer to the references mentioned. What concerns us here is the fact that the terms in Eq.4, in addition to ε and ξ_p include

two other parameters or "constants" that may still be open to debate. In C' and D there is a factor taking into account that in case of convective transport within the pores, the eluent and the solute have different velocity: this ratio is designated σ. In addition, there is a factor 2 in an exponential function in C' and in D, which may depend on column type: this factor 2 is designated φ. The refinement parameters mentioned are included in the band-broadening model: they are assigned sensible default values for normal use of the simulator, but the experienced user, within a certain range, can adjust them. Table 3 gives an overview of range and default values for these parameters.

Table 3. Dimensionless band-broadening refinement parameters.

Parameter	Description	Range (default)
ε	Surface roughness	0.4–2.2 (2.2)
ξ_p	Tortuosity of the pores	1.5–1.8 (1.5)
σ	Pore convection ratio	1–3 (1)
φ	Factor in exponent	1.9–2.1 (2)

Let us add some information about the program user interface. There is no mouse support and the menu structure is simple but effective and extremely fast. In spite of this, the program is extremely small (70 Kbytes source file, requires 500 Kbytes disk space) and runs on most PCs manufactured in the previous decade. The software works with single-key commands, such as H for Help and FLS for File Load Sample, etc. A number of multiple key commands are provided with a single key shortcut. The table gives an overview.

Table 4. Overview of shortcut keys.

Shortcut key(s)	Action
F1	Help
F2	Switch distribution
F3	Switch detection
F4	Switch column
F5	Change column oven temperature
F6 / F7	Change length & flow proportionally
F8	Switch off ex-column broadening
F9	Identify the Unknown (cheating)
F10	Restart with default settings
< / >	Change detector amplification
arrow left/right	Change molar mass
arrow up/down	Change distribution width

4. Conclusions

The software tool* resulting from this contribution enables a quantitative visualization of a large number of column, sample and detection effects in size exclusion chromatography, including column selection and optimization of operating conditions. In order to also practice a procedure of identification of an unknown polymer in an educational setting, a feature is included which temporarily enables to designate one of the polymers in the database as an unknown sample polymer. This of course would fit in with the primary objective of the paper, a hopefully valuable tool for educational settings such as lectures, demonstrations, practice by doing, dry-lab experimentation and as a first learning step in optimization. In this way it is envisaged that both students and those involved in teaching separation science will benefit.

The second objective expects to enable a useful tool in comparing and validating experimental results, especially with respect to the possible (unwanted) role that instrumentation can inflict upon precise and accurate interpretation of size exclusion chromatographic results. A refined, albeit user-adjustable on-column band broadening algorithm is included for that very purpose. Hidden for novice users, this refinement parameters menu is intended for polymer scientists and chromatographers who are anxious to find out what really happens in detail in the pores of the column packing.

* Details about software availability to be obtained from corresponding author, j.c.reijenga@tue.nl.

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Povzetek

Razvili smo računalniško simulacijo z namenom demonstracije in vizualizacije številnih učinkov na proces karakterizacije sinteznih polimernih mešanic z velikostno izključitveno (gelsko prepustnostno) kromatografijo. Na kromatografske rezultate in njihovo interpretacijo vplivajo številni parametri, ki izvirajo iz vzorca, kolone, instrumentacije (injekcija, detekcija itd.).
Ciljna publika za programsko opremo so raziskovalci na področju polimerov, učitelji separacijskih znanosti in študenti. Še posebej pri slednjih je potrebno poudariti, da programska oprema omogoča uvajanje namenskih napak, pri čemer se lahko iz napak učimo. Uporabnik lahko vizualizira (kvantitativno) učinke retencije in disperzije, eksperimentalno postavitev, preverjanje občutljivosti, instrumentalne nastavitve, ter na ta način opravlja hipotetične eksperimente. Prikazujemo več eksperimentov z izbiro kolon, primerjavo detektorjev in možnih neznank, ki nastopajo pri izračunih porazdelitev. Simulator je del družine sorodnih orodij za plinsko kromatografijo, tekočinsko kromatografijo, micelarno elektrokinetično kromatografijo in kapilarno elektroforezo. Njihovo uporabnost smo preverili pri učenju separacijskih ved na več evropskih univerzah.