# Facing the Challenge
## of
# Analyzing Complex Polymers



Chemical Composition

Molar Mass

A PRIMER IN 2-DIMENSIONAL LIQUID CHROMATOGRAPHY (2D-LC)
FOR
DETERMINING THE MOLECULAR WEIGHT AND COMPOSITIONAL
DISTRIBUTIONS OF COMPLEX POLYMERS

# INTRODUCTION

Advances in polymer synthesis techniques have resulted in a proliferation of new complex polymers (block copolymers, graft copolymers, blends etc.) with predetermined chemical composition, functionality and architecture. The task of characterizing these polymeric materials has now become a challenge, since no single analytical technique provides adequate information regarding all the different distributions. Analytical chemists have thus opted for hyphenated techniques such as two-dimensional liquid chromatography (2D-LC) in which two liquid chromatography methods are combined to achieve selective separations according to the various distributions.

The increased use of hyphenated techniques has prompted Polymer Standards Service (PSS) to publish this primer on the implementation of a 2D-LC technique. PSS has commercially pioneered the development of a unique software package that has the capability of performing 2D-LC analysis. WinGPC Unity allows data acquisition and control of fraction transfer between two liquid chromatographic systems.

This primer, **"Facing the Challenge of Analyzing Complex Polymers"** explains the basic concepts, the reasons to use 2D-LC, how it works, what you need, and what PSS offers in the way of equipment and supplies. "Facing the Challenge for Analyzing Complex Polymers" is divided into four main sections:

1. **Basic Concepts Review**

   Complex polymers

   High Performance Liquid Chromatography (HPLC), and the kind of information it yields

   Gel Permeation Cromatography (GPC),and the kind of information it provides

2. **Why use hyphenated techniques**

   When it is useful to combine GPC and HPLC into one technique

   Why use HPLC for 1st- Dimension Separation

3. **How 2D-LC works**

   Schematic diagram of a 2D-LC system

   How to collect and process data

   What the processed data look like

   Minimum Requirements for a 2D-LC System

4. **2D-LC Sources and Resources**

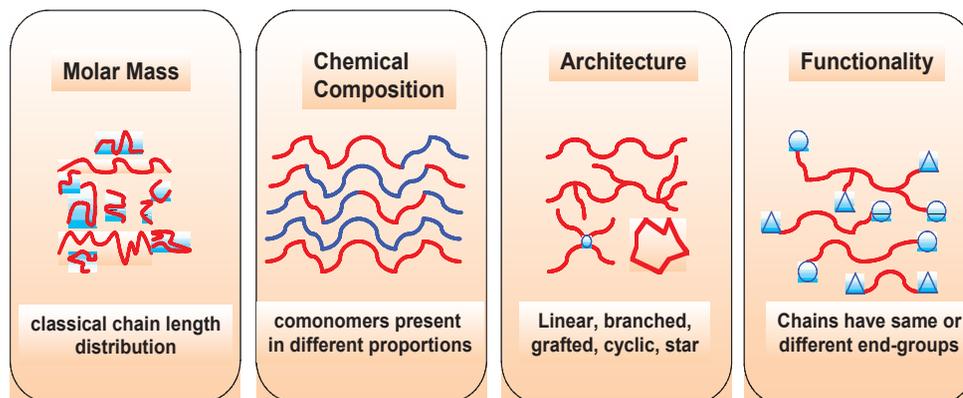   Reference publications about 2D-LC Analysis of Complex Polymers

   Where to purchase a 2D-LC System

   Who is PSS

# BASIC CONCEPT REVIEW

## COMPLEX POLYMERS

Complex polymers have more than one distribution. They are distributed according to MOLAR MASS, CHEMICAL COMPOSITION, FUNCTIONALITY, and ARCHITECTURE.



| Molar Mass | Chemical Composition | Architecture | Functionality |
|---|---|---|---|
| classical chain length distribution | comonomers present in different proportions | Linear, branched, grafted, cyclic, star | Chains have same or different end-groups |

A copolymer, for example, has a molar mass distribution (MMD) owing to the various chain lengths. It has a chemical composition distribution (CCD), reflecting the comonomer units that are present in varying amounts. It presents molecular architecture distributions (MAD), which are associated with the polymer structure: linear, star-shaped, comb-like, grafted or branched polymer. Finally, functionality type distributions (FTD) occur when polymer chains have differing end-groups.

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND THE KIND OF INFORMATION IT YIELDS

High Performance Liquid Chromatography, (HPLC), has been the technique of choice for many years to both identify and quantify chemical compounds that are usually solid, or liquids that have boiling points too high to analyze by gas chromatography.
The material to be analyzed is dissolved in a suitable solvent and injected onto a column that is usually silica-based, and most often contains a bonded phase that can interact (via partitioning or adsorption) with the components. Depending on the chemistry of these interactions between the eluting components and the bonded phase, the resulting chromatogram will show a clear separation of the component mixture. The materials that have little affinity or exhibit little interaction with the column will elute first, followed by the other materials that interact more strongly with the column (usually adsorption), and elute later. The stronger this interaction, the later the elution occurs.

Commonly, the mobile phase (or eluent) is not a single solvent that does not change composition throughout the analysis. Usually, a second (or more) solvent is brought in to change the polarity of the eluent and force later eluting species that interact strongly with the column to elute more quickly to shorten analysis time (gradient mode). The flow rate may also be changed during the course of the separation (flow programming), which will also help to optimize the analysis time and still maintain good peak capacity and resolution. An ultraviolet, (UV) or evaporative light scattering detector (ELSD) is usually used.

## GEL PERMEATION CHROMATOGRAPHY (GPC) AND THE KIND OF INFORMATION IT PROVIDES

Gel permeation chromatography, (GPC), also referred to as size exclusion chromatography (SEC), has been the technique of choice for more than 30 years to obtain the molecular weight distribution of macromolecules. Other techniques, such as osmometry, end-group analysis, mass spectrometry, etc., have also

been used for low molecular weight materials, but for polymers with molecular weights exceeding ~50,000, the GPC or SEC technique is still the best choice.

The GPC or SEC technique involves the separation of the polymeric material according to its hydrodynamic volume, which in turn gives information regarding the various molar mass averages (Mn, Mp, Mw and Mz) and polydispersity, (Mw/Mn), of the polymer.

The molar mass averages are simply statistical moments of the molar mass distribution, and can be used to make correlations with important physical properties and processing parameters. For example, Mn, the number average molecular weight, is the first statistical moment, and can be used to predict a polymer's flow properties and brittleness. The second statistical moment, Mw, (the weight average molecular weight), is directly related to the strength properties of a polymer, such as modulus, tensile strength and impact resistance. Mz is the third statistical moment of the distribution, and is used to make predictions about a polymer's flexibility and elongation, important parameters for elastomers. In SEC the polymer sample passes through a column, which is packed with porous beads. As the polymer chains pass through the column, the longer polymer chains are excluded from many of the pores, and travel between the beads and reach the column exit more quickly. The short polymer chains are capable of passing, (permeating) through

the pores of the beads and experience a longer residence time in the column and as a consequence elute later from the column. With SEC there is usually at least two columns connected in series to provide enough pore volume to do the analysis accurately. After exiting the last column, the separated polymer passes into a concentration detector, usually a differential refractometer. SEC is a relative method; that is, the column set has to be calibrated with standards of known molar masses. The standards are referred to as "narrow standards", which means that the polydispersities (Mw/Mn), are <1.20. The calibration curve is constructed using the Mp values, plotting *log molar mass versus elution volume*. . The molecular weight averages obtained are relative to the calibration molecule used.

The major advantage of the technique is its capability of analyzing polymers over a large molar mass range; from $10^2$ to $>10^7$ g/mol. The SEC technique works well when homopolymers are analyzed since for these the hydrodynamic volume is directly related to the molar mass.

*The analysis of copolymers is more complex. Since the hydrodynamic volume in the case of copolymers is related to both the molar mass and chemical composition, thus SEC alone is insufficient for providing chemical composition information within the copolymer.*
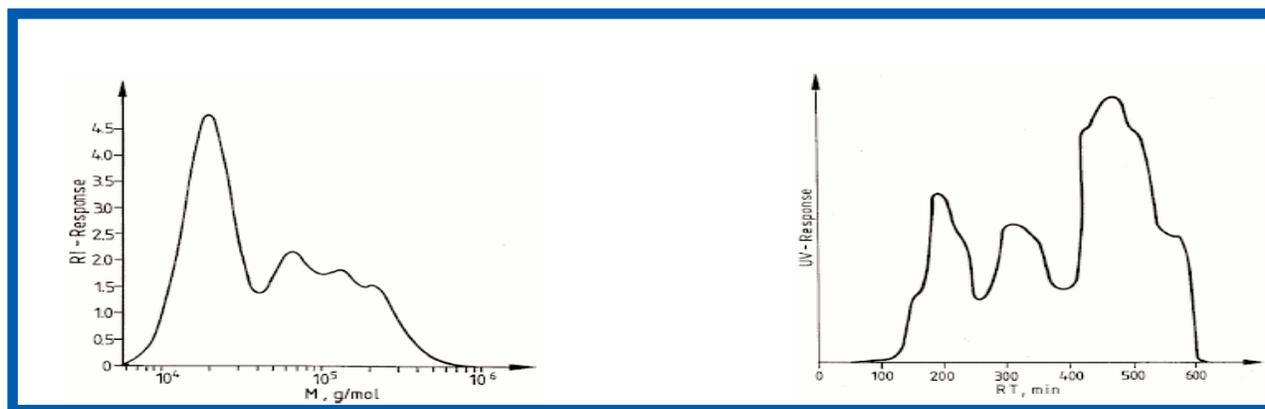


**SEC**          **SEPARATION OF STAR COPOLYMER**          **HPLC GRADIENT**

# WHY USE HYPHENATED TECHNIQUES

Complex polymer topologies, polymer blends and multi-component formulations require a different approach to perform a proper molecular characterization than simple polymers. In 2D chromatography different separation techniques are used to avoid co-elution of species and to measure molar mass and chemical composition in a truly independent way.

A comprehensible advantage of coupling two chromatographic techniques over performing two isolated methods is following: Even if the single runs show good separations, for example one according to composition and one according to molar mass, then it is still not possible to correlate e.g. one special composition to a certain molar mass. In fact, there are numerous possible combinations which give the same one-dimensional chromatograms. This is also depicted below, where schematic contour plots with different compounds show the same projections to the X- and Y-axes. Those contour plots can be easily produced when both separation techniques (e.g. HPLC and GPC) are coupled. Besides this easy visualization, the benefits of 2D chromatography versus one dimensional separation techniques can be quantified as well:

It is obvious that n independent molecular properties require n-dimensional methods for accurate (independent) characterization of all parameters. Additionally, the separation efficiency of any single separation method is limited by the efficiency and selectivity of this separation mode, i.e. the plate count N of the column and the phase system selected. Adding more columns will not overcome the need to identify more components in a complex sample, due to the limitation of peak capacities, n. The corresponding peak capacity in an n-dimensional separation is substantially higher due to the fact that each dimension contributes to the total peak capacity as a factor and not as an additive term for single dimension methods:

$$n_{total} = \prod_{i=1}^{n} n_i \cdot \sin^{(i-1)} \vartheta_i$$

$$\text{e.g. for a 2D system:}$$

$$n_{2D} = n_1 \cdot n_2 \cdot \sin \vartheta$$

where $n_{total}$ represents the total peak capacity, $n_i$ the peak capacity in dimension i and $\sigma_i$ is the "angle" between two dimensions; for orthogonal separations this angle will be 90° and the peak capacity will be maximized.
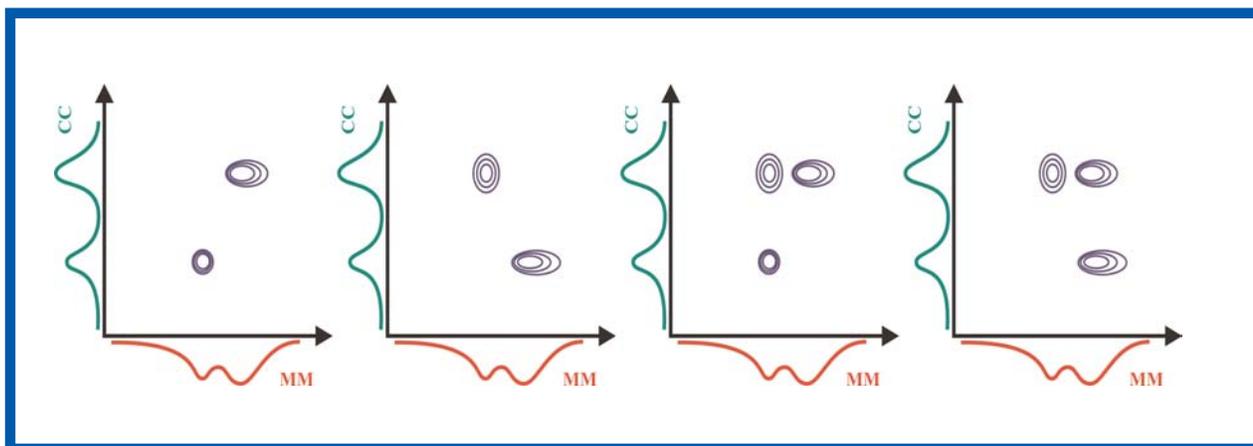


**CONTOURPLOTS OF DIFFERENT POLYMER BLENDS WITH THE SAME CHROMATOGRAPHIC BEHAVIOUR**

The angle between dimensions is determined by the independence of the methods; a 90 degree angle is obtained by two methods, which are completely independent of each other and will e.g. separate two properties solely on a single parameter without influencing themselves.

## WHEN IT IS USEFUL TO COMBINE GPC AND HPLC INTO ONE TECHNIQUE

It is useful to combine techniques when a single technique does not provide you with the entire picture. See the following example.

Two thermoplastic elastomers from different vendors were analyzed using SEC. The chromatograms and the SEC results don't show any differences between the two raw materials, but one of them gives a poor final product when used.

A 2D-LC separation performed on the thermoplastic elastomers show that they also differ in their chemical composition distribution, which influences the integrity of the final product.

|         | Vendor A | Vendor B |
|---------|----------|----------|
| Mn[kD]  | 99       | 90       |
| Mw[kD]  | 109      | 103      |
| Mw/Mn   | 1.08     | 1.14     |
| Mp[kD]  | 108      | 104      |
| by product | 0.8%  | 1.7%     |
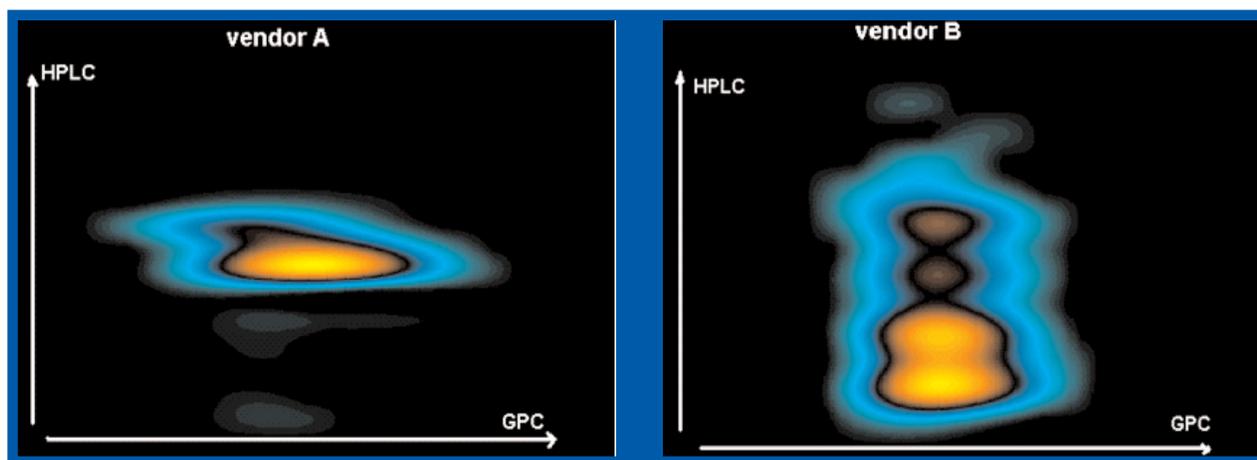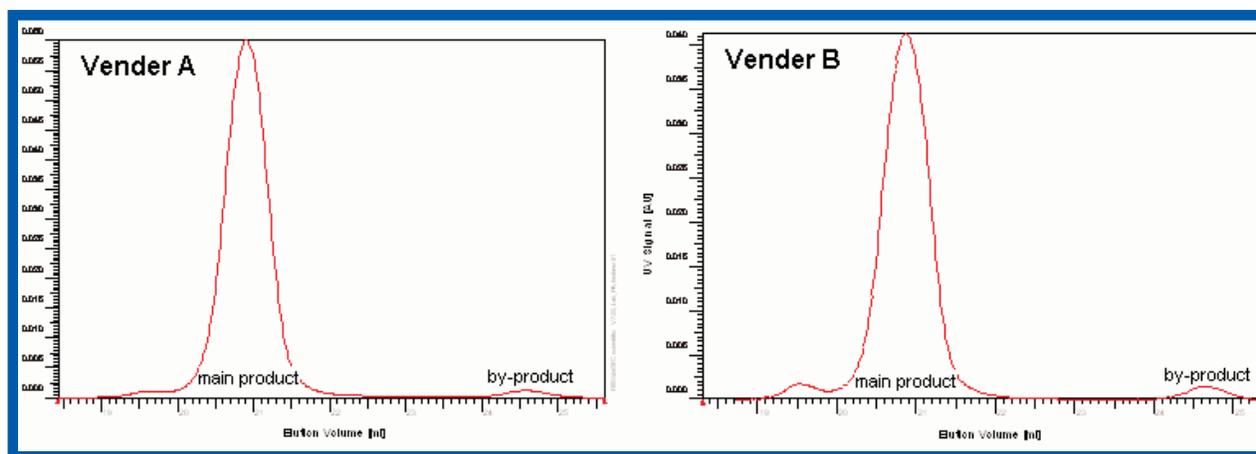




**VENDOR A SHOWS NARROW CCD**

**VENDOR B SHOWS TETRAMODAL CCD**

5

## WHY USE HPLC FOR 1ST- DIMENSION SEPARATION

HPLC is one of the most widely used analytical techniques owing to its capability of analyzing a diverse range of compounds according to chemical composition. Its popularity can be attributed to the large variety of commercially available columns having stationary phases with varying polarities, the choice of selecting a combination(s) of mobile phases for achieving the desired separation and the availability of literature collected over the past decades.

*HPLC is considered as the method of choice for separating polymers according to chemical composition; however, the technique does not provide information regarding molar mass averages and polydispersities for polymers.*

## ILLUSTRATION:

The SEC trace of a block copolymer shows a single peak with tailing at the low molar mass end. In other words there's polymeric material that's not being separated.

By using HPLC at critical conditions, two peaks that are attributed to the block copolymer and homopolymers are clearly resolved.

Using HPLC in the first method of separation allows greater flexibility for manipulating the liquid chromatographic conditions, i.e. HPLC

can be conducted at isocratic or gradient conditions or by using liquid chromatography at critical conditions (LCCC).

The various LC conditions are an advantage since this enhances probability of achieving good separation of complex polymers according to chemical composition and/or functionality.
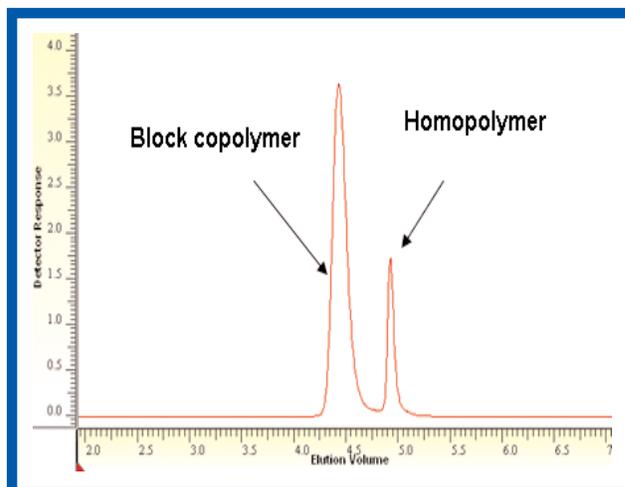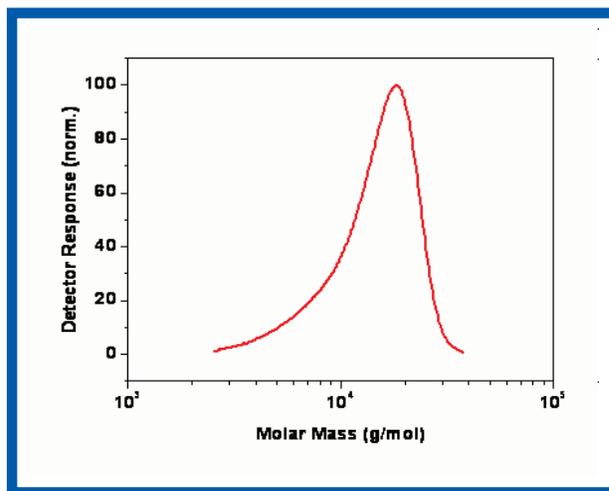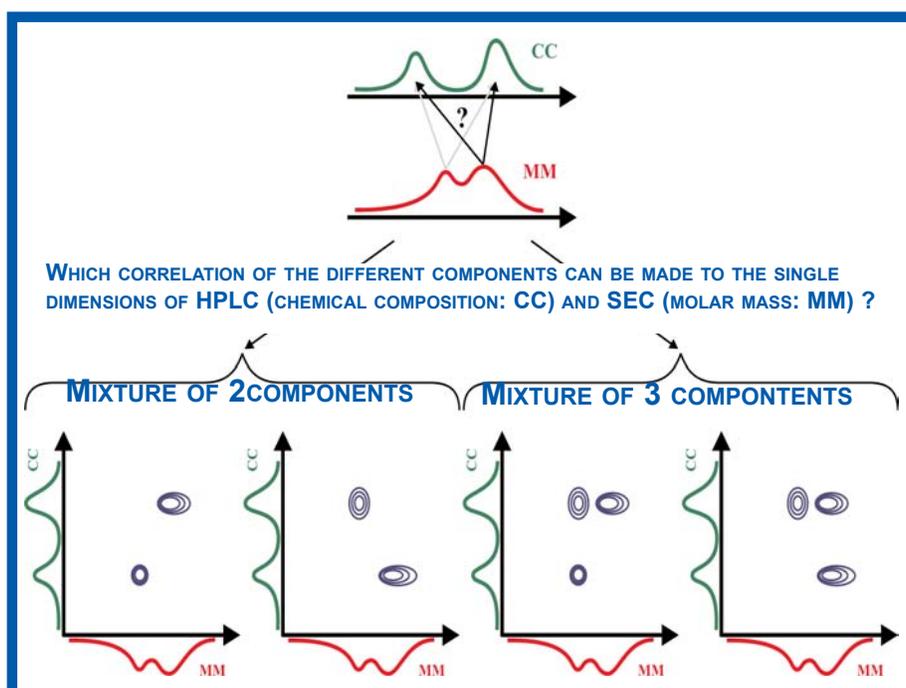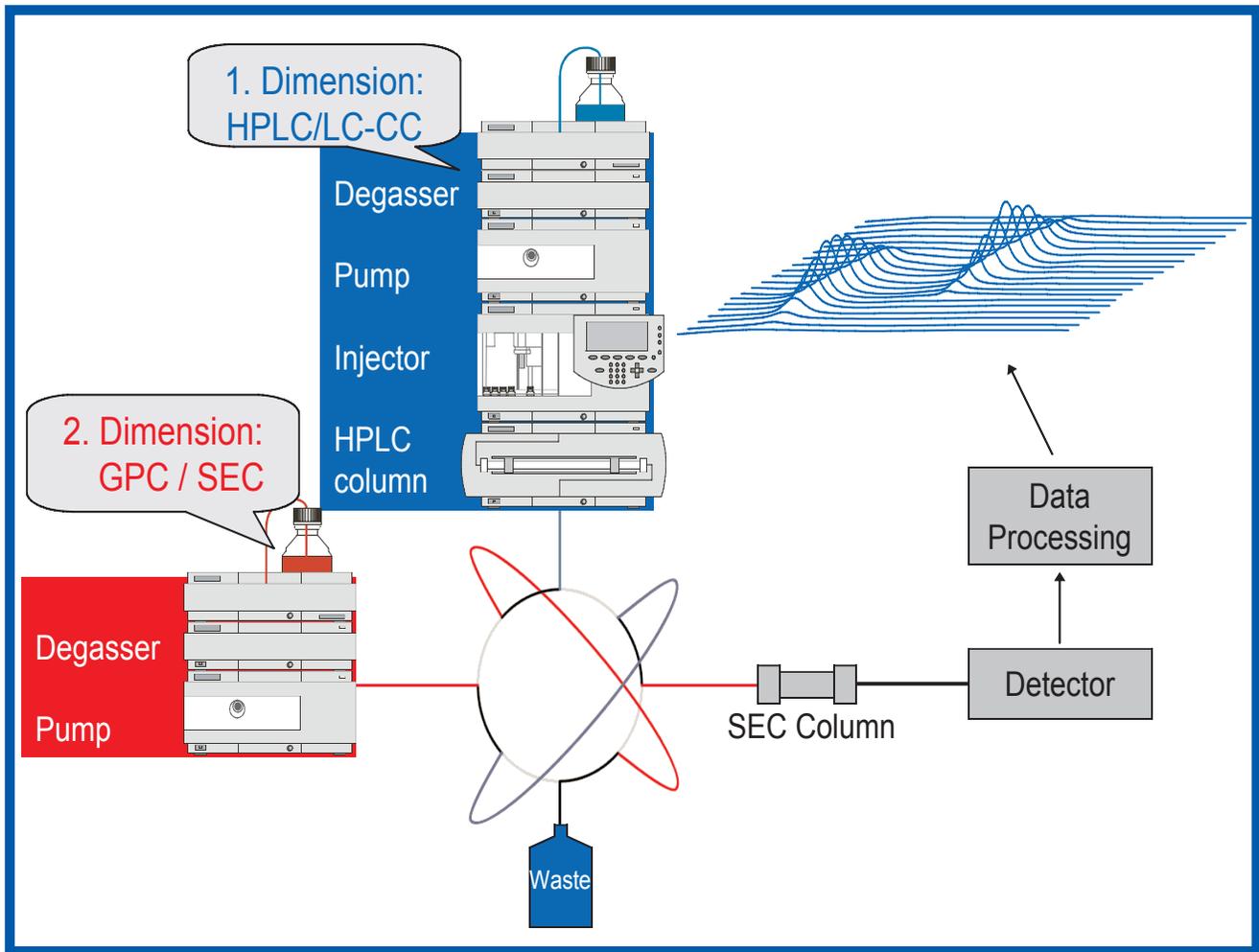
# HOW 2D-LC WORKS

A typical 2D-LC system consists of a High Performance Liquid Chromatography HPLC system (1st dimension) with its appropriate columns and mobile phases, and a (SEC) system (2nd dimension). These are coupled together via an automatic transfer valve, which allows the transfer of fractions from the 1st dimension to the 2nd dimension. In some cases, workers have used HPLC in both dimensions, such as reverse phase in the 1st dimension, and normal phase (or even ion chromatography), in the 2nd dimension. There is no restriction to just utilizing HPLC/GPC for the two dimensions, although that is the technique most commonly used.

In 2D-LC analysis a polymeric sample is injected into the HPLC system and passes through the HPLC column where it is separated according to its chemical composition i.e. homopolymers, copolymers and additives are separated depending on the degree of their interaction with the HPLC column. As the effluent leaves the HPLC column it is collected into storage loops (e.g. 100 µl or 200 µl) for a pre-determined collection time and then injected automatically into the SEC system. The fractions then pass through the SEC column where the polymeric material is separated according to its hydrodynamic volume (molar mass). As the polymeric material leaves the SEC column it is detected with a sensitive detector such as a Ultra-Violet (UV) and/or an Evaporative Light Scattering (ELSD) detector. Since this is an online technique, minimum operator time is required. For successful operation of the 2D-chromatographic system it is important that fractions (after being separated by the HPLC column) pass through the SEC system quickly before the next fraction is injected in order to avoid the overlapping of peaks of two chromatograms in the 2nd dimension. This requires that the flow rate in the 2nd dimension be extremely fast in comparison to the1st dimension flow. Since one conventional SEC column requires an analysis time of about 14 minutes (at a flow of 1ml/min) the analysis time for a single 2D-analysis can be as long as ~8 hours. However, with the advent of High-Speed SEC columns, flow rates of 5ml/min allow fractions to pass through the 2nd dimension within 2 minutes, resulting in much faster 2D-LC analysis. A 2D run can be completed in about one hour. The High-speed columns have lower back-pressures and use the equivalent amount of mobile phase as the conventional SEC columns.

The 2nd dimension shall be calibrated using appropriate standards of which narrowly disperse polystyrene or poly (methyl methacrylate) are commonly used.



WHICH CORRELATION OF THE DIFFERENT COMPONENTS CAN BE MADE TO THE SINGLE DIMENSIONS OF HPLC (CHEMICAL COMPOSITION: CC) AND SEC (MOLAR MASS: MM) ?

MIXTURE OF 2 COMPONENTS    MIXTURE OF 3 COMPONENTS

## HOW TO COLLECT AND PROCESS DATA

Polymer Standards Service has developed a unique software package that has a separate module especially for 2D-LC analysis. This easy-to-use software package, WinGPC Unity, allows data acquisition and control of fraction transfer between two liquid chromatographic systems. In addition, the raw data is presented as 2-dimensional contour or 3-dimensional surface plots, which upon integration yield molecular weight averages (Mn, Mp and Mw) and quantification of species present in complex samples. Other salient features in the software are:

1 Data acquisition from a variety of LC detectors

2 HPLC and SEC performed in a single run

3 Requires minimum operator intervention which saves time

4 Step-by-step 2D-Wizard guides you through the instrumentation set-up procedure

5 Saving and re-calling integration grids for future analysis

6 Calibration of both X- and Y-axes according to molecular weight, % composition etc.

7 360º angle rotation of 2D-contour and 3D-surface plots

8 The SEC and HPLC traces are projected along their respective axes

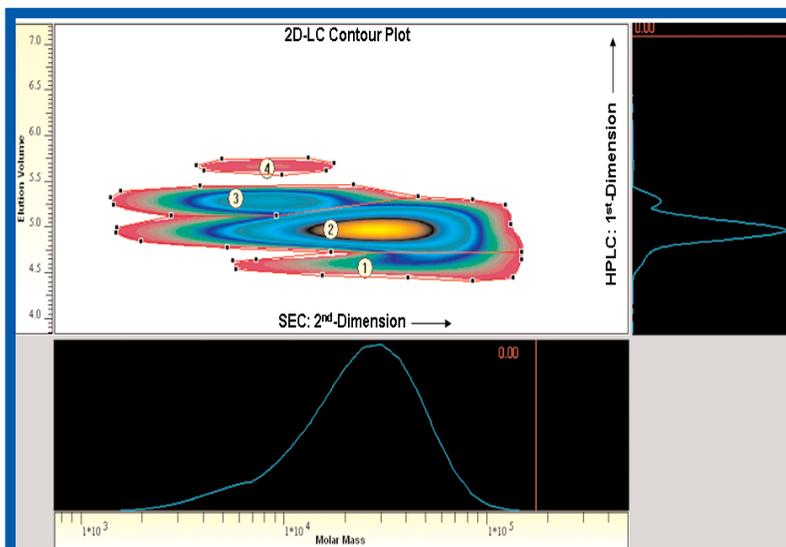9 Overlaying of 2D-contour plots from different analyses for comparison

## WHAT THE PROCESSED DATA LOOK LIKE

The contours represent the various species present in the copolymer sample. The SEC is plotted along the x-axis and represents the molar mass distribution for the copolymer sample, but does not provide information about the chemical composition of the sample. The HPLC chromatogram (representing chemical composition distribution) is plotted along the Y-axis and shows four chemically different species present in the sample. Each contour is integrated to yield the molar mass averages for each species; the polydispersity (PDI) for each species is calculated from the ratio Mw/Mn. The relative amounts of each species, in terms of area or volume, are also listed.



## Evaluation Results

| No. | Xmax | Ymax | Zmax | Area % | Vol. % | Mn 2.D | Mw 2.D | Mp 2.D |
|-----|-------|-------|-------|--------|--------|---------|---------|---------|
| 1 | 8.642... | 4.724... | 1.083... | 3.633... | 4.3071 | 34594.3 | 49727.1 | 55069.6 |
| 2 | 8.890... | 4.970... | 9.644... | 8.905... | 83.83... | 20291.5 | 31670 | 29914 |
| 3 | 9.391... | 5.281... | 1.809... | 3.419... | 9.2359 | 6224.65 | 8523.57 | 9131.07 |
| 4 | 9.472... | 5.6668 | 0.083... | 1.034... | 0.221... | 7702.15 | 8834.11 | 7642.21 |



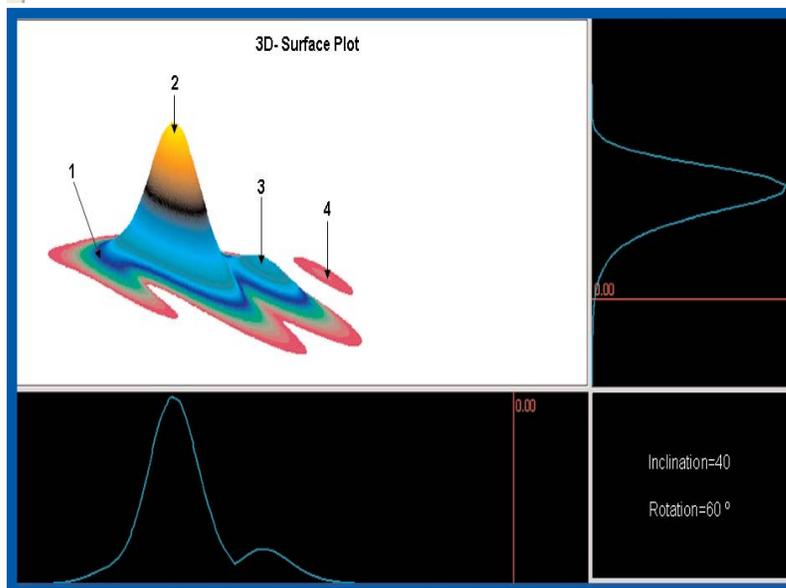## MINIMUM REQUIREMENTS FOR A 2D-LC SYSTEM

HPLC pump (isocratic or gradient)
Manual injector or autosampler
SEC/GPC pump (isocratic)
HPLC columns (PSS)
PSS high speed SEC/GPC columns (PSS)
Detector(s) (UV, ELSD or both)
Universal Dater Center UDC810, A/D converters, fiber optic cables, 8-port transfer valve with 2 storage loops and transfer valve actuator
WinGPC Unity software with 2D chromatography module, interface

(All equipment provided by PSS when not available)

# Sources and Resources

## Reference Publications about 2D-LC Analysis of Complex Polymers

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22  Pasch, H.; Adler, M.; Rittig, F.; Becker, S. *Macromol. Rapid Commun.* **26** (2005) 438–444.

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25  Pasch, H.; Adler, M.; Knecht, D.; Rittig. F; Lange, R. *Macromol. Symp.* **231** (2006) 166-177.

## Where to Purchase a 2D-LC System

PSS can provide you with all of the necessary components, including software, the column chemistry, valving and transfer loops, do

| Ordering Information | |
| --- | --- |
| WinGPC Unity Software | 400-0073 |
| Module for 2D Chromatography | 400-1008 |
| Universal Data Center UDC810 | 400-0018 |
| A/D Converter | 400-0006 |
| 8-Port transfer Valve | 409-9012 |
| Optional Report Designer | 400-1001 |

the installation, and show you how to operate the 2D-LC system. You need to provide the LC instrument for the 1st dimension, (injector and gradient pump), and a pump for the 2nd dimension (GPC separation), as well as a detector, (usually an ELSD, but a UV detector is also used on occasion.).

## Who is PSS

Polymer Standards Service GmbH (PSS) is certified DIN EN ISO 9001 to produce high quality Reference Polymers, GPC Columns and Software for the characterization of polymers by their molecular weight and their structural characteristics.   PSS employs the latest findings in polymer science for the synthesis and characterization of polymers, copolymers and biopolymers.

PSS operates a manufacturing facility equipped with a complete state-of-the-art characterization laboratory at the headquarters in Mainz (Germany), fully supporting customers working under stringent requirements, i.e. GLP, DIN, ISO 9000x certifications.

Polymer Standards Service-USA Inc. (PSS-USA, Inc.) is the sales and service partner of Polymer Standards Service GmbH (PSS) in North America for the US, Canada and Latin America customers since 1994.
PSS partners with other product manufacturers such as Agilent, Brookhaven, WGE, among others, to integrate the products necessary to place at your service the most comprehensive Gel Permeation Chromatography solution.  Contact us through phone, fax or e-mail

### PSS IS ONE OF THE WORLD'S LEADING COMPANIES OFFERING SOLUTIONS FOR POLYMER CHARACTERIZATION

Reference Polymer Standards
- GPC/SEC Standards
- Certified reference materials
- MALDI Kits
- Viscosity and light scattering validation kits
- ReadyCal kits
- Deuterated polymers
- Tailor made polymers

GPC/SEC Columns
- For organic and aqueous eluents
- For high and low molecular weight synthethic and bio-polymers
- Various dimensions
- For HighSpeed polymer and bio-polymer analysis

Software
- WinGPC Unity
- Porocheck- for pore size analysis

Analytical Services
- Molar mass determination
- Structuretnalysis
- Method development and transfer
- Complete product de-formulation

GPC Schools and Support
- GPC and software training
- GPC in-house training
- User meetings
- Net community & applications

GPC Instruments
- Complete systems and components
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- Viscosity detector
- FTIR/MALDI interface
- dn/dc Instrumentation

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