

# Supercritical Fluid Chromatography & Gel Permeation Chromatography for Characterization of Macromolecules

## *Evidence of the Formation of Micellar Nanostructures in GPC Columns*

*G.A. Mansoori*

*<mansoori@uic.edu>*

*Department of Chemical Engineering*



- *For presentation at the AIChE - Chicago Section, Seminar on Batch and Simulated Moving Bed (SMB) Design and Applications held at Pharmacia, Searle Parkway, Skokie, IL 60077*
  - *Wednesday March 14, 2001*

# Introduction

Supercritical fluids (SCFs) are increasingly replacing the organic solvents that are used in industrial purification and recrystallization operations because of regulatory and environmental pressures on hydrocarbon and ozone-depleting emissions. SCF-based processes has helped to eliminate the use of hexane and methylene chloride as solvents. With increasing scrutiny of solvent residues in pharmaceuticals, medical products, and nutraceuticals, and with stricter regulations on VOC and ODC emissions, the use of SCFs is rapidly proliferating in all industrial sectors.

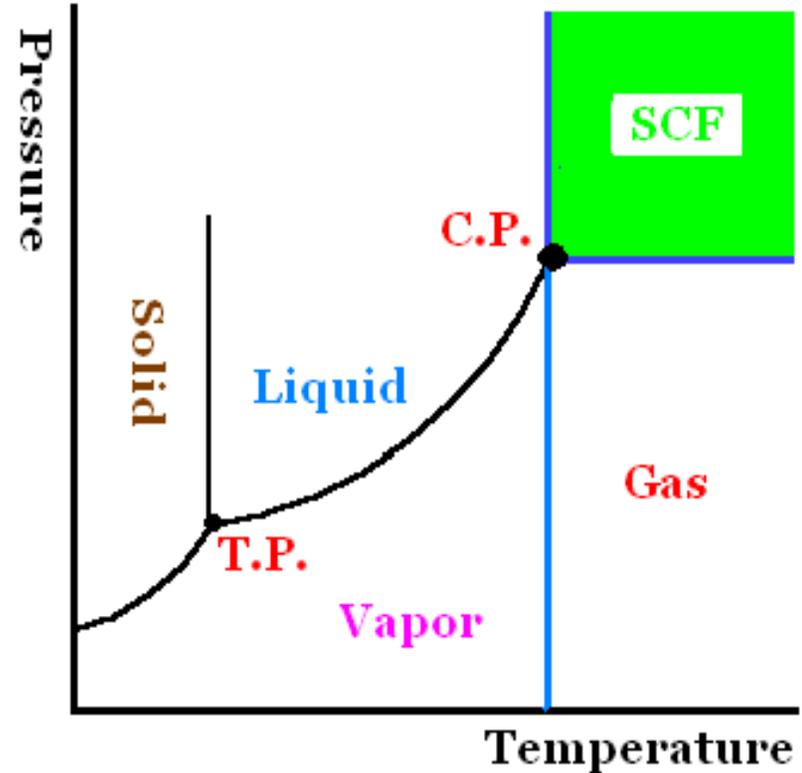
Supercritical fluid extraction (SFE) plants are operating at throughputs of 100,000,000 lbs/yr or more in the foods industry. Coffee and tea are decaffeinated via supercritical fluid extraction and most major brewers in the US and Europe use flavors that are extracted from hops with supercritical fluids.

SCF processes are being commercialized in the polymers, pharmaceuticals, specialty lubricants and fine chemicals industries. SCFs are advantageously applied to increasing product performance to levels that cannot be achieved by traditional processing technologies, and such applications for SCFs offer the potential for both technical and economic success.



# Supercritical Fluids

- A supercritical fluid (**SCF**) is characterized by physical and thermal properties that are between those of the pure liquid and gas. The fluid density is a strong function of the temperature and pressure. The diffusivity of SF is much higher than for a liquid and **SCF** readily penetrates porous and fibrous solids. Consequently, **SCF** can offer good catalytic activity.

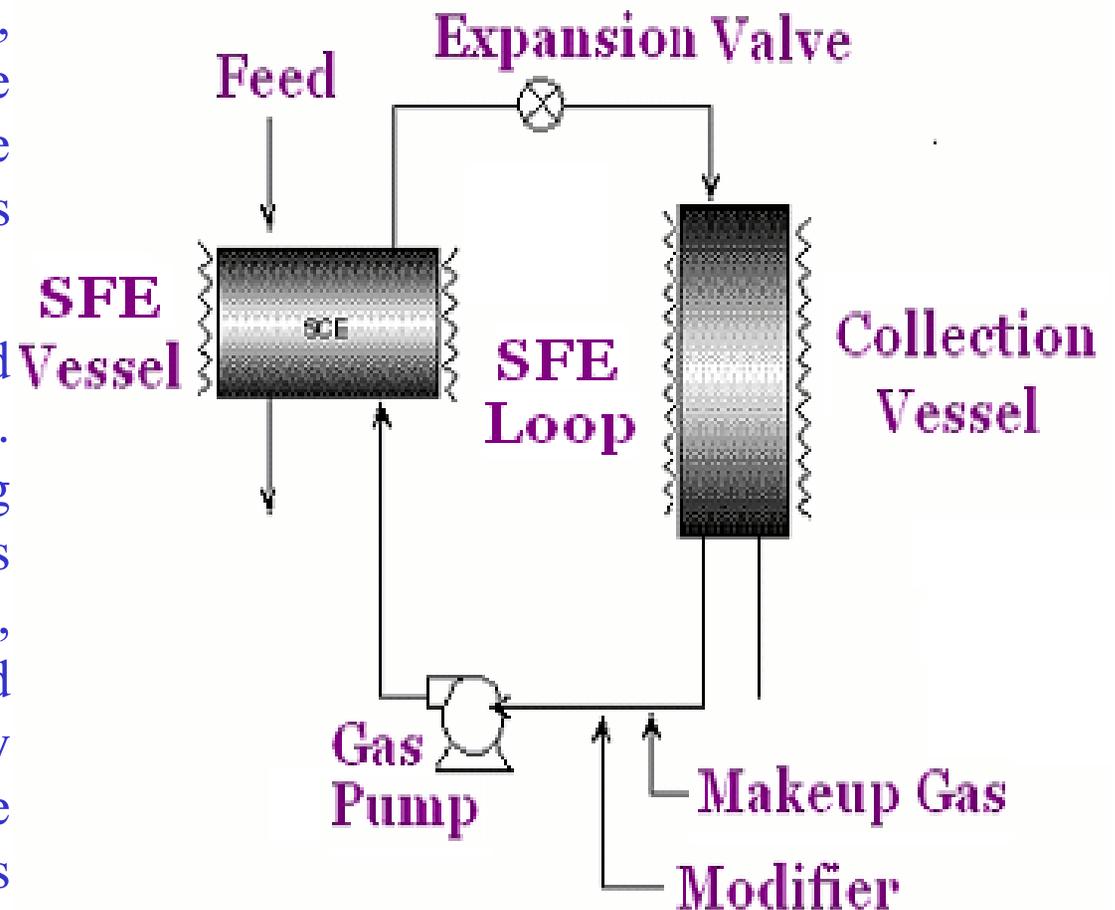


# Properties of Supercritical Fluids

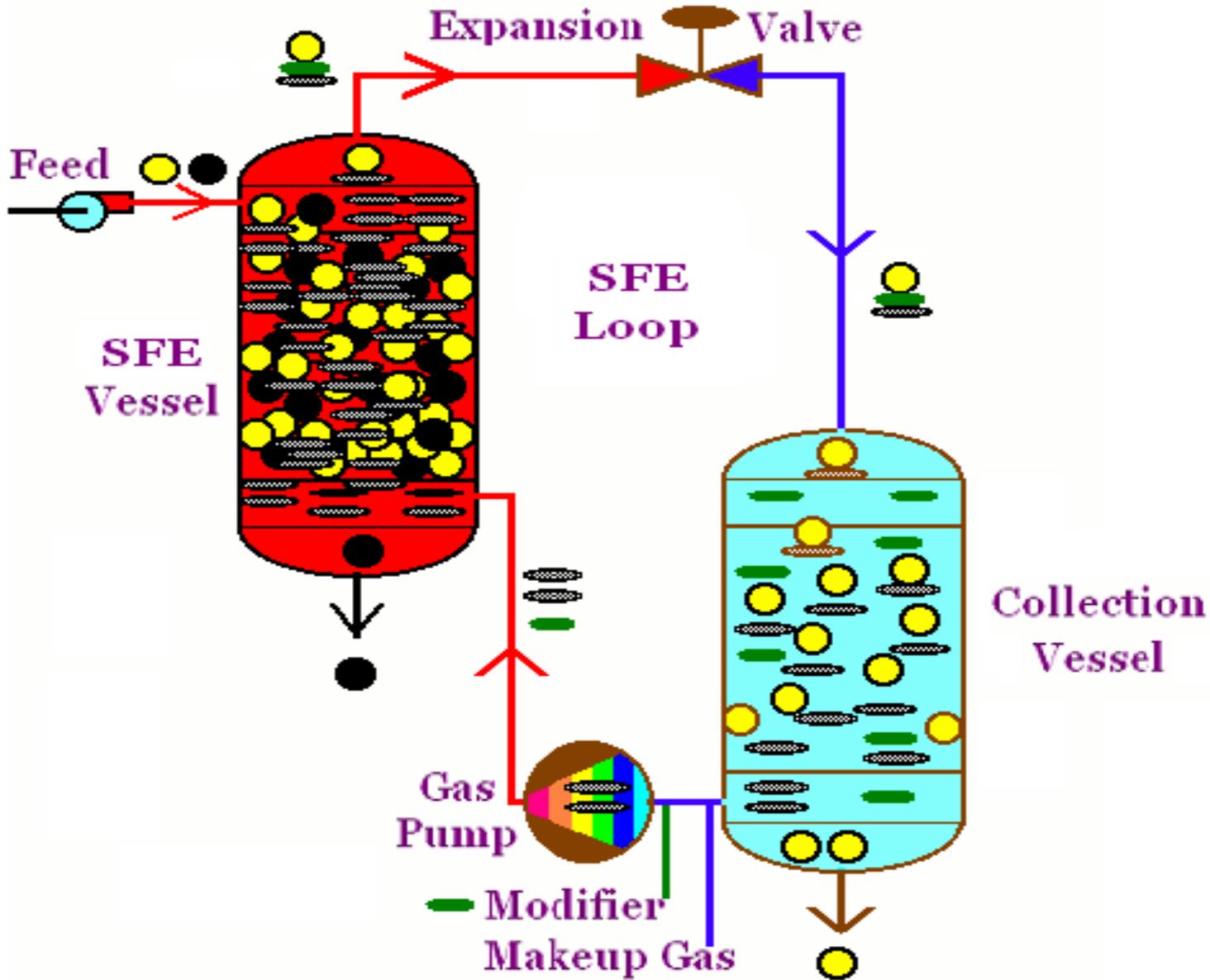
- There are drastic changes in some important properties of a pure liquid as its temperature and pressure are increased approaching the thermodynamic critical point. For example, under thermodynamic equilibrium conditions, the visual distinction between liquid and gas phases, as well as the difference between the liquid and gas densities, disappear at and above the critical point. Similar drastic changes exist in properties of a liquid mixture as it approaches the thermodynamic critical loci of the mixture.
- Other properties of a liquid fuel that change widely near the critical region are thermal conductivity, surface tension, constant-pressure heat capacity and viscosity. In comparing a liquid sample with a supercritical fluid (SCF) sample of the same fuel both possessing the same density, thermal conductivity and diffusivity of a SF are higher than the liquid, its viscosity is much lower, while its surface tension and heat of vaporization have completely disappeared. These drastic changes make a supercritical fuel appreciably preferred over that of a liquid fuel with the same density. Further, it is expected that the combustion phenomena resulting from that of a supercritical fuel will be quite different from that of a liquid fuel.
- Applications of SCF include recovery of organics from oil shale, separations of biological fluids, bioseparation, petroleum recovery, crude de-asphalting and dewaxing, coal processing (reactive extraction and liquefaction), selective extraction of fragrances, oils and impurities from agricultural and food products, pollution control, combustion and many other applications.

# Supercritical Fluid Extraction (SFE)

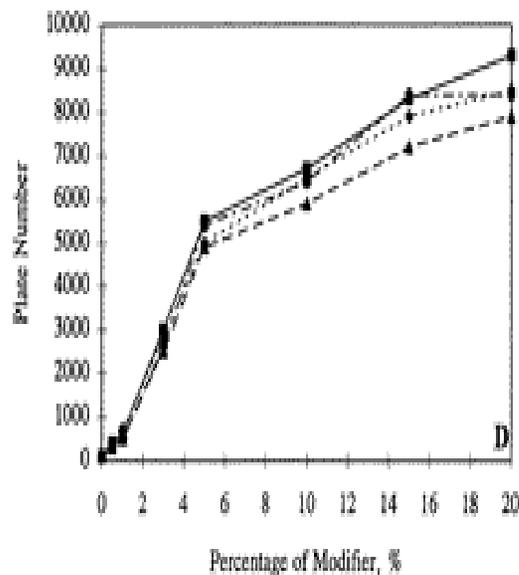
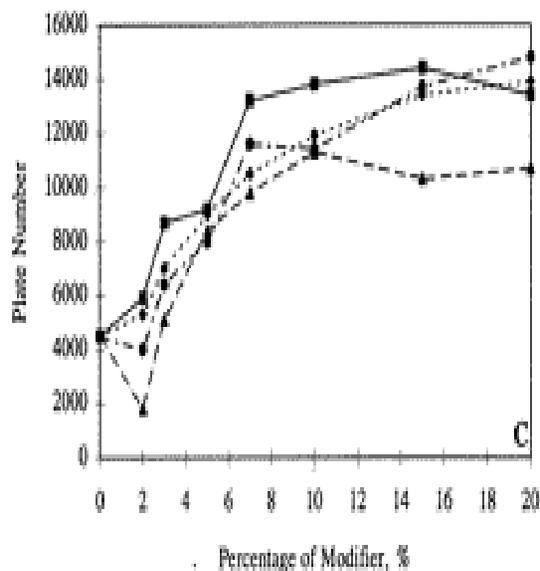
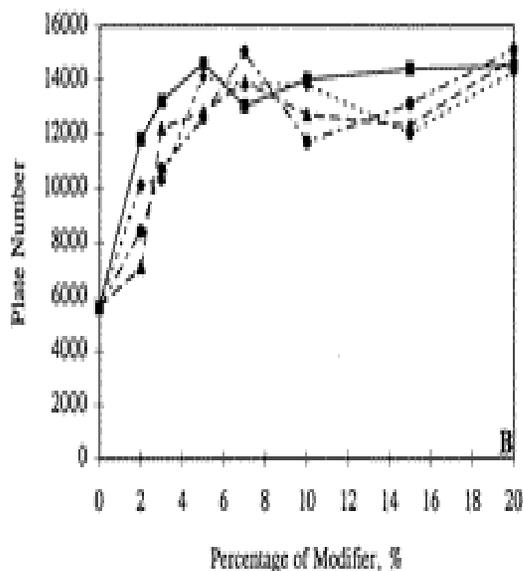
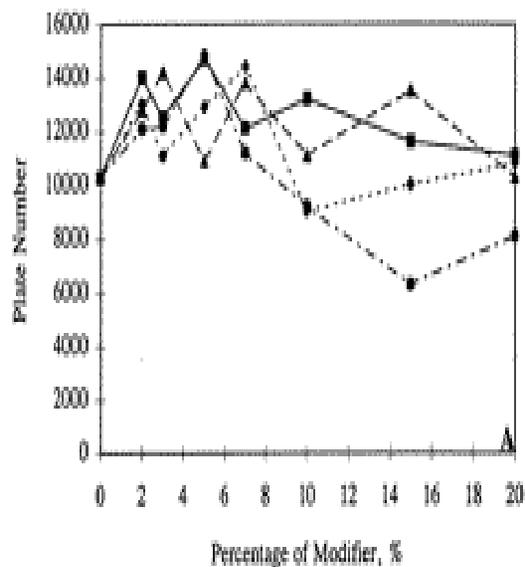
- Supercritical Fluid Extraction (SFE) is based on the fact that, near the critical point of the solvent, its properties change rapidly with only slight variations of pressure.
- Supercritical fluids can be used to extract analytes from samples. The main advantages of using supercritical fluids for extractions is that they are inexpensive, extract the analytes faster and more environmentally friendly than organic solvents. For these reasons supercritical fluid CO<sub>2</sub> is the reagent widely used as the supercritical solvent.



# Molecular Basis of SFE



# The role of Modifier on SFE



- Plots of modifier effects on efficiency for nonpolar solutes on C18 column at 80 °C and 210 bar, 2.0 mL/min. Solute: (A) naphthalene; (B) anthracene; (C) pyrene; (D) chrysene. Modifier: solid line with  $\square$  = methanol; broken line with  $\circ$  = ethanol; dotted line with  $\triangle$  = 1-propanol; dashed line with  $\diamond$  = acetonitrile [Zou & Dorsey, 2000]

# Advantages of SFE

- **1.** SCFs have solvating powers similar to liquid organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension.
- **2.** Since the solvating power can be adjusted by changing the pressure or temperature separation of analytes from solvent is fast and easy.
- **3.** By adding modifiers to a SCF (like methanol to CO<sub>2</sub>) its polarity can be changed for having more selective separation power.
- **4.** In industrial processes involving food or pharmaceuticals, one does not have to worry about solvent residuals as you would if a "typical" organic solvent were used.
- **5.** Candidate SCFs are generally cheap, simple and many are safe. Disposal costs are much less and in industrial processes, the fluids can be simple to recycle.
- **6.** SCF technology requires sensitive process control, which is a challenge. In addition, the phase behaviour of the mixture of solutes and solvents has to be measured or predicted quite accurately. Generally the phase behaviour in the critical region is rather difficult to control. Our recent research has provided much more insight into this phase behaviour. Our Thermodynamics Research Laboratory at UIC is well equipped to produce data and predictive schemes for the phase behaviour in the super- and sub-critical regions.

# Supercritical Fluid Chromatography

Berger SFC System



- **Supercritical fluid chromatography (SFC) is similar to gas chromatography (GC), except for a need for syringe pumps of high quality and facilities for density and pressure programming as well as for pressure reduction in the detector. In SFC the sample is carried through a separating column by a supercritical fluid (usually CO<sub>2</sub>) where the mixture is divided into unique bands based on the amount of interaction between the individual analytes and the stationary phase in the column. As these bands leave the column their identities and quantities are determined by a detector.**

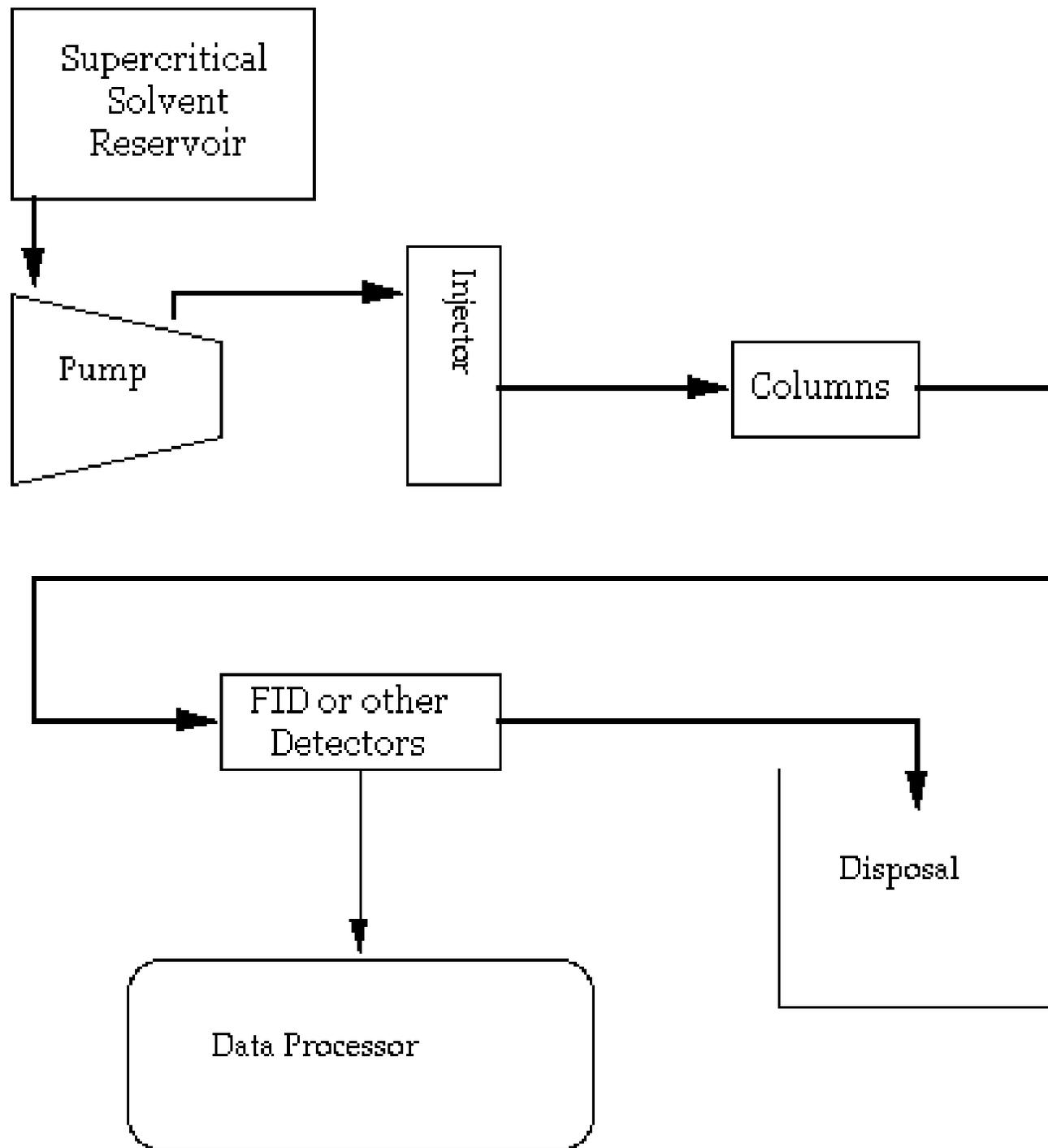
# SFC Components

- **SFC Columns:** There are two types of analytical columns used in SFC. Capillary columns of fused silica coated with cross-linked chemically bonded stationary phases, that are used in GC are equally applicable in SFC. Also packed columns developed for high-performance liquid chromatography (HPLC) are being used with SFC. The columns are conventionally stainless steel.
- **SFC Mobile Phase:** CO<sub>2</sub> is the primary mobile phase used in SFC. The advantage of CO<sub>2</sub> as the mobile phase is low cost, low interference with chromatographic detectors, nontoxicity, low critical temperature (31.1 °C), inflammability and that it can permit a flame ionisation detector to be used, with all the benefits in terms of ease of use, linearity and sensitivity that we have come to expect of this device in GC applications. Disadvantage of carbon dioxide is its inability to elute very polar or ionic compounds. By adding a small portion of a second fluid, modifier, this can be overcome. Modifiers are generally an organic fluid (such as alcohols and cyclic ethers and water) which are completely miscible with carbon dioxide. Modifiers improve the solvating ability of the SCF and sometimes enhance separation selectivity. A modifier fluid is commonly used, especially in packed column SFCs.
- **SFC Injection:** For packed SFC a typical LC injection valve is commonly used. In capillary SFC small sample volumes must be quickly injected into the column and therefore pneumatically driven valves are used. The ovens used in SFC are generally conventional GC or LC ovens.

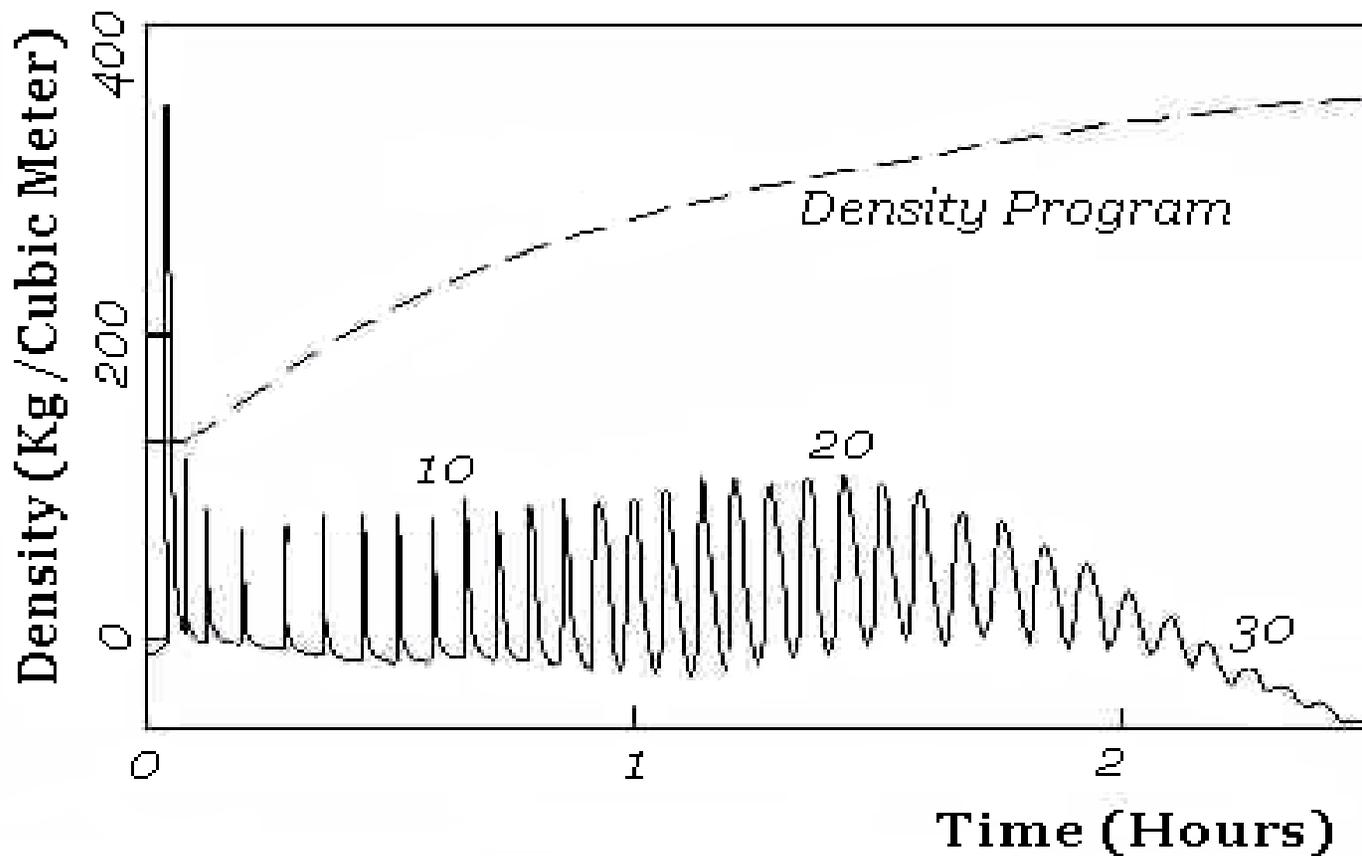
## SFC Components continued

- **SFC Pump:** The type of high pressure pump used in SFC is determined by the column type. For packed columns reciprocating pumps are generally used while for capillary SFC syringe pumps are used. Reciprocating pumps allow easier mixing of the mobile phase or introduction of modifier fluids. Syringe pumps provide consistent pressure for a neat mobile phase.
- **SFC Detector:** Optical detectors, flame detectors and spectroscopic detectors can be used. However, the mobile phase composition, column type and flow rate must be taken into account when the detector is selected. Some care must also be taken such that the detector components are capable of withstanding the high pressures of SFC. In practice, SFC operates at low to moderate temperatures and seems most suited to the analysis of heat-sensitive compounds of high molecular weight such biological fluids. The technique is therefore an alternative to high-temperature GC and to HPLC.
- **ASTM Guide E1449-92** is the Standard Guide for Supercritical Fluid Chromatography Terms and Relationships.

# SFC Flow Chart

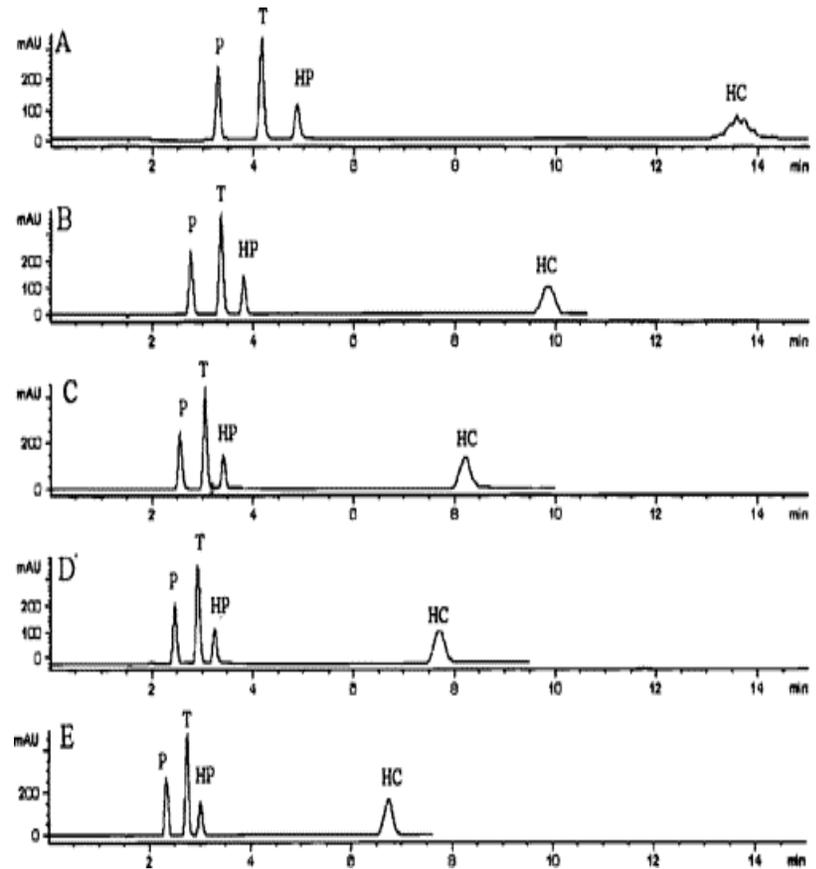


SFC is well established as an analytical technique and a large volume of information is available in the literature about it. Figure below is an example of an SFC chromatogram of polystyrene in supercritical pentane, where separation of individual oligomers up to a chain length of 30 is visible. Better resolution can be obtained if necessary. The density varied during the chromatography is shown by the dashed curve [*Express Separations Ltd.UK*].



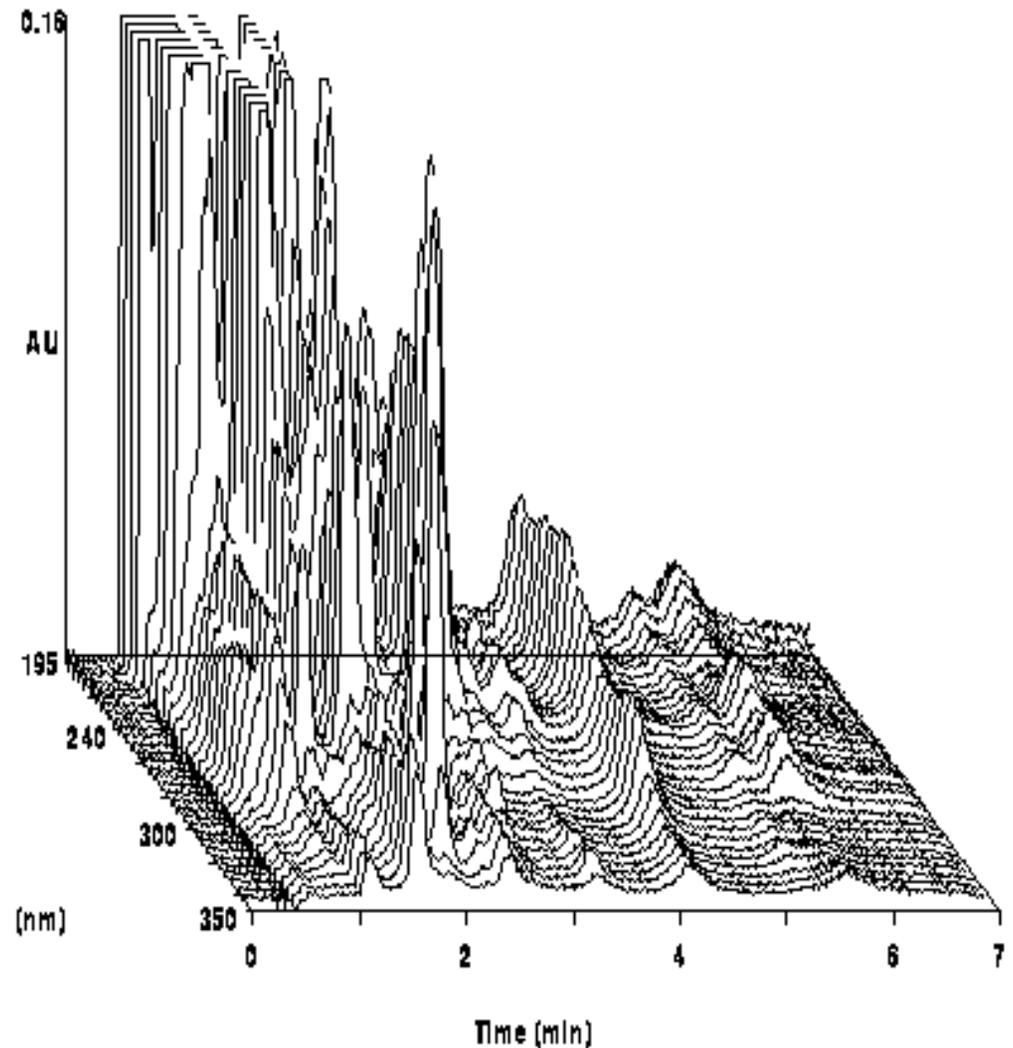
# The role of Modifier on SFC

- SFC separation of steroids using five different types of CO<sub>2</sub> containing 5% (v/v) methanol mixed in-line at the same pressure (200 bar): (A) level 1 HHS-CO<sub>2</sub>, (B) level 2 HHS-CO<sub>2</sub>, (C) level 3 HHS-CO<sub>2</sub>, (D) level 4 HHS-CO<sub>2</sub>, and (E) level 5 pure CO<sub>2</sub>. Peak identity: (P) progesterone, (T) testosterone, (HP) 17 $\alpha$ -hydroxyprogesterone, and (HC) hydrocortisone [Leichter *et al*, 1996].



# SFE/SFC

The direct connection of supercritical fluid extraction and supercritical fluid chromatography (SFE/SFC) has found many applications recently. SFE/SFC has the advantages of not requiring a concentrating procedure or a cleanup procedure before analysis. SFE/SFC is applied for various compounds from different matrixes, including caffeine extraction, tocopherol enrichment, flavors extraction and analysis of pesticide residues. The direct coupling of SFE with GC, GPC, etc. in an on-line approach is conceptually straightforward, assuming quantitative deposition of the extracted analyte into the chromatographic inlet. SFC-MS has found several useful applications in recent years.



Three-dimensional chromatogram of the extract from the petroleum residue with supercritical carbon dioxide obtained by direct introduction into SFC [*JASCO Corp*].

# Gel Permeation Chromatography (GPC)

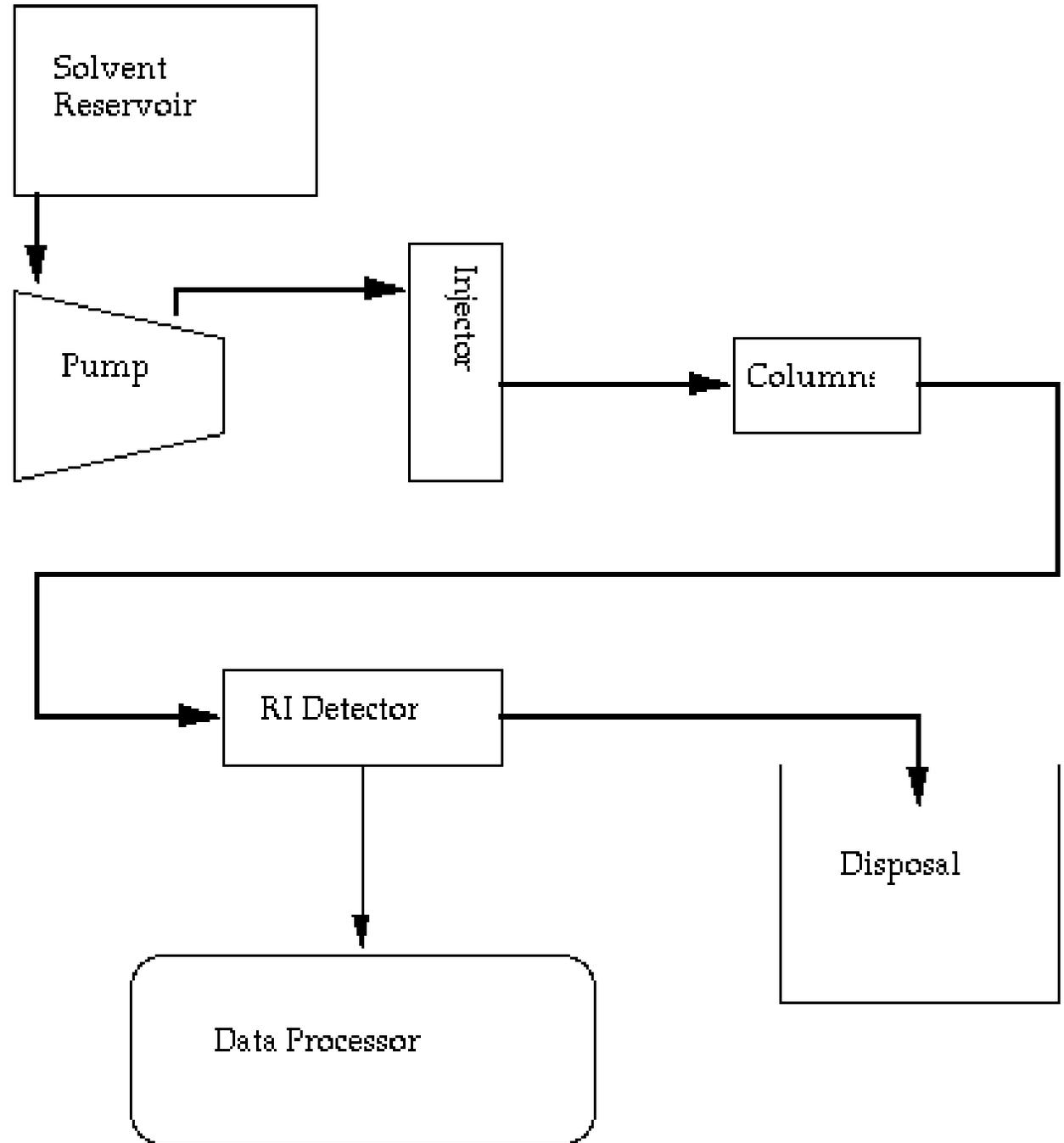


- Gel permeation chromatography (also called molecular exclusion chromatography and size exclusion chromatography) separates chemical matrices according to molecular sizes. The primary use of GPC is in the removal of natural, biological macromolecules.
- GPC is a powerful technique for understanding and predicting the performance of materials. Molecular weight and polydispersity analysis by GPC can help in understanding macromolecule properties such as strength and viscosity. In macromolecules, GPC can also determine low molecular weight species which may affect bulk properties, such as the presence of plasticizers, oligomers, and monomers.

# GPC Components

- **GPC Columns:** Act as a molecular filtration system. The columns are packed for organic or water soluble polymers as well as aqueous GPC and consist of a crosslinked gel, which contain surface pores. The columns are usually heated to some elevated temperature. Column systems are either Extended Range / Mixed Bed or series of individual pore size columns. Column packing material vary.
- **GPC Mobile Phase:** Consist of a liquid solvent in which the sample is dissolved in. There are *isocratic* (single solvent) and *gradient* (two or more solvent) GPC systems.
- **GPC Injection:** Consist of a manual or autosampler injector.
- **GPC Pump:** Is a sophisticated and precise liquid pumping device with better than 0.10% precision.
- **GPC Detector:** Differential refractometer is most widely used detector. UV, or possibly PDA (photodiode array), detectors are used for styrenic type polymer and in *gradient* analysis. Light scattering detector, coupled with refractometer, is another powerful mode of advanced detection for GPC analysis.

# GPC Flow Chart

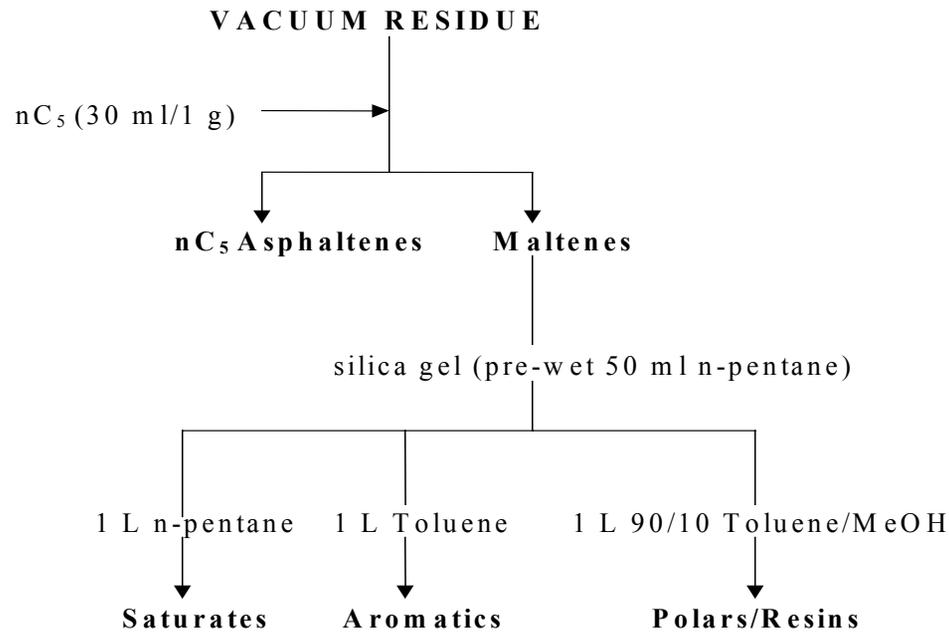
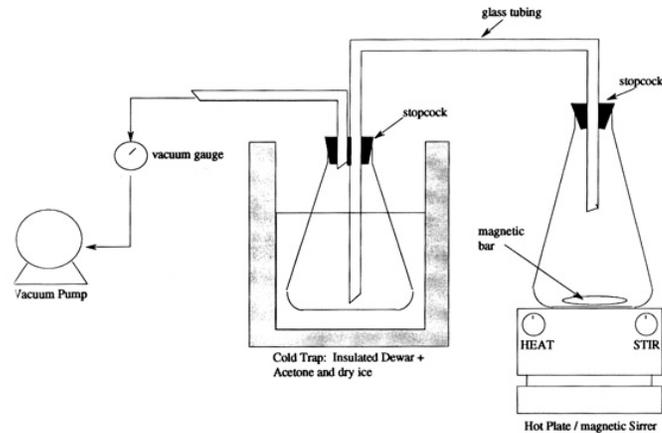


# Petroleum Heavy End Chromatography

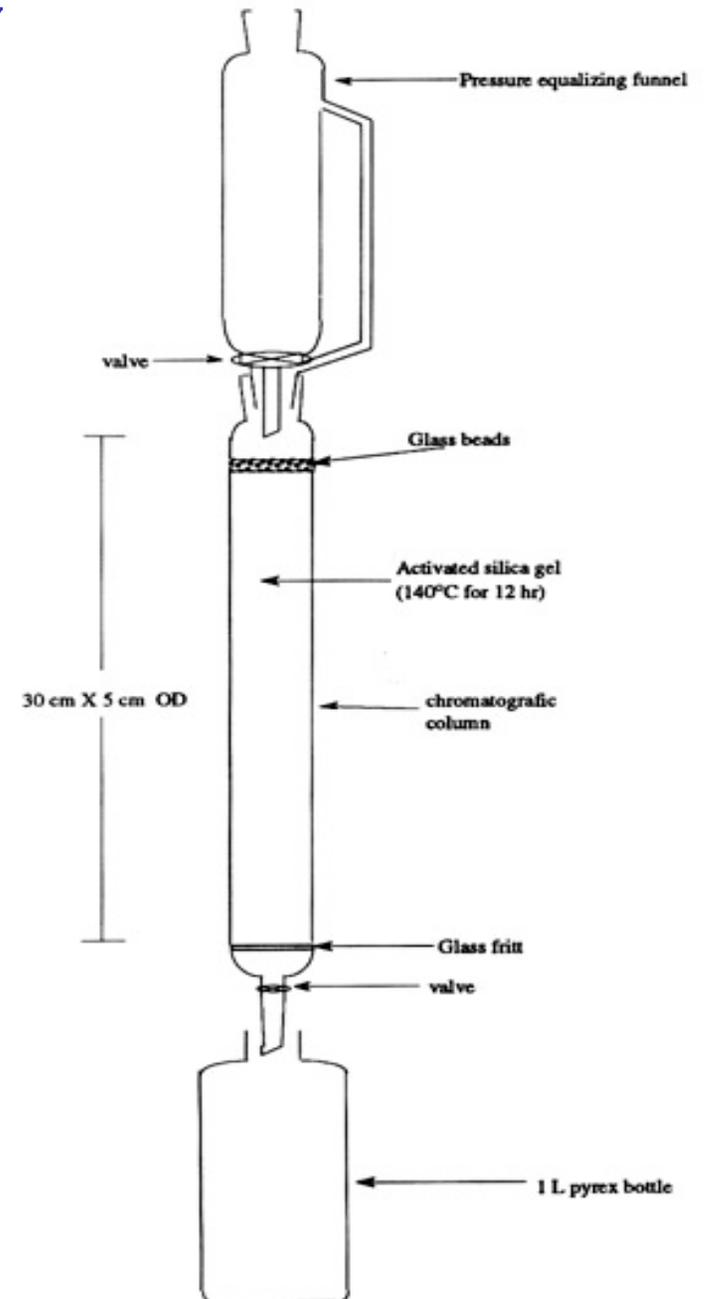
For mixtures such as petroleum, whose composition and properties vary continuously from the simplest structures to macromolecules, the characterization is a key first step to understand its properties. The common problem one must face consists then on the identification of the discrete components of the mixture and the definition of families or fractions of similar components using chromatography.

In the simplest case, petroleum is considered to be composed of four major fractions of similar species which are mutually soluble forming one phase as long as certain conditions are maintained in the mixture. Once separated, the polar molecules may start to aggregate by hydrogen bonding or other polar group interactions and form larger particles that precipitate out of the mixture as solid deposits which in many cases plug wells, pipelines and processing equipment .

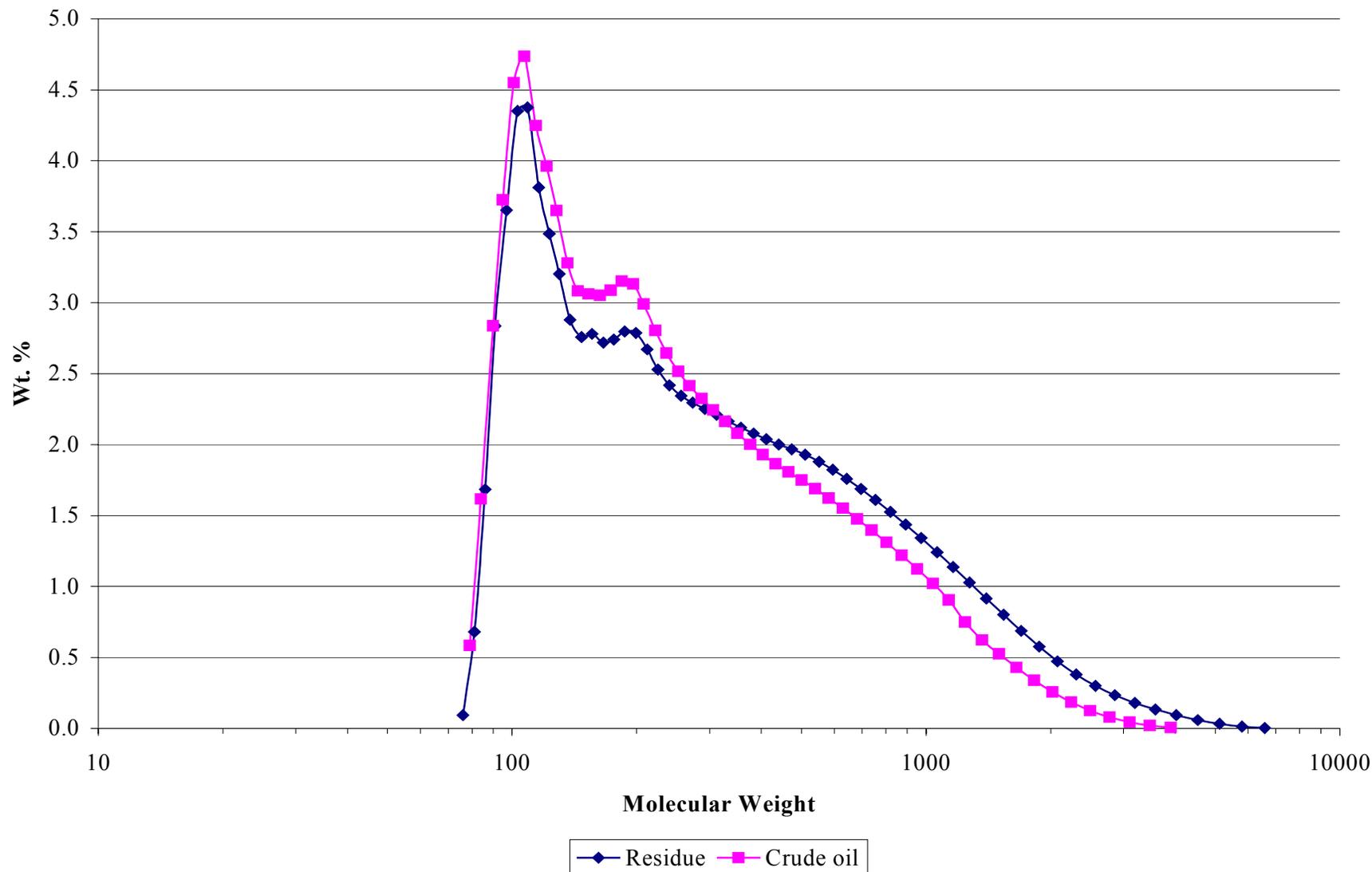
# Liquid Chromatography



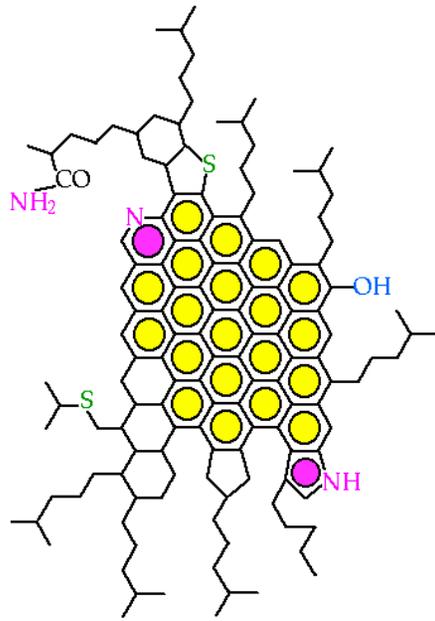
- The SARA procedure is one of the standard methods widely used to achieve the separation of the heavy fraction of petroleum based on liquid chromatography and solvent treatment techniques. This method provides an excellent tool to compare a variety of different petroleum fluids. Four classes of compound are separated, namely saturates, aromatic, resins and asphaltenes.



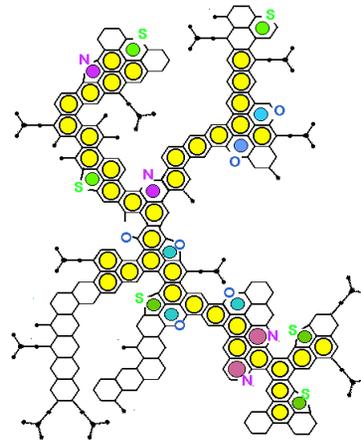
# GPC of the Oil & its Vacuum Residue



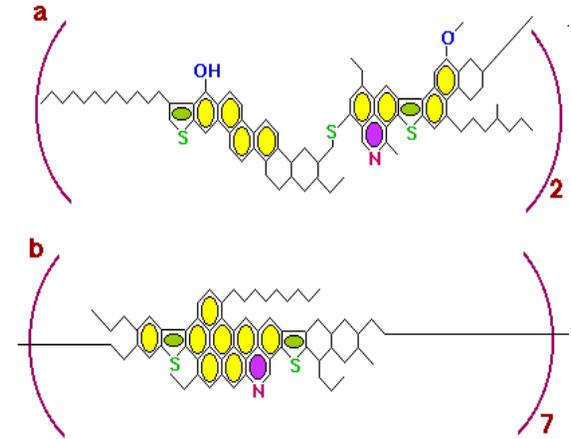
**Asphaltene** is a highly active non- hydrocarbon molecule which is often present, along with many variety of hydrocarbons and non- hydrocarbons, in natural gas and petroleum systems. Variations of the molecular structures of asphaltenes from natural sources is well documented



**VENEZUELAN**



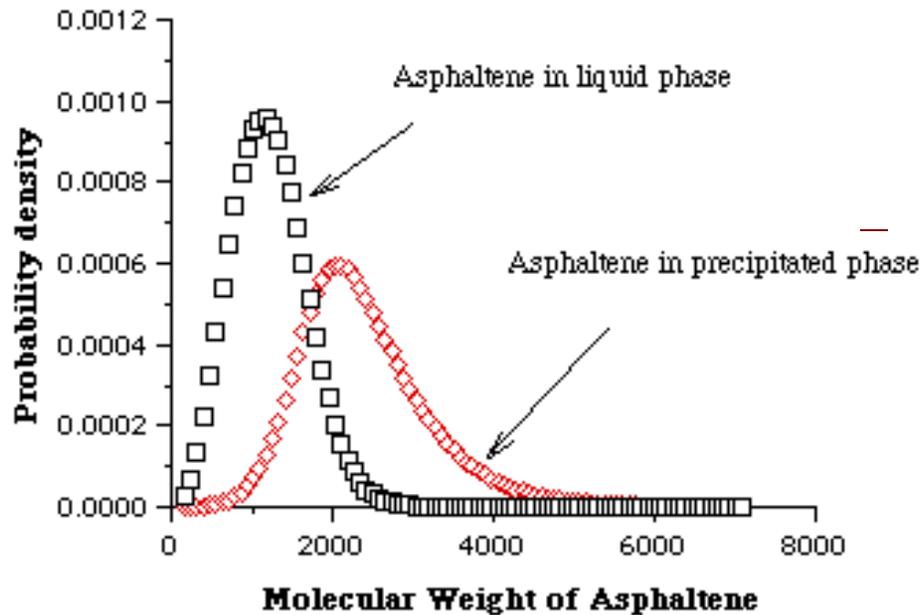
**MEXICAN  
ASPHALTENE**



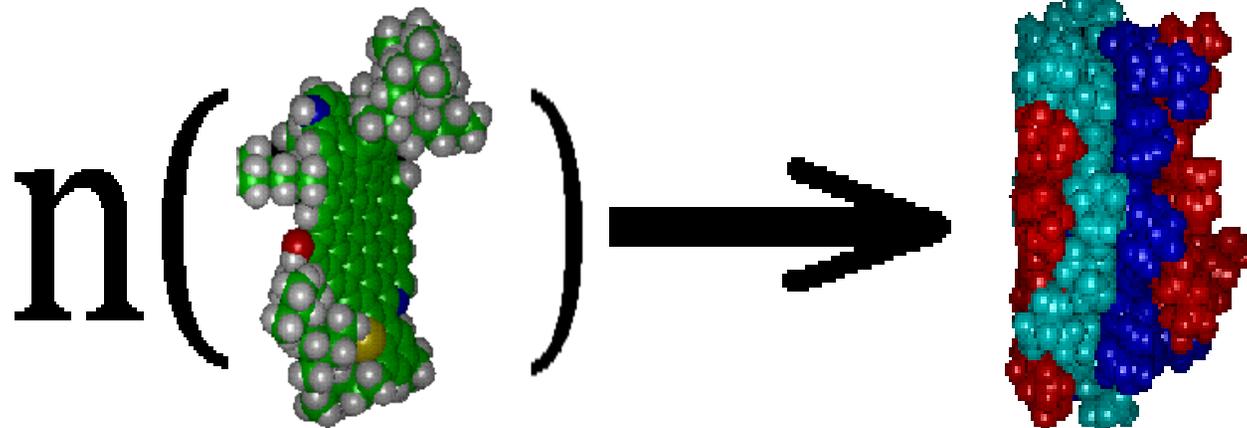
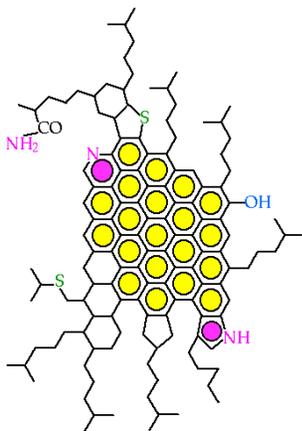
**CANADIAN**

Asphaltenes, considered to be the major difficulty for fouling in the flow of petroleum fluids, are polynucleus aromatic molecules with surfactant characteristics. During refining of petroleum asphaltene, other heterocyclic compounds, heavy hydrocarbons and non-volatile products will remain in the bottom of the refinery's fractionation column as a very complex mixture known as resid.

# Properties of Asphaltene

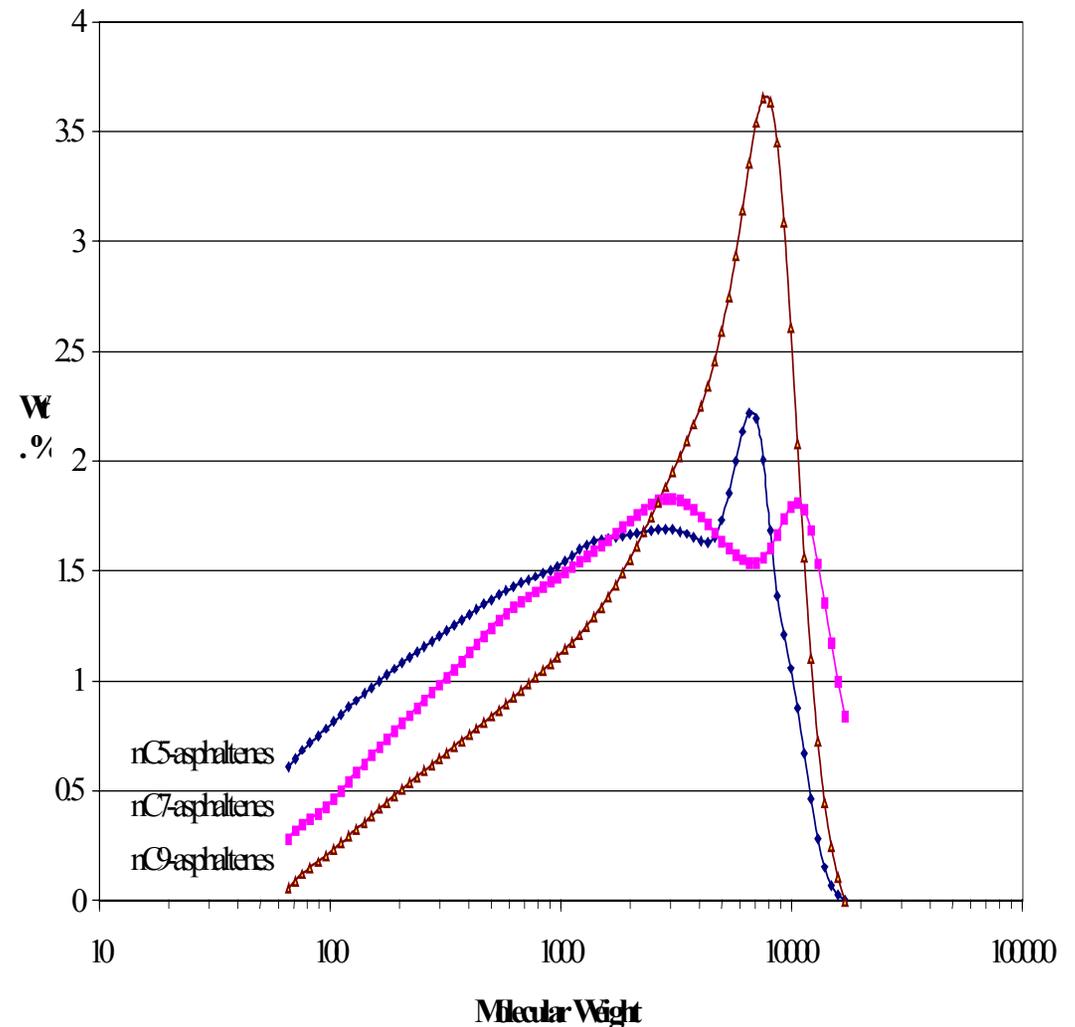


- Asphaltenes are polar, polydisperse and surface active agents, and are of the category of associating compounds.
- Variables which are known to affect asphaltene stability in crude oil are temperature, pressure, the nature of the oil container, conduit through which oil flows, oil thermal and electrical conductivities, oil viscosity, and oil flow regime.

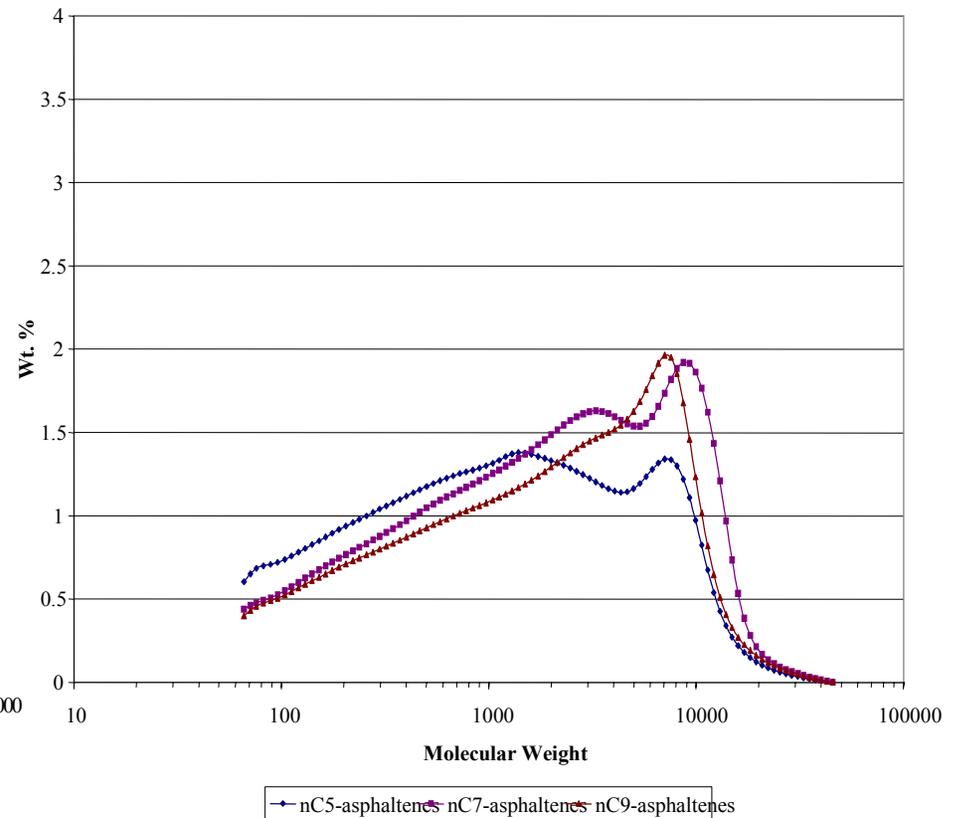
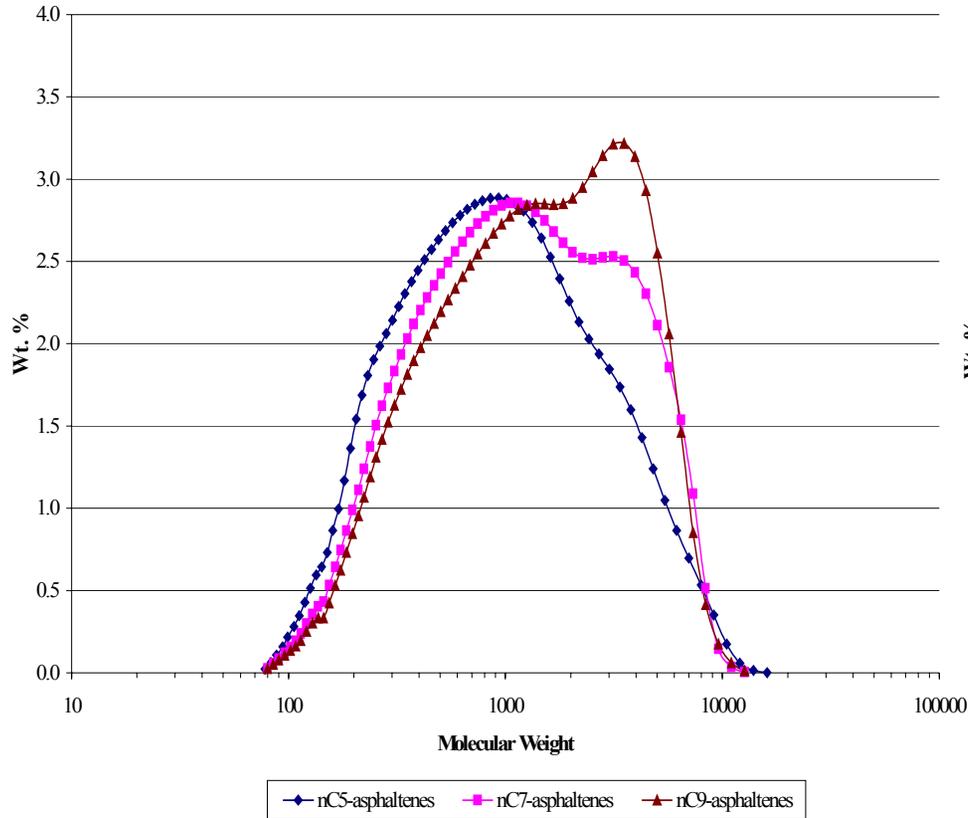


# GPC STUDIES OF SEPARATED ASPHALTENES FROM A CRUDE OIL

- The most widely used method for determining the MW and polydispersity of asphaltenes is GPC. It is understood, however, that GPC provides relative molecular weights and depends entirely on the calibration standards used. The bimodal distributions are indicative of micelle and micelle coagulate formation in the columns.
- MOLECULES  $\leftrightarrow$  MICELLES
- MICELLES  $\leftrightarrow$  MICELLE AGGREGATES
- [*Vasquez & Mansoori, 2000*].



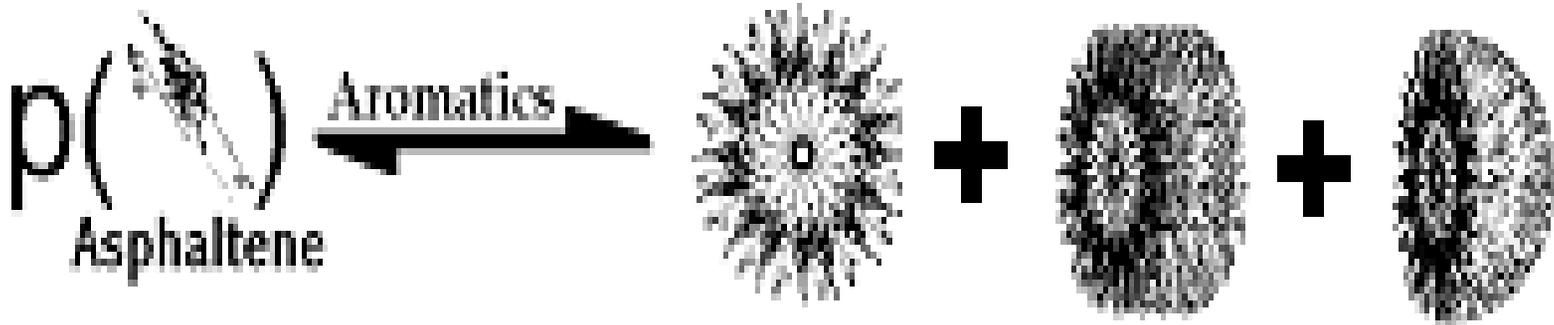
# Two other GPC Data



# Micellization

Micellization is a phenomenon originally observed due to the self-association process of the surface active materials in aqueous solution due to van der Waals attractive forces. The same van der Waals forces that are found to be sufficiently strong to bind C60 and carbon nanotube molecular solids and to allow the reproducible fabrication of free-standing C60 membranes on silicon wafers have the role of forming asphaltene micelles. The surface active asphaltene (surfactant) molecule tends to self-assemble into geometric shapes (disks, spheres, cylinders) and become suspended in the solution. This phenomenon occurs only when the surfactant asphaltene concentration exceeds a threshold known as critical micelle concentration (CMC).

On washing the asphaltene precipitate with aromatic solvent it will be dissolved and will form asphaltene micelles:



# Coacervation



If one starts with a solution of asphaltene micelles in an appropriate solvent, then as a result of increase in asphaltene micelle concentration a large part of the micelles can be separated out into a new phase. The original single phase system becomes two phases. One is rich and the other is poor in micelle concentration. The micelle-rich phase in a dispersed state appears as amorphous liquid droplets called coacervate droplets. Upon standing these coalesce into one clear homogenous micelle-rich liquid layer, known as the coacervate layer which can be deposited. The Coacervation process was originally discovered and developed into practical applications by Green and Schleicher in 1957. Coacervation and phase separation from organic and aqueous media involve many properties, materials and processes such as phase inducing agents, stirring rates, core to wall ratios, polymer characteristics, core characteristics (wettability, solubility), cooling rates and rates of addition.

# Conclusions

Several interesting industrial processes using carbon dioxide as supercritical solvent are developed such as decaffeination of coffee and hop extraction.

Due to the special solvent properties of supercritical fluids, supercritical fluid chromatography now form part of industrial practice.

These methods are particularly useful in separating compounds that are thermally unstable, nonpolar, or nonvolatile.

Applications of Supercritical Fluid Chromatography and Gel Permeation Chromatography are complimentary to each other in characterization of macromolecules and their mixtures.

# BIBLIOGRAPHY

- Christie, W.W. (1989) *Gas Chromatography and Lipids*, The Oily Press, Dundee.
- Ekhtera, M.R., Mansoori, G.A., Mensinger, M.C., Rehmat, A. and Deville, B. "Supercritical Fluid Extraction for Remediation of Contaminated Soil" in "Supercritical Fluids: Extraction and Pollution Prevention" M. Abraham and A. Sunol (Editors), ACSSS Vol. # 670, pp. 280-298, American Chemical Society, Washington, D.C., 1997.
- Kwak, T.Y. and Mansoori, G.A. "Van der Waals Mixing Rules for Cubic Equations of State - Applications for Supercritical Fluid Extraction Modelling", *Chemical Eng. Science*, 41, 5, pp.1303-1309, 1986.
- Kwak, T.Y., Benmekki, E.H. and Mansoori, G.A. "Van der Waals Mixing Rules for Cubic Equations of State (Applications for Supercritical Fluid Extraction Modeling and Phase Equilibria Calculations)", *ACS Symposium Series 329*, pp.101-114, American Chemical Society, Washington, D.C., 1987.
- Kwon, Y.J. and Mansoori, G.A. "Solubility modeling of solids in supercritical fluids using the Kirkwood-Buff fluctuation integral with the hard-sphere expansion (HSE) theory", *The Journal of Supercritical Fluids*, Vol. 6, pp. 173-180, 1993.
- Leichter, E., Thompson, J., Strode, B., Taylor, L.T. and Schweighardt, F.K. "Effect of Helium in Helium Headspace Carbon Dioxide Cylinders on Packed-Column Supercritical Fluid Chromatography" *Anal. Chem.*, 68 (5), 894 -898, 1996.
- Mansoori, G.A. and Ely, J.F. "Density Expansion (DEX) Mixing Rules (Thermodynamic Modeling of Supercritical Extraction)", *J. Chem. Phys.*, 82, 406, 1985.
- Mansoori, G.A., Schulz, K. and Martinelli, E. "Bioseparation Using Supercritical Fluid Extraction / Retrograde Condensation", (Feature Article) *BIO/TECHNOLOGY*, Vol. 6, pp.393-396, 1988.

# BIBLIOGRAPHY

## *Continued*

- Martinelli, E., Schulz, K. and Mansoori, G.A. "Supercritical Fluid Extraction / Retrograde Condensation (SFE/RC) with Applications in Biotechnology", pp. 451-478, in "SUPERCRITICAL FLUID TECHNOLOGY", Edited by T.J. Bruno and J.F. Ely, CRC Press, Boca Raton, FL, 1991.
- Park, S.J., Kwak, T.Y. and Mansoori, G.A. "Statistical Mechanical Description of Supercritical Fluid Extraction and Retrograde Condensation.", *International J. Thermophysics*, 8, pp. 449-471, 1987.
- Shearer, R.L. and Skelton, R.J. "Supercritical fluid chromatography of petroleum products using flameless sulfur chemiluminescence detection", *J. High Res. Chromatogr.*, 17 (1994) 251-254.
- Smith, R.M. (ed.) (1988) *Supercritical Fluid Chromatography*, Royal Society of Chemistry, London.
- Touba, H. and Mansoori, G.A. "Structure and Property Prediction of Sub- and Supercritical-Water", *Fluid Phase Equilibria*, Vol. 151, pp. 459-468, 1998.
- Hartono, R., Mansoori, G.A. and Suwono, A. "Prediction of Molar Volumes, Vapor Pressures and Supercritical Solubilities of Alkanes by Equations of State" *Chemical Engineering Communications*, Vol. 173, pp. 23-42, 1999.
- Vasquez, D. and Mansoori, G.A., "Identification and Measurement of Petroleum Precipitates". *J. Petrol. Sci. & Engineering*, Vol. 26, Nos. 1-4, pp. 49-56, 2000.
- Zou, W. and Dorsey, J.G. "Modifier Effects on Column Efficiency in Packed-Column Supercritical Fluid Chromatography" *Anal. Chem.*, 72 (15), 3620 -3626, 2000.