Characterization of Chromatographic Stationary Phases using Raman Spectroscopy

Introduction

Reverse-phase liquid chromatography (RPLC) is arguably the most common mode of liquid chromatography used. Not just unique to academic research laboratories, RPLC is used by government agencies to prove regulatory compliance, medical laboratories to identify drug metabolites, and industry to monitor quality control. Despite the wide use of RPLC, a fundamental mechanism of separation has yet to be obtained through investigation of stationary phases with chromatography, NMR, fluorescence, and even IR. The ultimate purpose of this research is to elucidate the molecular basis of a mechanism of separation with Raman spectroscopy.

Raman spectroscopy has the advantage of direct conformational information about the alkyl chain of the stationary phase. In particular, spectra of stationary phases are affected by the presence of all-trans, single-gauche, end-gauche, double-gauche, and kink conformations, Figure 1. Ratios of the intensities associated with ordered and disordered conformations yield tangible measures of order (or disorder). The data presented here uses the ratio of the methylene asymmetric to symmetric stretch modes. The higher this ratio, the more ordered the stationary phase.

Systematic investigations of temperature and mobile phase solvent have been initiated. Figure 2 shows the order of a...
polymeric C18 stationary phase as a function of temperature. As one might expect, the order of the alkyl chains decreases as the temperature increases. Figure 3 shows the order of a polymeric C18 stationary phase as a function of solvent. Note that with the exception of DMSO and water, stationary phases are more ordered in polar than non-polar solvents.

Figure 4 shows the order of a polymeric C18 stationary phase as a function of percent methanol in water.

These few experiments are just the beginning of a systematic investigation of temperature, solvent (binary and tertiary solvent systems), and pressure effects on stationary phase conformation order. The various combinations of temperature, solvent, and pressure will also be investigated as will the effect of surface coverage and surface distribution of stationary phase. Once such effects on conformation are catalogued, separation efficiency can be studied. Effectively, these results should lead to an enhanced understanding of the mechanism of separation.