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The question of how to assess bioavailability has received much attention. Bioavailabilty is most often approximated by the distribution of the solute in question between two phases, most often bulk phases, of water and an immiscrible organic solvent. Since the inception of reversed phase liquid chromatography there have been many attempts to correlate chromatographic retention with bioavailability and the most often used bulk measure, the octanol-water partition coefficient. An entire field has developed around this research, referred to as Quantitative Structure Activity Relationships (QSAR), or where chromatographic retention is the measured parameter, Quantitative Structure Retention Relationships (QSRR). Yet with present technology, these attempts are inevitably doomed to failure. On the one hand, bulk phases are not appropriat for modeling a partitioning process in an interphase such as biological membranes, and while chromatographic stationary phases can be argued as having similar structure to a membrane because of chain organization, the density of the grafted chains is much too low to provide a suitable model. It is these problems which we have come to understand and propose to address.

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"Improved Chromatographic Bioavailability Estimations"

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The question of how to assess bioavailability has received much attention. Bioavailability is most often approximated by the distribution of the solute in question between two phases, most often bulk phases, of water and an immiscible organic solvent. Since the inception of reversed phase liquid chromatography there have been many attempts to correlate chromatographic retention with bioavailability and the most often used bulk measure, the octanol-water partition coefficient. An entire field has developed around this research, referred to as Quantitative Structure Activity Relationships (QSAR), or where chromatographic retention is the measured parameter, Quantitative Structure Retention Relationships (QSRR). Yet with present technology, these attempts are inevitably doomed to failure. On the one hand, bulk phases are not appropriate for modeling a partitioning process in an interphase such as biological membranes, and while chromatographic stationary phases can be argued as having similar structure to a membrane because of chain organization, the density of the grafted chains is much too low to provide a suitable model. It is these problems which we have come to understand and propose to address.

Recent statistical mechanical theory developed by Dill has shown that the partitioning of solutes between a bulk phase and an interphase, such as a bilayer membrane, is controlled by the entropy of mixing, the configurations of the chains, and the contact interactions between solute and chains and solute and bulk solvent. We have previously developed new synthetic methodology which yields reversed phase stationary phases of significantly higher bonding density than commercially available phases, and we have shown partitioning of solutes into these phases follows the theory developed by Dill. In our "parent" grant (Thermodynamically Correct Bioavailability Estimations, AFOSR 91-0254), we are investigating the utility of these high density phases for the modeling of bioavailability of various solutes. Instead of the traditional correlations between chromatographic retention and octanol-water partition coefficients, we are investigating correlations between chromatographic retention and physiologically relevant events, such as bioaccumulation. We are greatly encouraged with the results shown to date, and feel that these high density phases provide a significantly better estimation of bioavailability and bioaccumulation than other models.

We have investigated *in situ* methods of increasing reversed-phase stationary phase rigidity, with the hypothesis being that this would increase the entropic contribution to retention, and would make commercial stationary phases more "membrane-like". We investigated a series of mobile phase additives, and found the best results with n-hexanol. Thermodynamic analysis of the chromatography resulting from these experiments showed that the addition of a strongly solvating agent to the mobile phase can provide selectivity changes which result from specific solvent/solute interaction, and that the stationary phase does not undergo a significant structural change.

In an effort to provide increased surface ordering, cholesterol, which is more structurally rigid than n-hexanol and has been shown to order lipid bilayers, was also evaluated. The results of this study showed that added cholesterol in the mobile phase coated readily onto the stationary phase, and the resulting chromatographic experiments showed dramatically improved shape selectivity for a wide variety of differently shaped molecules. This additive was used in both methanol/water and acetonitrile/water mobile phases with equal success. The selectivity of these systems could be controlled by adjusting the cholesterol concentration, the system temperature, or both. When mobile phases of less than 70% methanol were used, the cholesterol coating was stable.

In summary, we have met the goals of our proposal, and these results have been published in the open literature. Following is a list of refereed papers which acknowledge AFOSR support.

1. "Microscopic Order as a Function of Surface Coverage in Alkyl Modified Silicas: Spin Probe Studies", Paul B. Wright, Edward Lamb, John G. Dorsey and Robert G. Kooser, *Anal. Chem., 64,* 785-789 (1992).

2. "Temperature Dependence of Retention in Reversed Phase Liquid Chromatography: Stationary Phase Considerations", Lynn A. Cole and John G. Dorsey, *Anal. Chem., 64,* 1317-1323 (1992).

3. "Temperature Dependence of Retention in Reversed Phase Liquid Chromatography: Mobile Phase Considerations", Lynn A. Cole, John G. Dorsey and Ken A. Dill, *Anal. Chem., 64,* 1324-1327 (1992).

4. "Liquid Chromatography: Theory and Methodology", John G. Dorsey, Joe P. Foley, William T. Cooper, Robert A. Barford and Howard G. Barth, *Anal. Chem., 64,* 353R-389R (1992).

5. "Accurate Determination of log k'_W in Reversed Phase Liquid Chromatography: Implications for Quantitative Structure Retention Relationships", Mei-Ming Hsieh and John G. Dorsey, *J. Chromatogr., 631,* 63-78 (1993).

6. "The Effect of Stationary Phase Solvation on Shape Selectivity in Reversed Phase Liquid Chromatography", Steven R. Cole and John G. Dorsey, *J. Chromatogr., 635,* 177-186 (1993).

7. "Phase Transitions of Reversed Phase Stationary Phases: Cause and Effects in the Mechanism of Retention", John F. Wheeler, Thomas L. Beck, S. J. Klatte, Lynn A. Cole and John G. Dorsey, *J. Chromatogr., 656,* 317–333 (1993).

8. "Hydrophobicity Estimations by Reversed Phase Liquid Chromatography: Implications for Biological Partitioning Processes", John G. Dorsey and Morteza G. Khaledi, *J. Chromatogr., 656,* 485–499 (1993).

9. "Retention Mechanisms of Bonded Phase Liquid Chromatography", John G. Dorsey and William T. Cooper, *Anal. Chem., 66,* 857A-867A (1994).

10. "Liquid Chromatography: Theory and Methodology", John G. Dorsey, William T. Cooper, John F. Wheeler, Howard G. Barth and Joe P. Foley, *Anal. Chem., 66,* 500R-546R (1994).

11. "Bioavailability Estimation by Reversed-Phase Liquid Chromatography: High Bonding Density C-18 Phases for Modeling Biopartitioning Processes", Mei-Ming Hsieh and John G. Dorsey, *Anal. Chem.*, *67*, 48-57 (1995).

12. "n-Octanol-Water Partition Coefficient Estimation by Micellar Electrokinetic Capillary Chromatography", Bradford J. Herbert and John G. Dorsey, *Anal. Chem.*, *67*, 744-749 (1995).

13. "Silver (I) Mediated Separations by Capillary Zone Electrophoresis and Micellar Electrokinetic Chromatography: Argentation Electrophoresis", Paul B. Wright and John G. Dorsey, *Anal. Chem.*, *68*, 415-424 (1996).

14. "The Informational Orthogonality of 2-Dimensional Chromatographic Separations", Patrick J. Slonecker, Xiaodong Li, Thomas H. Ridgway and John G. Dorsey, *Anal. Chem.*, *68*, 682-689 (1996).

15. "Liquid Chromatography: Theory and Methodology", John G. Dorsey, William T. Cooper, Barbara A. Siles, Joe P. Foley and Howard G. Barth, *Anal. Chem.*, *68*, 515R-568R (1996).

16. "Mobile Phase Additives for Enhanced Peptide Separations in Reversed Phase Liquid Chromatography", Steven R. Cole and John G. Dorsey, *Biomed. Chromatogr.*, in press.

17. "Characterization of Conventional Chemically-Bonded Liquid Chromatographic Stationary Phases by Raman Spectroscopy: The Effect of Ligand Type", Charles A. Doyle, Thomas J. Vickers, Charles K. Mann and John G. Dorsey, *J. Chromatogr.*, in press.

18. "Reduction of Total Analysis Time in Gradient Elution, Reversed-Phase Liquid Chromatography", Deanna L. Warner and John G. Dorsey, *LC-GC*, in press.