Subcritical Carbon Dioxide for Ultrafast and Enantioselective Analytical Separations

M. Farooq Wahab*
Department of Chemistry & Biochemistry, University of Texas at Arlington, Arlington, Texas, USA.
*E-mail: mfwahab@uta.edu

ABSTRACT

Introduction: Liquid chromatography imposes a nontrivial environmental, health, and safety burden because it consumes large volumes of synthetic or fossil-derived organic solvents. Analytical chemists have been promoting alternatives such as sub/supercritical fluid chromatography (SFC) and enhanced fluidity liquid chromatography (EFLC) as greener methods, yet that claim lacks consistent, quantitative validation. This presentation focuses on three aspects of using compressed carbon dioxide as an economical solvent for chemical separations. First, we will address the solvatochromic properties of neat carbon dioxide, then a biomass-friendly SFC concept that replaces methanol with minimum-boiling azeotropic ethanol, and, lastly, digital techniques for denoising, baseline correction, and peak modeling to obtain quantitative information about a sample, rather than wasting time and energy on chemical separations.

Objectives: To show that it is highly feasible to reduce the amount of solvents such as hexane, heptane, chlorinated solvents, acetonitrile, and use more friendly compressed carbon dioxide for analytical separations. This presentation will also demonstrate several challenging case stories, including enantioseparations of tobacco alkaloids to identify the truly greenest mode on a per-task basis.

Methodology: Specialized equipment that can pump liquid carbon dioxide and mix it with small amounts of organic solvents is used. Methanol was replaced with 190-proof ethanol in test cases. Macrocyclic glycopeptide-bonded silica and chiral polysaccharide columns were evaluated for enantioselective separations. Additive effects and the nonideal behavior of methanol–acetonitrile mixtures were investigated. In our study, the test probes included tobacco alkaloid enantiomers (nicotine, nornicotine, anabasine, anatabine) spanning four distinct retention patterns representative of small molecule enantioseparations; (ii) 23 ionic Ru(II)/Os(II) polypyridyl complexes, separated as Δ/Λ pairs, and (iii) many chiral/achiral molecules of biological relevance.

We also introduce the concept of digital signal processing as another dimension of modern separations to add greenness. Signals can be denoised by the Bohlmann-Whittaker smoother with area preservation. Baselines were estimated using asymmetric reweighted least squares and curvature-weighted schemes to improve robustness to signal variations. Peak modes were localized using numerically stable second derivatives from Savitzky–Golay filtering. Nonlinear least squares with a trust-region reflective algorithm fit two skew-capable models: a numerically stable bi-directional exponentially modified Gaussian and a twice-generalized normal with third- and fourth-moment parameters.

Key Findings: Replacing methanol with azeotropic ethanol reduced retention and increased efficiency, with gains up to an order of magnitude in effective plates under matched backpressure and temperature. Azeotropic ethanol, distilled at constant composition, simplifies solvent recycling and lowers costs. Compatibility was confirmed across macrocyclic and chiral polysaccharide stationary phases, providing a practical route to greener routine and preparative SFC. Contrary to the prevailing view that ionic species are inaccessible to SFC, cyclofructan-bonded column chemistry enabled the first chiral SFC separations of 23 Ru/Os polypyridyl complexes relevant to anti-cancer activity. Enantioselectivity correlated with increased

conjugation and rigidity of the coordination framework. Additives modulated retention and selectivity; mixing methanol and acetonitrile produced unexpected nonidealities requiring dedicated method development. High-efficiency, baseline-resolved separations were achieved with short analysis times suitable for screening and quantitation.

Conclusion: Compressed carbon dioxide mixed with 5-40% organic solvent is an excellent solvent system that can reduce solvent consumption by tons and minimize environmental hazards. Sub-critical CO_2azeotropic ethanol replaces methanol without sacrificing performance and, in many cases, improves efficiency while enabling convenient solvent recycling.

Keywords: Green Chemistry, Carbon Dioxide, Chemical Separations, Enantioselective Analysis, Subcritical Fluid Chromatography..

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