Supercritical or not, pressurized fluids at Pittcon'98

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Increasingly stringent requirements for "high-throughput", "mass spectrometry compatible", and "environmentally correct" molecular separations boost the popularity of pressurized fluids technologies, whether or not these fluids are implemented in conditions academically defined as supercritical or subcritical. At Pittcon'98, this was particularly true for laboratory-scale preparative work: off-line sample preparation for analytical purposes and chromatographic purification.

A paradox is a truth which walks on its head to draw our attention. Thus we can say: supercritical fluids (SFs) represent the general fluid state, liquids and gases being its extreme forms, those which exist at atmospheric pressure. This stimulating outlook [1] unifies and opens up a diversity of techniques taking advantage of the rapid mass transfer occurring in sub- and supercritical phases, mainly according to the temperature of the pressurized fluid. Whether they use liquefied gases, common solvents, or mixtures of both, all these techniques require a pressurized-outlet instrument: for chromatography (SFC), extraction (SFE, and accelerated or enhanced solvent extraction – ASE or ESE), reaction (SFR), and various industrial processes such as spraying, infusion, dying, coating and material cleaning. Supercritical carbon dioxide (SC-CO_2) and subcritical water (sc-water) are the prominent components of pressurized fluids because of their unique array of physical, economical and ecological properties.

Daily sessions

Starting on Sunday, one hundred and thirty people attended the SF reception organized by the Midwest SFC Users Group and the Tri-State SF Discussion Group. This vendor-sponsored gathering of "friends", sharing knowledge, exchanging ideas and keeping up with new technology, listened to Jerry King (ARS-USDA/NCAUR, Peoria, IL) presenting a lecture entitled "Utilizing SF-based instrumentation to save time, money and labor in analytical and process research and development". The application he emphasized was transesterification of triglycerides in the presence of SC–CO_2 and a lipase-based catalyst to produce fatty acid methyl esters (FAMEs) from vegetable oils, then extract them for analysis by enzymo-immunoassays (EIA).

In this year's program, SFs and related topics were presented as two short courses and four sessions: food analysis by SFE, SFE-SFC, SF separations, and sc-water in environmental sample preparation. An overview of at least 46 communications (32 lectures and 14 posters) follows, by sector of activity.

Environmental pollutants

For nonpolar organics (PAHs, PCBs, PCDDs, PCDFs, Cl- and P-containing pesticides) in soils, optimum conditions for maximum extraction using modified SC-CO_2 were determined for a specific porosity of the matrix, in the range 14 – 46 Å [1880P]*. In water, for ultra low concentration levels (0.1 – 1 pg/mL), SFE results were more accurate, fast and reproducible, than those of liquid-liquid extraction (LLE) and solid phase extraction (SPE) [2196]. SFE was investigated to transfer water samples on high-performance thin layer chromatography (HPTLC) plates coupled with laser microprobe mass spectrometry (LMMS) [1873P].

For polar organics and inorganics (phenols, benzenediols, carboxylic acids, organometallics, metal ions, and explosives) in soils, the solvent used in ASE or ESE should have a solubility parameter equivalent to that of the polymeric matrix (e.g. humic organic matter) [1212]. SFE with modified CO_2 selectively extracted organomercury species, then mercury ions, using lithium bis(trifluoroethyl)dithiocarbamate - Li(FDDC) - as a chelating agent [774, 775]. For explosives containing nitro (NO_2), nitramine (N–NO_2), or nitrate ester (O–NO_2) functionalities, the vapor pressure of these analytes gave a rough indication of their solubility in SF–CO_2 [777].

For most of the above pollutants, extraction using sc-water, without organic solvent, provided a simple and rapid way to extract them from soils (Fig. 1). Sc-water extraction was coupled with SPE [965], solid phase microextraction (SPME) [1878], or directly followed by GC-MS [1260]. Ambient water is too polar to efficiently dissolve hydrophobic organics. However, heating water to 200 °C reduces its dielectric constant (polarity), surface tension, and viscosity to values similar to those of methanol and acetonitrile, which increases the solubilities of hydrophobic organics by several orders of magnitude. In general, raising the temperature of water by 50 °C increases the solubility of organic solutes by an order of magnitude [1258, 1255]. In contrast with SC–CO_2, pressure has little effect on the polarity of sc-water as long as the liquid state is maintained [965, 1254].

Food and feeds

Food products are now being labelled for total fat content. For the determination of fat/oil content in agriculturally-derived products, such as oilseed and ground beef [410],

* Refers to lecture or poster number in the Pittcon'98 book of abstracts.

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animal feed [2197], snack foods [411, 415], chocolate products [412], milk-based products [409], SFE, sometimes used in conjunction with SFR, is a suitable replacement technique for traditional organic solvent-based methods. As compared with gravimetry, GC-FAMEs analysis not only gives comparable recovery of lipids, but allows the analyst to chemically speciate the extract [410]. Free fatty acids, markers for age, were analyzed in fat using SFE coupled with FTIR [413]. The use of analytical scale SFR/SFE in support of process development research was illustrated by the production of FAMEs from dissolved vegetable oils with transesterification yields higher than 98% [1059].

At the pilot plant scale, oils from saw palmetto berries were produced using preparative SFE with CO_2 [2199P].

Polymers and petrochemicals

The quantitative analysis of antioxidants from high-density polyethylene (HDPE) using off-line SFE and HPLC was illustrated for the case of Ethanox 330 [752]. The quantitative analysis of various other standard additives (BHT, BHEB, Isonox 129, Irganox 1076, Irganox 1010) from low-density polyethylene (LDPE) was carried out by on-line SFE/SFC with a flame ionization detector (FID) and produced results comparable with those from off-line SFE/HPLC [772]. The decomposition process of polyethyleneterephtalate (PET) was monitored using sc-water and laser Raman spectroscopy [1865P].

There was a presentation of the SFC analysis of petroleum fractions, especially aromatics in diesel and jet fuels according to the ASTM method D5186-96, using FID and SFC-tested silica columns [778, 779].

Biomedicals and pharmaceuticals

Five phospholipids were analyzed by SFC with an evaporative light-scattering detector (ELSD) [2200P]. Cholesterol and five cholesterol oxides were analyzed in biological matrices using SFE and a sorbent trap, followed by SFC with FID and MS [629].

For drug products, several examples of purification by preparative SFC were presented, including chiral substances such as ibuprofen [2201], propranolol and oxprenolol [1576P].

For drug candidates, laboratory-scale preparative SFC using 4 to 20 mm bore columns can produce 10 to 500 mg/h of purified substance, collected as a concentrated liquid fraction from repeated injections. This fits the needs of medicinal chemists involved in drug discovery, both at the research stage to isolate substances for preliminary activity testing, and at the development stage to develop a preparative method to be implemented at the process scale. Practical aspects of preparative SFC were presented to maximize sample loading (Fig. 2) as well as fraction purity and recovery [1576P]. For screening, developing and scaling-up of separations from an analytical to the pilot scale, SFC was described as addressing the issue of throughput in the pharmaceutical industry [362].

Industrial process feasibility

The use of SC–CO_2 in the chemical process industries is expanding at an ever increasing rate. Method development

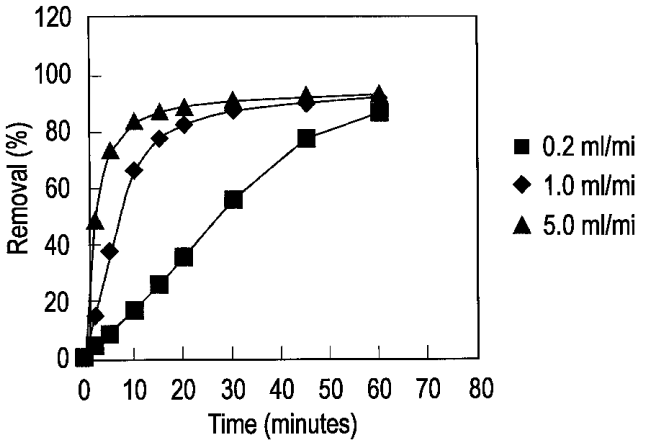

Figure 1. Effect of the flow rate of subcritical water on the extraction rate of metolachlor from a 1-g sample of contaminated soil at 100 °C.
Courtesy of Steven Hawthorne, Arnaud Lagadec, David J. Miller and Boris Jansen, Energy and Environmental Research Center, University of North Dakota, Grand Forks ND, USA.

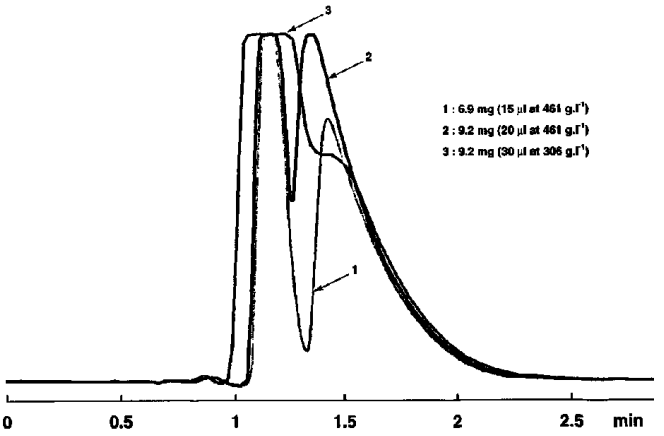

Figure 2. Effect of the injected sample volume and concentration on the preparative separation of oxprenolol racemate.

Sample: solid oxprenolol dissolved in water-ethanol (3-1), 306 and 461 mg/mL. **Injections:** 15, 20 and 30 µL in 100 µL loop (40 µL front air gap), 6.9 and 9.2 mg. **Column:** Chirose-Bond C1 (Chiralsep), 5 µm, 4.6 × 150 mm. **Mobile phase:** CO_2 with 40% isopropanol containing 2% diethylamine. **Flow-rate:** 3 mL/min. **Column outlet pressure:** 15 MPa (inlet: 22.5 MPa). **Temperature:** 19 °C. **Detection:** 254 nm. **Collection solvent:** ethanol, 0.15 mL/min. **Preparative throughput:** > 100 mg/h (12 injections per hour).

Courtesy of Régis Boutant, Gilson S.A., Villiers le Bel, France.

in one-liter extraction vessels was carried out for applications such as: removal of solvent impurities from styrenedivinylbenzene, fractionation of citrus oils, regeneration of activated carbon, fractionation of HDPE [2198P]. For recycling spent bleaching earth, a phase equilibrium analyzer (PEA) was able to determine the solubility of the compound of interest by recording its dew point [776]. Phase distribution equilibria of SC–CO_2 in polymethylmethylmethacrylate (PMMA) was determined by tracer pulse molecular probe chromatography [2034P]. Fluorescence was used for probing solute-fluid interactions at a solid interface [251]. The extent of conformational changes in polymers dissolved in

SFs was described by differences in the radius of gyration [246]. Finally, solubility studies were applied to the purification of diverse chemical reagents with SC–CO_2 [2202].

Chemical reactions and detection

Derivatization reactions of carbamate pesticides in SC–CO_2 were much more efficient than in an organic solvent [414]. Sc-water was implemented for the synthesis of monodispersed silver colloid particles to be used in heterogeneous catalysis and as substrates for surface-enhanced Raman spectroscopy (SERS) [150]. SC–CO_2 acted as the aerosolization agent in the synthesis of nanoparticles with diameters of less than one micrometer [151].

In situ spectroscopy under SF conditions was highlighted: Fourier-transform infra-red (FTIR) used a newly designed optical cell to measure hydrogen bonds [1570P], to monitor SFE, or to measure the uptake of modifier solvent by SFC stationary phases [773]; UV–Vis absorbance measured partitioning coefficients of solutes between SF and polymer phases representing stationary phases of SFC. For instance, at 40 °C, the partition coefficient of 2-naphtol varied more than two orders of magnitude from 8 to 18 MPa [773]. Finally, the response of an ELSD used in the LC mode and SFE/SFC modes showed slightly better sensitivity in the LC mode, but a consistently wider linear range in SFE and SFC [771].

A well-attended exhibition

Over fifty exhibiting companies - more than in previous years [2,3] - were listed in the Guide of SFs at Pittcon'98: six for SFC systems (Berger, Gilson, Jasco, Sensar Larson-Davis, Thar Designs, and ABB Process Analytics), seven for SFE systems (Isco, Applied Separations, Thar Designs, Leco, Laballiance, Supercritical Fluid Technologies, and Autoclave Engineers Group), and the others for detectors, components and supplies. The main products of the major exhibiting manufacturers are briefly described below.

Berger Instruments (Newark, DE) presented analytical SFC systems: manual-injection with ChemStation PC software, and automated-injection with ChemStation and carbon dioxide tank switching; 35 MPa, 150 °C, cryogenic option; UV detectors, FID, nitrogen-phosphorus and electron capture detectors (NPD and ECD).

Gilson (Villiers le Bel, France; Middleton, WI) exhibited the Series SF3 system with dual analytical and preparative capability, extensive data processing and graphic tracking of samples and fractions from UniPoint PC software; 60 MPa, 200 °C, 1 to 20 mm bore columns, up to 500 mg/h; UV detectors and ELSD. Two conferences on preparative SFC were held in a private seminar room.

Jasco (Tokyo, Japan) exhibited an SFC system and presented a new optical cell for SFE, with temperature regulated by a heater, and selection for stationary or flow measurements. Combined with FTIR, this cell allows the analysis of solvents and solutes under SF conditions.

Sensar Larson-Davis (Provo, UT) introduced the Series 3000, a new generation of SFC instrumentation for wall-coated and packed capillary columns; 10 mL syringe pump, 250 µL/min, 42 MPa, 250 °C, FID, optional autosampler, fixed restrictor with frit-filtered orifice. A range of SFC-tested fused-silica columns was presented: 0.25 µm film, 50 µm diameter, 10 m length including a 3 m retention gap; and 5 µm silica, 75 cm length, for the ASTM method D5186.

Thar Designs (Pittsburgh, PA) presented systems and equipment for diverse SF applications: pilot to plant scale SFE and SFC systems, phase equilibrium analyzers, variable-volume view cells (5 – 25 mL, 135 °C, 70 MPa), high pressure pumps, high pressure fingertight vessels, extractors, cyclone separators, back pressure regulators, reaction vessels and valve actuators. The company also provides services such as consultation, toll processing and feasibility studies.

ABB Process Analytics (Lewisburg, WV) offered the Vista PSFC process supercritical fluid chromatograph, 48 MPa, pneumatic-amplifier pump, carbon dioxide and FID.

Isco-Suprex (Lincoln, NE) displayed a wide range of lab-scale SFE instruments. For food and agricultural products: AutoPrep 44 automated 44-sample system; and FatMaster 200 gravimetric fat analyzer. For environmental sample preparation: SFX 3560DM dual mode extractor for automated SFE and ESE of 24 samples. For polymers and pharmaceuticals: SFX 220 dual-chamber extractor with direct solvent trapping of analytes, and PrepMaster with AccuTrap sorbent/cryotrap for volatile analytes.

Applied Separations (Allentown, PA) presented the Spe-ed SFE range for food, environmental, pharmaceutical, natural products, and polymer/chemical applications: Spe-ed SFE-4 processing four samples simultaneously, and Spe-ed Fat processing 44 samples in 3 hours; 69 MPa and 250 °C, heated-valve outlet block for pressure control and collection into SPE cartridges, vessels from 1 mL to 2 liters, or pilot plant systems up to 100 liters.

Dionex (Sunnyvale, CA) displayed ASE 200, for the extraction of micro-pollutants in environmental matrices using solvents at high temperature under pressure; 200 °C, 20 MPa, 24 cells of 11 mL, 22 mL and 33 mL capacity, with 40 mL or 60 mL collection vessels.

Leco (Saint Joseph, MI) displayed FA-100, SFE module for gravimetric fat analysis, 69 MPa, 150 °C, three samples simultaneously, capable of the remote control of two other identical modules.

Pressure Products Industries (Warminster, PA) exhibited an SFE process development unit, 70 MPa, 110 °C, recycling, up to a 10-liter extractor; plus stirred reaction vessels, 14 MPa, 350 °C, 0.3–2 liter capacity.

Supercritical Fluid Technologies (Newark, DE) presented their activities: design, manufacture and servicing of SFE and SFR systems.

Antek Instruments (Houston, TX) exhibited the chemiluminescence nitrogen detector (CLND) for GC, SFC and HPLC. The response factor of this detector is proportional to the number of nitrogen atoms in the detected substance, and the limit of detection was claimed to be 0.3 ng of nitrogen (i.e. femtomoles for proteins).

Bourne Scientific (Newtonville, MA) presented an FTIR detector for GC, SFC and HPLC, producing real time chromatographic and spectral data at the highest sensitivity levels available today.

The calendar of the year

March 23-25: 5th Meeting on SFs (materials and natural products processing), Nice, France.
May 11-15: SFC lecture-laboratory course organized by the American Chemical Society.
June 15-19: SFE lecture-laboratory course organized by the American Chemical Society.
July 12-16: 8th International symposium on SF chromatography and extraction, Saint Louis, MI, USA.
September 30-October 1: 3rd Symposium on SFs (high pressure extraction), Siegen, Germany.

References

1. Chester, T. *Anal. Chem. News Features* **1997**, 165A-169A.
2. Vérillon, F. *Analusis* **1996**, *24*(3), 17-19.
3. Vérillon, F. *Analusis* **1997**, *25*(4), 21-23.

Photo 1. Nouvel objectif ATR pour microscope FTIR (Bio-Rad).