Conclusion

Coupled SFE–SFC has shown itself to be a very useful technique for those samples for which it is applicable. The ability to transfer all the extract to the analytical column without manipulation increases sensitivity, reduces contamination and sample handling. The overloading of capillary columns is avoided. Now that methods for using modifiers in the extraction solvent and SFE sample injection methods have been developed, there is every likelihood that SFE–SFC will become a more widely used technique.

See also: **II/Chromatography: Supercritical Fluid:** Fourier Transform Infrared Spectrometry Detection; Historical Development; Instrumentation; Large-Scale Supercritical Fluid Chromatography; Theory of Supercritical Fluid Chromatography.

Further Reading

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SUPERHEATED WATER MOBILE PHASES: LIQUID CHROMATOGRAPHY

R. M. Smith, Loughborough University, Loughborough, Leics, UK

At room temperature, water on its own is an unattractive solvent in liquid chromatography. In reversed-phase chromatography, water is a weak eluent and is often regarded as an inert component of the mobile phase. It is mainly used to dilute a stronger organic component and thus control the overall eluent strength. In contrast, in normal-phase chromatography, water is a powerful eluent and interacts strongly with the stationary phase, often deactivating it. Even trace amounts in a nonpolar eluent (or even in a sample) will markedly alter the retention properties of a silica surface. In separation methods aqueous eluents are used primarily for ion exchange chromatography or for the size exclusion separation of biological molecules.

However, this represents the situation at room temperature and atmospheric pressure. When liquid water is heated under pressure, its dielectric constant, viscosity and surface tension all decrease. These changes in the properties of water are well known but have largely remained the province of the physical chemist and chemical engineer. They have been widely studied because of the importance of water as a heat transfer agent and they play their part in the design and construction of steam power generation plant and in related areas. Above 374°C under a pressure of 221 bar, a single supercritical phase is obtained. Although these conditions seem extreme for the laboratory, they occur in nature in the ocean depths at the spreading points in the earth's crust where water issues from fumeroles at 350–400°C and 250 bar.

In recent years organic chemists have been attracted by the possibility of using superheated or supercritical water to achieve clean solvent-free conditions and to generate novel reaction conditions which are not available at room temperature. It has also been employed as a solvent for the high temperature oxidation for waste remediation or for the destruction of hazardous materials such as nerve gases and explosives as an alternative to high temperature incineration. In inorganic chemistry, supercritical water has been used as a solvent to enable high temperature reactions to be carried out without the inconvenience of using molten salts.

However, the analytical chemist has made little use of water under pressure, although the potential of supercritical water as a fluid solvent for chromatography was recognized by Lovelock in 1958. Some work has exploited steam as a mobile phase in gas chromatography, but the condensed phase has largely been ignored. Although liquid chromatographers have used elevated temperatures to improve separations or efficiencies, in almost every case the composition of the organic–aqueous eluent was kept

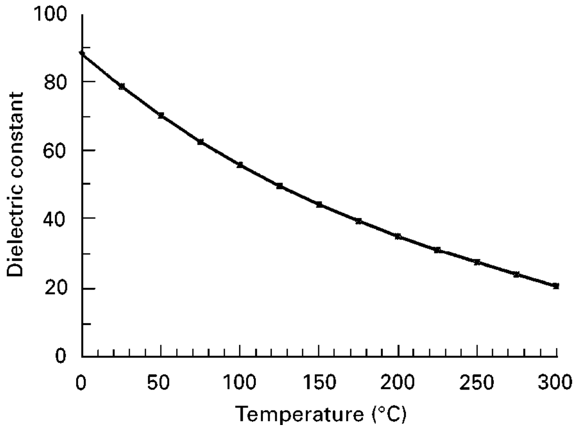

Figure 1 Change in dielectric constant of water with temperature.

constant and only the effect of the temperature was studied, typically up to 70–80°C.

Recently, the changes in the properties of liquid water above 100°C have attracted the interest of the analytical chemists, who have recognized that these low polarities might provide an environmentally clean solvent for extraction. Subsequently, the use of superheated water has also been applied to liquid chromatography. Some of the published papers have referred to this region of the phase diagram as subcritical water but this could imply any temperature less than 374°C. This review will instead employ the expression superheated water, which is defined as water held as a liquid under pressure between 100°C and the critical point.

Properties of Superheated Water

As the temperature of water is raised under a sufficiently high pressure for it to remain in the liquid state there is a steady decrease in its dielectric constant (**Figure 1**) from about 80 at room temperature to less than 25 at 300°C. These changes represent a marked change in the solvent polarity of the water. By 250°C the dielectric constant of water is about 30, which is less than that of methanol at room temperature, so that even under conditions well below its critical point, water will resemble the polarity of common organic solvents used as eluents in reversed-phase liquid chromatography. There is also a decrease in the viscosity of water from 1.0 cp at room temperature to 0.28 cp at 100°C, as well as in the surface tension.

The pressure conditions usually needed to carry out supercritical fluid chromatography (SFC) with carbon dioxide can be as high as 300–500 bar in order to achieve a sufficiently high density to provide a reasonable solvent strength. However, the vapour pressure of water (**Figure 2**) is modest and even by 200°C only reaches 15 bar, so that only moderate pressures are required to maintain a liquid state. In addition, the density of hot liquid water changes by only a small amount with changes in the applied pressure. The solvation properties are thus effectively independent of pressure. This is in marked contrast to supercritical carbon dioxide where pressure control is critical because of its marked effect on density.

Application in Analytical Extractions

The first serious analytical chemistry interest in the potential of water under pressure above 100°C came in 1994, when Hawthorne and colleagues investigated the extraction of organic pollutants from environmental solids with supercritical and subcritical water. They had been prompted by reports that the solubility of benzo[*a*]pyrene increases from 4 ng mL^{-1} under ambient conditions to approximately 10% by weight

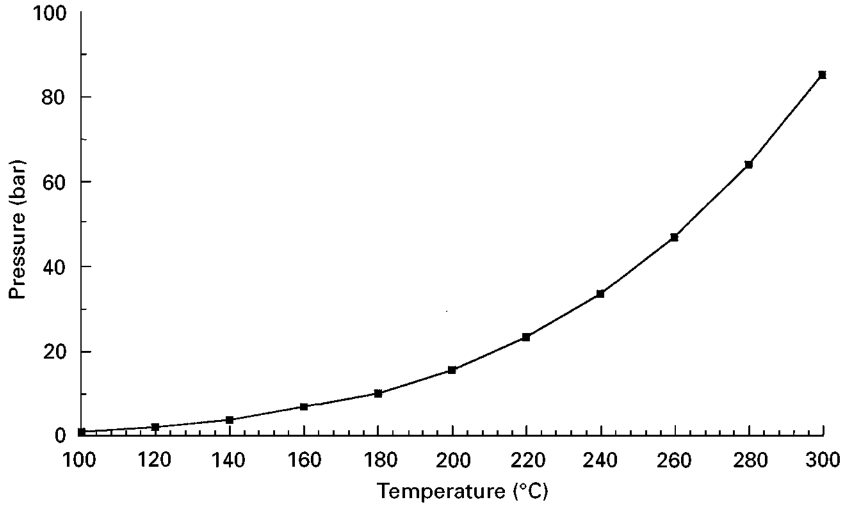

Figure 2 Change in vapour pressure of water with temperature.

at 350°C and 100 bar. This represents an increase in solubility of about 25 million. They found considerable solubility for polar analytes, such as chlorinated phenols, and even a significant solubility for nonpolar analytes, such as naphthalene at 50°C. Raising the temperature resulted in an increased solubility for polynuclear aromatic hydrocarbons (PAHs) and by 250°C all their test compounds except the *n*-alkanes had been completely extracted. The alkanes required supercritical conditions and were completely extracted at 400°C. These results confirmed that superheated water had a sufficiently high solubilizing power to be used as a solvent for even nonpolar analytes. They then demonstrated that subcritical conditions of 250°C and 50 bar would also efficiently extract PAHs from soil samples. In addition, good recoveries were obtained from air particulates. These results also showed that pressure was not an important factor, in marked contrast to the pressure dependence of supercritical fluid extraction with carbon dioxide.

Subsequently they studied the extractions further and showed that polychlorinated biphenyls (PCBs) could be extracted from soils and sediments with subcritical water. At 300°C and 50 bar, complete extraction could be achieved in pure water. Other workers have found that water can be used for the extraction of PCBs from a range of matrices in good yield. In a similar study, the pesticides Dacthal and acid metabolites have been extracted from soil with superheated water.

Chromatography using Superheated Water as the Mobile Phase

It was realized that if superheated water could extract PAHs from soils and dissolve PCBs, then it was behaving as a less polar solvent than the methanol– water and acetonitrile–water mixtures conventionally used as mobile phases in reversed-phase liquid chromatography. It should therefore be possible to use superheated water as a mobile phase and achieve typical reversed-phase liquid chromatographic separations. Many studies have examined the effect of increasing the temperature on separations and have shown that there is a consistent drop in retention with an inverse relationship to the absolute temperature (k varies as $1/T$ K). However, most of this work has either looked at temperature effects using constant eluent composition or has been limited to 80–90°C by the volatility of the organic components of the mobile phase.

In 1996, Smith and Burgess demonstrated that under a modest pressure it was possible to carry out the reversed-phase separation of phenols using superheated water at 160°C on a polystyrene-divinylbenzene (PS-DVB) column. The equipment was a combination of high performance liquid chromatography (HPLC) and gas chromatography (GC) systems (**Figure 3**) with some components from a packed-column SFC system. The water mobile phase was pumped using a single reciprocating pump but, unlike SFC, there was no need to cool the pump heads to condense the mobile phase. As the mobile-phase polarity can be controlled by temperature, no modifier pump was needed. The column was heated in a GC column oven which enabled the temperature to be controlled up to 350°C. To maintain the pressure a SFC back-pressure regulator was used. A detector fitted with a high pressure flow cell was originally employed but, because the back-pressures required are relatively low, in later studies standard HPLC spectroscopic flow cells were used for fluorescence

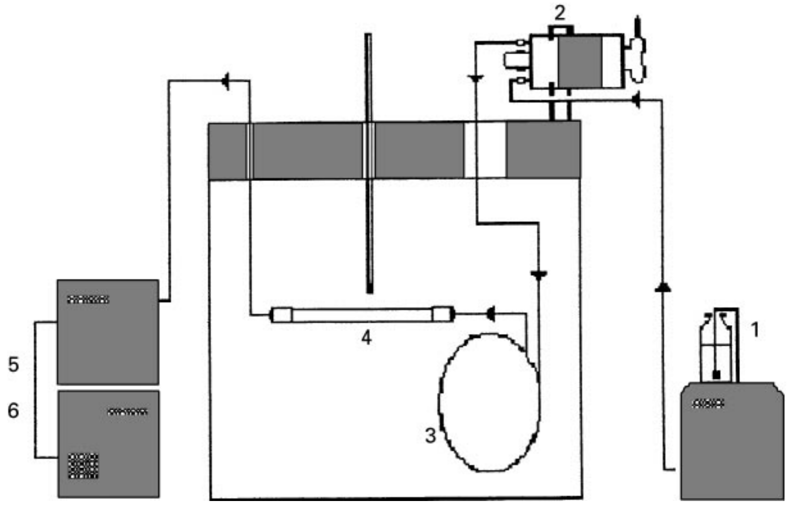

Figure 3 Superheated water chromatograph. Components: 1, pump; 2, injection valve; 3, preheating coil; 4, column; 5, detector; 6, back-pressure regulator.

and ultraviolet-visible spectroscopy. It is also possible to replace the back-pressure regulator with a length of narrow-bore PEEK tubing.

A PS-DVB packed column was used in the first studies because this material can be used without problems in size exclusion chromatography at 160°C and is thermally stable at this temperature. Phenols were examined with a methanol–water eluent and then the methanol content was reduced while raising the temperature in steps. With each increase in temperature the retention times decreased. By 180°C in the absence of methanol, the samples had similar retention times to those in 20 : 80 acetonitrile–water at room temperature.

The relative retention of a wide range of analytes from phenols, amides, esters to simple aromatic compounds demonstrated that the retention followed a similar pattern to conventional reversed-phase liquid chromatography. The separation followed the hydrophobicities of the analytes and the homologous parabens eluted in order of increasing chain length. They were also stable, showing neither hydrolysis nor oxidation. The separations, as expected, were insensitive to the back-pressure applied to the column.

It was also realized that programming the temperature of the column during the separation would systematically reduce the eluent polarity. This produced an effect similar to gradient elution, and would speed up and focus later peaks. Inorganic buffers could also be added to control the pH without causing any problems.

Stationary Phases for Superheated Water Chromatography

Most of the work that has been reported has employed PS-DVB columns which have shown reasonable temperature stability. They can be used up to about 220°C before softening appears to reduce their lifetime. As in ordinary HPLC, these columns show a marked difference in the retention of nonpolar and hydrogen-bonding analytes. The latter, including alcohols and phenols, have markedly lower retentions than nonpolar analytes such as alkylbenzenes and nitrobenzene. These latter compounds cannot be easily eluted even at 230°C.

Porous graphite carbon (Hypercarb) has been examined as an alternative thermally stable stationary phase. No instability was observed and the separations of mixtures of phenols, anilines and aryl amides were similar to those obtained at room temperature with conventional eluents (**Figure 4**).

There is particular interest in octadecylsilica (ODS)-bonded silica phases because of their widespread use in conventional liquid chromatography. However, although a number of different silica-based bonded-phase materials have been examined, they all show quite rapid degradation at temperatures greater than 100°C. Even though they have a lower retention capacity than the PS-DVB columns and do not require such high temperatures to obtain elution, it appears that this matrix is insufficiently stable for routine use.

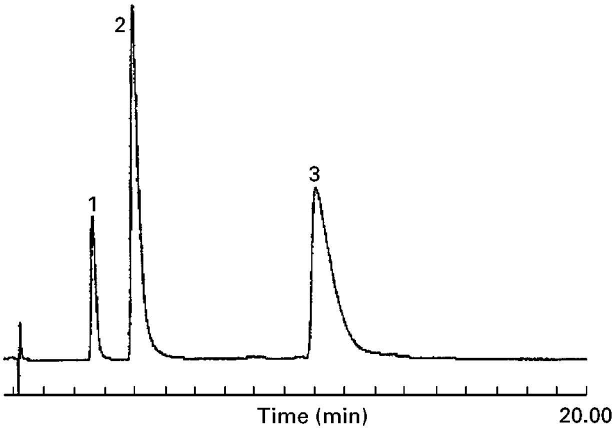

Figure 4 Separation of amides on porous graphitic carbon column at 190°C. Analytes: 1, benzesulfonamide; 2, benzamide; 3, *m*-toluamide.

A second problem was that, when highly end-capped ODS-bonded materials were examined with 100% water as the eluent, the octadecyl chains collapsed on to the silica surface on cooling the column to room temperature. As a result, the retention capacity of the column dropped markedly and this was not restored by heating. Instead, the column had to be treated with methanol–water mixtures. Similar problems have been reported in reversed-phase liquid chromatography at room temperature when the mobile phase contains less than 2% methanol.

A promising alternative stationary phase is polybutadiene-coated zirconia, a relatively new material, which is reported to be stable in water at 200°C. It gives good separations and peak shapes. The order of elution is similar to that on ODS phases in conventional reversed-phase liquid chromatography.

The elevated temperatures would be expected to reduce mass transfer effects in the mobile phase because of higher diffusion rates and this would result in improved separation efficiencies. Van Deemter curves of column plate heights against mobile-phase flow rate of water at elevated temperature determined for the PS-DVB and Hypercarb columns have been compared with acetonitrile–water separations at room temperature. In both cases, at the optimum flow rates the height equivalent to one theoretical plate (HETP) values are similar, but in superheated water the efficiency decreases rapidly at lower flow rates. This effect can be ascribed to a higher diffusion rate in the mobile phase at the higher temperature.

Detection in Superheated Water Chromatography

One of the advantages of superheated water chromatography is that it increases the possible number of detection methods that can be employed compared to liquid chromatographic methods using an organic solvent. However, with some detectors the mobile phase had to be cooled to room temperature to avoid baseline instability due to refractive index effects. So far no problems have been experienced due to the analytes coming out of solution between the column and detector, probably because the concentrations are generally low and the transfer time to the detector is brief.

Because usually only low back-pressures (less than 50 bar) are required to maintain the liquid state in the column, standard liquid chromatography flow cells can frequently be employed for ultraviolet-visible and fluorescence spectroscopic detection. Alternatively, high pressure ultraviolet-visible flow cells designed for SFC application can be used. In both methods of detection, one advantage of water as an eluent is that is it is transparent down to 190 nm. This enables low wavelength detection of unconjugated double-bond chromophores without solvent interference. However, some fluorescence detection is reduced compared to organic solvents because of quenching by the polar aqueous solvent.

The absence of an organic modifier raised the possibility that the eluent could be passed to a flame ionization detector. This could provide a simple method of universal detection for liquid chromatography, which previously has only been obtainable using mass spectrometry. This possibility was realized by Miller and Hawthorne, who demonstrated the use of the FID in 1997 to detect alkanols, phenols and amino acids, and confirmed by others.

Another detector that has problems in conventional liquid chromatography, because of mobile phase interference, is on-line nuclear magnetic resonance spectroscopy (LC-NMR). Many of the problems can be overcome by employing superheated heavy water (deuterium oxide) as the mobile phase. Compared to deuterated organic modifiers, deuterium oxide is relatively cheap and unlike supercritical fluid chromatography the flow cell can be at room temperature and pressure. This makes stop-flow detection easier and characteristic proton-NMR spectra have been obtained for a range of compounds, including barbiturates, sulfonamides and a number of pharmaceuticals and natural products.

It has also been demonstrated that superheated water chromatography can be linked to mass spectrometry using a standard LC-interface to give a superheated water LC-NMR-MS system. These separations using superheated deuterium oxide have also provided some interesting exchange reactions which are more selective and specific than those reported with supercritical deuterium oxide.

Application of Superheated Water Chromatography

A wide range of analytes (**Table 1**) has been examined by superheated water chromatography. They have generally been moderately polar and could be characterized as analytes where conventional liquid chromatography would employ a mobile phase with 60% or less organic modifier. Less polar analytes, such as alkylbenzenes, can currently cause problems because they require a mobile-phase temperature above the limit of the polymeric stationary phases primarily used so far.

The principal groups of compounds examined so far have been phenols (**Figure 5**), alcohols, amino acids, esters, pharmaceuticals, water-soluble vitamins and lactone natural products. The method is still relatively new and further applications are constantly being developed. Although there was concern that the separation conditions might cause sample oxidation, hydrolysis or degradation, so far few compounds have caused problems. Not surprisingly, aspirin is hydrolysed but this occurs readily even at room temperature. Nitrobenzene appears to degrade and there is some suggestion that other nitro-compounds are also thermally unstable in hot water. In contrast, compounds such as the parabens (4-hydroxybenzoate

Table 1 Typical compounds which have been separated by superheated water chromatography

Aryl aldehydes
Amino acids
Aryl alkyl ketones
Aryl amides
Aryl amines
Arylsulfonamides
Parabens
Pharmaceuticals, including:
Barbiturates
Caffeine
Paracetamol
Phenacetin
Sulfonamides
Phenols, including:
Cresols
Guaiacol
Methoxyphenols
Phenol
1,2,3-Trihydroxybenzene

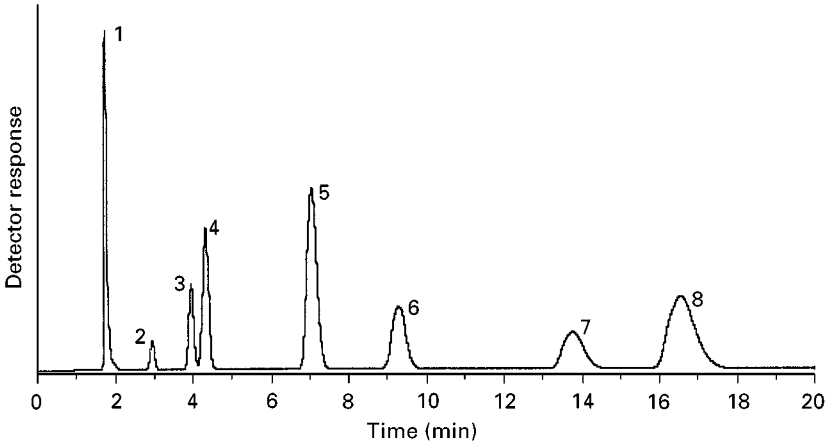

Figure 5 Functional group selectivity of PS-DVB column. Conditions: column, PLRP-S; temperature, 200°C. Solutes: 1, hydroquinone; 2, *p*-cyanophenol; 3, phenol; 4, *p*-methoxyphenol; 5, *p*-cresol; 6, *p*-bromophenol; 7, 3,5-xylenol; 8, 2,4-xylenol.

esters) which might be thought to be susceptible both to oxidation and to hydrolysis, have been separated without difficulty. One reason may be that, as the temperature is raised and the water becomes less polar, it also becomes a weaker hydrolysis agent.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases; Nuclear Magnetic Resonance Detectors. **Extraction:** Supercritical Fluid Extraction. **III/Environmental Applications:** Pressurized Fluid Extraction. **Porous Polymers: Liquid Chromatography.**

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SURFACTANTS

Chromatography

J. G. Lawrence, Unilever Research
Bebington, Merseyside, UK

Introduction

'Surfactant' is a contraction of 'surface active agent'. It has come to be used interchangeably with detergent, particularly when applied to cleaning products such as fabric washing powders, soaps, hard-surface cleaners and the many other products used for cleaning in and around the home. Solutions of surfactants exhibit one or more of the properties of detergency, foaming, wetting, emulsifying, solubilizing and dispersing.

This article will deal with the main classes of surfactants used as commercial detergents, which are not single compounds but mixtures of compounds of the same general structure but having a range of alkyl chain lengths. Surfactants have the follow-