# SOLUTIONS TO RETENTION LOSS OF RPLC COUMNS USING FULLY AQUEOUS MOBILE PHASES

## Waters
### THE SCIENCE OF WHAT'S POSSIBLE.™

*Fabrice G. Gritti, Thomas Walter, Martin Gilar*
*Waters Corporation, Milford, MA, 01757, USA*

## PROBLEM

Due to the instability of the liquid/vapor water system in confined (ex: 100 Å mesopores) and hydrophobic (ex: $C_{18}$) geometries, RPLC columns suffers from severe retention loss when run with 100% aqueous mobile phases. This is explained by the dewetting of water from the internal surface of the mesoporous silica-bonded particles (see Figure 1). New generations of RPLC stationary phases are then developed to cope with this problem. In order to design the most appropriate RPLC phase, the impact of chemical and physical parameters on the dewetting kinetics of water should be investigated. This work proposes a new approach to answer these fundamental questions.
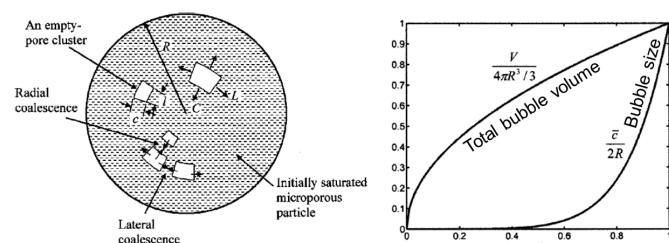
Figure 1. **Dewetting kinetic mechanism of water from a mesoporous particle.** (Left) The process starts with the nucleation of a microbubble followed by the growth and radial/lateral coalescence of these bubbles. (Right) Variation of the total bubble volume and bubble size from beginning to end of the dewetting process.
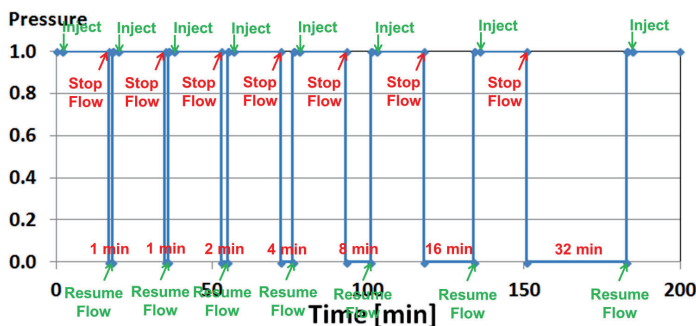
Figure 2. **Experimental protocol used to measure accurately the dewetting kinetics of water from RPLC columns.** The column is first equilibrated with methanol then with water at a flow rate for which the inlet and outlet column pressures are smaller and larger than the intrusion and extrusion pressures of water.

## Waters
## INNOVATION

## METHODOLOGIES

An experimental protocol was designed to follow in real time the dewetting kinetics of water from RPLC columns. The protocol is based on sequentially stopping and resuming the flow rate at different times (see Figure 2) and to monitor the retention times of a weakly (thiourea) and strongly (thymine) retained compound in pure water under flow conditions. During flow conditions, the inlet and outlet column pressures must be kept smaller and larger than the intrusion and extrusion pressures, respectively, of water (see Figure 3), which are measured under static conditions using flow restrictors placed downstream the column outlet.
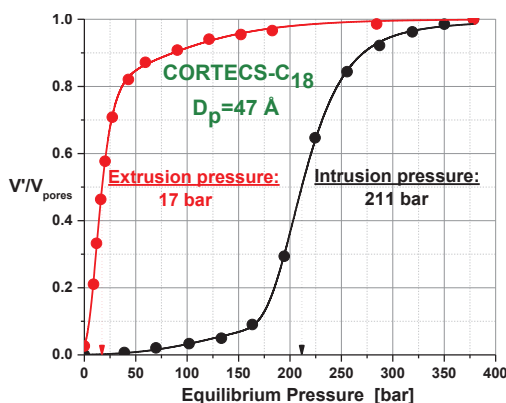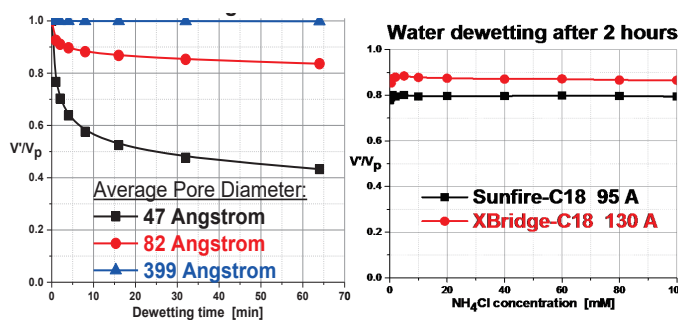
Figure 3. **Water porogram (pore volume fraction $V'/V_{pore}$ accessible to water as a function of the applied water pressure) of the superficially porous 90Å Cortecs-$C_{18}$ particles.** Note the extrusion and intrusion pressures measured during the increase (black symbols) and decrease (red symbols) of water pressure from 1 to 400 bar.
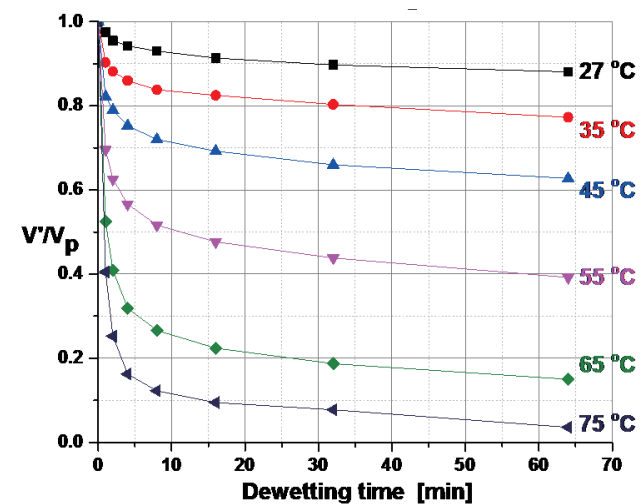
## RESULTS and CONCLUSIONS

**Impact of pore size (Left, Cortecs-$C_{18}$) and salt concentration (Right) on water dewetting kinetics.** Large pore size are needed to minimize water dewetting while ionic strength is not relevant.
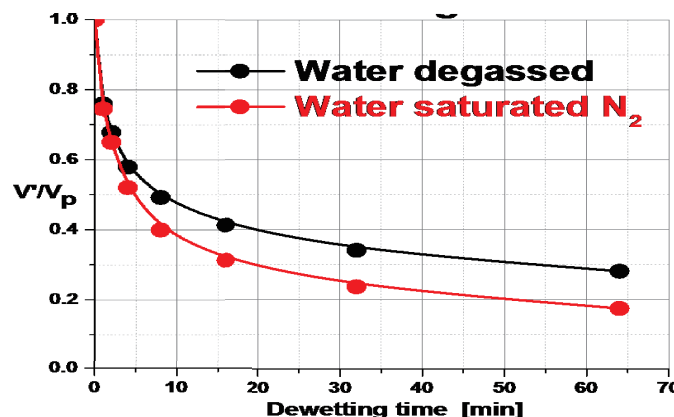
**Impact of dissolved nitrogen in water (Cortecs-C18).** Water should be degassed to minimize water dewetting.

**Impact of temperature on water dewetting kinetics.** High temperatures speed up dramatically the dewetting process.
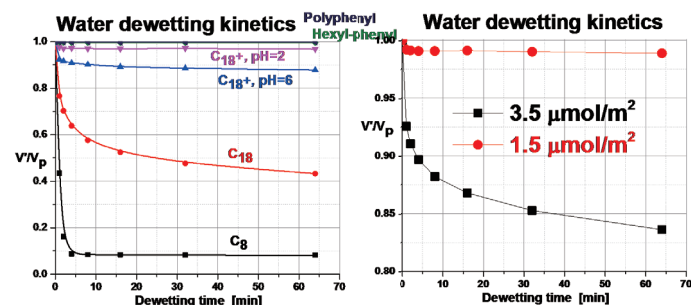
**Impact of surface chemistry (left) and surface coverage (right).** $C_{18}$+ means a doped surface with small amount of positive charges. Long alkyl chains, low surface coverage and doped surface are required to minimize water dewetting.
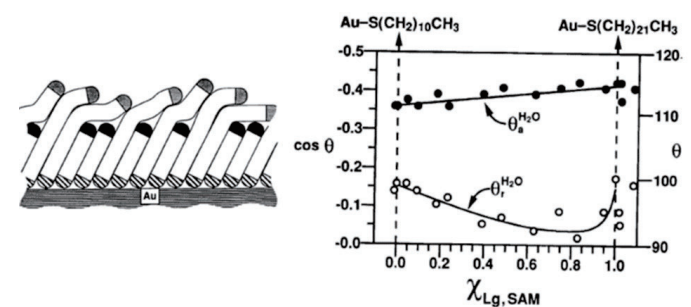
**Impact of surface heterogeneity and disorder.** Mixed bonding surface reduces the receding contact angle q from 100° to 92°. Disorder is required to minimize water dewetting.
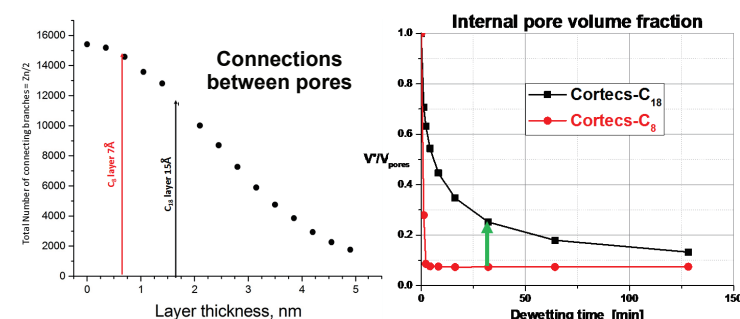
**Impact of pore connectivity (high for $C_8$, lower for $C_{18}$).** Decreasing pore connectivity is required to reduce water dewetting.