How to degas your eluents

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TECHNICAL NOTES

The degassing of eluents in HPLC is an important step to ensure a high sensitivity and detection limit in your HPLC method. The existence of excess oxygen dissolved in the eluent promotes the formation of small bubbles that, when passing through the detection cell, can cause erroneous readings of the absorbance (UV-VIS), fluorescence or refractive index and, in the case of electrochemical detectors, interfere with the redox reaction of the analytes on the electrode. In more extreme cases, the bubbles can be lodged in the detection cell, a problem that becomes a challenge to remedy. The 4 methods used for degassing in HPLC are: sonication, vacuum, helium sparging and in-line degassing.

The most used materials are the following :

- **Sonication**
  Graphite ferrules are made from high-purity graphite, being particularly soft, so they must be handled with care. The main advantages of graphite are that they seal without applying too much force and do not change its shape when subjected to high temperature, therefore there is no need to overtighten the nuts. Since they do not adhere to the column, they can be reused whenever it is disassembled and the column is re-installed, but care must be taken not to deform them by excessive tightening. Graphite ferrules are the most widely used, both in connection of the column to the injector and to the detector, supporting up to 450 ° C. Since graphite is permeable to air, it should not be used in GC-MS systems.
- **Vacuum**
  Vacuum degassing consists of submitting the eluent to vacuum in a closed reservoir. Since all eluents must be filtered before use on HPLC, vacuum filtration is an excellent and easy solution, combining two solutions for two problems. In the '70s and' 80s, it was also common to perform vacuum filtration with the solvent collection flask immersed in an ultrasonic bath, increasing efficiency. Like sonication, these are methods of limited effectiveness and mostly suitable for isocratic methods.
- **Helium sparging**
  In the late 1980s, the popularization of solvent gradient methods led to a demand for systems that would allow it to be carried out. Although in the beginning the gradients were obtained at high pressure by combining two synchronized HPLC pumps in parallel, some manufacturers began to develop gradient systems at low pressure, in which the solvent mixture posed a tremendous challenge for generating bubbles caused by the mixture of solvents of different viscosities at atmospheric pressure. The solution was to provide the equipment with an inlet for a highly diffusible gas – helium – which saturated the solvents thus replacing the dissolved air and, when subjected to the compression of the pump, was expelled without creating bubbles. As the most effective solution, its cost and the need for a gas cylinder or line has become an obstacle to widespread use.

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- **In-line degassing**
  In the late 1990s, most manufacturers begin to incorporate in-line solvent degassers as a standard option. In-line degassers are portable units with 1 to 6 channels/ solvent lines in which the eluent passes through a tube of chemically inert material with high gas permeability, which is subjected to a vacuum atmosphere. The vacuum removes the dissolved gas in each line, resulting in a gas-free eluent. These units can also be supplied as stand-alone units.

In short:

- Sonication and vacuum filtration are methods of limited effectiveness and intrinsically suitable for isocratic methods.
- Helium sparging is the most effective method, but in the long run it becomes costly.
- In-line degassing is highly effective and currently stands as the industry standard.