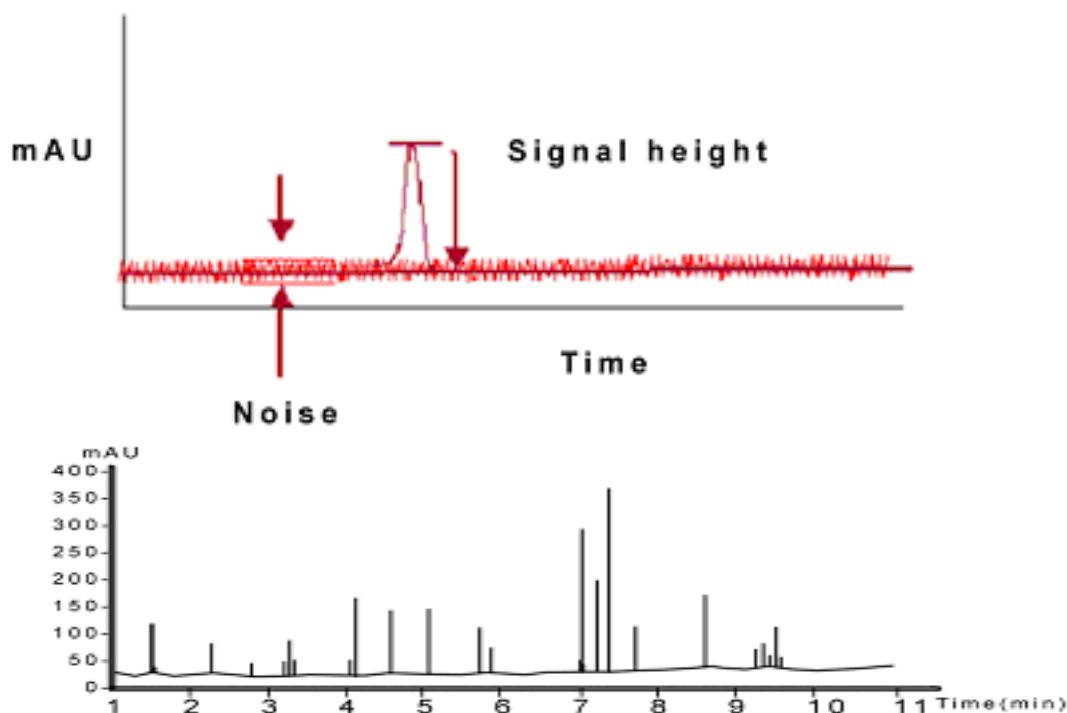


Gas bubbles introduced into the HPLC system will also affect the chromatographic baseline, which will become noisy with the generation of spurious peaks.

Additionally, if a gas bubble becomes trapped in the detector flowcell, high absorbing noise spikes will additionally be produced randomly throughout the recorded baseline. The net effect will be a decrease in the signal-to-noise (S/N) ratio, with a corresponding increase in the method's limit-of-detection (LoD) and limit-of-quantitation (LoQ).

As the mobile enters the detector flow cell it undergoes a drop in pressure. As a result of this pressure drop small quantities of air trapped in the mobile phase can be liberated in the flow cell. This outlet-degassing often results in an increase in baseline noise:



Increased baseline noise (top) and Baseline Spikes (bottom) caused by trapped air or outlet degassing in the detector flow cell

It is common practice to fit backpressure regulators at the exit of detector flowcells, in order to maintain a reasonably high pressure to minimise such outgassing, until the mobile phase flow is sufficiently distant to prevent such entrapment of any air bubble formed. If a backpressure regulator is not an integral fitting of the detector, then a 50cm length of 0.17mm i.d. PEEK tubing, to which the eluent waste line is then attached, will suffice.

Additionally a poorly degassed mobile phase will result in poor pump priming and gradient reproducibility, leading to variable analyte retention times.

Methods of Degassing

There are four accepted ways of degassing a mobile phase:

- Helium sparging
- Vacuum degassing
- Ultrasonication
- Refluxing

Filtering mobile phases under vacuum, a procedure typically performed when removing particulate material from solution prior to use, indirectly degasses mobile phases. Whilst a necessary step, especially when using buffers or mobile phases containing ion pair reagents, care must be taken to avoid selective loss of any organic solvent leading to analyte retention time variability.

Helium Sparging

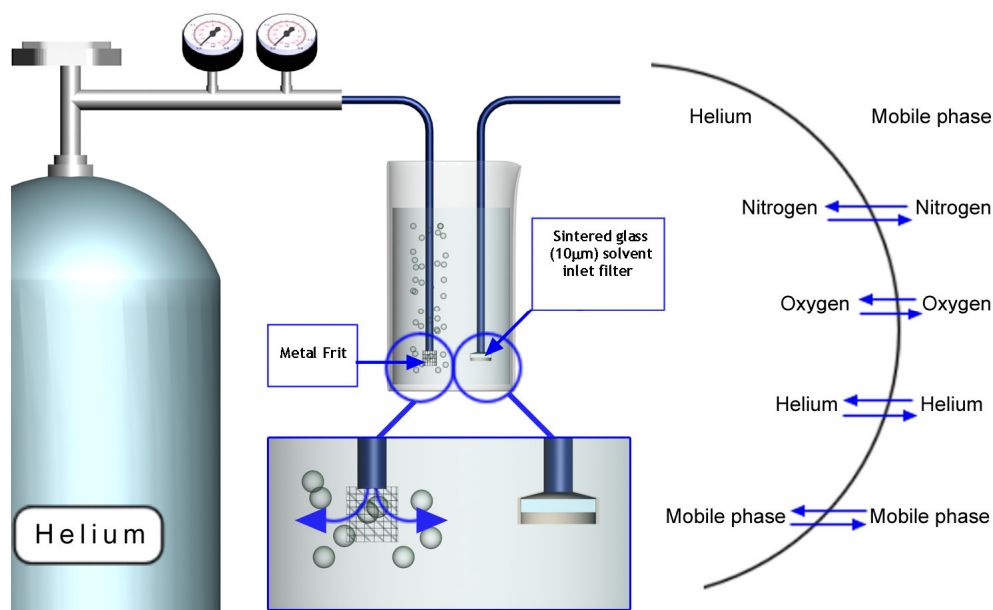
Helium sparging removes approximately 80% of the dissolved gases. Helium spargers can be simply constructed by connecting a length of PTFE tubing with a frit at one end to a helium cylinder. Immerse the frit into the mobile phase and adjust the regulator to release a gentle stream of bubbles.

In general one litre of mobile phase will be fully degassed with one litre of helium. Excessive degassing with helium can cause the loss of the more volatile mobile phase components, leading to retention time variability.

The principle of helium sparging is that dissolved N_2 and O_2 are drawn out of the mobile phase, due to their increased solubility in the introduced helium. As helium itself has a very low solubility in most mobile phases, it leaves taking the dissolved N_2 and O_2 with it, and the resultant solution is left in a relatively gas-free state.

When utilising helium sparging be aware that potential changes in the mobile phase composition may result, due to the selective volatilisation of any mobile phase additives i.e. Trifluoroacetic acid (TFA).

Avoid “vigorous” helium sparging, which can lead to excessive disturbance at the mobile phase surface, with the result that more air is introduced than is removed.



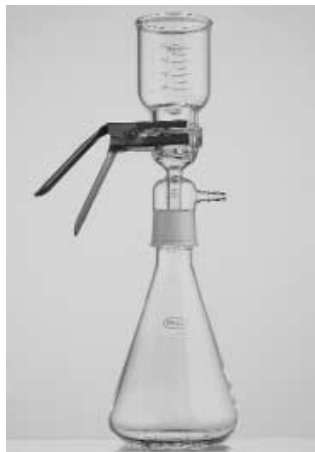
Vacuum Degassing.

Vacuum degassing can be performed in one of three ways:

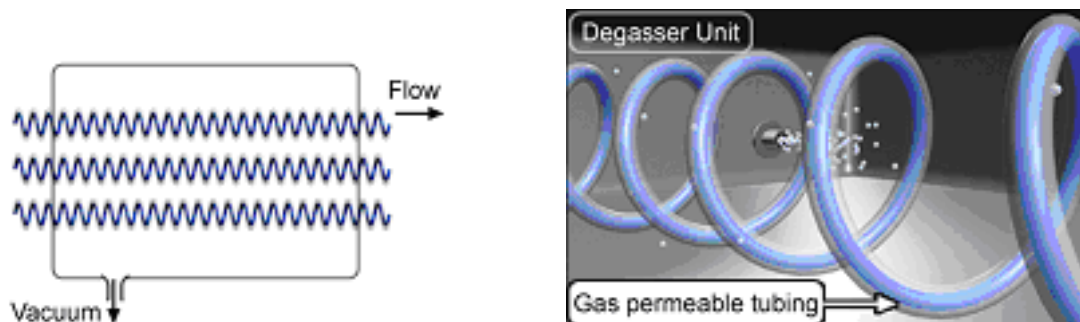
- Batch-wise
- Incidental
- On-line degassing

Batch-wise degassing occurs as the mobile phase contained in a vacuum flask is subjected to a continuous vacuum, either via a water aspirator or simple vacuum pump.

Incidental degassing occurs as the mobile phase passes through a membrane filter. Since the liquid is finely dispersed and exposed to a slight vacuum during this process degassing will occur. Incidental degassing is a secondary feature when filtering mobile phases to remove particulate material under vacuum prior to use.



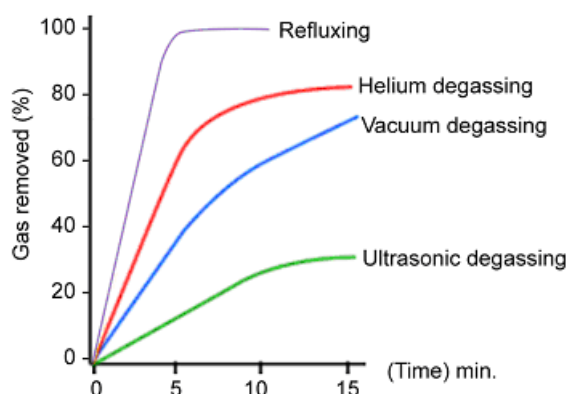
On-line degassing is now the most popular way to degas the mobile phase. The mobile phase is contained within gas permeable tubing as it passes through the degasser unit. The vacuum gradient generated results in any dissolved gases permeating out of the mobile phase and across the tubing membrane.



Ultrasonication

This is the least efficient way to degas a mobile phase, and will only remove approximately 30% of the dissolved gases. The mobile phase is degassed as the sonic vibrations cause any dissolved gas to agglomerate into bubbles large enough to float to the liquid surface. This degassing technique is effectively limited, due to it being performed off-line, and that during sonication heating of the mobile phase makes it possible for some of the more volatile mobile phase components to be lost through selective evaporation. Its advantages of simplicity and cost-effectiveness are generally outweighed by these described limitations.

Ultrasonication is therefore not recommended, and if performed should be for the minimum time possible, and preferably within a temperature controlled water bath.



The figure above illustrates the relative efficiency of various degassing methods, and is based on the percentage of oxygen removed from a given volume of methanol in the times shown.

3.4 Mobile Phase Filtration

Filtration removes dust and other physical particulates and contaminants, and is an essential step during the preparation of any buffered mobile phase. Particulates can lodge in the solvent delivery system, causing wear in the pumphead components, resulting in leakage, irreproducible flowrates and mobile phase compositional changes. Particulates can also lodge in the sample injector switching valve generating crossport leaks, resulting in irreproducible peak areas and/or peak heights as well as shortening its effective lifetime.

Mobile phase filtering also helps prevent any particulates clogging any system in-line filter and guard column if present, with their resultant continuous replacement and associated expense. To be correctly positioned an in-line filter should be placed after the injection system *but* before the analytical column. In this way any particulates generated from wear of the rotor seal component of the injector switching valve will also additionally be effectively retained.

Without mobile phase filtering, and without the added protection of an in-line filter, particulates introduced in to the HPLC system can accumulate on the head of the analytical column resulting in a variety of chromatographic problems.

A partial blockage on the column's inlet frit results in high backpressures, and a subsequent deterioration in chromatographic peak shape, evidenced as peak splitting, fronting or tailing. The figure below illustrates the affect a partially blocked inlet frit has on the subsequent introduction of the sample onto the head of the column. With no blockage the sample emerges from the connective tubing, distributing itself evenly within the inlet frit and then onto the head of the column. With a partial blockage this even distribution is effectively prevented, and consequently sample introduction is both inhibited and split, resulting in the described peak shape abnormalities.

