Sample Deoxygenation for Reductive LCEC

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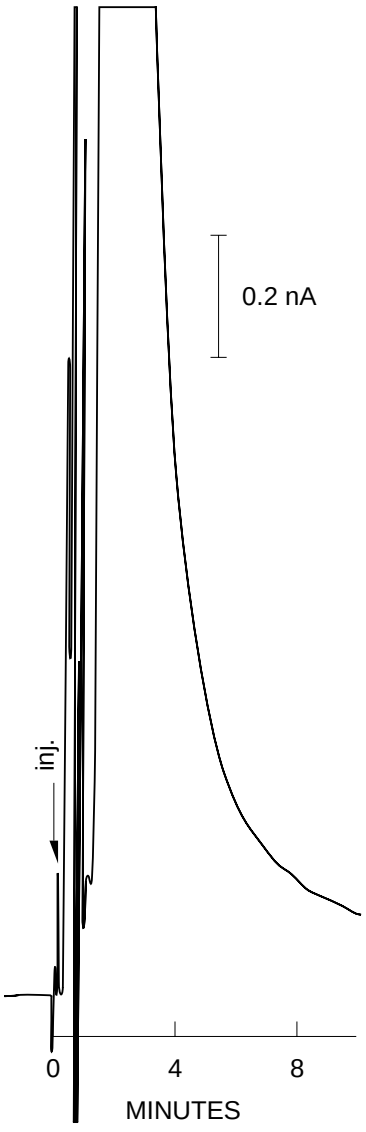
Sample deoxygenation is a necessity for most reductive LCEC applications. The BAS-200B has a unique geometry that allows for simple and effective sample deoxygenation with only minor modification to the system.



F1

Injection of 300 pg benzoquinone without sparging the sample. The benzoquinone peak is completely hidden under the large oxygen peak. Compare to F5.

Oxygen can be reduced at a glassy carbon electrode at potentials more negative than about -300 mV (*vs.* Ag/AgCl). This poses a two-fold problem for reductive LCEC. First, oxygen must be removed from the mobile phase. Otherwise, high backgrounds (in the µAmp range) will cause increased baseline noise and make rezeroing difficult. The solution here is simple: vigorously sparge the mobile phase with helium to remove oxygen, then pressurize the system to prevent its reentry. All plastic tubing, which is permeable to oxygen, must be replaced with stainless steel. The BAS-200 series of liquid chromatographs have employed this solution for years.

The second problem posed by oxygen is its presence in the sample. Dissolved oxygen will be retarded on-column by a size-exclusion mechanism, and produce a characteristic tailing peak. At sensitive gains this oxygen peak can obscure the analyte peaks (***F1***), and it may take many minutes for the detector's response to come back to baseline. Removing oxygen from samples is difficult, however.

One scheme for deoxygenating samples was proposed by Lloyd (1). BAS simplified the process, and described it in 1983 (2). The idea is to bubble helium through the injector valve into the sample, which sits in a modified syringe (***F2***). The major drawback of this scheme was that the injector had to be mounted vertically, so gas could bubble up through the syringe. Since most injectors are mounted horizontally, this meant major modifications to the system.

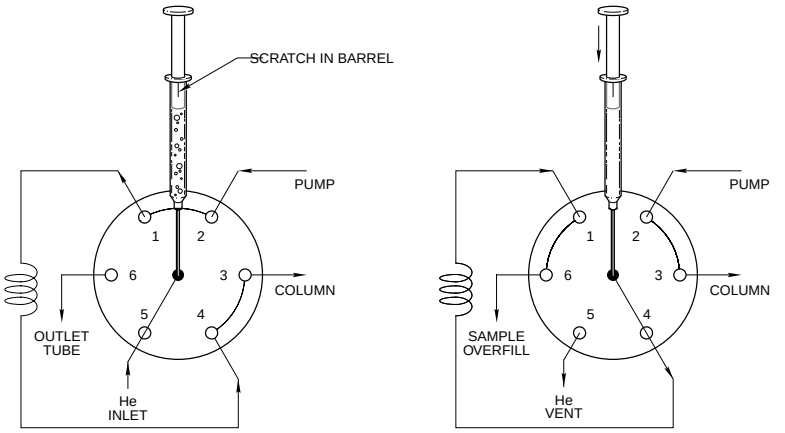
Injector valves on the BAS-200B Liquid Chromatograph are mounted at a 45° angle rather than horizontally (***F3***). This design change permits direct connection of microbore columns between the injector and the detector. But the new mounting angle also permits on-line dexoxygenation of samples, with only minor modification to the system.

System Modification

A 0.040" i.d. stainless steel tube was connected between the gas exhaust of the BAS-200B (***F3***) and port 5 of the injection valve (in this case, a Rheodyne 8125, but any 125-series valve will work). The connectors were pieces of PTFE tubing of appropriate diameter to slip over the steel tubing.

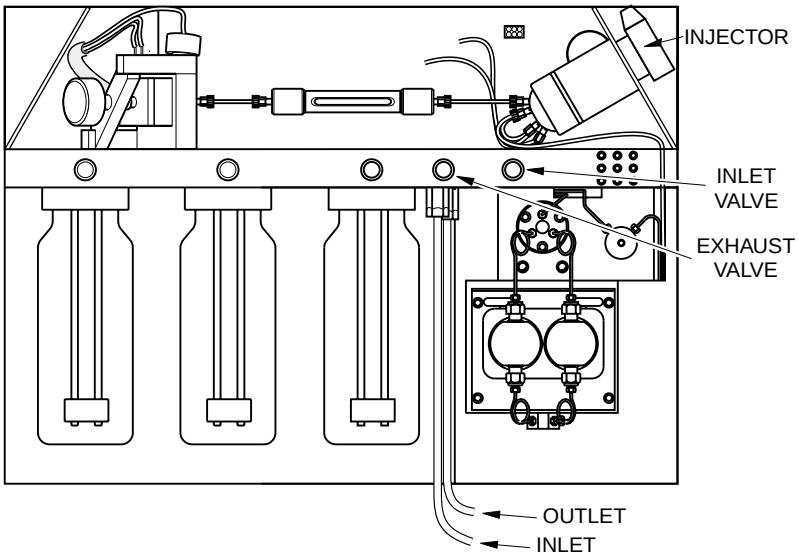
F2

Sample deoxygenation procedure. Left: degassing in 'inject' position. Right: loading loop in 'load' position.



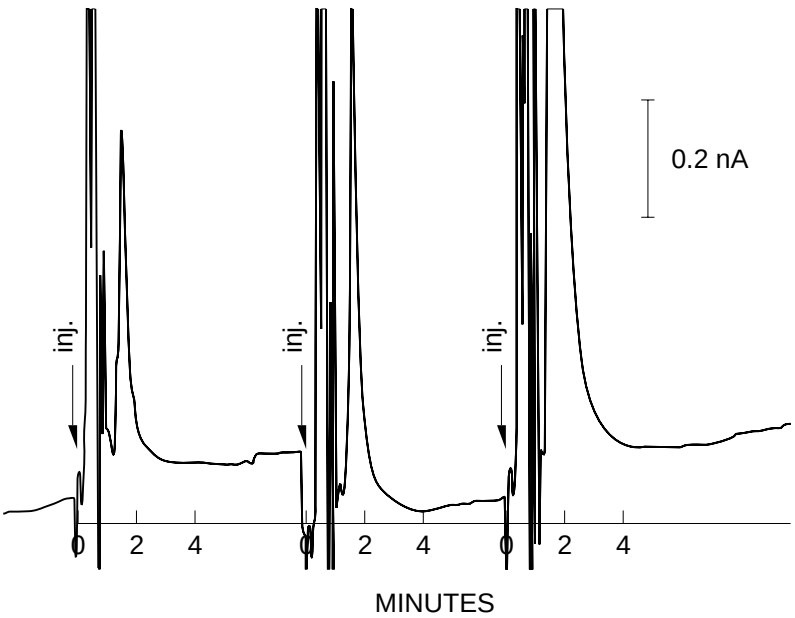
F3

Diagram of BAS-200B, showing 45 degree mounting angle of injector. Also note the helium outlet tube, and exhaust valve, which can be used to route and control helium flow for sample deoxygenation.



F4

Injection of water that had been sparged with helium for five (left), two (middle), and one (right) minute.



A 2-mL glass syringe with glass luer tip (#5006, Popper and Sons) was used to deoxygenate and inject the sample. A 2-cm-long scratch was made with a file, along the inside of the barrel where the plunger enters. The scratch allows the gas to escape when the plunger is pulled most of the way back. When you depress the plunger to inject the sample, it passes the scratched region and then seals normally.

Conditions

System: BAS-200B Liquid Chromatograph
Injection Valve: Rheodyne 8125, with 5 µL stainless steel loop
Column: C_{18}, 100 x 3.2 mm, 3 µm particle size (MF-6213)
Electrode: Glassy carbon, cross flow, 3 mm (MF-1000)
Potential: -600 mV *vs.* Ag/AgCl
Range: -2 nAfs
Analyte: 1,4-Benzoquinone.
Flow rate: 1 mL/min.
Temperature: 35° C.

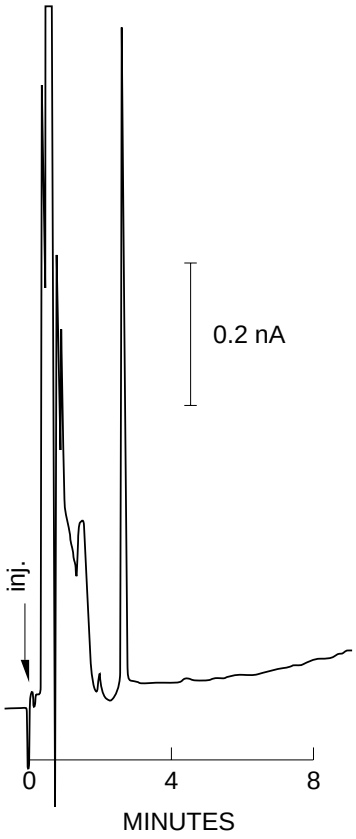
Method

First, set up the LC system. Deoxygenate the mobile phase and column thoroughly for several hours, then close the exhaust valve to pressurize the bottles with helium. Finally, open the exhaust valve to obtain a very slow bubbling rate in the mobile phase. Equilibration of the system may take overnight. Attach the luer needle adapter provided with the Rheodyne valve to the glass syringe. Load the sample into the syringe, then insert the syringe into the valve. The valve should be in the 'inject' position during deoxygenation.

To begin deoxygenating the sample, attach the stainless steel tube between the exhaust port of the BAS-200B and port 5 of the injection valve. Slowly open the exhaust knob on the BAS-200B. This sends humidified helium from the

F5

Injection of 300 pg benzoquinone after five minutes of sparging with helium. Compare to F1.



sparging system through the valve, and it will bubble through the sample. The gas will push the syringe plunger out to the level of the scratched barrel (you may have to stop its travel manually).

Allow the sample to deoxygenate for several minutes. The exact time will depend on the gas flow and the level of deoxygenation needed. ***F4*** shows the effects of five-, two-, and one-minute sparging times on the size of the oxygen peak.

To inject the sample, first turn the valve to the 'load' position. Now comes the tricky part. You have to load the loop by depressing the syringe plunger, but you will be pushing on the blanket of helium above the sample, which compresses, and in turn pushes the sample forward. You want to push as much sample as possible through the loop, because oxygen will be diffusing in from the waste port. But be careful not to push so hard that the sample goes completely through and helium enters the loop.

Results and Discussion

F5 shows the injection of a deoxygenated sample under the same conditions as in ***F1***. The oxygen peak was reduced dramatically and no longer interfered with the analysis.

This procedure required a minimum sample volume of 200 µL. Smaller sample sizes were difficult to manipulate with the 2-mL syringe. I tried a 1-mL syringe, but the barrel diameter was too narrow—helium pushed the sample out rather than bubbling through it.

References

1. *Lloyd, J.B.F., J. Chromatogr. 256 (1983) 323.*
2. *Current Separations 5 (1983) 53.*