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Note

On-line dynamic flowing reference compensation for high-performance liquid chromatographic detectors

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In many types of high-performance liquid chromatography (HPLC), compensation for background response and instrument variation by a detector reference channel becomes advisable and at times essential. With commercial detectors which have incorporated only limited offsetting capability in the electronics, high background response of a mobile phase containing detectable components can become a formidable obstacle in attaining baseline stability and background response suppression. For refractive index (RI) detectors, compensation using the reference side of the detector is usually performed with a static solution of the mobile phase. With ultraviolet (UV) absorption detectors an air reference is usually used. In cases where the mobile phase exhibits a high detector response, this reference compensation is especially critical.

Existing methods of using mobile phase for reference compensation are inconvenient, cumbersome, or unsatisfactory. This note describes a simple and effective means by which dynamic reference compensation can be used without additional hardware. Our novel configuration is to connect the inlet to the HPLC pump through the reference channel so that the mobile phase is continuously sucked through (Fig. 1). This approach has been used with success with UV and conductivity detectors.

EXPERIMENTAL

Apparatus

For indirect UV visualization studies an Altex (CA, U.S.A.) solvent metering pump (Model 110A) and a 254-nm UV monitor (Model 153) were used. For the conductivity detection an LDC model 701 conductometer was used. An LDC constametric II pump (Laboratory Data Control, Riviera Beach, FL, U.S.A.) and a pulseless ACS Model 400/01 pump (Applied Chromatography Systems, State College, PA, U.S.A.) were used.

Chemicals and reagents

Naphthalene-2-sulfonic acid (Eastman Kodak, Rochester, NY, U.S.A.) was recrystallized from water before use. Phosphoric acid used to acidify the mobile phase was Baker Analyzed Reagent (J. T. Baker, Phillipsburg, NJ, U.S.A.).

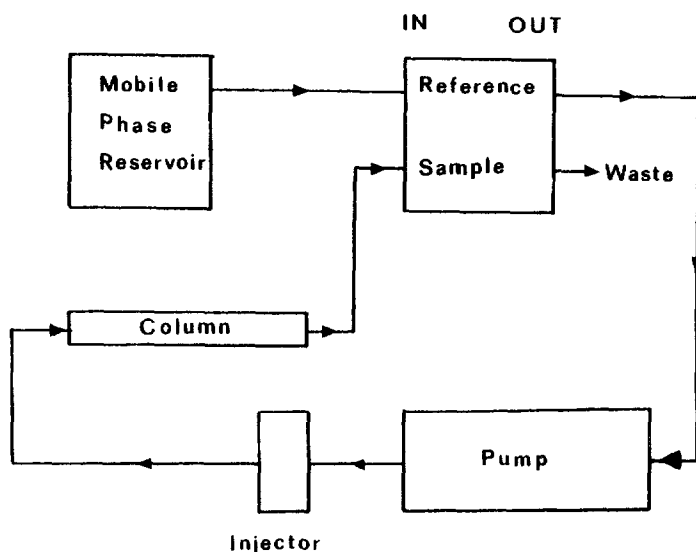


Fig. 1. Block diagram of the on-line dynamic flowing reference set up for HPLC detectors. The mobile phase is fed into the HPLC pump via the reference channel of the UV detector. Note that no additional piece of hardware is required to achieve dynamic on-line reference compensation.

RESULTS AND DISCUSSION

Presently there are three techniques which employ the mobile phase for reference compensation. A static solution is most commonly used but bubbles resulting from inadequate filling or from the mobile phase being heated by the detector source cause noisy, unstable baselines. Photodecomposition in the static mobile phase can cause changes in the reference signal over a period of time. Changing mobile phase in the static loop is inconvenient.

Splitting the pump output to provide a dynamic flowing reference requires a pressure drop fitting or second column or a high pressure cell and does not easily facilitate matching the flow-rates in the two cells. The third approach is to use a second low pressure pump for the reference channel. This is inconvenient, expensive and can cause problems in exactly matching the flow-rates and temperature in the two independent fluid pathways.

The HPLC system was configured as shown (Fig. 1) with the mobile phase drawn into through the reference cell and into the pump inlet. There is no need of excessive tubing. The solvent reservoir can be raised above the level of the pump to provide the pump with adequate solvent. Pump starvation or erratic performance were not encountered.

Initially the suction side is filled with the mobile phase manually with a syringe. Air leaks should be carefully avoided just as when plumbing the suction side for any HPLC system.

Indirect UV visualization

When studying indirect UV visualization under reversed-phase HPLC condi-

tions¹⁻⁶ the high UV absorption of the mobile phase severely limited the usable concentrations of UV absorbing counterions. Baseline zero offsetting was not possible for mobile phase containing more than 0.4 mM naphthalenesulfonic acid in 0.05 M phosphoric acid. Under these conditions only the 1.28 a.u.f.s. detector setting could be used⁷. Using reference suction it was possible to zero the detector on the 0.04 a.u.f.s. scale. This 32-fold increase in workable sensitivity was accomplished while maintaining satisfactory baseline stability.

Conductivity detection

With 0.05 M phosphoric acid as the mobile phase the highest sensitivity obtainable in the absolute mode of the conductivity detector was $10^3 \mu\text{mho}$ full scale. In the reference suction setup we could achieve a sensitivity up to 30% Δ in the differential mode. Also it was found that the differential mode of operation is inherently more sensitive than the corresponding absolute mode scale. Comparative studies showed that this is more than a 33-fold increase in the operable sensitivity range.

The disadvantage is that the cyclic flow pattern from the reciprocating pump was transmitted to the conductivity detector in the form of short term pulsation noise (Fig. 2A) mainly from the reference channel because in the sample path the column serves as a pulse dampener. High sensitivities for aliphatic alcohols were achieved even with this periodic noise⁸. That work is detailed elsewhere⁹. However, this periodic noise pattern due to the discontinuous pump inlet flow can be completely eliminated by using a totally pulseless pump which has a very rapid pumping cycle (Fig. 2B). Triple-headed reciprocating pumps with continuous duty cycle on inlet stroke can also overcome this problem.

A 

B _____

Fig. 2. Comparison of typical baselines with conductivity detector in differential detection mode under maximum attainable sensitivity with reference suction. Mobile phase is 0.05 M phosphoric acid. Conductivity detector is at 30% Δ in the differential mode of operation and chart speed at 30 cm/h. (A) Baseline with LDC constametric pump. Note the regular periodic pulsation noise of the pump. (B) Baseline under exactly identical conditions with a pulseless ACS pump.

Although we have demonstrated the utility of this technique in the cases of ultraviolet and conductivity detection, it can be used with any dual-channel HPLC detector or in flow injection analysis¹⁰ with no extra hardware.

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