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MOBILE PHASE DELIVERY IN SUPERCRITICAL-FLUID CHROMATOGRAPHY

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SUMMARY

The properties of two syringe pumps and of two reciprocating piston pumps in supercritical-fluid chromatography (SFC) have been compared. One of the syringe pumps was not compatible with CO_2 as the mobile phase, due to leaks at the piston seal. The piston pumps were modified with extra check valves and with cooling at the pump-heads and at the check valves. At controlled flow-rates the piston pumps delivered CO_2 with good reproducibility, but low flow-rates reduced the reliability of the check valves. In the constant-flow mode, the noise of a flame ionization detector caused by the piston pumps was found to be twice the noise from all other sources. In the constant-pressure mode, the noise was found to be significantly higher due to pulses produced in the check valves.

Even though positive-displacement pumps do have some problems with compressed gases, syringe pumps, in general, may be considered superior to reciprocating pumps for SFC. One advantage is that pressure gradients are easy to realize, but the main advantage is probably that pumps without check valves give a higher day-to-day reliability. However, piston pumps would be extremely valuable in SFC if the check valves could be replaced by fast-working, active valves.

INTRODUCTION

The first paper on supercritical-fluid chromatography (SFC) was published by Klesper *et al.* almost 25 years ago[1]. The development of methods and applications then proceeded slowly, due to technical difficulties, competition from modern liquid chromatography, and lack of commercially available instruments. With the renaissance of SFC, which is mainly connected with the development of capillary columns[2] and with new detection strategies[3], a closer look at the properties of the instrumentation may be of value. Since still only few instruments are available commercially and since many technical solutions are far from the state-of-the-art of modern gas chromatography, the entrance ticket to the supercritical club easily becomes too expensive for the newcomer battling instrument problems that nobody ever mentioned.

One of the main components of SFC systems is the pump, which is supposed to deliver compressed gases, like CO_2 and N_2O, reliably and reproducibly. So far, this has been accomplished by using modified HPLC pumps, mainly syringe pumps

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(positive displacement pumps) and reciprocating diaphragm pumps, but recently also by using a reciprocating piston pump[4]. Reciprocating piston pumps as well as diaphragm pumps are equipped with check valves, and both pump types suffer from problems caused by the check valves. This paper contains a discussion of the properties of syringe pumps used for SFC partly based on noise levels which have been measured with different instrument modifications. The use of diaphragm pumps, at relatively high flow-rates with packed columns, has been described elsewhere[5–8].

EXPERIMENTAL

Materials and columns

Liquid CO_2 (standard grade) and liquid N_2O (medical grade) were obtained from AGA Norgas (Oslo, Norway).

The 250 × 1.3 mm I.D. CP-Spher silica (8 μm) columns were purchased from Chrompack (Middelburg, The Netherlands), 30 × 2.1 mm I.D. Spheri-5 cyano (5 μm) MPLC cartridge columns were purchased from Brownlee Labs. (Santa Clara, CA, U.S.A.) and a 150 × 1 mm I.D. Novapak C_{18} (4 μm) column was received as a gift from Millipore/Waters (Milford, MA, U.S.A.).

Instruments

Liquid CO_2 was transferred from the flask to the pump by an eductor tube and a stainless-steel transfer line. The N_2O tank which could not be obtained with the eductor tube, was turned upside down. A 2-μm filter was inserted in the transfer line.

The pumping systems used were a Waters 6000 A pump, modified for SFC as previously described[4] (hereafter called the W-6000 pump), a micro Metric metering pump from LDC/Milton Roy (Riviera Beach, FL, U.S.A.), a μLC-500 Micropump from Isco (Lincoln, NB, U.S.A.) (hereafter called the I-500 pump) and a Waters Model 590 pump (hereafter called the W-590 pump).

The LDC pump contained a piston seal which evidently was not compatible with SFC and resulted in gross leaks of CO_2.

The I-500 pump was used without any modifications, except that a cooling coil around the cylinder was made from 1 m of stainless-steel tubing, connected to a Julabo F 20-VL (Julabo labortechnik, Seelbach, F.R.G.) circulation bath with methanol at −15°C. Cooling was turned on only during the filling procedure, since leaks were observed through cold piston seals.

Samples were injected with a Rheodyne 7520 injector or a Valco CI 4W injector at room temperature.

Two Hewlett-Packard 5790 A (Hewlett-Packard, Avondale, PA, U.S.A.) gas chromatographs functioned as column ovens and were equipped with a flame ionization detector and a nitrogen/phosphorus detector. The flame ionization detector was kept at 325°C and the thermoionic detector at 350°C.

Pump-head cooling unit

The clamp-on cooling unit for the Waters-590 pump was made from aluminium. The dimensions and the flow of the cooling liquid are shown in Fig. 1. To allow the pump-head unit to be made in three pieces, the center part was precision-cut to

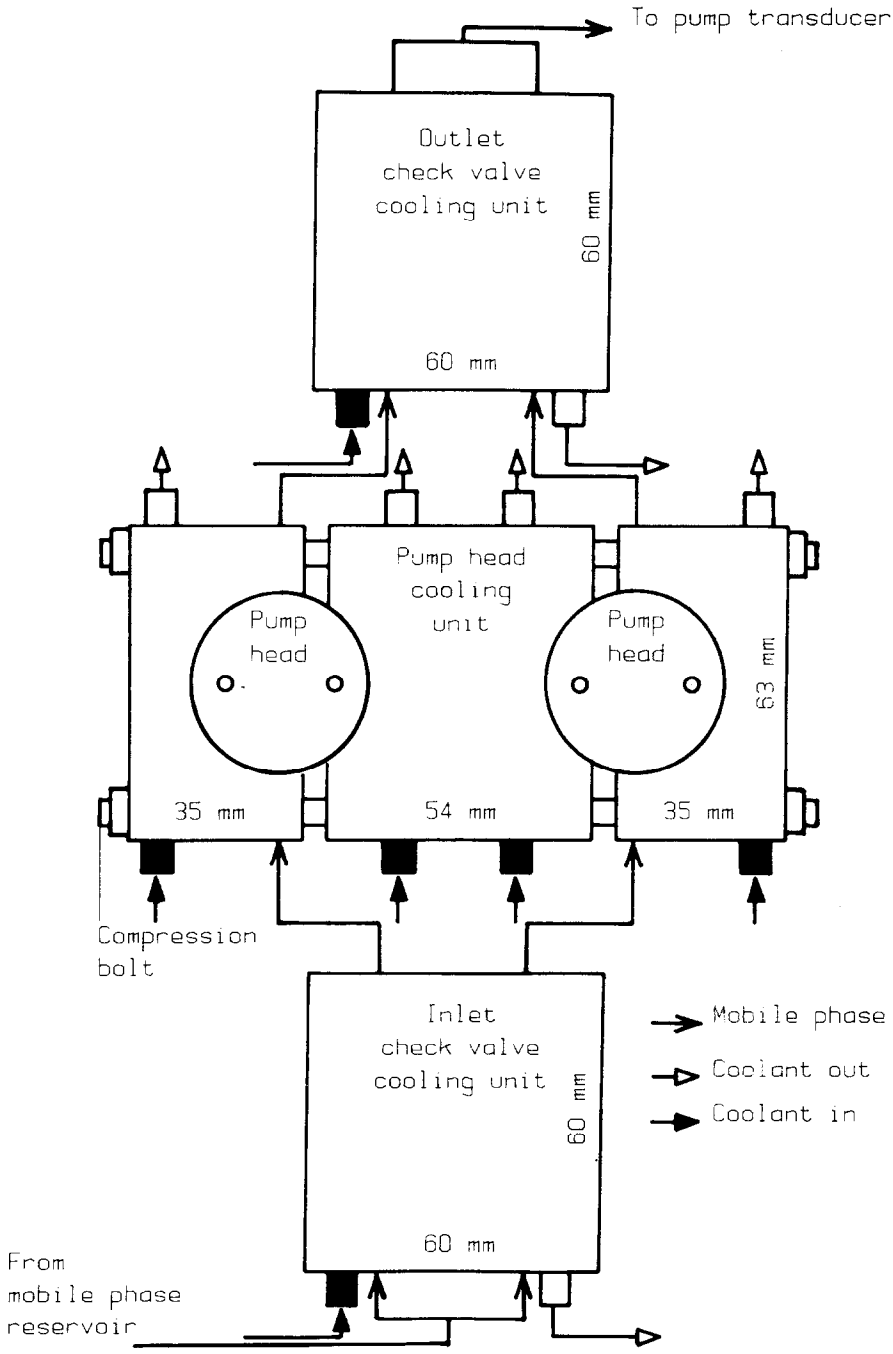

Fig. 1. Dimensions and flow diagram of pump head and check valve cooling units.

fit accurately between the two cylindrical pump heads. Through each separate part, three parallel 1/8 in. I.D. channels were drilled and the 1/4 in. I.D. entrance and exit copper tubes were interconnected with poly(vinyl chloride) (PVC) tubing. Cold methanol (−5°C) was pumped through the units by the Julabo circulation bath. Circulation tubes connected to the cooling bath were 0.5–1 m long and insulated with expanded polystyrene hoses. The PVC tubes were not insulated.

The check valves were equipped with cooling blocks made of aluminium, containing cooling channels, as previously described[4], and connected to the circulation system described above.

Since the W-590 pump contained a flow-programming unit, flow–pressure gradients could be constructed without any external units.

RESULTS AND DISCUSSION

Reciprocating piston pumps

In publications on instrumentation for SFC it is not unusual to see the authors write off reciprocating piston pumps as useless for SFC. This is actually quite surprising, since reciprocating diaphragm pumps have been used for years in SFC[5–8]. A common misunderstanding seems to be that compressed gases will leak through the piston seals. After more than three years of frequent use of piston pumps in SFC, we have never observed measurable leaks at the piston seals. Even in HPLC this record would be quite impressive. The reason is, of course, that leaks are mainly caused by damage to the seals by solid particles, which can effectively be filtered out of mobile phases like CO_2. Furthermore, the viscosity of cold liquid CO_2 is close to the viscosity of solvents, which can be pumped by piston pumps without any problems.

The second objection against piston pumps is the noise which supposedly is created by the pulsating pistons. With a UV detector and a piston pump in constant-flow mode, the pulsation was actually determined to be less than 10^{-4} a.u.[4]. With a flame ionization detector, which is mass-sensitive, the contribution to the overall noise from pulsation could be expected to be higher. Not surprisingly, flow–pressure programs have often shown an increasing baseline noise at high pressures. At moderate pressures (below 200 bar) the flow-rate-related changes in noise are not significant (Table I). At pressures of 300 bar or above, higher pressure pulses are observed.

TABLE I

BASELINE NOISE, MEASURED WITH PISTON PUMPS AT CONSTANT FLOW IN COMBINATION WITH A FLAME IONIZATION DETECTOR

The noise was measured in mV, which should be related to the 1 V output full scale. Mobile phase: CO_2.

Pump	*Restrictor*	*Pump flow (μl/min)*	*Pressure (bar)*	*Noise (mV)*
W-6000	Pt-1	200	138	0.2
W-6000	Pt-1	300	193	0.2
W-590	Si-1	100	110	0.2
W-590	Si-1	200	186	0.2

TABLE II

BASELINE NOISE, MEASURED WITH A PISTON PUMP AT CONSTANT FLOW WITH FLUID SPLIT BACK TO THE PUMP IN COMBINATION WITH A THERMOIONIC DETECTOR

Mobile phase: CO_2, pump: W-6000, restrictor: Pt-4.

Pressure (bar)	*Noise (mV)*
145	0.5
207	0.2
310	0.1

With a thermoionic detector, optimized for the best signal-to-noise ratio, the noise was higher at lower pressures (Table II). This effect is possibly related to the temperature of the bead in the detector and, obviously, was not a function of pulsations at the pump.

The first piston pump that was modified for SFC[4] could not be operated in the constant-pressure mode. A W-590 pump, with the choice of flow or pressure control, has now been modified, as described in the Experimental section. At constant flow, the noise was approximately the same as with the other piston pump (Table I). At constant pressure, the noise was considerably higher, with relatively large fluctuations. The piston-stroke pattern, which was apparent, is thought to be caused mainly by cross-flow from an outlet check-valve through the other valve during its closure, resulting in pressure pulses up to 200 p.s.i., which is more than ten times as high as the pulses that were measured at constant flow. In the constant-flow mode the W-590 pump was used at flow-rates down to 100 μl/min of liquid CO_2. At this flow the check valves malfunctioned quite often. However, at 300 μl/min, reproducibility of the retention time was determined to 0.2% (measured during one day's runs). Based on the measurement of the noise within each piston stroke, the noise caused by check valve pulses was estimated to be twice the noise from other sources.

The check valves caused more problems with another fluid: with N_2O as the mobile phase, a stable flow could not be obtained at any time with the W-6000 piston pump. Since the viscosity of N_2O is almost identical to that of CO_2, this suggests a different extent of swelling of the O-ring in the soft-seat check valves.

If a pump does not function properly at low flow-rates, but better at high flow-rates, an alternative is to use high flow-rates combined with a split. The split can be installed at the injector, going to waste, thereby splitting the injected sample. The split can also be installed ahead of the injector with a transfer line (and a needle valve), leading the split-out fluid back to the pump. Finally, the split can be installed after the column. With splits installed at the injector or after the column, the major part of the injected sample is lost, which naturally is a problem when the amount of sample is very limited. However, with open-tubular capillary columns the injector split is a necessity to prevent overloading of the column. The most economic split arrangement, both for sample and for fluid, is the back-up transfer line. A W-6000 pump, equipped with this arrangement functioned properly most of the time, but unfortunately the needle valve was too sensitive to allow easy flow control. Connected to a thermoionic detector, injections of 0.1 ng of azobenzene gave a detectable peak, with the noise levels shown in Table II.

It should be realized that the nominal flow control is lost with this system, as with all split systems. However, the main problem with splits is not the loss of nominal flow control, but the difficulties in obtaining good reproducibilities.

On this basis packed capillary columns, large enough to receive the whole injected sample, are still of considerable interest in SFC. For columns containing silica-based packings, modifiers are often needed. If modifier gradients or several modifier steps are required, good reproducibility has been obtained with two low-volume piston pumps, one for the pure fluid and one for the modifier[9,10]. As already mentioned, a problem with double-headed pumps equipped with check valves is the long closing time of the valve, which results in occasional malfunctioning and in high pulses in the constant-pressure mode. The apparent solution to this problem is to replace the outlet check valves with rapidly working active valves, synchronized with the piston movements. Even for HPLC applications at flow-rates suitable for narrow columns, active valves would be an improvement compared to check valves. To make such valves available at reasonable prices should be a challenge to the manufacturers.

Another valve problem is connected with the use of materials in soft-seat check valves which swell in the fluids and cause the valves to malfunctions[4]. So far no check valves have been found that work properly over extended periods of time at the flow-rates required for capillary columns.

Syringe pumps

The majority of SFC users today utilize syring pumps in order to obtain pulseless flow at controlled pressure. An important part of the syringe pump is the piston seal. Of the two syringe pumps that were examined in this study, one could not be used with CO_2, due to large leaks. The inner O-ring of the seal was displaced after swelling in CO_2. This weakened the seal and resulted in a leak large enough not to allow constant pressure to be obtained. It should be added that the manufacturer of the pump never recommended this system for SFC.

With constant pressure or with pressure gradients, small leaks can be tolerated. The only effect will be that the cylinder needs to be refilled more often. With the I-500 pump tested in this study, the filling procedure took 45 min. When the pump cylinder was kept at room temperature, no leaks were observed. With a cold cylinder, 10–30% of the content could be lost overnight. Thus, the cylinder was cooled down only during filling (with CO_2 and N_2O), and was brought back to room temperature as soon as possible by shutting down the circulating cooling liquid. In order to minimize the instrument down-time the filling should be done at the end of the day.

With an average column flow of 5 μl/min through a 50-μm capillary column and an injector split of 1:20, the pump will have to deliver 100 μl/min, which requires one filling each day with the 50-ml cylinder of the I-500 pump. With smaller pumps, a split-closing valve must be installed in order to ensure one day's work without refilling, unless two pumpheads are connected with alternating pressurizing and filling. The 250-ml cylinders found in some syring pumps used for SFC assure longer periods between fillings, but have potentially more reproducibility problems, due to the large volumes to be compressed. Unfortunately, few data can be found in the literature on the reproducibility of pressure programs with such pumps.

One difficulty with constant-pressure pumps is the uncertainty about the flow through the column. In order to examine whether constant pressure really resulted

TABLE III

BASELINE NOISE MEASURED WITH SYRINGE PUMP AT CONSTANT FLOW

Other conditions as in Table I. Mobile phase: CO_2, pump: I-500.

Restrictor	*Pump flow (μl/min)*	*Pressure (bar)*	*Noise (mV)*
Pt-2	100	204	0.05–0.1
Pt-2	200	331	0.05–0.1
Pt-3	20	124	0.05–0.1
Pt-3	50	198	0.05–0.1

in constant flow, a single experiment was performed with the I-500 pump. With two different restrictors, one that gave approximately 55 μl/min of CO_2 at 4100 p.s.i. and one that gave 110 μl/min at 4100 p.s.i., the flow was registered by the flowmeter of the pump during the day for two consecutive days. All other parameters were kept constant. The result was that the nominal flow increased by 6–22% each day. The increasing nominal flow is suggested to be caused by variations in room temperature during the day, and does not reflect increasing CO_2 flow through the restrictor, since retention times did not vary significantly.

In order to compare the noise of a syringe pump–flame ionization detector combination with the noise of a piston pump–flame ionization detector combination, as described above, the baseline noise was measured with the I-500 pump at constant flow (Table III) and at constant pressure (Table IV), at different flow-rates and with different restrictors. It is concluded that the noise was approximately constant within the variations caused by different restrictors and that the level was approximately half the level measured with piston pumps at constant flow. This result is in complete agreement with the estimates above, based on studies of the baseline flow pattern. The conclusion is that further reductions in noise will depend on the development of restrictors that create less noise than the present ones.

Noise contribution from other sources

With an improper restrictor, the contribution from the pump to the overall noise is completely insignificant. When a steel restrictor was used with N_2O as mobile phase, the nitrous oxides which are formed by combustion of N_2O[11] evidently at-

TABLE IV

BASELINE NOISE, MEASURED WITH A SYRINGE PUMP AT CONSTANT PRESSURE

Other conditions as in Table I. Mobile phase: CO_2, pump: I-500.

Restrictor	*Pump flow (μl/min)*	*Pressure (bar)*	*Noise (mV)*
Pt-2	80	172	0.05–0.1
Pt-2	163	276	0.05–0.1
Pt-3	42	172	0.05–0.1
Pt-3	75	283	0.05–0.1
Steel-1	130	283	0.1

TABLE V

BASELINE NOISE, MEASURED WITH A SYRINGE PUMP AT CONSTANT PRESSURE

Other conditions as in Table I. Mobile phase: N_2O, pump: I-500.

Restrictor	*Pump flow (μl/min)*	*Pressure (bar)*	*Noise (mV)*
Steel-I	130	283	57.0
Si-2	87	283	0.1
Si-3	38	138	0.1
Si-3	55	228	0.1

tacked the restrictor and increased the noise by 2–3 orders of magnitude (Table V). The noise increased with increasing flow-rate. Platinum restrictors gave a high noise as well (with N_2O) and rapidly ended up being clogged. Fused-silica restrictors were unaffected by N_2O.

With CO_2, the platinum restrictors gave little noise and no spiking. With fused-silica restrictors, the noise (and spiking) increased at lower detector temperatures. This has also been observed by others[12] and has led to the construction of a detector with an extra heating element.

With fused-silica restrictors the baseline noise with N_2O was comparable with the noise obtained with CO_2. However, the background current was much higher with N_2O than with CO_2, and the signal became severely quenched. Compared to CO_2 as the mobile phase, which resulted in detectable peaks (signal-to-noise ratio equal to 3) from 0.1–1 ng of injected hydrocarbons, the detection limit with N_2O was closer to 1 μg (injected). At present the actual reason for the reduced sensitivity is not known.

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