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Freeze-thaw flow management: a novel concept for high-performance liquid chromatography, capillary electrophoresis, electrochromatography and associated techniques

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Abstract

A new method for managing flow in capillaries or narrow channels is described. The fluid is made to act as its own shut-off valve by freezing the contents of a small section of the tube. In particular flow can thereby be diverted to further tube(s), forming the basis of an innovative flow switching device that requires no moving parts and contributes no dead volume. The principle of the method is demonstrated, and it is shown that full exploitation of the technique will require the development of junctions with very low dead volumes. Some advantages of this advance are discussed.

1. Introduction

Sequential chromatographic techniques are used widely and their advantages have been documented thoroughly [1]. These benefits derive primarily from the very high specificity and information content obtained with coupled orthogonal methods [2]. In addition to the sequential approach, coupled techniques such as liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–nuclear magnetic resonance spectroscopy (LC–NMR) have also contributed to the development of highly selective and sensitive analytical methods [3].

Advantages of performing separations in open tubular columns have been apparent for many years, and indeed capillary gas chromatography has been employed with great success for over three decades [4]. However, significant transfer of capillary technology to liquid chromatography and electrophoresis had to await the appropriate developments in instrumentation and in column chemistries. Many important advances have been made in the last decade and as a result, both capillary LC and capillary electrophoresis (CE) are well established.

Despite the successes of sequential chromatography and capillary separation science, the potential inherent in their combination has yet to be exploited fully. Again, considerable technological barriers impede further progress. One constraint is the design of conventional switching valve technology. The need to handle nanolitre volumes of analyte solutions imposes severe limitations on the specifications of switching devices. For example, the best commercial rotary valves have effective dead volumes no smaller than about 40 nl and it is unlikely that

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this type of design can furnish the order of magnitude scale of improvements that are necessary to maintain the resolution obtainable with capillary technology. Some aspects of this general problem have been encountered by several workers [5–10].

Lemmo and Jorgensen documented the severe limiting restrictions encountered when using a combined size exclusion–CE system [5] and have described the construction of a transverse flow gating interface that enables coupling of micro-column LC with CE [6].

Fraction collection at the micro- and nano-scales further highlights the problems associated with the management of very small volumes. Nashabeh et al. [7] designed a post-column multi-capillary device to address this issue.

Fluids used in electrophoresis can be directed into specified channels by voltage control and this principle has been applied in the design of miniaturised CE systems micro-machined on chips of silicon or glass [8–10]. Reduction of convective leakage in these devices requires independent control of the potential of all buffer reservoirs connected to the intersection. Furthermore, it may not always be convenient to control the potential at the end of each limb of an electrically driven sequential system. Hence alternative means of switching merit consideration.

In 1991, we were studying unnatural oligonucleotides that contained multiple chiral phosphoramidate bridges. In an extreme case a sample comprising a set of 512 possible diastereoisomers gave a broad envelope of incompletely resolved peaks when subjected to micellar electrokinetic chromatography (MEKC) [11,12]. The potential problems involved in the identification, isolation and quantification of any single one of these components prompted us to consider means of achieving higher resolution than that obtained by MEKC. This in turn prompted our interest in sequential CE.

We now introduce an idea that represents a radical departure from conventional switching using valves or voltage control. The fluid control method described in this report appears to be particularly well suited to solving the problems outlined above, and should also prove to have a widespread utility in areas other than separation science. We show that the idea of using the fluid as its own shut-off valve to control and divert flow can simply and elegantly be realised by freezing and thawing. The advantages of freeze-thaw control (FTC) and freeze-thaw switching (FTS) will be discussed. Integrated use of FTC and FTS is termed freeze-thaw flow management (FTFM).

2. Experimental

2.1. Apparatus

Electrophoresis was performed using an Applied Biosystems (ABI, Warrington, UK) Model 270A capillary electrophoresis system. The column compartment was normally air thermostatted at 30°C. Various voltages between 10 kV and 30 kV were employed. Samples were introduced by applying a vacuum of 127.0 mm Hg (ca. 16.9 kPa) to the cathodic end of the column. The UV spectrophotometric detector was used at wavelengths indicated in the text.

Polyvinylchloride (PVC) tubing (0.25 mm I.D. × 2.8 mm O.D) was obtained from Ormantine International (Winchester, UK). Fused-silica capillaries (720 mm × 50 μm I.D. × 375 μm O.D.) were obtained from ABI. New capillaries were conditioned by flushing for 30 min with 1.0 *M* sodium hydroxide, rinsing with deionised water for 10 min, 0.1 *M* sodium hydroxide for 30 min and the electrophoretic buffer for 30 min. Capillaries used for the analysis of fractions had an effective length of 500 mm from injection point to detector window. Capillary assemblies were constructed as indicated schematically in Fig. 1, using this tubing or UV-transparent 75 μm I.D. × 375 μm O.D. fused-silica tubing (Supelco, Bellefonte, PA, USA).

The individual silica limbs were connected using a 'Y'-piece of either fused-silica (Composite Metal Services, UK) or glass (Supelco) and cemented in place with epoxy resin glue. This construction resulted in an estimated total dead volume between the three capillary ends of about 200 nl. The assembly (Fig. 1) was installed

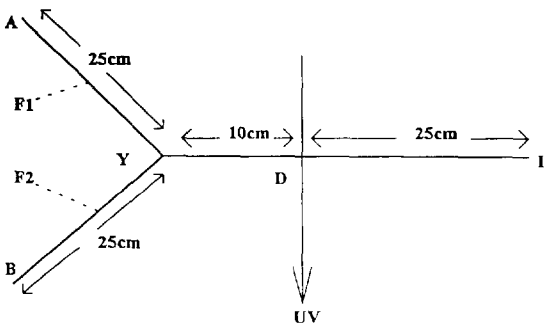

Fig. 1. Schematic diagram of the capillary assembly used to demonstrate FTS. I = Injection end (anode); A and B = cathodic limbs; D = detector; Y = Y-piece; F1 and F2 = freeze points, arbitrarily located approximately 10 cm from Y.

into the electrophoretic instrument such that both limbs AY and BY were dipped into the cathodic buffer and the detector D was located between the Y-piece and the anodic buffer at I. Freezing of capillary contents at points F1 and F2 was achieved by controlled direction of a carbon dioxide jet from a cylinder of the compressed liquid. The jet was a mixture of liquid and gaseous carbon dioxide fluids, and was delivered through 0.5 mm I.D. polyether ether ketone (PEEK) tubing.

2.2. Materials

Reagents used in preparation of buffers were of Analytical Reagent or Electrophoresis Purity grades. Crocein Orange G and *m*-Cresol Purple were obtained from Aldrich (Gillingham, UK). Reagents and chemicals were used without further purification. Water was purified by passage through an Elgastat Spectrum system (Elga, High Wycombe, UK). The electrophoretic buffer was 50 m*M* sodium dodecyl sulphate (SDS) in a solution obtained by adjusting the pH of 50 m*M* disodium tetraborate to 7.0 with 50 m*M* sodium dihydrogenphosphate. Buffers and rinsing solutions were filtered using disposable 0.45 μm syringe filter units (Anachem, Luton, UK) and degassed with helium before use.

3. Results and discussion

3.1. Freeze-thaw control

Hydraulically driven flow is usually controlled and switched by valves that require moving parts to block or divert the fluid. Valve components may contribute to dead volume and potentially be subject to abrasion and corrosion, resulting in leaks and contamination. These defects become worse as the internal diameter of the tube decreases. However, the advantages of using the flowing liquid as its own shut-off valve increase with decreasing tube I.D. because it becomes more practicable to rapidly freeze the contents of a short section of narrow tubing. The blockage produced may be expected to stop instantaneously all liquid flow inside tubing of suitable dimensions until thawing is allowed to occur. Managed cycles of freezing and thawing will control the presence or absence of flow in a narrow tube (FTC). It will be possible to divert or switch flow into specified tubes (FTS) by using FTC to block a tube or channel selected from within a system of interconnected tubes.

To test the feasibility of this idea, it was necessary to select an experimentally convenient device capable of rapidly freezing small flows of liquid. We chose to use liquid carbon dioxide to produce a fine spray of cold fluids. This cold spray was directed onto the outer wall of the tubing at the point where freezing was required. Clearly other methods, for example devices using thermoelectric means of heat transfer such as Peltier [13] devices, are also attractive. We were able almost instantaneously to stop hydraulically driven flows of water–acetonitrile (100:0 to 40:60 by volume) of up to at least 1.0 ml/min by using cold fluids sprayed from a nozzle of I.D. 0.5 mm, onto 1.6 mm O.D. stainless steel tubes having I.D.s of 0.7–1.1 mm. Smaller flows of up to about 90% (v/v) acetonitrile could also be halted, as could mixtures of up to about 20% (v/v) methanol in water. The technique is also applicable to fluids in fused-silica capillaries, but is less effective when used with tubes of PEEK, which is a good thermal insulator. The potential to halt flow can be

gauged by the fact that the temperature of the jet of fluids was found to be around −65°C. Using published [14] values of the respective thermal capacities of quartz and water of 0.74 J g^{-1} K^{-1} and 4.22 J g^{-1} K^{-1} (at 0°C), thermal conductivity of vitreous silica of 1.20 W m^{-1} K^{-1} (at −50°C), and enthalpy of fusion of water of 334 J g^{-1}, calculation shows that the 20 nl of water contained at 30°C in a 1 cm section of 50 μm I.D. × 375 μm O.D. fused-silica tubing will be frozen in a few tenths of a second by rapidly flowing carbon dioxide fluids at −65°C. The frozen plug of ice weighs about 20 μg and so only releases about 10 mJ on formation from water at 30°C. About 200 mJ more are required if it is assumed that the silica wall and ice cool to −65°C.

Freezing also proved effective when used to halt mass transport in electrically driven systems. Fig. 2 shows the trace produced by electrophoresis of a synthetic mixture in a 720 mm fused-silica column, through the detector zone and past a freeze point. After 5.9 min, the column contents at this point were frozen. The loss of current indicates the instantaneous formation of an insulating plug of ice. Freezing was maintained for 5 min, during which period the voltage was reversed. On subsequent thawing, rapid restoration of current was recorded, as was a 'mirror image' of the 'frozen' electropherogram of the components passing in reversed order through the detector zone. Very little evidence of diffusive resolution loss can be seen. Freezing halts the flow as effectively as does removal of electromotive force as illustrated in Fig. 3.

3.2. Freeze-thaw switching

We next performed a simple demonstration of the ability of FTC to switch analytes (FTS) to a second capillary via a fused-silica Y-piece (Fig. 1). First, a solution of Cresol Purple (10 mg/ml) was sampled into the assembly and allowed to electrophorese into limb IY for 1 min. Power was then turned off and limb BY was blocked by sleeving it with 0.25 mm I.D. PVC tubing plugged with copper wire. Section AYI was then flushed with electrophoretic buffer (ca. 10 μl) into an Eppendorf tube using gentle pressure from a syringe attached at A. The contents of the tube were blown gently to dryness and reconstituted in deionised water (10.0 μl). This solution served as a reference standard and was

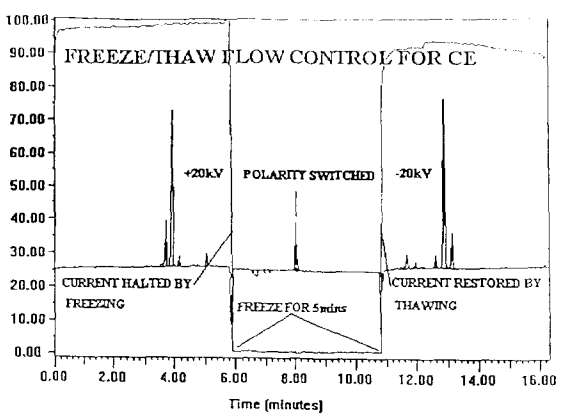

Fig. 2. A demonstration of the effect of freezing and thawing on the process of capillary electrophoresis. Sample: synthetic mixture in deionised water, vacuum injection (1.5 s). Column: 720 mm (500 mm to detector), 50 μm I.D. × 375 μm O.D. fused-silica. Electrolyte: 50 m*M* SDS in pH 7.0, 50 m*M* phosphate–borate buffer. Detection at 220 nm; response shown in arbitrary units. Voltage: between + and −20 kV as described in the text.

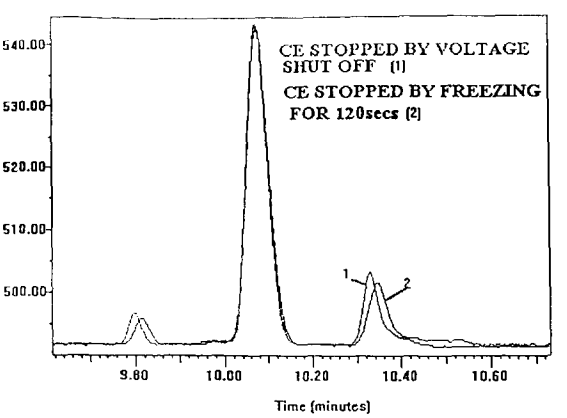

Fig. 3. Comparison of peak profiles produced when electrophoresis was halted for 120 s by (1) voltage shut-off and (2) freezing whilst maintaining −20 kV across the column. Sample and conditions as for Fig. 2. Response in arbitrary units. Note: migration time is 3 min shorter than in Fig. 2 because electrophoresis was interrupted for a correspondingly shorter time.

analysed (3.0 s injection, detection at 210 nm) using a 720 mm column operated at 30 kV as indicated above. To demonstrate FTS, a second aliquot of the Cresol Purple solution was sampled at I and electrophoresis allowed to continue until the sample zone was in section DY, when limb AY was frozen after 7.1 min with a jet of cold carbon dioxide fluids. Freezing was maintained until the sample zone was judged to be several centimetres into limb BY, when AY was allowed to thaw (t = 12.7 min). Fig. 4 shows the electropherogram obtained; note the reduction in current that occurred when only one of the limbs AY and BY (i.e. the latter) was open circuit. Limbs AY and BY were simultaneously flushed with electrophoretic buffer from a syringe at I into separate Eppendorf tubes at A and B. The contents of both tubes were dried, reconstituted and analysed as before. The experiment was repeated and the mean relative peak areas (reference standard peak area defined as 100%) were 10.4% in frozen limb AY and 64.5% in open limb BY. Thus 75% of the dye was recovered and had been split at Y with a ratio of 86:14. Although this result is a demonstration of the validity of the FTS concept, recovery was incomplete and totally clean switching was not obtained. Both these effects can be directly attributed to the use of a Y-piece of relatively large dead volume and with a potential for stagnant zones. It is difficult to take full advantage of having a shut-off valve (i.e. the frozen plug) that contributes no effective dead volume, because the internal volume of the Y-piece used (ca. 200 nl between column ends) is much greater than the volume occupied by a typical analyte zone.

Fig. 5 illustrates the peak broadening due to the Y-piece. This trace was produced by injection of a mixture of two dyes, Cresol Purple (1.0 mg/ml) and Crocein Orange (0.1 mg/ml) and allowing Cresol Purple to pass through Y into the limbs AY and BY. Power was turned off after 8.5 min, i.e. after the Crocein Orange zone had passed the detector but before it had reached Y. The voltage was reversed after 8.8 min and the power restored, driving the Crocein Orange zone back through the detector. The Cresol Purple peak shows gross broadening following two passages through the Y-piece. Note that on its second passage through the detector, the Crocein Orange peak had narrowed slightly and its area had decreased by about 7%. These observations both indicate a small Poiseuille flow towards I. After allowing

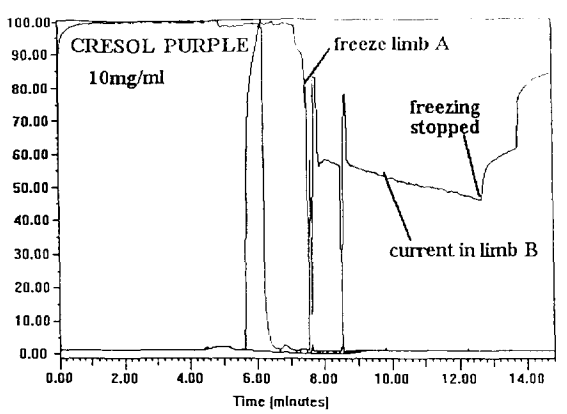

Fig. 4. Freeze-thaw switching. Sample: Cresol Purple, 10 mg/ml in deionised water, vacuum injection for 1.5 s. Column: construction shown in Fig. 1 (UV-transparent tubing and fused-silica Y-piece). Electrolyte as in Fig. 2. Detection at 300 nm; response in arbitrary units. Voltage: 17 kV applied continuously. Other details as given in the text.

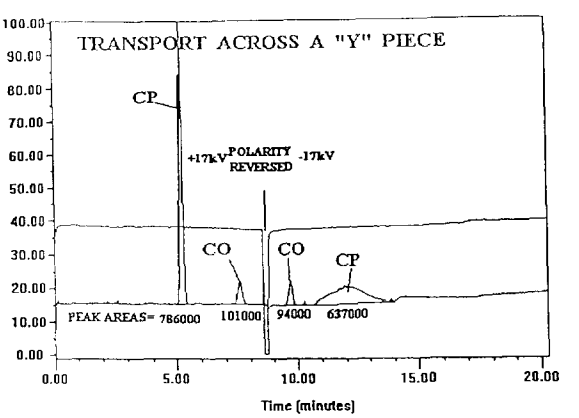

Fig. 5. The effect of the Y-piece on bandwidths and recoveries in the absence of freezing. Sample: Cresol Purple (1.0 mg/ml) (CP) and Crocein Orange (0.1 mg/ml) (CO) both in deionised water. Vacuum injection for 0.5 s. Column construction, electrolyte and detection as in Fig. 4. Voltage: 17 kV, reversed at 8.5–8.8 min. Response in arbitrary units.

for this effect, the loss of Cresol Purple in the Y-piece was estimated at 13%. This result confirms that the Y-piece caused substantial losses and peak broadening.

As expected, the experimental conditions employed here do not help to confine the analyte zone [15]. The Y-piece will cause more peak dispersion in hydraulically driven systems. Clearly, the design of the connector is critical. Conventional means of forming capillary junctions incur considerable void volume arising from the abuttal of three or more capillary walls (e.g. ca. 30 nl contained in the void between three tubes of 375 μm O.D. abutting at 120° to each other as shown in Fig. 6). We have therefore designed a connector of minimal void volume that we believe will help to realise fully the potential of FTS. This device is currently under development.

3.3. Advantages of freeze-thaw flow management

Controlling and switching flow in this manner affords a number of significant advantages of

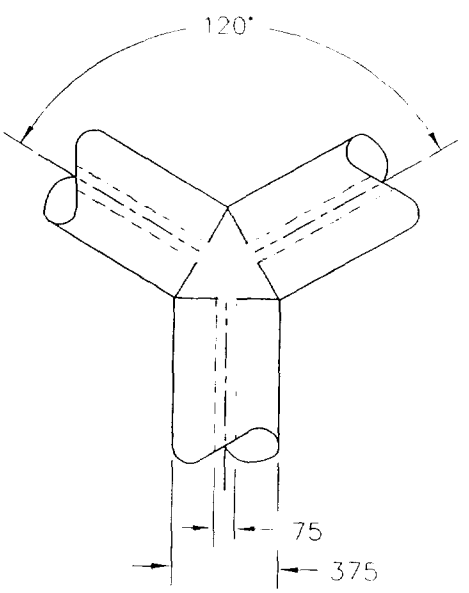

Fig. 6. Illustration of the void volume produced by abutting three columns at 120° to each other. Dimensions in μm.

relevance to micro-column separation technology:

(i) Freezing is extremely rapid due to the very small thermal capacities of the tubing and its contents. There is potential for 'fine-tuning' by automated remote control of both the flow of the cryogen and of warm gas for thawing.

(ii) Control is non-invasive. This is a direct consequence of using the liquid in the tubing as its own shut-off valve. Therefore there are no moving parts to contribute to peak dispersion arising from extra dead volume or turbulence. This is especially relevant in packed columns where there is inevitably extra void volume upstream of any conventional valve owing to the need for an in-situ sinter barrier. Similarly, FTFM should be suited to liquids containing suspended solids. Elimination of moving parts removes the possibility of the shut-off mechanism altering the chemical composition of the liquid stream, whether by mechanical abrasion, adsorptive losses and carry-over, contamination by valve components or lubricants, or by attack by corrosive liquids.

(iii) Carbon dioxide is economical, effective and safe to use, being both non-flammable and an electrical insulator. It can achieve temperatures of below −50°C on evaporation; if liquids freezing below this temperature are to be controlled, then suitable alternative cryogens could be substituted.

(iv) The FT principle has no inherent requirement for precision engineering and can be expected to work on any fluid that can be frozen.

(v) The shut-off unit (the frozen plug) has no extra-column connections and so external leakage at this point is impossible. With aqueous systems, the water will expand to ice and self-seal against any internal creep leakage. This sealing, which utilises friction between the plug and the internal wall, will become increasingly effective with decreasing column I.D.

(vi) Column coatings should remain unaffected by FTFM.

(vii) FTFM offers further advantages when the liquid is driven electrically. During the separation process, electroosmosis and ion transport are maintained across what is essentially a shut-

off valve in the open configuration. There is no electrical contact between column and switch, so the high voltages cannot be earthed through the switch. Additionally, the insulating properties of carbon dioxide and ice should be noted; the former maintains safety and the latter instantaneously halts electroosmosis and electrophoresis in the column both upstream of and downstream from the freeze point The minute size of the ice plug ensures rapid thawing with concomitant restoration of current.

There would appear to be considerable scope for the application of FTFM to the separation sciences. Briefly, any valve in conventional chromatographic and electrophoretic systems is in principle replaceable with an FTS device in the corresponding miniaturised systems. We believe the technique is applicable to many areas and therefore we demonstrated its principles by construction of an elementary switching device. FTC and FTS should facilitate heart-cutting, sequential and coupled techniques, in-line reactions and derivatisation schemes, and preparative applications on a miniaturised scale. The latter would be achieved by switching fractions into the desired limb which would then function as a microstorage vessel. A particular area that interests us is the potential of the technique for expanding the range of mobile phases and buffers compatible with a mass spectrometer. An example of this would be an in-line micellar CE–MS interface. Such a device is currently under development. FTFM becomes increasingly attractive with diminishing column dimensions. Therefore we anticipate use of this technology in the design of miniaturised separation systems such as CE devices incorporated within single plastic [16], silicon or glass [8–10] micro-chips. Electrothermal (e.g. Peltier) cooling is an attractive possibility in this context.

FTFM should also find applications in other areas of analytical chemistry (for example flow injection analysis) and in technologies where there is a requirement for remote control of fluids in narrow channels.

We have not attempted to define the practical limitations of this approach since clearly these will depend on the sizes, flows and physical properties of the materials used. Whilst in principle it is possible to freeze eluant from a 50 mm I.D. preparative column for example, there is in this case no advantage in replacing a conventional valve with an FTFM device. On the other hand, FTS would seem to be a method of choice for sequential micro-column separations.

4. Conclusions

We have introduced here a novel means of managing fluid flow in narrow tubes and channels. The method, termed freeze-thaw flow management, appears to have wide applicability. This paper has demonstrated freeze-thaw control and freeze-thaw switching, outlined some of their advantages, and discussed some potential applications. FTFM is an advance applicable not only to micro-liquid chromatography, electrochromatography, capillary electrophoresis, sequential and coupled techniques, but also to other areas of science and technology.

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