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Parallel segmented outlet flow high performance liquid chromatography with multiplexed detection

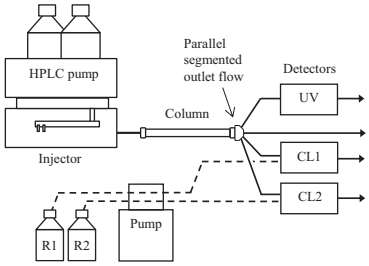
Michelle Camenzuli[a], Jessica M. Terry[b], R. Andrew Shalliker[a,**], Xavier A. Conlan[b], Neil W. Barnett[b], Paul S. Francis[b,*]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Science and Health, University of Western Sydney (Parramatta), Sydney, NSW, Australia
[b] Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Deakin University, Geelong, Victoria 3216, Australia

HIGHLIGHTS

- Multiplexed detection for liquid chromatography.
- 'Parallel segmented outlet flow' distributes inner and outer portions of the analyte zone.
- Three detectors were used simultaneously for the determination of opiate alkaloids.

GRAPHICAL ABSTRACT



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ABSTRACT

We describe a new approach to multiplex detection for HPLC, exploiting parallel segmented outlet flow – a new column technology that provides pressure-regulated control of eluate flow through multiple outlet channels, which minimises the additional dead volume associated with conventional post-column flow splitting. Using three detectors: one UV-absorbance and two chemiluminescence systems (tris(2,2′-bipyridine)ruthenium(III) and permanganate), we examine the relative responses for six opium poppy (*Papaver somniferum*) alkaloids under conventional and multiplexed conditions, where approximately 30% of the eluate was distributed to each detector and the remaining solution directed to a collection vessel. The parallel segmented outlet flow mode of operation offers advantages in terms of solvent consumption, waste generation, total analysis time and solute band volume when applying multiple detectors to HPLC, but the manner in which each detection system is influenced by changes in solute concentration and solution flow rates must be carefully considered.

1. Introduction

The scope of liquid chromatography as an analytical tool depends on the capabilities of both separation and detection [1]. Innovation in stationary phase technologies [2–5] and the application of multidimensional separations [6–9] continue to expand the limits of chromatographic selectivity and separation power, but further advances in the speed and comprehensiveness of analysis can be achieved by combining multiple modes of detection with distinct selectivities. Moreover, this approach enables quantitation to be combined with assessments of the molecular structure [10,11] or (bio)activity [12,13] of individual sample components. Non-destructive detectors (e.g. absorbance or fluorescence) can be connected in series with a single destructive mode of detection (such as mass spectrometry [14] or chemiluminescence [15]). However, each successive detector suffers from the cumulative band broadening imparted by all previous components and the extra column connections must be considered in order to correctly align

* Corresponding author. Tel.: +61 3 5227 1294; fax: +61 3 5227 1040.
** Corresponding author. Tel.: +61 2 9685 9951; fax: +61 2 9685 9915.
E-mail addresses: r.shalliker@uws.edu.au (R.A. Shalliker), paul.francis@deakin.edu.au, psf@deakin.edu.au (P.S. Francis).

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the chromatographic response. Alternatively, by splitting the post-column stream using a T-piece [13], several modes of detection can be performed in parallel at the expense of greater sample dilution and extra-column band broadening.

We have recently described a new column technology that separates the mobile phase eluting from the radial centre of the chromatographic bed from that eluting near the column wall [16,17]. The new design comprises an annular frit located at the column outlet inside a multi-port outlet end fitting. The frit has a solid PEEK ring that divides two permeable frit zones (central and peripheral) which direct the flow to either a single central outlet, or three peripheral exit ports depending on the region traversed by the mobile phase. The ratio of flow (central/peripheral) can be controlled by the relative pressure at each port. This 'parallel segmented outlet flow' has several major benefits: (1) the portion of each solute that has migrated through the more efficient central region of the column is separated from the trailing portion in the peripheral zone that is influenced by the wall effect. As a result, solutes that exit the central port elute with a higher number of theoretical plates compared to the band as a whole, producing narrower bands [17,18]; (2) without dilution from the peripheral zone, the leading portion of solute from the central region is more concentrated, which can improve detection sensitivity [17,18]; and (3) flow-limited detectors such as mass spectrometers can be employed in conjunction with high separation flow rates without the losses in sensitivity and efficiency associated with traditional post-column stream splitting [18].

To date, parallel segmented outlet flow columns have been used with a UV-absorbance or mass spectrometry detector connected through the central outlet port, with the solution from the peripheral region either directed to a collection vessel or monitored with a second UV-absorbance detector. However, the ability to control the solution flow through four separate outlets, without additional extra-column dead volume or post-column flow splitters, offers great potential for the incorporation of multiple detection systems, particularly considering that the portion of solute eluting from the peripheral region exhibits less on-column band broadening than the solute in its entirety [19]. In this paper, we explore the use of parallel segmented outlet flow for multiplexed detection, using the determination of six opium poppy (*Papaver somniferum*) alkaloids as a model system that exploits the complementary selectivity of two widely used chemiluminescence reagents [20–22]. Coupling these reagent systems is of interest in applications such as monitoring the extraction of opiate alkaloids from poppy straw [23,24], and rapid screening for heroin in suspected drug samples [25–27].

2. Materials and methods

2.1. Chemicals and reagents

Deionised water and analytical grade reagents were used unless otherwise stated. Chemicals were obtained from the following sources: sodium polyphosphate (+80 mesh) and trifluoroacetic acid from Sigma–Aldrich (NSW, Australia); sodium thiosulfate from Fluka (NSW, Australia); potassium permanganate from ChemSupply (SA, Australia); lead dioxide and sodium perchlorate from Ajax Finechem (NSW, Australia); methanol and sulfuric acid from Merck (Vic., Australia); glacial acetic acid and perchloric acid (70%, w/v) from Univar (NSW, Australia); acetonitrile from Burdick & Jackson (MI, USA) and tris(2,2′-bipyridine)ruthenium(II) dichloride hexahydrate from Strem Chemicals (MN, USA). Heroin (3,6-diacetylmorphine), codeine, morphine, oripavine, papaverine and thebaine were provided by GlaxoSmithKline (Vic., Australia). Stock solutions of the opiate alkaloids (1 mM) were prepared in acidified deionised water. Heroin (1 mM) was prepared in 0.1% (v/v) acetic acid and diluted in 0.05% (v/v) acetic acid.

The permanganate reagent was prepared by dissolving potassium permanganate (1.9 mM) in 1% (m/v) sodium polyphosphate, adjusting to pH 2.5 with sulfuric acid, and then adding sodium thiosulfate (0.6 mM), using a small volume of a 0.1 M solution [28]. The tris(2,2′-bipyridine)ruthenium(III) reagent was prepared by treating $[Ru(bipy)_3]Cl_2$ with sodium perchlorate in aqueous solution to yield a bright orange $[Ru(bipy)_3](ClO_4)_2$ precipitate, which was collected by vacuum filtration, washed twice with ice water, and dried over phosphorus pentoxide for 24 h [29]. The $[Ru(bipy)_3](ClO_4)_2$ crystals (1 mM) were then oxidised with lead dioxide (0.2 g/100 mL) in acetonitrile containing 0.05 M perchloric acid, which was observed as a change in the colour of the solution from orange to blue-green. The excess solid oxidant left in the reagent was prevented from entering the chemiluminescence detector by a filter (consisting of a small Pasteur pipette packed tightly with glass wool) fitted to the end of the tubing in the reagent reservoir.

2.2. High performance liquid chromatography (HPLC)

Analyses were carried out on an Agilent Technologies 1200 series liquid chromatography system, equipped with a quaternary pump, solvent degasser system and autosampler, using a reversed phase Hypersil GOLD chromatography column (100 mm × 4.6 mm i.d., 5 μm, ThermoFisher Scientific, Cheshire, UK), with an injection volume of 20 μL, flow rate of 2.5 mL min^{-1}, and gradient elution using deionised water adjusted to pH 2.5 with trifluoroacetic acid (solvent A) and methanol (solvent B) as follows: 0–1 min: 5–10% B, 1–2 min: 10–25% B, 2–6 min: 25–35% B, 6–6.5 min: 35% B, 6.5–8 min: 35–5% B, 8–10 min: 5% B. Solvents and sample solutions were filtered through a 0.45 μm nylon membrane.

2.3. Conventional detection

Separations were conducted with the column connected to each detection system individually. This included UV-absorbance at 280 nm (G1314A variable wavelength detector with standard flow-cell; 10 mm path length, 14 μL volume; Agilent Technologies), and two chemiluminescence detectors with permanganate and $[Ru(bipy)_3]^{3+}$ reagents, in which the column eluate (2.5 mL min^{-1}) and reagent (1.0 mL min^{-1}) merged immediately prior to or within the detection zone of the respective flow-cell (Fig. 1a). For the experiments with the permanganate reagent, we used a GloCel detector (Global FIA, WA, USA) with dual-inlet serpentine-channel flow-cell (fabricated from Teflon impregnated with glass microspheres) [30,31]. For experiments with the $[Ru(bipy)_3]^{3+}$ reagent, we used an in-house fabricated detector comprising a coil of transparent PTFE-PFA tubing (0.8 mm i.d.) that was glued into a spiral channel in an aluminium plate [31]. The flow-cell was mounted against an extended range photomultiplier module (Electron Tubes model P30A-05; ETP, NSW, Australia) within a light-tight housing. All tubing entering and exiting the detector was black PTFE (0.76 mm i.d., Global FIA).

2.4. Multiplexed detection

Using the parallel segmented outlet flow column end-fitting (ThermoFisher Scientific), the column eluate (2.5 mL min^{-1}) was divided in the following manner: 30% (0.74 mL min^{-1}) was directed to the first chemiluminescence detector (permanganate reagent) via peripheral port 1; 30% (0.76 mL min^{-1}) to the second chemiluminescence detector ($[Ru(bipy)_3]^{3+}$ reagent) via peripheral port 2; 27% (0.68 mL min^{-1}) to the UV-absorbance detector via peripheral port 3; and the remaining 13% (0.32 mL min^{-1}) to a collection vessel via the central port (Fig. 1b). The chemiluminescence