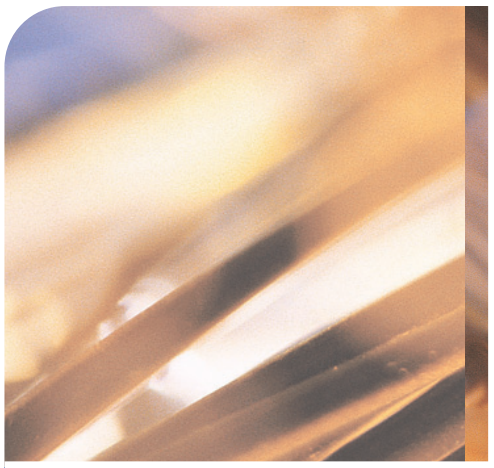


Spectroscopy Focus

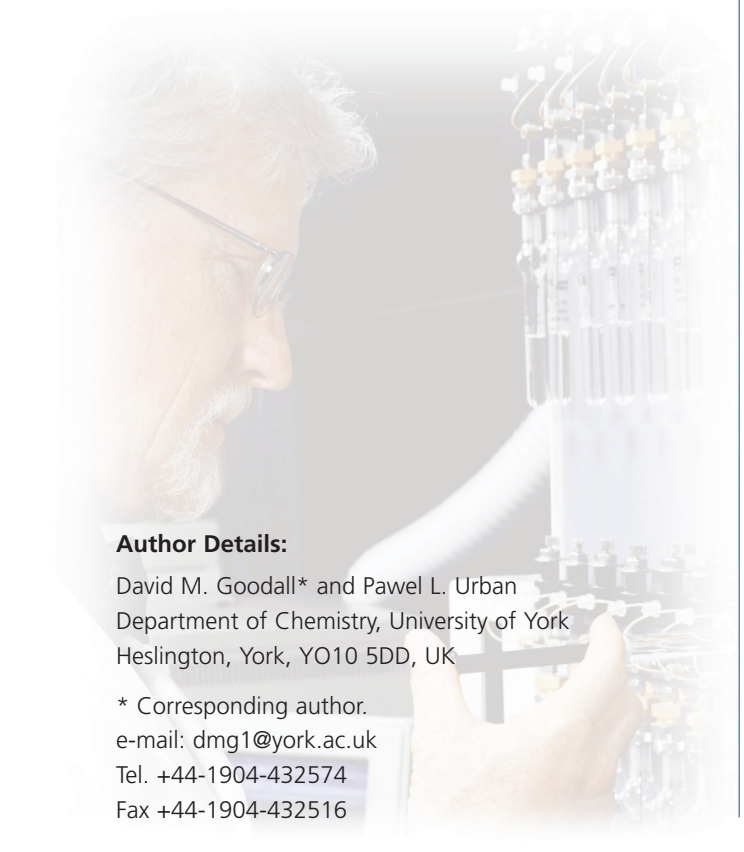
LAB-ON-CAPILLARY: A VERSATILE FORMAT FOR NANOLITRE SCALE CHEMISTRY AND BIOCHEMISTRY

David M. Goodall* and Pawel L. Urban

In the past 20 years, lab-on-a-chip and micro total analysis systems have opened the way for miniaturization in analytical science.

The aim of this article is to show how separations in capillaries can be readily integrated with other in-capillary processes such as mixing and reaction, laying the foundations for a versatile and widely applicable lab-on-capillary format.

"THIS METHOD CAN ALSO BE USED IN SCREENING ENZYME SPECIFICITY AGAINST A RANGE OF SUBSTRATES."



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BENEFITS OF IN-CAPILLARY PROCESSES

Numerous procedures can be carried out in a capillary format. We are particularly interested in combining reaction, separation and detection since many applications of interest to chemists and biochemists involve complex mixtures of reactants and products.

Attractive features include being able to adapt readily available components in assembling integrated systems, and carrying out reactions on a nanoscale with low consumption of expensive materials such as enzymes. Various steps of analytical protocols that can be combined with a lab-on-capillary platform are summarized in *Table 1*.

Table 1. Processes for lab-on-capillary platform.

Process	Method
Injection	Loop switching Hydrodynamic or electrokinetic
Preconcentration	Stacking, sweeping Solid phase extraction (SPE)
Splitting/combining streams	Y or cross connectors Manifolds Microfluidic valves
Mixing	Diffusion Turbulent mixers Electrically driven mixing Pressure shuttle
Reaction	Heterogeneous: flow through reactor bed or coated tube Homogeneous: Incubation of reactants after mixing Photochemical: UV irradiation
Separation	Nanobore liquid chromatography (nanoLC) Capillary electrophoresis (CE)
Detection	On capillary: UV-Vis, fluorescence Off capillary: MS
Fraction collection	Output stream deposition, e.g. onto 2D MALDI plate

Capillaries are typically made of fused silica and their dimensions are in a range 20 to 500 µm i.d. Originally introduced for gas chromatography, polyimide coated fused silica capillaries are now used in many analytical procedures. Other materials used include PTFE, quartz and glass.

EXAMPLES

In recent work a range of lab-on-capillary approaches have been developed for carrying out low volume enzyme assays involving in-line mixing and reaction, electrophoretic separation of products from reactants and enzyme, and detection.

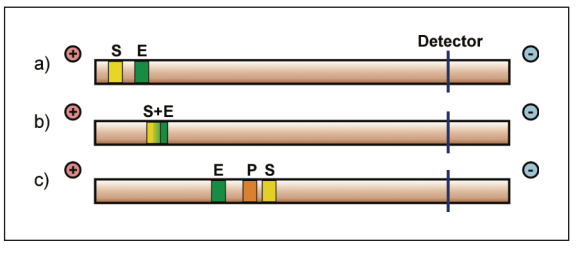

Figure 1. Electrophoretically mediated microanalysis (EMMA): homogeneous reaction/separation sequence using mobility differences. (a) Sandwich injection of reactants, (b) zone overlap, (c) separation of reactant and product.

In the plug-plug mode of electrophoretically mediated microanalysis (EMMA), represented in *Figure 1*, nanolitre samples of reactant and enzyme solutions are injected into a capillary, reaction occurs when the plugs cross each other on application of voltage, and separation of products and unreacted substrates follows. The key advantage of this application is that capillaries can be multiplexed, i.e. several capillaries can be used at the same time. In the simplest parallel arrangement, with two capillaries (*Figure 2a*), one capillary is used for the reaction and the other one without enzyme is used as reference.

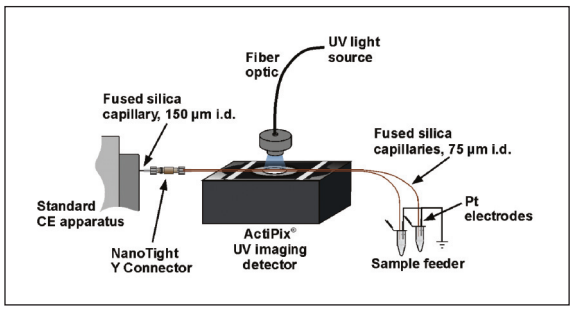

Figure 2a. Parallel capillary electrophoresis: coupling of two capillaries to a commercial CE system using a low volume Y-connector (Upchurch)

By comparing the two electropherograms in a single run (*Figure 2b*) one can easily evaluate substrate conversion. This method can also be used in screening enzyme specificity against a range of substrates.

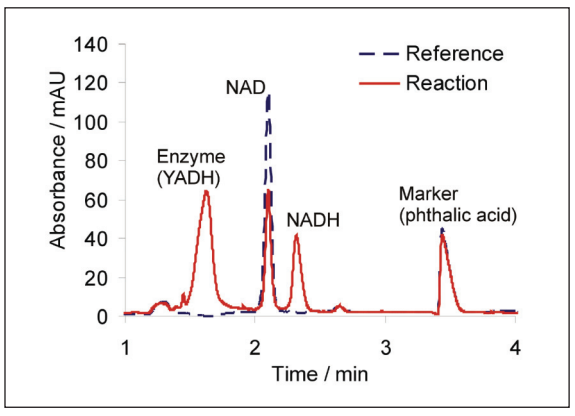

Figure 2b. On-capillary electrophoretic enzyme assay: oxidation of ithanol catalyzed by yeast alcohol dehydrogenase (YADH) involving the cofactor NAD which is reduced to NADH. One capillary used as reactor and the other for reference.

A mixture of potential substrates is injected into both capillaries while the enzyme solution is injected into the reaction capillary only. Comparison of two sets of electropherograms highlights compounds amenable to the biotransformation studied. Other on-capillary methodologies implemented in biochemical assays include electroinjection analysis (EIA), where both components are injected from opposite ends of a capillary. An alternative method to mix plugs of reactants injected into a capillary is to use a sequence of pressure reversals; a "pressure shuttle". This approach does not require differences of electrophoretic mobilities to bring the species together, hence it can be used as a generic tool in mixing. A well-established method for initiating reactions uses continuous or segmented flow where reactant streams are brought together via Y connectors.

Multiplexed detection of reactions and separations in parallel and looped capillaries can be carried out using the ActiPix® D100 UV imaging detector recently commercialized by Paraytec Ltd (*Figure 3*).

Figure 3. Miniature capillary UV imaging detector (ActiPix® D100) for lab-on-capillary applications. Courtesy of Paraytec Ltd.

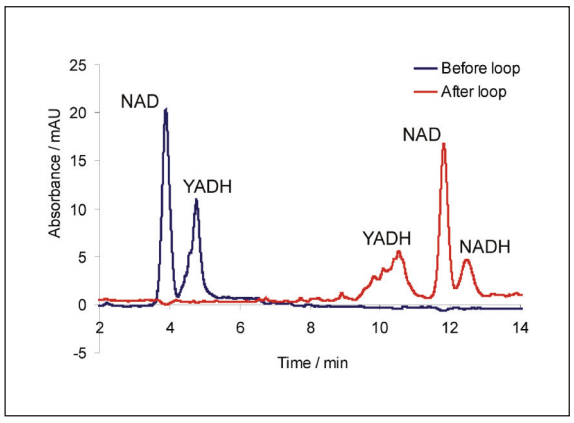

Figure 4. Electropherograms obtained in the single run, before and after the reaction loop, showing conversion of NAD to NADH in the process catalyzed by yeast alcohol dehydrogenase (YADH).

This images an area rather than a line as is characteristic of diode array detectors and can be used with any capillary system, e.g. CE, nano liquid chromatography (nanoLC), micro flow injection analysis (μFIA). A simple way of driving flow in multiplexed capillaries is via a single capillary using a low dead volume manifold as interconnect.

With such a set-up, different concentrations of an enzyme can be assayed in parallel, increasing throughput and work efficiency. In a study with alcohol dehydrogenase (*c.f. Figure 2*) we have also demonstrated an example of CE carried out in a loop. In this arrangement the substrates were visualized before and after reaction, which occurs in the loop, in a double pass through the ActiPix® D100 detector (*Figure 4*). This simple miniaturised detector allows for efficient on-line monitoring of the reaction.

Lab-on-capillary methods can readily be used with reactions catalyzed by immobilized enzymes. Biocatalysts can be attached to the surface of the capillary wall or onto beads packed into it. An applications example is for protein digestion prior to separation and this approach has already been used in several proteomic studies.

In this case MS detection at the end of capillary is also advantageous (see *Table 1*) and allows for rapid identification of the analyzed peptides. A similar protocol has also been used in the screening of inhibitors of an enzyme immobilized in situ by adsorption on a modified capillary wall (*Figure 5*).

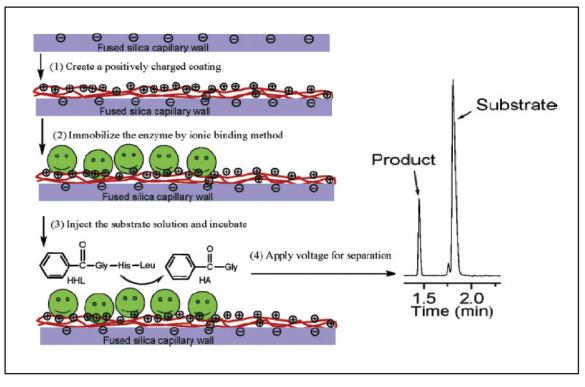

Figure 5. High throughput screening of inhibitors. Schematic representation of on-column immobilized enzyme microreactor prepared by an ionic binding method. Reprinted with permission from Z. Tang and J. Kang, Anal. Chem. 2006, 78, 2514. Copyright (2006) American Chemical Society.

Such on-capillary protocols have great potential for early stage screening in drug discovery. Whilst the previous example uses an electrophoretic separation, reaction and separation can be initiated and carried out using pressure-driven flow of eluent and LC separation of the products of the reaction, as shown schematically in *Figure 6*.

Such a duplex arrangement makes use of hyphenation between a packed reactor and a packed column. This can be achieved using low or zero dead volume connectors, which in some cases can be made in-house using pieces of PTFE tubing with suitable inner diameter.

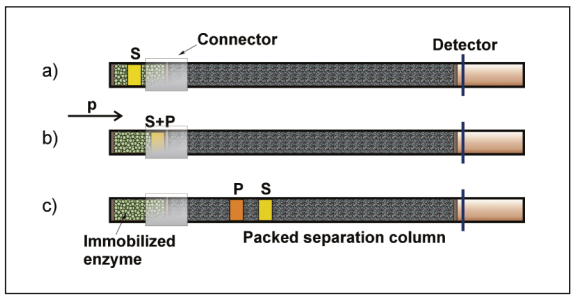

Figure 6. Pressure-driven reaction and separation. Sequence of flow through a packed reactor followed by a packed-bed separation stage: (a) substrate plug injected into the immobilized enzyme zone, (b) pressure-driven flow – substrate/product zone moving towards the separation column, (c) LC separation of substrate and product. PTFE tubing used as zero dead volume connector.

Figure 7. Microfluidic proteomic reactor for on-line protein concentration, reduction, alkylation and digestion. (M. Ethier et al., J. Proteome Res. 2006, 5, 2754; picture from: J. Proteome Res. 2006, 5, 2750) Courtesy of Daniel Figeys.

An inexpensive capillary-based microfluidic proteomic reactor (*Figure 7*) has recently been reported for on-line protein concentration, reduction, alkylation and digestion. The proteins and trypsin are extracted from solution at low pH onto particles of strong cation exchange resin, then reduced and alkylated; they are subsequently released from the resin following increase of pH, and the activated trypsin digests the proteins. The total volume of the device is approximately 50 nl and the low cost (~$1) allows the proteomic capillary reactor to be a disposable item. The reactor can be coupled via a frit with another capillary used for cell loading and lysis, allowing separation of proteins from cell debris prior to preconcentration of proteins and digestion.

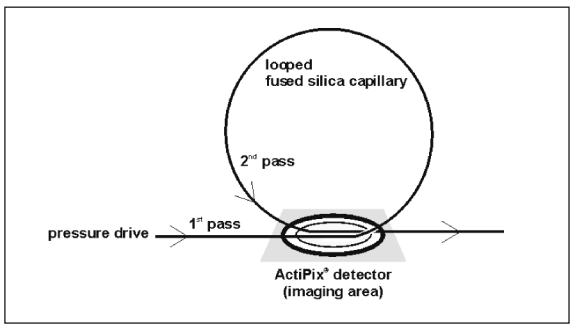

Figure 8a. Capillary loop arrangement for simultaneous UV quantification and sizing.

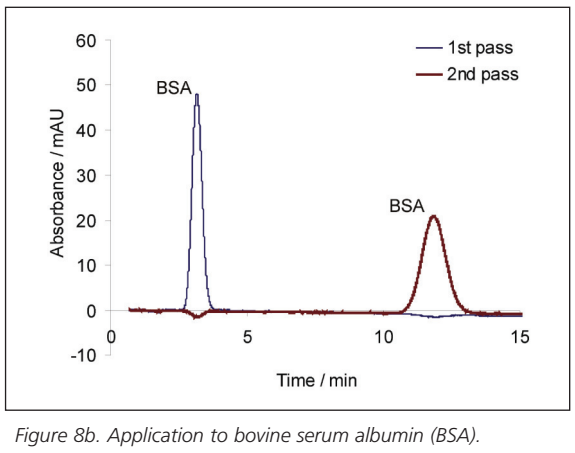

Figure 8b. Application to bovine serum albumin (BSA).

Another application of a capillary loop is sizing of proteins. In this mode one injects a sample hydrodynamically and uses pressure to drive it through both detection windows (*Figure 8a*). Due to the Taylor dispersion and diffusion taking place during the passage of the analyte plug in the loop, the analyte zone gets wider (*Figure 8b*). Since the magnitude of this additional band broadening is inversely proportional to the square root of the diffusion coefficient, and the diffusion coefficient is inversely proportional to the hydrodynamic radius, this approach provides a simple way of measuring the size of molecules using nanolitre amounts of samples. Sizing in this way is equally applicable to small and large molecules, unlike dynamic light scattering. The method is particularly well suited to be used in a sequence of steps in a lab-on-capillary assembly, for example (i) reaction and (ii) separation (*Figures 1 or 6*) followed by (iii) sizing (*Figure 8a*), and allows simultaneous sizing and quantification (via UV peak area) of all separated components in a nanolitre sample.

CONCLUDING REMARKS

We have been working on a range of novel applications for low-volume enzyme assays which could be used for rapid and cost-effective evaluation of biocatalytic activity and characterization of biochemical samples. *Table 2* compares lab-on-capillary with the lab-on-a-chip format, and shows its huge utility. Unlike channels in chips, capillaries have well defined cross section over the long lengths required to provide high resolution separations of complex mixtures, e.g. in proteomics. Furthermore, lab-on-a-chip devices are difficult to fabricate and not generally amenable to direct UV detection of reactants or products.

Table 2. Comparison of lab-on-capillary with lab-on-a-chip format.

	Lab-on-capillary	Lab-on-a-chip
Volume	nanolitres	nanolitres
Parallel runs	standard	possible
Detection	numerous on-line and off-line; UV absorbance down to 190 nm	different modes, on- and off-line, normally fluorescence; UV < 240 nm not possible on PDMS
Availability and ease of use	readily assembled from inexpensive components	tailor-made for certain applications, e.g. protein & DNA characterization
Capital cost of associated instrumentation	low to moderate	moderate to high
Running cost	low	low

Our approach uses readily-available fused silica capillaries and connectors, together with a novel UV area array detector to visualise the reactions. While the lab-on-a-chip platform offers miniaturization of many procedures carried out using standard formats, it is still expensive and there are considerable obstacles preventing widespread implementation. By contrast, manufacture of capillary reactors can be carried out in any laboratory and no clean room is required. Lab-on-a-chip devices are sometimes difficult to interface to other components in a total system, whereas lab-on-capillary sections can be easily assembled and integrated with other devices (pumps, power supplies, detectors) using commercially-available adaptors and fittings.

Standard protocols already available for steps such as separations can then be applied to the assembled lab-on-capillary system. One thing that requires attention is the quality of the capillary connectors which should always have low dead volume to assure low band dispersion. In conclusion, we believe lab-on-capillary offers many benefits of sequential processes in a nanoscale format without the costs and complications of lab-on-a-chip, and that the approach could have significant impact in nanoscale chemistry and biochemistry.

ACKNOWLEDGEMENTS

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