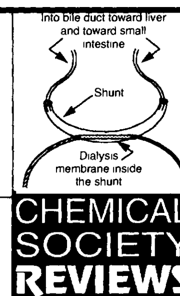
Microdialysis sampling coupled on-line to microseparation techniques



CHEMICAL SOCIETY REVIEWS

Malonne I. Davies[a] **and Craig E. Lunte**[b]

[a] *Bioanalytical Systems Kansas Research Laboratory, University of Kansas, Lawrence, KS, USA*
[b] *Department of Chemistry and the Center for Bioanalytical Research, University of Kansas, Lawrence, KS 66045-0046, USA*

Microdialysis sampling is a powerful tool for continuously monitoring the extracellular concentration of compounds in tissues *in vivo.* In order to fully utilize the high temporal resolution but small sample volume of the microdialysis technique, several approaches to coupling microdialysis sampling on-line to microseparation techniques have been developed. This article will describe the analytical challenges of microdialysis sampling. Direct coupling of the microdialysis system to the analytical system can provide many benefits. The application of on-line microseparation techniques will be reviewed.

1 The process of microdialysis sampling

Microdialysis sampling is a powerful technique for investigating biochemical events in the extracellular fluid of virtually any tissue, organ or biological fluid. Development of the technique from a long-term dialysis sac implantation[1] through push-pull cannulas[2] to its present form as a continuous sampling technique[3] was accomplished largely by researchers in the neurosciences. Today, microdialysis sampling is a standard technique in the neurosciences and tertiary and secondary literature has begun to appear in the form of books and reviews.[4–8] The success of the technique in the study of neurotransmitter release has led to the extension of microdialysis for general pharmacokinetic, toxicology and ADME studies. Reviews focusing on the use of microdialysis sampling in these area have also been published.[9–11]

Microdialysis sampling is accomplished by implanting a probe consisting of a hollow fibre dialysis membrane into the organ or biological fluid of interest. The short length of dialysis fibre is affixed to pieces of narrow bore tubing which serve as inlet and outlet tubes. A solution, termed the perfusate, is pumped slowly through the probe. The perfusate is an aqueous solution which closely matches the ionic composition and pH of the surrounding sample matrix. For sampling *in vivo* from tissue, the sample matrix is the extracellular fluid (ECF). When the perfusate is correctly matched to the sample matrix, there should be no net exchange of ions across the membrane.

Microdialysis is a diffusion controlled process. The perfusion rate through the probe is generally in the range 0.5–5.0 μl min^{-1}. At this flow rate there is no net transport of liquid across the dialysis membrane. The driving force for mass transport is then the concentration gradient existing between the ECF and the fluid in the probe lumen. Low molecular mass compounds, such as analytes of interest, diffuse into (recovery) or out of (delivery) the probe lumen. Large molecules such as proteins and small molecules bound to proteins are excluded by the membrane. A diagram of the microdialysis process is shown in Fig. 1. Those molecules entering the lumen of the membrane are swept along by the perfusate and exit the probe. The solution leaving the probe, called the dialysate, is collected for analysis.

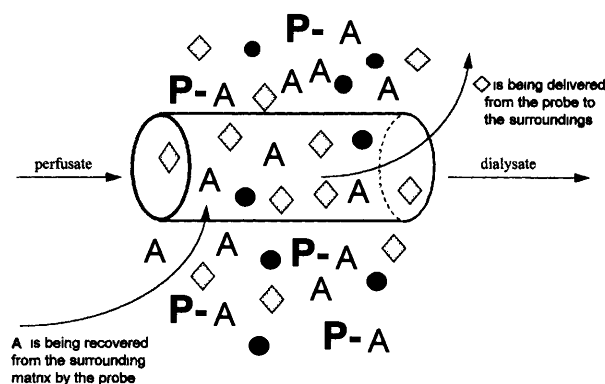
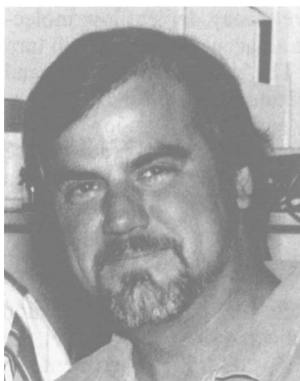


Fig. 1 Diagram of the microdialysis process. **A** represents the analyte of interest in the surrounding medium. **P–A** represents analyte bound to protein and excluded by the membrane. ♦ is a small molecule in higher concentration in the perfusate solution than in the surroundings and ● represents another compound present in both.



Craig E. Lunte is Professor of Analytical Chemistry at the University of Kansas, Lawrence, Kansas, USA. Professor Lunte received the BS in Chemistry at the University of Missouri–Rolla in 1979 and the PhD in Analytical Chemistry at Purdue University in 1984. His research interest is in bioanalytical chemistry, with projects in microdialysis sampling, electrochemistry, capillary electrophoresis and microbore liquid chromatography.

Craig E. Lunte

Malonne I. Davies is a Senior Scientist with Bioanalytical Systems, Inc., West Lafayette, Indiana, USA. She holds a BS degree in Biology and MS degrees in Biology and Chemistry from Emporia State University and a PhD in Chemistry from the University of Kansas. She is currently the Manager of the BAS-Kansas Research and Development Laboratory in Lawrence, Kansas. Her research focuses on in vivo *applications of microdialysis.*

Malonne I. Davies

1.1 Advantages of microdialysis sampling

Microdialysis samples represent a local profile of low molecular mass hydrophilic substances in the matrix surrounding the probe. The sampling process excludes small molecules bound to proteins. For compounds of pharmaceutical interest, the dialysate reflects the free fraction of the compound of interest, that is, the therapeutically active portion of the dose. Enzymes are excluded from the dialysate sample so there will be no further enzymatic degradation of the sample. The small size of the dialysis membrane, nominally 300 μm outer diameter (o.d.) and 4–10 mm in length, causes minimum perturbation to the tissue. The technique can be used in awake animals allowing the integrity of tissues, organs and systems to be maintained. Several different probe geometries have been developed to facilitate *in vivo* sampling from various sites. The diagrams in Fig. 2 illustrate the general probe geometries commonly used.

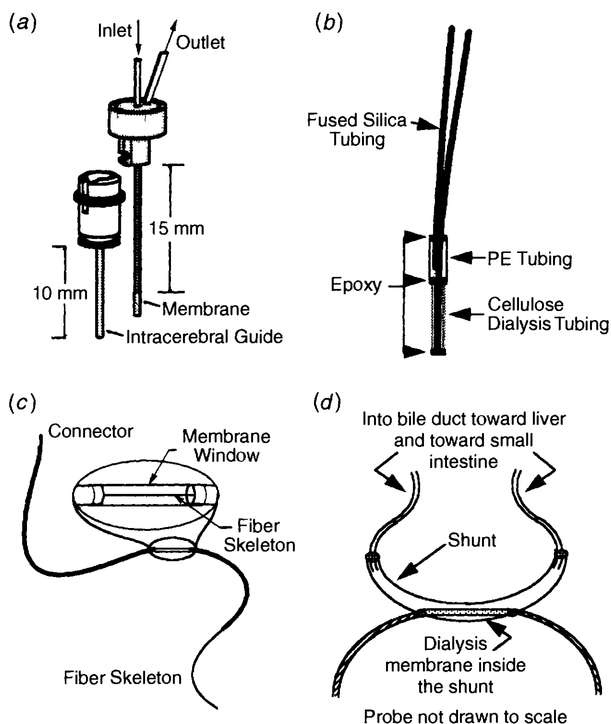


Fig. 2 Typical microdialysis probe geometries.(*a*) Rigid cannula probe typically used for brain microdialysis. (*b*) Flexible cannula probe for implantation in a blood vessel of the rat. (*c*) Linear probe especially suited for peripheral tissue such as skin, muscle or liver. Loop probes, intended for *in vitro* or subcutaneous sampling, are essentially linear probes with longer membrane windows. (*d*) Shunt or by-pass probe consists of a linear probe inside a larger tube and is used for sampling from the bile duct of a rat.

Since there is no net fluid loss, samples can be collected continuously for hours or days in one animal. The microdialysis probe acts as an artificial blood vessel in that it can both deliver and remove compounds from the local area. Delivery of the parent compound *via* the probe allows study of local metabolism without systemic involvement. Since the membrane excludes proteins and other macromolecules, the dialysate can usually be analysed without further sample clean-up. Each animal serves as its own control and the number of experimental animals needed is reduced.

1.2 Limitations of microdialysis sampling

While the small size of the microdialysis probe causes minimal perturbation to the tissue, the surgery to implant the probe is invasive. The experimental animals are anaesthetized during probe implantation and the anatomical location of the target tissue dictates the duration of anaesthesia and the severity of the surgical invasion. For example, probe implantations in the dermis or muscle are much less invasive to the animal as a whole than implantation in the liver. Experiments in which the actual tissue concentration of the analyte is desired, as opposed to changes in concentration, require an *in vivo* determination of the probe's extraction efficiency, which is generally a time-consuming procedure. Microdialysis typically results in low volume samples. The process inherently dilutes the samples as they are collected. Small volume samples often with low analyte concentration present a considerable challenge to the analytical techniques.

2 Relationship between the microdialysis experiment and the analytical methods

At typical perfusion rates, equilibrium is not established across the microdialysis membrane. The concentration of analyte determined in the dialysate is some fraction of the actual concentration in the surrounding sample matrix. The relationship between the analyte concentration in the dialysate and that in the sample matrix may be thought of as the extraction efficiency of the probe. Among the parameters that influence extraction efficiency are temperature, perfusate flow rate, chemical and physical properties of the dialysis membrane, probe geometry, membrane surface area and properties of the analyte. Diffusion rate within the matrix also affects extraction efficiency. *In vivo* uptake into cells, metabolic rate, extent of tissue vascularization and blood flow will influence diffusion through the matrix. Fortunately, under normal conditions of microdialysis sampling these parameters remain constant so that although equilibrium is not established, a steady-state is rapidly achieved. Thus, the extraction efficiency of the probe for a given set of parameters is constant and the direction of net flux of the analyte across the membrane is determined by the concentration gradient of the analyte.

From the analytical perspective, there are two important issues with respect to extraction efficiency. First, the dialysisate concentration will be less than the actual tissue concentration of the analyte. Thus, the limit of detection must be lower than the lowest *in vivo* concentration expected. Secondly, extraction efficiency increases as perfusion rate decreases. The slower the perfusion rate, the closer the dialysate concentration of the analyte will be to that in the tissue surrounding the probe.

When microdialysis sampling is applied *in vivo*, three previously independent systems become interlinked: the animal, the microdialysis sampling system, and the analytical system. The experimentalist must be aware that once these systems are linked, the conditions that were optimal for each independent system must now be considered in relationship to the other systems. Frequently, the analytical method's sample volume requirement necessitates increasing the microdialysis perfusion rate which in turn lowers the probe's extraction efficiency and thus provides samples containing lower concentration of analyte. Using a lower perfusion rate to increase probe efficiency and analyte concentration results in longer sampling times. Some degree of temporal resolution is lost by this compromise. The increased recovery may deplete low molecular mass compounds in the tissue adjacent to the probe in turn perturbing the biological system. The anatomical location and the spatial resolution needed for obtaining the desired information influence the probe design and active window length. While the implantation of the microdialysis probe may cause little disruption of the target organ, the necessary anaesthesia and extent of the surgical procedure also impact the biological system. The successful use of microdialysis sampling *in vivo* will depend on achieving a suitable balance among these systems.

The trade-offs among perfusate flow rate, concentration detection limit and sample volume requirement will set the temporal resolution that can be achieved for the experiment. An important consideration in balancing these parameters is the *in vivo* event being investigated and what information about the event is of primary importance. A clear statement of the

experimental question should dictate the balancing of the microdialysis sampling and analytical method parameters.

2.1 The analytical challenge of microdialysis sampling

The analytical method ultimately determines the sensitivity for the substances recovered by microdialysis sampling. Although the sampling step is physically separate from the analysis step, the two are connected by several experimental parameters. Microdialysis is a continuous sampling method in which the response time is determined by the permeability of the microdialysis membrane. On the other hand, most analytical methods require discrete samples of some finite volume. The time needed to collect the discrete sample is far longer than the microdialysis probe's response time. The dialysate is therefore collected over some appropriate time interval to provide this discrete sample. The sample volume requirement of the analytical method typically determines the overall temporal resolution and not the properties of the sampling itself.

For off-line analysis, the injection volume requirement and the perfusate flow rate determine the fastest temporal resolution that can be achieved. Since the small volume of microdialysis samples precludes preconcentration of the sample, the analytical method must have detection limits below the lowest concentration expected in the dialysates. If this condition is not met, samples must be collected over longer time intervals resulting in poorer temporal resolution in the experiment. The analytical method with the lowest concentration detection limit and smallest sample volume requirement provides the best temporal resolution for a microdialysis experiment. Using microbore chromatographic systems injection volumes of 1 μl or less are common. Capillary electrophoresis (CE) requires only a few nl be injected. Clearly, a difficulty encountered with microdialysis sampling is the collection, handling and injection of the small volume samples. Any derivatization, extraction or other manipulation of the sample will likely result in considerable loss of precision in the data. Loss of sample due to transfer and evaporation are reduced by the use of sophisticated fraction collectors and autosamplers.

2.2 On-line approaches

Because microdialysis samples are protein-free, the technique is amenable to on-line coupling with the analytical system. The physical devices required to implement on-line analysis vary with the analytical technique used and are discussed below. Regardless of the technique, on-line analysis has several advantages as well as some limitations. A typical on-line system for microdialysis sampling from an awake animal is shown in Fig. 3.

Such coupling can eliminate problems associated with transferring small volume samples. Direct transfer from the microdialysis sampling system to the analytical system prevents evaporation of samples. When necessary, the dialysate stream can be joined with other solution flow streams to achieve derivatization of the sample prior to injection into the analytical system. In many cases, on-line systems can provide near-real time analysis of microdialysis samples.

The temporal resolution of the experiment is a function of the analysis time when microdialysis samples are analysed on-line. The next sample cannot be injected until the previous one has been completely eluted because there is no provision for sample storage. Clearly, a major limitation for coupling microdialysis on-line with an analytical method is analysis speed. One must consider whether the temporal resolution that can be achieved by the analysis is sufficient for the kinetic or physiological events of interest.

Continuous analytical techniques such as enzyme reactors and sensors have been coupled to microdialysis sampling to provide near-real time data for analytes such as lactate, glucose, acetylcholine and ethanol. However, as these are generally not separation based methods, they are beyond the scope of this review.

3 Microseparation techniques

This review focuses primarily on separation techniques coupled on-line to microdialysis sampling and we have further narrowed the emphasis to microseparations, that is modifications of traditional separation methodologies which accommodate the small volume, low concentration samples typical of microdialysis. Microbore liquid chromatography (LC) and open tubular CE, also called capillary zone electrophoresis (CZE) feature most prominently in the microdialysis literature.

3.1 Microbore liquid chromatography

The dialysate is a continuous sample stream with low molecular mass compounds in protein-free aqueous solution of high ionic strength. Microdialysis samples are amenable to various analytical chemistry techniques. These include CE, immunoassay, ion selective electrodes, mass spectrometry and some specific clinical analysers. Liquid chromatography is, however,

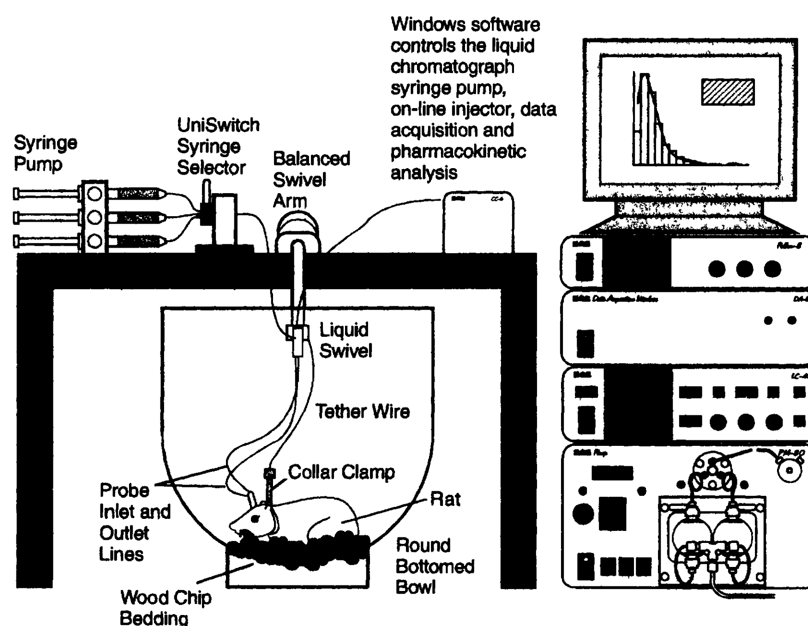


Fig. 3 An on-line microdialysis-LC system. (Figure used with permission from BAS, Inc. West Lafayette, IN, USA)

by far the most common choice for the analysis of microdialysis samples.[12] A major consideration in favour of LC is its wide availability and its ability to determine more than one analyte at a time. LC is also inherently compatible with high ionic strength aqueous samples such as dialysates. For *in vivo* experiments, determining multiple analytes provides a clearer picture of the *in vivo* event, for example, following the changing levels of several neurotransmitters in response to a specific stimulus or providing the concentration time profiles of metabolites in addition to that of the parent compound.

The modes of LC most compatible with direct injection of aqueous microdialysis samples are reverse phase and ion-exchange, the choice being dependent on the physicochemical properties of the analyte or analytes of interest. Column parameters (length, internal diameter and particle size) are determined by the sampling interval desired (temporal resolution needed for the experiment) and the sensitivity required. Equivalent separations can, in theory, be obtained with columns of the same length but different internal diameters (i.d.) because column efficiency and analysis time depend on the linear velocity of the mobile phase. Thus, no resolution advantage is gained by the use of narrow bore columns compared to conventional i.d. columns. However, since peak dispersion is proportional to the square of the column diameter, the use of microbore columns yields a tremendous increase in sensitivity. To realize the advantages of high sensitivity and rapid analysis offered by microbore columns, extra column contributions to band-broadening, dead volumes in the injector and flow cell, along with the length of connecting tubing must be minimized. Ref. 13 contains a particularly good discussion of the trade-offs that must be made for any application coupling microdialysis sampling on-line with microbore LC. Other discussions of microdialysis sampling and microbore LC may be found in refs. 12 and 14.

On-line injection systems using a six-port valve have been used for monitoring various substances. With this approach, however, valuable information is lost as the dialysate is shunted to waste during the separation. Possible sample carryover due to incomplete flushing of the injection valve is another consideration when using on-line analysis. For a conventional LC column with 1 ml min^{-1} flow rate, a 5 μl sample loop is completely flushed (ten volumes) in approximately 3 s. When a microbore column is used with a flow rate of 50 μl min^{-1}, the time required to flush the 5 μl loop with ten volumes of mobile phase will be 1 min. Even if the separation time is not the limiting factor, 1 min of chemical information is lost during the flushing process. Multiple sample loop systems have been described to overcome this limitation.[15]

3.2 Capillary electrophoresis

Because of the low sample volume requirement of CE, it is particularly attractive for analysis of the small volume microdialysis samples. Since injection volumes of 1–10 nl are typical for CE, it is possible to increase microdialysis probe efficiency by lowering the perfusion flow rate while maintaining the temporal resolution of the experiment. CE with UV detection can be used for some analytes at high micromolar concentration, however, optical detection methods suffer from poor concentration detection limits because of the extremely short optical pathlength available with CE. More sensitive methods are generally necessary for many biologically important compounds at normal physiological concentrations. In such cases, laser induced fluorescence (LIF) or electrochemical detection are employed for analysis of microdialysis samples by CE. For a recent review of CE separations applied to microdialysis samples see ref. 16.

Coupling microdialysis sampling on-line with CE requires an interface to convert the continuous dialysate stream to discrete samples for CE analysis. The interface also serves to protect the experimental animal from the high potential used for CE separations. Additionally, the interface serves to make the microdialysis flow rate compatible with CE. Typical arrangements of microdialysis–CE systems are shown in Fig. 4.

On-line coupling of microdialysis sampling to CE was first described by Hogan *et al.*[17] In this system a commercially available rotary microinjection valve and a specially designed injection interface were combined. The microinjection valve segments the dialysis stream into discrete plugs (60 nl) which are carried to the injection interface by a stream of CE run buffer continuously pumped through a transfer line into the buffer reservoir by electroosmotic flow. A diagram of the injection interface is shown in Fig. 5. The sample plug exits the transfer line into the reservoir directly across from the injection end of the separation capillary. The CE separation voltage is applied continuously throughout the experiment and buffer is continuously drawn into the separation capillary from the reservoir. Electrokinetic injection of the sample plug into the CE takes place as the analyte passes near the orifice of the separation capillary. Any sample not immediately injected is swept away from the injection site by run buffer being pumped through the transfer line behind the sample plug. Careful positioning of the transfer capillary relative to the separation capillary and proper adjustment of the intervening gap are essential for the proper operation of this injection interface.

A second microdialysis–CE interface, as reported by Lada and Kennedy, is a modification of the flow-gated interface developed by Lemmo and Jorgensen for coupling capillary chromatography with CE.[18] The interface consists of a 75 μm thick PTFE spacer sandwiched between two steel plates (Fig. 6). A slit in the spacer produces a small volume flow channel between the plates. The outlet of the microdialysis probe and the inlet of the CE separation capillary are positioned directly opposite one another at a distance nominally defined by the spacer. Two additional ports are used for introduction and

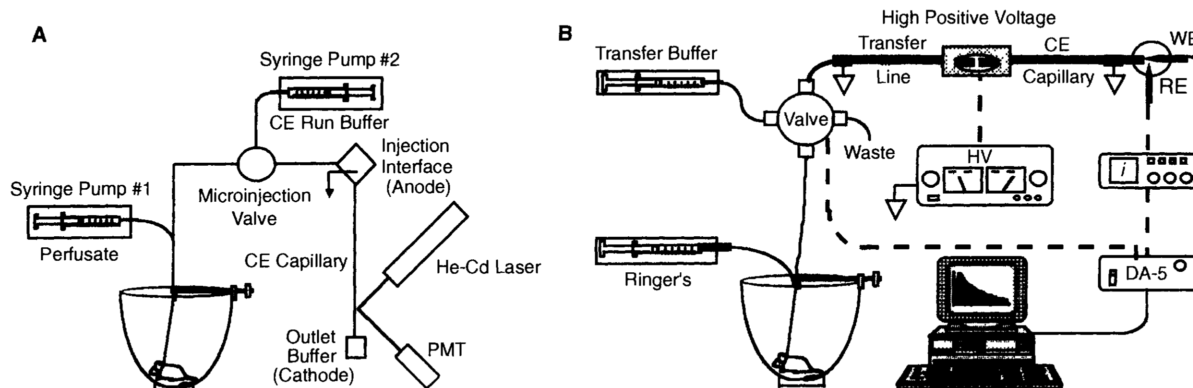


Fig. 4 Typical on-line microdialysis–CE systems. **A**. Arrangement of an on-line microdialysis–CE system with laser induced fluorescence detection. **B**. On-line microdialysis–CE system with electrochemical detection.

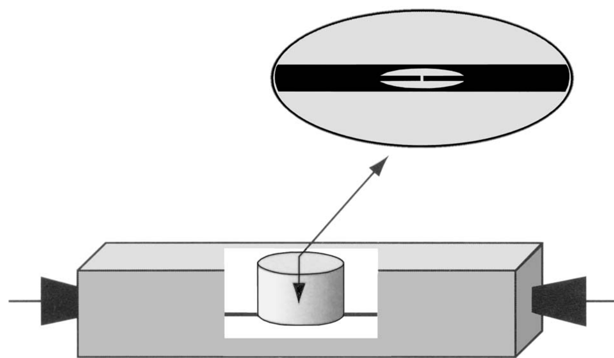


Fig. 5 Detail of the gap design injection interface used with an electrically actuated valve. A buffer reservoir is machined in a kelef block. A channel drilled the length of the block and threaded for LC fittings contains a length of PEEK tubing with an opening as shown in the close-up. The opening exposes the fracture between the transfer line from the valve and the separation capillary to the running buffer.

removal of electrophoretic buffer in a flow pattern perpendicular to the dialysis–CE capillary flow. During the CE run, the gating flow rinses away the dialysate from the inlet of the CE and provides the electrophoretic buffer necessary for the separation. Injection of analyte into the CE is accomplished by stopping the gating flow, turning off the separation voltage thus allowing dialysate to build up near the CE inlet. After an appropriate delay period, a low voltage is applied for a few seconds to inject the sample. The voltage is turned off, the gating flow is resumed sweeping away excess dialysate, and the separation voltage is applied to the capillary. The very low dead volume of this interface allows low nanolitre flow rates through the microdialysis probe while maintaining good temporal resolution. As with the microinjection valve–interface coupling, correct positioning of the dialysis and CE capillaries, adjustment of the gating flow rate and delay time are necessary for proper operation of the interface.

A disadvantage of both designs is that only a portion of the sample stream is utilized in the analysis. As a result, rapid changes in concentration that occur between runs may not be observed. Additionally, the voltage switching required with the flow gated interface can cause baseline drift and could be a major problem for electrochemical detection.

3.3 Other techniques

Mass spectrometry (MS) can be a useful tool for quantitative analysis of polar molecules in aqueous environments. However, coupling MS directly on-line with microdialysis sampling presents significant challenges. Microdialysis samples, especially those collected *in vivo*, are collected in physiological saline or other high salt content solution. This high salt content is not compatible with the MS inlet. The sensitivity of MS may not be adequate for all analytes of interest. Despite these challenges, tandem MS has been used on-line with microdialysis sampling.

4 Review of applications in the recent literature

The examples of microdialysis sampling coupled on-line to the analytical system cited in this review are intended to provide an overview of the current scope of applications. Most of the work noted here was published between 1993 and 1996. Earlier reviews of microdialysis sampling coupled on-line with analytical techniques can be found in refs. 19 and 9. A searchable bibliography of reports on microdialysis sampling is also available on computer disk.[20] While microdialysis sampling has proved to be an excellent technique for *in vitro* applications such as drug dissolution studies, kinetics in enzyme incubations and monitoring bioprocessors, we have further limited the applications discussed here to *in vivo* studies. We have chosen to separate the applications on the basis of the analytical method used rather than the tissue sample or the analyte.

4.1 Microbore liquid chromatography

As noted above, refs. 12–14 contain helpful discussions of the implementation of microbore liquid chromatography for the on-line analysis of microdialysis samples. A summary of applications with information about microdialysis and analytical parameters is provided in Table 1.

Two loops and a multifunction ten-port valve were used by Wang and co-workers for on-line LC–UV analysis to study the distribution of zidovudine using microdialysis probes simultaneously sampling from cerebrospinal fluid and thalamus of rabbits.[21] Dialysate from the probes collected into separate sample loops (5 μl) for sequential injection onto a small bore column (2.1 × 200 mm). The sampling interval for each probe was 20 min. The report also compared *in vivo* calibration of the microdialysis probes using retrodialyis and the zero net flux method.

Wang and associates used a small bore column (2.1 × 200 mm) with fluorescence detection for the on-line analysis of microdialysis samples in a study of the effect of cyclosporin A on the distribution of rhodamine-123 in rat brain.[22] Perfusate flow rate was 0.5 μl min^{-1} into the loop and the injection interval was 40 min.

In a summary of their recent results, Lambás-Señas and colleagues characterize on-line microdialysis sampling coupled to LC with electrochemical (EC) detection and *in vivo* voltammetry as complementary techniques for monitoring monoamine metabolism in rat brain stem.[23] The LC separation of DOPAC and catecholamine was performed on a 2.1 × 100 mm column with an interval of 15 min. While *in vivo* voltammetry provided better spatial and temporal resolution than microdialysis, microdialysis sampling with separation based analysis provided more selective and precise identification of compounds from the extracellular fluid.

Michelsen and Pettersson analysed contrast agents, such as those used in X-ray and magnetic resonance imaging, to demonstrate an on-line microdialysis–LC–MS system.[24] Dialysate from a probe implanted in the carotid vein was collected directly into the loop of the electrically actuated injection valve. Separation was achieved using a narrow bore column (2 × 150

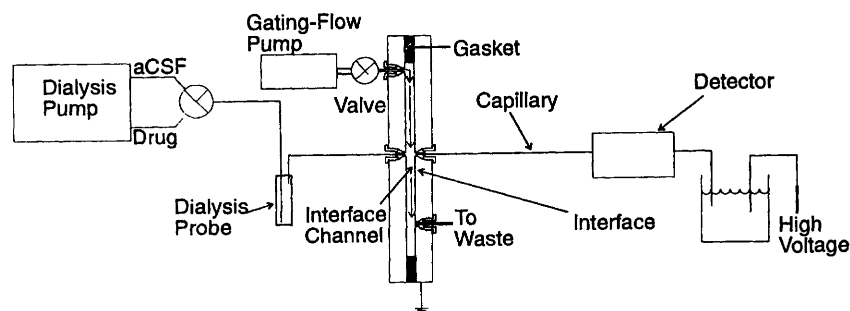


Fig. 6 Diagram of the on-line microdialysis–CE system based on the flow-gated interface showing the details of the flow-gated interface. (Reproduced with permission from *Anal. Chim. Acta.*, 1995, **307**, 217.[18]

Table 1 Applications of microdialysis coupled on-line to microbore liquid chromatography[a]

	Microdialysis parameters					Analytical system details					
			Membrane								
Analytes	Tissue	Probe Design	MWCO	Length	Perfusate Flow rate (µl/min)	Loop Volume	Injection Interval	Column I.D. × length (mm)	Detection Mode	Notes	Ref.
Rhodamine-123	frontal cortex of rat brain	RC	20000	3 mm	0.5	n.g.	40 min	2.1 × 200	FL		21
Zidovudine (AZT) AZdU (as an internal standard)	rabbit brain, thalmus and ventricle	RC	20000	3 mm	1	2 loops, each 5 µl	20 min alter-nating loops	2.1 × 200	UV	Used 2 probes in different location, alternating injections	22
DOPAC catacholiamine	rat brain	RC	6000	2 mm	1	15 µl	15 min	2.1 × 100	EC		23
Omnipaque (350 mg iodine/ml)	carotid vein of rat	RC	20000	10 mm	2	5 µl	10 min	2 × 150	MS and photo-diode array		24
dopamine and metabolites	rat brain	RC	n.g.	4 mm	various	n.g.	5 min	1 × various	EC	push-pull perfusion of the microdialysis probe	13
dopamine	rat brain	RC	n.g.	4 mm	0.2	0.5 µl	5 min	1 × 100	EC	push-pull perfusion of the microdialysis probe	25
[1] acetylcholine [2] 8 monoamines	striatum of rat brain	RC	20000	3 mm	2	5 µl	20 min	[1] 1 × 530 1 × 55 [2] 1 × 100	EC	2 injection loops in series injected microdialysis samples simultaneously into two LC systems	26
serotonin (5-hydroxytryptamine)	jugular vein of anaesthetized rats	FC	20000	10 mm	0.5	10 µl	20 min	1 × 100	EC		27
APAP APAP-*O*-sulfate APAP-glucuroinide	jugular vein of awake rats	FC	5000	4 mm	1	2 loops, each 7 µl	5 min	1 × 100	UV	Dual 6-port and 8-port valves compared	15
APAP and metabolites caffeine and metabolites	jugular vein of awake rats	FC	5000	n.g.	1.5	0.5 µl internal loop	1 min	1 × 14	UV	APAP and 2 metabolites were resolved in $<$ 1 min Caffeine and 2 metabolites also resolved in $<$ 1 min	28
Substance P	striatum of rat brain	RC	20000	4 mm	0.3	10 µl	30 min	0.05 × 50	MS		29

[a] Probe Designs: FC—flexible cannula; RC—rigid cannula. Detection Modes: FL—fluorescence; UV—ultraviolet; EC—electrochemical; MS—mass spectrometry.

mm) with a mobile phase flow rate of 300 µl min^{-1}. Flow from the LC column was split to achieve a flow rate of 80 µl min^{-1} before entering the ion pneumatically assisted electrospray source.

On-line analysis of microdialysis samples using 1 mm i.d. columns and EC detection has been used to quantify neurotransmitters.[19] Wages *et al.* used various dopamine metabolites to illustrate the impact of microdialysis sampling and chromatographic system parameters on experimental results when using on-line microbore systems.[13] Their study characterized the system performance both *in vitro* and *in vivo*. Church and Justice demonstrated the use of such a system for studying neurochemical responses to pharmacological agents.[25] Extracellular dopamine in the brain of awake rats was monitored on-line at 5 min intervals following administration of haloperidol (increased extracellular dopamine) or apomorphine (decreased extracellular dopamine).

Tsai and Chen measured acetylcholine and monoamines using two microdialysis on-line systems with two analytical systems simultaneously.[26] Dialysate samples were collected into two automated injection valves (5 µl loops) connected in series and injected at 20 min intervals onto the two LC systems. Acetylcholine (ACh) was determined using a prepacked BAS ACh microbore column (1 × 530 mm) and post-column reactor (1 × 55 mm) with EC detection at a platinum electrode. Monoamines were analysed on the second system using a microbore column (1 × 100 mm) and EC detection at a glassy carbon electrode. In another study, Tsai and Chen studied collagen-induced serotonin release in the blood of anaesthetized rats.[27] The microdialysis probe was implanted in the jugular vein and dialysate collected directly into the 10 µl loop of an on-line injector. With a sampling interval of 20 min, serotonin and its major metabolite, 5-hydroxyindoleacetic acid, were determined using a 1 × 100 mm column and EC detection.

Steele and Lunte demonstrated the potential of microdialysis sampling coupled to on-line microbore LC analysis for pharmacokinetic studies.[15] The separation of acetaminophen and its two conjugated metabolites was achieved using a microbore column (1 × 100 mm). Injector precision, on-line system response time and system delay time were evaluated for two on-line systems, a dual six-port valve system and an eight-port valve system. The eight-port valve system was used on-line with flexible microdialysis probes implanted in the jugular vein of rats to study the *in vivo* pharmacokinetics of an intravenous dose of acetaminophen. The dialysate was collected directly into the sample loops (7 µl in all cases) for injection into the LC–UV system. The LC injection interval was 5 min.

Fast microbore liquid chromatography was coupled on-line with venous microdialysis sampling by Chen and Lunte.[28] Using a 1 × 14 mm microbore column, analysis times of less than 1 min were achieved for acetaminophen and its conjugate metabolites and for caffeine and two metabolites. In this case, the electrically actuated injection valve had a 0.5 µl internal sample loop. The chromatographic detector output directly shows the concentration–time profile of the drug with 1 min temporal resolution (Fig. 7).

LC–MS with micro-electrospray ionization has also been coupled to *in vivo* microdialysis sampling to study the metabolism of Substance P.[29] Dialysate samples were collected directly into the loop of a ten-port valve for loading onto a capillary column (0.05 × 50 mm). Following washing with a weak mobile phase to remove most ionic compounds, a stronger mobile phase was used to elute Substance P and its metabolite fragments into the ion source.

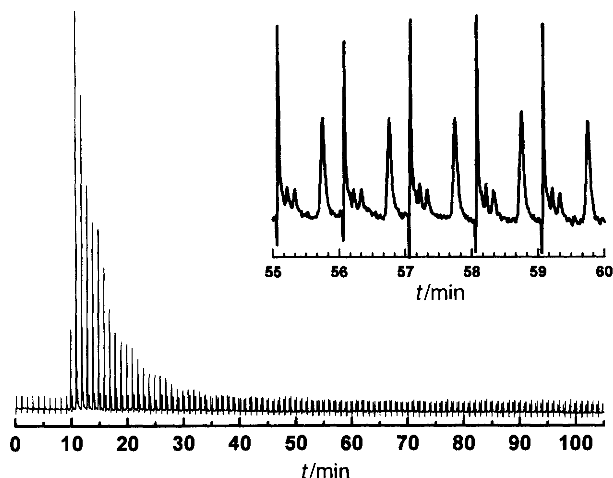


Fig. 7 Detector output from the on-line microdialysis sampling-fast microbore LC system following the plasma concentration of caffeine and its main metabolites. Inset shows expanded scale of output from 55–60 min. (Reproduced with permission from *J. Chrom. A*, 1995, **691**, 29.[28]

4.2 Capillary electrophoresis

Table 2 presents a summary of microdialysis and analytical parameters for applications coupling microdialysis on-line with capillary electrophoresis. For additional applications using CE analysis with microdialysis studies, including off-line analysis, the reader should consult ref. 16.

The first reported on-line microdialysis–CE system was based on an electrically actuated microinjector.[17] Using LIF detection, Hogan *et al.* investigated a potential antineoplastic agent, SR 4233, and its main metabolite, SR 4317. A high-speed micellar electrokinetic chromatographic (also called micellar electrokinetic capillary chromatography) separation provided 90 s temporal resolution for *in vivo* experiments.

Zhou and colleagues modified the system to include on-line NDA/CN derivatization of amino acids.[30] Although the free zone electrophoretic separation of glutamate and aspartate required less than 2 min, the overall temporal resolution of the system optimized for the derivatization was 5–7 min. Continuous monitoring of glutamate and aspartate in the brains of rats was carried out. Concentration changes of the analytes, triggered by infusing potassium *via* the probe, demonstrated the feasibility of the system for neurochemical applications.

Lada *et al.*, using a flow gated interface, already discussed, coupled microdialysis with CE–UV for the determination of ascorbate in rat brain.[18] The migration time for the ascorbate under the conditions of the *in vivo* experiment was *ca.* 100 s with the experiment having a temporal resolution of 110 s. Using the same system, Lada and Kennedy studied ascorbate and lactate *in vivo* in rats monitoring concentration changes in response to administration of anaesthesia or to elevated potassium levels.[31] Incorporating *o*-phthaldehyde/β-mercaptoethanol derivatization with the on-line system and using a micellar electrokinetic chromatography with LIF detection, they have also monitored aspartate and glutamate in rat brain dialysates.[32]

Although CE–EC has been used off-line for a variety of substances in microdialysis samples, the only report coupling microdialysis sampling on-line with CE–EC determined nicotine from a dermal patch in the skin of a rat.[33] The system requires an interface at each end of the separation capillary to shield both the experimental animal and the EC detector from the high separation potential. If the separation current is not grounded before the detector, the relatively small analytical signal generated at the electrode will be lost in the separation current.

Coupling MS detection to CE currently results in substantial loss of sensitivity compared to LC due to the small amounts of analyte that are delivered to the MS by the CE. The literature searches conducted for this review found only one report of CE–MS for the analysis of microdialysis samples. Although the microdialysis system is not on-line with the CE–MS, Takada *et al.* recently reported the detection of γ-aminobutyric acid (GABA), a well studied neurotransmitter, in samples collected *in vivo* from rat brain by microdialysis.[34] Previous experiments by the group had determined the limit of detection of GABA for the CE–MS system was 10^{-5} M using standard solutions. However, GABA in microdialysis samples was not detected under the same analytical conditions. After modification of the analytical conditions they were able to obtain a signal for GABA. Although this signal was insufficient for quantitation, the results do suggest that coupling microdialysis sampling on-line to CE–MS may eventually be successful.

Table 2 Applications of microdialysis coupled on-line to capillary electrophoresis[a]

	Microdialysis parameters					Analytical system details					
			Membrane		Perfusate flow			Capillary I.D. × total : working			
Analytes	Tissue	Probe design	MWCO	Length	rate/ µl min^{-1}	Injection Regime	Injection Interval	lengths (µm × cm : cm)	Detection Mode	Notes	Ref.
SR4233, a benzotriazine SR4317, metabolite	jugular vein of rat	FC	5000	n.g.	1	60 nl plug delivered to interface, electrokinetic injection	90 s	50 × 40 : 15	LIF		17
glutamate aspartate	hippocampus of rat brain	RC	20 000	3 mm	1	60 nl plug delivered to interface, electrokinetic injection	2 min	25 × 30 : 14	LIF	on-line derivatization	30
ascorbate	rat brain	linear, U	6000	2 mm	0.1	10 s at 1 kV, 10 s delay	110 s	25 × 50–60 : 15	UV		18
ascorbate lactate	rat brain	linear, U	6000	2 mm	0.079	5 s at 1 kV	50 s to 2 min	25 or 50 × 50 : 15	UV		31
aspartate glutamate	rat brain	linear, U	6000	2 mm	0.079	5 s at 1 kV or 2 s at 100 V	0.5 to 3 min	25 × 20 : 15 or 10	LIF	on-line derivatization	32
nicotine	dermis of awake rat	linear	30 000	15 mm	1	60 nl plug delivered to interface, electrokinetic injection	10 min	n.g.	EC		33
GABA	striatum of rat brain	RC	20 000	3 mm	1	5 nl		50 × 40 : 40	MS	microdialysis to CE on-line, detection off-line	34

[a] Probe Designs: FC—flexible cannula; RC—rigid cannula. Detection Modes: LIF—lasar induced fluorescence; UV—ultraviolet; EC—electrochemical; MS—mass spectrometry.

4.3 Other analytical approaches

Tandem mass spectrometry (MS–MS) is another separation based analytical approach that has been coupled on-line to microdialysis sampling.[36] Caprioli and Lin directed the dialysate flow into the MS by way of the continuous-flow–fast-atom bombardment (FAB) interface.[35] Dialysate samples from a microdialysis probe implanted in the jugular vein of a rat were analysed on-line to provide a concentration–time profile of penicillin following an intramuscular dose Deterding and co-workers also used a FAB interface to couple microdialysis on-line to MS–MS Using tris(2-chloroethyl) phosphate as the model compound they compared the blood pharmacokinetics obtained by the on-line microdialysis–MS–MS system with those from conventional methods (serial blood sampling) [36]

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