New Options for Collecting Small Volume Samples: Implications for Microdialysis and In Vivo Ultrafiltration

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UPDATE!

The HoneyComb can now collect into two vials via dual sampling cannulae.

Two new additions to the BAS Bee™ family of products for in vivo sampling provide useful alternatives for the collection of small volume samples. These samples range from perfusates flowing from microdialysis probes to filtrates flowing from implanted ultrafiltration probes. Post-column collection of individual peaks from a microbore liquid chromatograph is also possible.

Microdialysis sampling is accomplished by washing the interior surface of an implanted, semi-permeable dialysis membrane with a suitable perfusion solution, at a constant rate of flow. The membrane is in direct contact with a tissue or fluid to be sampled. The gradient between the concentration of the analyte in the tissue and the concentration of the analyte in the perfusion solution drives low molecular weight analytes across the membrane. When the analyte travels from the tissue into the probe, it is swept away from the membrane by the constant flow of perfusion fluid. The final step in a microdialysis sampling experiment involves the collection of a sample for analysis. Typically, small sample volumes on the order of 5 to 20 µL are collected.

F1

The new BAS HoneyComb™ is a refrigerated fraction collector for microdialysis and ultrafiltration sampling. No computers, extra boxes, fluid lines or coolants are required. This compact device, as shown, will cool to 4°C within 20 minutes. Open architecture makes it possible to remove and replace vials during collection.

Ultrafiltration sampling is simpler than microdialysis sampling. The extracellular fluid is filtered through a similar semi-permeable membrane that has been implanted in the tissue, and the protein-free ultrafiltrate is collected in small vials or glass tubes. A vacuum pulls fluid from the tissue, through the probe, and into the collection vessel. The source of the vacuum is either a simple vacutainer attached to the ultrafiltration probe or a peristaltic pump, which continuously delivers the ultrafiltrate to vials in a fraction collector.

Collection of microdialysis samples is usually accomplished by routing the dialysate to a small glass or plastic vial. When samples are collected manually, they may be analyzed immediately or capped, stored, and frozen for later processing. Since sampling intervals are usually on the order of minutes, and sampling experiments tend to last for several hours, manual fraction collection can become tedious. This process can also be automated through the use of a fraction collector.

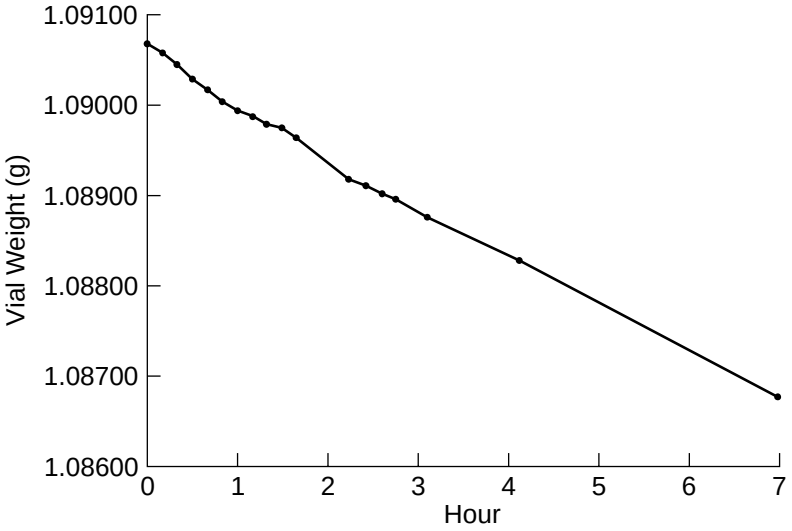
Common fraction collectors are unsuitable for this purpose if they rely on collection of drops falling from a cannula poised over an open vial. The amount of fluid required to form a drop that falls freely from a cannula can range from 10 to 100 µL depending on fluid viscosity, humidity, flow rate, cannula type, etc. At microdialysis flow rates of 1 or 2 µL/min, the minimum sampling interval would range from 5 to 100 minutes. At such low flow rates, the forming drop would also be subject to evaporation as would any sample residing in an open vial (***F2***).

The new BAS HoneyComb™ fraction collector (***F1***) was designed to collect small volume fractions from microdialysis or ultrafiltration probes with a high degree of reproducibility. It delivers fluid to capped/sealed vials, open vials, or a combination of open and closed vials. The sampling needle touches the bottom of each vial, as illustrated in ***F3***, so fluid flows into the vial instead of forming a hanging drop, allowing smaller volume samples to be collected. The sampling needle in the HoneyComb has an internal volume of only 7.1 µL, so the residence time of dialysate flowing through the needle amounts to only a few minutes.

The BAS HoneyComb uses a mechanical needle assembly instead of relying on delicate electronic microsensors, which require careful calibration and frequent adjustment. This simple yet reliable

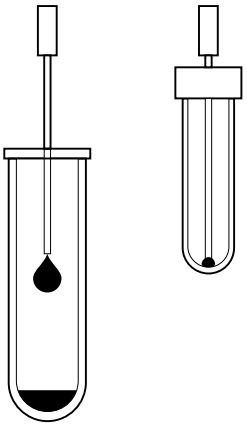
F2

The evaporation of 5 µL of Ringer's Solution in an open 300 µL glass vial was monitored by maintaining the vial in still air within an analytical balance, for a period of 6.98 hours. The room temp. fluctuated from 20.9-21.2°C and the relative humidity was 45%. Within an hour, 14.8% of the sample was lost. After 7 hours, the loss was 78.2%.



F3

Collection of drops from an open cannula at low flow rates may expose the sample to evaporative loss and contamination. At least 10 µL of fluid must flow before the drop will fall. The BAS HoneyComb delivers small volumes of fluid into capped and sealed vials. The sample cannula touches the bottom of each vial so that fluid flows instead of dropping so that smaller sample volumes can be collected.



mechanism ensures that the needle pierces the vial seal and also touches the bottom of each vial, despite normal variations in glass thickness. Needle tension is mediated by a spring, which allows the needle to touch the bottom of each vial regardless of the thickness of the vial bottom or lip. Since the mechanism is mechanical instead of electronic, needle travel is unaffected by mixtures of open and closed vials in the vial carousel. An "air bleed" cannula on the needle releases pressure that may build up while the sealed vial is filling, so there is no impediment to flow. After the needle is withdrawn, the vial septum reseals to protect the sample against evaporative loss.

The reliability of needle travel and vial advance operations in the fraction collector has been proven during continuous duty cycles exceeding 500,000 repetitions without failure. A simple replacement procedure allows the average user to quickly change a needle that fails due to normal wear or mishap.

The HoneyComb has a change-over time (bottom of one vial to bottom of next vial) of less than 4 seconds. It takes half this time to clear the seal of one vial and pierce the next. The fast rate of change-over makes it possible to pierce vial seals without significant sample loss, at flow rates ranging from 1 to 125 µL/min.

Refrigeration is beneficial during the collection of dialysates or ultrafiltrates for a variety of reasons. Some analytes, specifically catecholamines, degrade at room temperature and physiological pH (1,2). Acidification of dialysates may retard this process but does not eliminate it completely. The addition of acid may be detrimental to the chromatography or even cause degradation of other components in the dialysate, such as serotonin (1). Refrigeration is more protective of labile samples than acidification alone (1,2). The control of bacterial growth provides a very compelling reason to refrigerate both types of samples during sample collection. Bacteria grow at logarithmic rates and proliferate in nutrient-rich dialysates and ultrafiltrates. It takes very little time for bacteria, incubated at room temperature, to alter concentrations of amino acids, glucose, and other potential analytes in these samples.

The HoneyComb is preset to a temperature of 4°C. At average room temperature and humidity, it achieves this temperature within 20 minutes after power start-up (***F4***). No cooling lines, water, or other fluids or gases are required to achieve cooling. The HoneyComb uses an electronic device and highly efficient fan to chill the vials sus-

T1

At a typical microdialysis flow rate of 2.0 µL/min. – delivered by a Baby Bee Syringe pump with a MD-0050 Bee Stinger syringe (500 µL) – the BAS HoneyComb fraction collector was able to collect 5 µL samples in capped and sealed vials with an RSD of 2.4 %. The HoneyComb refrigerated the vials at a temperature of 4°C. Vials were weighed on an analytical balance calibrated to 0.0001 g.

Vial #	Net Wt. (g)	Vial #	Net Wt. (g)	Vial #	Net Wt. (g)
1	.0048	21	.0050	41	.0049
2	.0048	22	.0049	42	.0049
3	.0047	23	.0049	43	.0051
4	.0049	24	.0049	44	.0048
5	.0048	25	.0049	45	.0050
6	.0047	26	.0049	46	.0048
7	.0050	27	.0049	47	.0049
8	.0052	28	.0050		
9	.0048	29	.0049	Mean	0.0049
10	.0048	30	.0052	Std. Dev.	0.0001
11	.0048	31	.0049	% RSD	2.4
12	.0051	32	.0049		
13	.0049	33	.0049		
14	.0049	34	.0048		
15	.0048	35	.0051		
16	.0049	36	.0050		
17	.0049	37	.0048		
18	.0050	38	.0049		
19	.0050	39	.0049		
20	.0048	40	.0052		

pended in a "cold canal". Because of the nature of the cooling device, the only restriction on the placement of a HoneyComb is access to electrical power.

The reproducibility of fluid collection by the HoneyComb is demonstrated by ***T1*** and ***T2***. Data in ***T1*** came from the collection of 5 µL volumes of deionized water at a flow rate typical for microdialysis. ***T2*** demonstrates the feasibility of collecting larger fluid volumes at the higher flow rates typical of microbore LC. In both cases, the HoneyComb demonstrated a high degree of reproducibility.

The HoneyComb can be used for basic fraction collection without the need for an external device, such as a PC. A front panel keypad and backlit LCD provide local control. The user can set a *delay time* in anticipation of the time required to fill or purge connecting tubing, *a collection interval*, a total *number of samples*, and a choice of whether the *cooling* is turned on or off. Control of the HoneyComb can also be surrendered to a remote device, such as a PC, through a terminal strip. This feature enables the BAS Queen Bee™, an intelligent syringe pump, to deliver fractions at variable time intervals.

The HoneyComb has 47 vial positions and one waste position, which can also be used for an additional vial. Because of the open architecture of the instrument, there is no need to restrict fraction collection to 48 vials. Vials can be easily removed from the collector while the instrument is still running and replaced with new vials. For this reason, the maximum number of vials that can be set is 999.

The standard vial holder on the HoneyComb was designed to hold 300 µL glass sample vials (6 x 32 mm). An optional holder for plastic sample vials (6 x 35 mm) is available. Either vial holder can be used for long-term storage of collected samples. For cold storage, the vial holders fit into covered plastic containers available from BAS.

On-Line Injection

Fraction collection is not limited solely to the use of sample vials. Another alternative is to collect dialysate in the loop of an injection valve that is on-line with a liquid chromatograph. This approach offers several advantages. Sample order is maintained since there are no vials to mislabel or lose. Analytical information is generated while the microdialysis experiment is still in progress! Photo-labile or oxygen-labile samples are protected inside stainless steel loops.

The Pollen-8™ On-Line Injector (***F7***) is another new addition to the BAS Bee product line. This device has a simple controller, a versatile 10-port injector, and a compact valve-actuator. It may be used in a variety of ways. When two *matched* and *calibrated* loops are installed, partial filling of each loop captures *all* of the dialysate (***F8A***). While one loop fills, the other injects the sample onto the LC, and vice versa. Since the loops are filled by precise syringe pumps at low flow rates, the fill volume can approach 95% of the loop volume. Loop sizes range from 2 to 50 µL. The same valve and loops can be used to *divide* a dialysis stream between two individual chromatographs (***F8B***). This approach can also feed the output of two separate microdialysis probes into one LC system (***F8C***) to monitor, for example, the blood-brain barrier through probes at each site. With only one loop installed, the Pollen-8 can also split a dialysate between on-line injection and collection in the BAS HoneyComb (***F8D***). This would enable half of the fractions to be monitored on-line while the other half are collected in vials for later processing. This method is useful when some analytes require a derivatization reaction (e.g., amino acids).

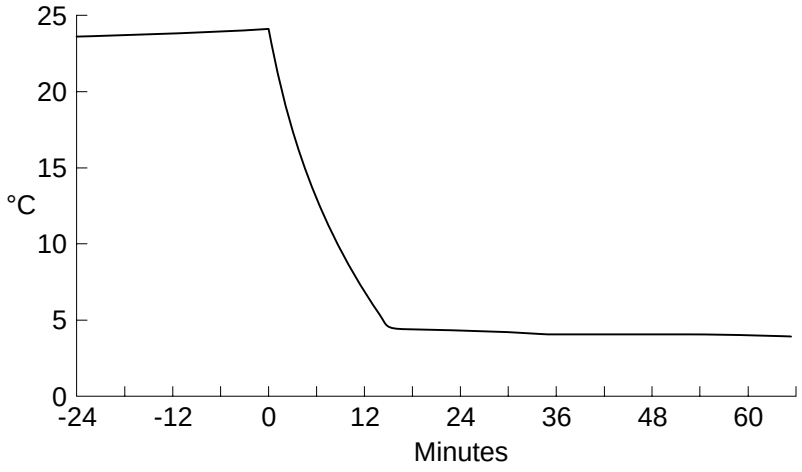
T2

At a 125 µL/min flow rate typical of microbore LC, the HoneyComb was able to reproducibly collect 125 µL samples in capped and sealed vials as demonstrated by a 1.0 % RSD. Such performance makes the HoneyComb a good candidate for collection of peaks from a microbore LC system – a useful technique prior to RIA analysis of peptides.

Vial #	Net Wt. (g)	Vial #	Net Wt. (g)
1	.1189	11	.1227
2	.1207	12	.1235
3	.1203	13	.1225
4	.1197	14	.1223
5	.1209	15	.1219
6	.1202	16	.1226
7	.1201	17	.1211
8	.1224	18	.1214
9	.1228	Mean	0.1214
10	.1219	Std. Dev.	0.001
		% RSD	1.04

F4

A cooling curve for the "cold canal" in the BAS HoneyComb demonstrates both how rapidly this instrument achieves the 4°C setpoint and how well it maintains it. Starting from ambient temperature of 24.4°C, the canal reached 4°C in 16 minutes without the use of liquid or gas coolants.

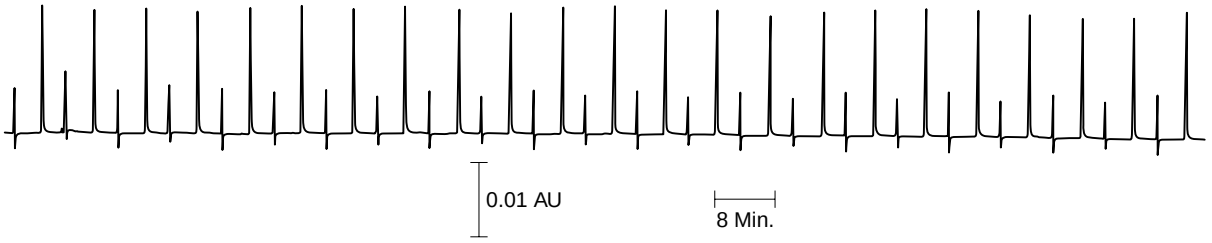


F5

The vial holder on the HoneyComb is easily removed for storage or transfer of vials. A détente on the bottom of the holder allows vials to rise slightly out of the holder for easy access when the holder is placed on a tabletop.

F6

The performance of the Pollen-8™ is demonstrated using a Neubron standard solution delivered to two 10 µL sample loops by a BAS Bee syringe pump set at 1.0 µL/min flow. The injection/collection volume was 7.0 µL into a BAS UniJet microbore column, 100 x 1 mm, 3 µm ODS.



F7

The Pollen-8 can be used with any LC system or microdialysis syringe pump. The package includes a 10-port injection valve, electric actuator, fittings, and a compact controller, which stacks on top of other BAS LC instruments. The matched and calibrated loops are purchased separately and are available in a variety of sizes.

The dialysate collected in vials is neither contaminated nor diluted by LC mobile phase. The HoneyComb advances to a new vial after receiving a signal from the Pollen-8.

The Pollen-8 package includes the controller and valve-actuator. Accessories provided with the package include stainless steel nuts and ferrules, a Teflon®-lined microdialysis probe port, two waste ports, cable, power cord, and manual. Injection loops are not included. These loops are sold as matched and calibrated pairs.

The Pollen-8 controller sets the injection/collection timing. There is only one time interval to set since injection occurs from one loop while collection occurs simultaneously. When it is necessary to control injection from each loop at two different timed intervals, the Pollen-8 can also be controlled by the Queen Bee Intelligent Syringe Pump.

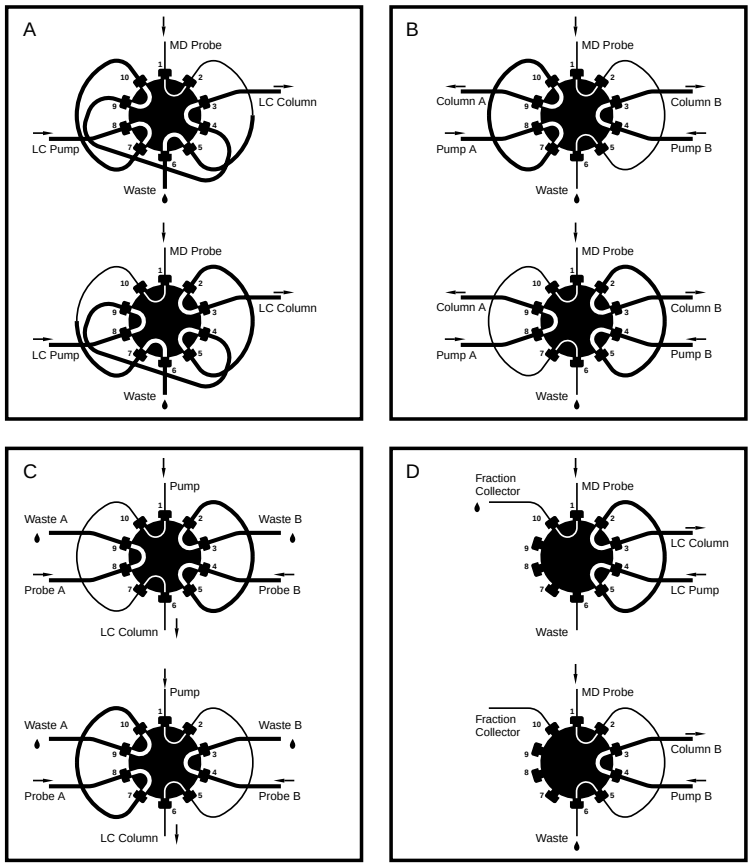
Using the Pollen-8 front panel controls, it is also possible to set a limit on the total number of injections made. Since samples are delivered to the loop by a syringe pump, the normal limit would be determined by the volume of the syringe pump. However, it is also possible to extend the perfusion indefinitely using two BAS Bee syringe pumps and a UniSwitch (3).

The Pollen-8 valve-actuator accepts two types of valves: microbore or conventional LC. The valves differ according to the ID of the tubing and channels. It also accepts other 2-position valves such as 6- and 8-port versions. The Pollen-8 controller recognizes the type of valve installed upon powerup. Applications to diversion of void peaks in LC/MS as well as column switching LC are also feasible. Use of BAS UniJet microbore columns requires the use of the microbore version. All valves use flat rotor seals; therefore, maintenance is simple.

These two, second-generation products overcome the disadvantages of earlier models to make microdialysis an easier and more reliable process.

F8

The versatile Pollen-8 adapts to various on-line microdialysis sampling situations through simple changes in valve plumbing. A., Partial filling of loops collects all of the dialysate – while one loop fills, the other njects. B., The dialysate flow stream is split between two LC systems. C., Two dialysis probes can be monitored by a single LC system. D., Inject and collect as the flow stream from one probe is divided between on-line injection and the HoneyComb fraction collector.



References

1. *F. Hefti, Current Separations, 2 (1980) 3.*
2. *T. Huang and P.T. Kissinger, Current Separations, 14 (1996), 114.*
3. *UniSwitch User's Guide, BAS Part No. (A-1871), (1995) 2.*

Acknowledgment

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