Autolab SPR

Benefits Cuvette versus Flow cell:

The technique SPR is a perfect tool to study molecular interactions. One way to administer a sample to the sensor is the use of a Flow system. Here we describe the benefits of the Cuvette system to apply a sample to the sensor. Due to the flexibility of the cuvette system, it supports a huge application range.

In advantage of the cuvette, the main differences between the sample induction techniques include:

- Fixed sample volume in combination with different flow rates and interaction incubation time settings
- Wide range of inorganic and organic applications
- Lack of contamination
- Decreased risk of clogging with clotting proteins, bacteria, viruses and particles
- Kinetic assessment; Low/high affinity interaction studies
- Combinatorial techniques, like in situ characterization (ESPR) and sequential (MS) analysis

Cuvette system

The Autolab SPR instruments are set up with a cuvette configuration. This open configuration allows injection of the solution directly to the sensor surface automatically, semi-automatically or even manually. The whole sample solution is instantly injected onto the surface to start the binding process. During the interaction a constant mix flow rate will be maintained, of which the incubation time is independent of the sample volume and the flow rate. By design, not only aqueous solutions but also organic solutions to a
maximum refractive index of 1.52 can be measured.

Flow cell system

In a flow cell configuration, the sample is injected into a tubing system that transports it at a constant flow rate to the sensor surface where the binding process can be monitored for as long as the sample is in contact with the sensor surface. The measurement time depends on the sample volume and the flow rate. More details are given in the low/high affinity studies section further in this application note. The application ranges are only for aqueous sample solutions up to refractive index of 1.40.

The cuvette, inertness of material, flexibility of classes of solvent, and the open structure gives control of every step at any moment in the interaction experimental setup.

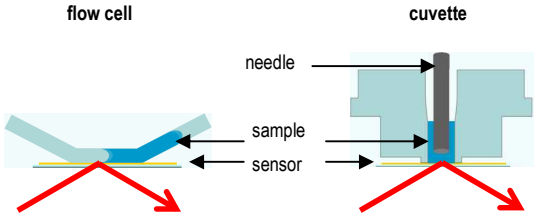

Figure 1. Schematic representation of flow cell (left) and cuvette (right) configuration

Contamination

In the cuvette system, the sample has a small contact area in the needle before injected to the sensor. Therefore, any composition of solution can be used for measurements. The small contact area will be washed (with cleaning solutions and/or high flow rates) between different steps in an experiment to avoid altogether the contamination problem. Non-specific interactions effect needs to be reduced with additives due to biochemical properties of the molecules.
In a flow cell, when a sample is transported through tubing to the sensor, there will be a problem with loss of target molecules. Especially since the flow rate is usually very low, the tubing will get contaminated. Necessary to add are additives, like detergents, high salts, or chelating agents. However, the additives not only affect the delivery of target molecules to the sensor but also the chemical interaction rate.

The cuvette system increases the accuracy of the reaction rates, with decreased likelihood of contamination.

Molecular size

The cuvette system has a 0.5 mm diameter tube to handle the sample. This diameter easily handles all kind of molecular sizes.
To increase the interaction incubation time, tubing in a flow setup have a small diameter. This can cause clogging in case of, bigger particles, clotting of molecules, and use of bacteria as a sample.

The cuvette has advantages for big molecular size measurements: less clogging.

Low affinity interaction/low sample concentration
The cuvette handles the same sample volume for a measurement of 1 minute as well as for 1 day. This is profitable for very slow binding processes, which demand a long incubation time to be able to reach the equilibrium phase. For reliable kinetic analysis, it is known to have at least 85% of the binding been proceeded. Calculated reaction rates will be biased up to 30% without equilibrium SPR data, which is not a problem for the cuvette system.
The flow cell demands exorbitant sample volume to handle this extended incubation time. (See application note: *Affinity constants from SPR equilibrium analysis. appl032)*.

High affinity interactions
High affinity biological interaction systems need only minutes to reach the equilibrium phase of the experiment. In the cuvette the target molecules altogether have simultaneously the availability to bind to the sensor.
In the flow cell setup it is necessary to have a high flow rate. Due to the fact that molecules normally enter the flow cell sensor slower than they bind, the flow rate will be measured, not the binding rate. The higher flow rates will increase the sample volume to be greater than the necessary volume in the cuvette.

The cuvette handles a smaller constant sample size for every quality/quantity biomolecular interaction.

Mass transport
As mentioned before, the cuvette handles measurements with a constant sample size, which is an advantage for mass transport limited measurements.

Mass transport is a physical phenomenon where molecules are transferred in a solution from one area to another within that solution, passing a concentration gradient. Binding of target molecules to the sensor within the diffusion layer, results in a local decrease in concentration. If the binding process proceeds at a higher speed than that of the mass transfer, the solution will temporarily be depleted of free molecules. The binding process will be delayed and biased.
In the situation of the flow cell configuration and not enough sample volume to reach the equilibrium phase of the interaction, it is impossible to have a good evaluation of the SPR data.

The cuvette's small constant sample size eliminates the mass transport effect due to the equilibrium phase measurement.

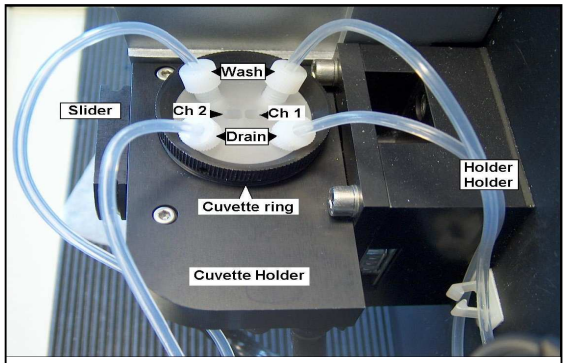

Figure 2 An overview of the cuvette holder.

Hyphenated techniques, E-SPR, SPR-MS
The cuvette configuration is able to recover samples from the sensor at any point of the experiment. This enables a sequential technique like MS. Secondly, the open cuvette structure allows simultaneous SPR and Electrochemical measurements. The Autolab SPR instruments are provided with a special electrochemistry cuvette (ESPR cuvette, figure 3) that has build in electrochemical electrodes. This ESPR set up in combination with the Autolab potentiostats/ galvanostats, provides electrochemical techniques together with SPR. The Autolab SPR instruments are therefore also very well suited for biosensor development, the development of modified electrodes, electropolymerization, etc.
In contrast of a flow system, the cuvette system is better suited for many applications.


Figure 3
The electrochemical Cuvette.

The simplicity of sample recovery and the straight forward combination with electrochemical techniques are tools to make the cuvette design a professional SPR research instrument.