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AUTOLAB

Surface *plasmon* resonance *instrument*

THE ESPRIT

THE HARDWARE

THE SOFTWARE

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Autolab Esprit liquid handling unit.

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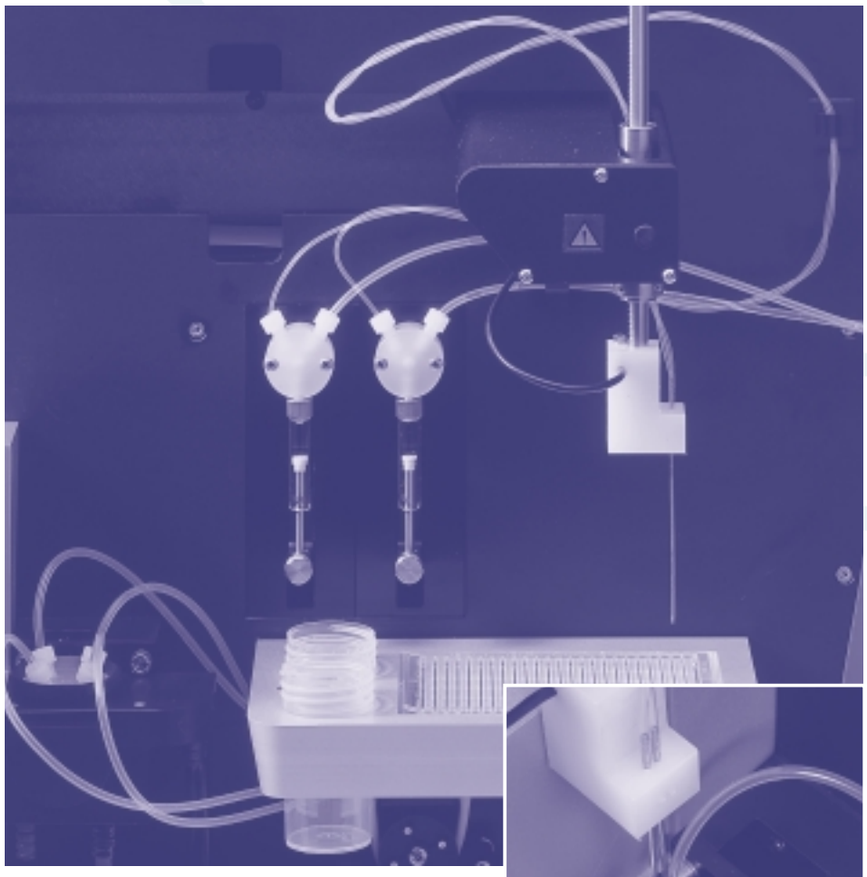

Sample is injected in the cuvette.



Electrochemical SPR cuvette.



Sensor disks with slider.

Eco Chemie B.V. was founded in 1986 by two *electrochemists* from the University of Utrecht, *The Netherlands,* and has since then been *focussed* on the development and production of *Autolab* electrochemical instruments and *accessories.*

More recently, the company also started developing and producing (Electrochemical) Surface Plasmon Resonance instrumentation, which resulted in the Autolab Esprit.

The surface plasmon resonance technique has become a standard tool in life sciences and pharmaceutical research laboratories. It is essential to study and characterize molecular interactions, to explore the structure-function relationships of biomolecules and to understand biological systems in life sciences.

The Autolab Esprit is a surface plasmon resonance (SPR) instrument with the unique possibility of simultaneous electrochemical and SPR measurements.

In order to enable electrochemical SPR measurements, the Autolab Esprit needs to be connected to an Autolab potentiostat/galvanostat. The Autolab Esprit allows the scientist to analyse bi-molecular, such as antigen-antibody, interactions in real time without labelling requirements. Other interactions where a modified surface interacts with molecules in solution can also be studied.

Combining electrochemistry with SPR makes the Esprit perfect for biosensor and DNA- or RNA- or protein chips research.

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FIELD OF OPERATION

Surface plasmon resonance measurement techniques can be used to:

- Obtain quantitative information.
  - Total concentration of a biomolecule in solution.
- Determine reaction kinetics.
- Determine affinity constants for molecular interactions.
- Determine the active concentration of biomolecules in solution.
  - Discrimination between active and non-active molecules.
- Determine which molecules interact, ligand screening or epitope mapping.
- Perform complex assembly studies;
  - Are there any steric/allosteric effects.
- Study biocompatibility of coatings.
  - How does a molecule interact with a coating.

The combination of electro-chemistry and SPR can additionaly be used to study:

- Enzymes.
- Ion channels.
- Membrane proteins.
- Polymer layers.
- Polymerisation.
- Polymer-biomolecular interactions.
- Preparation of biosensors.
  - Combining impedance or ampero-metric detection with SPR.

The Autolab Esprit is designed with an open cuvette system, giving the user access to the cuvette as well as the gold disk. It comes with a flexible software package allowing the user to fully control the experiments.

- **Electrochemical techniques**

The GPES software package provides the user with almost all electrochemical techniques and is, in combination with one of the Autolab instruments, an electrochemical workstation that can be used in many different areas.

The most commonly used techniques in combination with surface plasmon resonance are:

- Cyclic voltammetry.
- Chrono-amperometry.
- Impedance spectroscopy.



THE HARDWARE

- **Optical unit**

The instruments optical unit is designed to measure in two channels simultaneously, one sample channel and one reference channel.

The very fast sampling rate allows averaging of many data points per second, resulting in low noise

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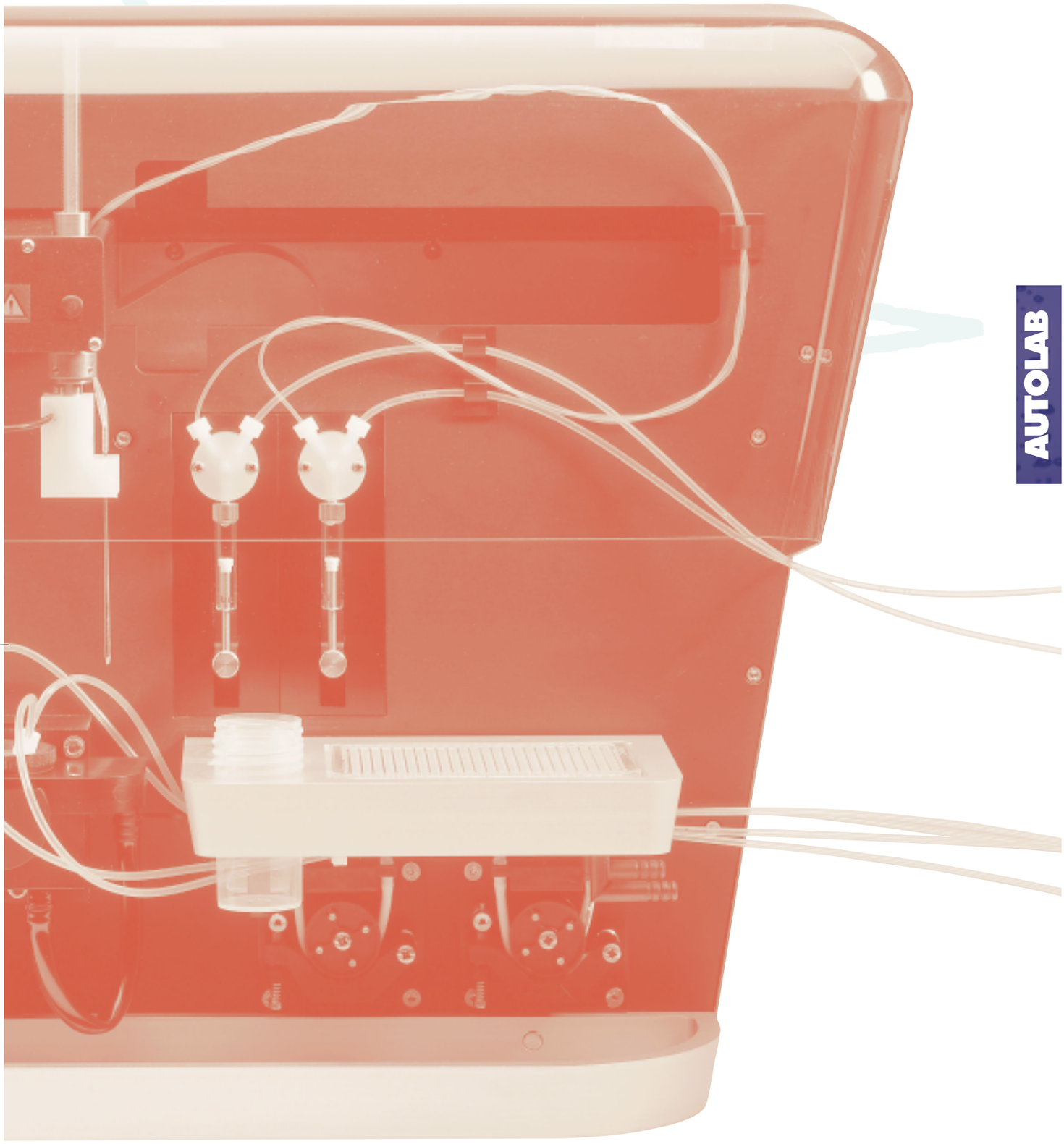

measurements. The scanning range is 4 degrees around the offset angle.

The offset angle can be manually controlled, providing a large dynamic angle range.

The laser spot has an area of 2 mm² on the gold sensor disk. Measured data will be an average of all events in that area. This has the advantage that any inhomogeneity in, or defects of any of the additional layers on top of the gold surface will be eliminated.

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- **Measurement cuvette**

The hydrodynamic parameters of the cuvette are:

- Mixing volume.
- Mixing rate.
- Distance of the needle to the sensor surface.
- Volume of the solution in the cuvette.
- Diameter of the cuvette.
- Geometry of the needle.
- Viscosity of the solution.
- Temperature of the solution.

Except for the diameter of the cuvette and geometry of the needle, all other parameters can be controlled by the user, making the instrument very suitable for research purposes.

The open cuvette structure and the flexible software give the user a number of benefits:

- Multiple sample addition is easy.
- Cuvette, cuvette slider and sensor disk can be cleaned easily.
- Consumption- and maintenance costs are relatively low.

The electrochemical cuvette has a 3-electrode system;

- Fixed contact point to the gold layer of the sensor disk (working electrode).
- Solid state Ag/AgCl reference electrode.
- Platinum counter electrode.

When SPR is combined with electrochemistry, both signals will be available in the Autolab GPES software.

- **Liquid handling unit**

Liquid or sample handling can be done manually or semi- or full automatically. The two channels of the cuvette use independent syringe pumps to assure reproducible experimental conditions. Chemically inert tubing is used throughout the whole system.

- **Fully automatic**

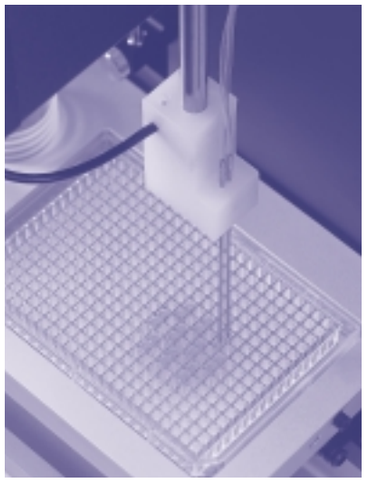
The autosampler is controlled by the software. Samples are taken from the microtiter plate and injected in the cuvette.

The automatic control allows the user to run measurements continuously.

- **Semi automatic control**

The sample is aspirated from the vial into the needle or pipetted in the cuvette manually.

Dispensing, draining, washing, mixing of sample and measurement are performed automatically.



- **Manual control**

After the sample is pipetted into the cuvette, each following step like mixing, draining, washing, starting of measurement, rinsing etc. is controlled from the software.

The general configuration of the liquid handling unit makes sample recovery possible at any time.

- **Spincoater**

The spincoater is an accessory to the Esprit system that can be used to develop customer specific surfaces on gold coated glass disks.



The Esprit software package contains a program for data *acquisition* and a separate program for *kinetic* evaluation. Both programs can be *used* with the operating systems Windows 98, 2000 *and* XP.

- **The data acquisition software**

The data acquisition software contains all necessary tools to control the instrument. Automatic control is done through so-called measurement sequences.



The sequencer window allows the user to create new or modify existing sequences. A sequence is a list of commands that is used to automate the control of the instrument.



Sample positions on the microwell plate and incubation times can be selected in the autosampler control window.

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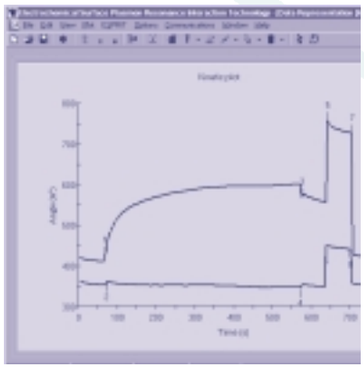
The data presentation window shows all measured data; the association, dissociation and regeneration phase. The temperature is also recorded at the same interval.

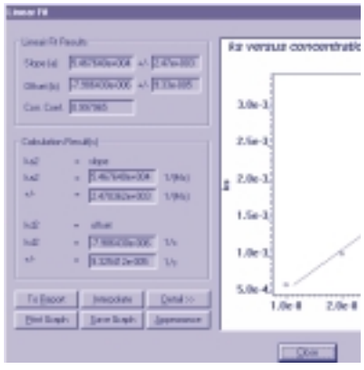


The user can add markers to any datapoint on the plot, so that comments on special events or specific areas of interest can be marked and logged into an event recorder.

- **The kinetic evaluation software**

Data generated by the data acquisition software can be analysed with the kinetic evaluation software.

With the kinetic evaluation software the following parameters can be determined:
- Kinetic rate constants.
- Affinity constant.
- Thermodynamic parameters.



Using multiple curves measured with different concentrations allows the user to obtain kinetic constants.

Actual curves can be fitted to different kinetic models.

AUTOLAB ESPRIT

• Measuring principle	surface plasmon resonance
• Transducer principle	scanning mirror
• Liquid handling	cuvette system
• Parallel channels	2
• Fixed wavelength	670 nm
• Sample loading and injection	manual-, semi-automatic-, or automatic control
• Mixing	continuous with autosampler needles
• Autosampler	integrated
• Pumps	syringe and peristaltic pumps
• Flow rate range	0.8 - 227 µl/s
• Sample volume	20 - 100 µl
• Offset of SPR angle by spindle	68° - 78°
• Dynamic range	4000 m°
• Angle resolution	< 0.5 m°
• Refractive index	1.33 - 1.43
• Refractive index resolution	$< 2 \cdot 10^{-6}$
• Measuring frequency	76 Hz
• Time interval range	0.1 s - 300 s
• Baseline noise	0.05 m° (0.5 RU)
• Sensor disk	gold coated glass disk (25 mm ∅)
• Individual surfaces modification	easy
• Standard supplied cuvette	combined Electrochemistry-SPR cuvette (ESPR)
• Additional cuvette	optional for normal SPR
• Biacore® sensor chip adaptor	optional
• Weight	40 kg
• Dimensions (H × W × D)	51 × 60 × 45 cm³
• Interface	RS 232 C
• Power requirements	170 W, 100 ... 240 V, 50/60 Hz

SPINCOATER

• Standard sample size	25 mm ∅ disk
• Motor speed range	100 - 10,000 RPM
• Manual speed setting	100 - 10,000 RPM in 100 RPM steps
• Acceleration/decelleration	4000 RPM/s
• Weight	2.8 kg (controller) 1.0 kg (spincoater)
• Dimensions (H × W × D)	77 × 196 × 245 mm³
• Power requirements	100 ... 240 V, 50/60 Hz

Specifications are subject to change without notice.

AUTOLAB

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AUTOLAB

THE HARDWARE

THE SOFTWARE

ECO CHEMIE 05/2003



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