

AWACSS - Automated Water Analysers Computer Supported System

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The AWACSS project (EVK-CT-2000-00045, <http://barolo.ipc.uni-tuebingen.de/awacss/index.html>) is supported by the European Union under the Fifth Framework Programme. It contributes to the implementation of the Key Action “Sustainable Management and Quality of Water” within the “Energy, Environment, and Sustainable Development”. The three-years project is coming to its end and the major outputs including prototypes of the multi-analyte on-line water monitoring biosensor will be presented to an interested audience on 26 March 2004 in Tübingen, Germany.

Despite the fast developments of chromatography-based techniques over the past decades, up till now almost no technology exists that can monitor trace levels of organic micropollutants in water in real-time and at reasonable costs. Therefore the project focused on the development of a robust, cost-effective water-monitoring device based on immunochemical technology that can measure several tens of organic pollutants at low-ng/l level in a single few-minutes analysis without any prior sample concentration step. Having in mind actual needs of water-sector managers related to the implementation of the Drinking Water Directive (98/83/EC) and Water Framework Directive (2000/60/EC), drinking, ground, surface and waste waters were major media used at the evaluation of the system performance and the instrument was equipped with remote control and surveillance facilities. Target compounds were selected mainly from the groups of modern pesticides, endocrine disrupting compounds and pharmaceuticals. Four water-monitoring groups from the project team were extensively assessing various fields of potential application of the system and measurement sites that could accommodate the final instruments for testing. Also, an in-depth evaluation of the performance of the AWACSS biosensor has been made in comparison to conventional analytical techniques, such as liquid and gas chromatographic systems with diode-array UV and mass spectrometric detectors. The experience of water laboratories has been utilised at the design of the instrument's hardware and software in order to make the system rugged and user-friendly. Several

market analysis surveys were conducted during the project to assess the applicability of the final system.

Principle of operation

Immunochemistry

The immunochemistry utilised in the project takes advantage of a binding inhibition test that requires antibodies directed against specific analytes and analyte derivatives that can be covalently bound to a transducer surface. Such polyclonal antibodies and their corresponding analyte derivatives have been produced for a variety of organic micropollutants. After being purified and labelled with a fluorescent marker, they were developed into immunoassays and used in the project.

Detection

The AWACSS instruments are based on Total Internal Reflection Fluorescence (TIRF) technology (Figure 1). Laser light is coupled into an optical transducer and guided down the transducer by total internal reflection. The transducer surface is chemically modified with analyte derivatives. Analyte-specific antibodies are labelled with a fluorescent marker, which upon binding to the transducer surface are excited in the evanescent field. The emitted light is then collected for detection. The design allows for the simultaneous measurement of multi-analyte spots (Figure 2).

Analyte recognition is based on a binding inhibition assay (Figure 1). Analyte derivatives are immobilised onto the transducer surface prior to the assay. Next, analyte-specific antibodies labelled with fluorescent markers are incubated with the analyte samples. After the short incubation period, the analyte solution flows over the transducer. Only analyte-specific antibodies with free paratopes will bind to the transducer surface whereas, at the same time, antibodies that have two analyte molecules bound to each epitope will not bind to the surface. The surface bound labelled antibodies are excited in the evanescent field and the fluorescence is detected. As a result, an inverse analyte signal is measured, with samples having low analyte concentrations giving rise to high fluorescence signals and samples with high analyte concentrations resulting in low fluorescence.

Innovations

A previous EU-funded project entitled, “RIver ANalyser (RIANA)” successfully utilised the biosensor technology. AWACSS was building on the achievements of the RIANA device with major improvements in three critical areas:

- 1) Expanded multi-analyte analysis capability allowing for simultaneous measurements of up to 30 analytes. A set of new immunochemical reagents was developed for a number of organic micropollutants to be analysed in water.
- 2) Novel design approaches to the optical detection and fluidics including miniaturized integrated optics and microfluidics.
- 3) Intelligent remote surveillance and control that allows for unattended continuous monitoring.

Results

Two fully automated AWACSS systems consisting of the newly developed optical biosensor with measurement control and assay programming software have been constructed and successfully tested. The software allows also for the Internet-based networking between the measurement and control stations for global management, trend analysis and early-warning applications. An easy-to-use web-based AWACSS database was created for automated evaluation and storage of the obtained data in a format compatible with major databases of environmental organic pollutants in Europe, such as Trans-National Monitoring Network and Joint Danube Survey databases at the International Commission for the Protection of the Danube River (ICPDR, 14 European countries). A built-in multimedia tutorial was developed for low-cost training of system operators.

So far more than 20 different polyclonal antibodies have been isolated and their corresponding analyte derivatives were synthesised. The target analytes were selected mainly from the classes of pesticides, endocrine disrupting compounds and antibiotics. Project partners of the water-monitoring group have systematically investigated various types of water around Europe and addressed possible water matrix effects related to the AWACSS immunoassay chemistry. Immunoassay measurements of estrone, atrazine and bisphenol-A by AWACSS system showed good reproducibility even at concentrations below 10 ng/l in both Milli-Q water and industrial wastewater. The overall performance of the AWACSS system in

comparison to the conventional analytical techniques was successfully tested in a recent inter-laboratory collaborative trial among the project partners. Results of the marketing survey and the fact that many of the AWACSS compounds are being frequently detected in real water samples all over Europe give good perspectives to the system to be placed among the current state-of-the-art analytical instruments.

Partners in the project:

University of Tübingen, Institute of Physical and Theoretical Chemistry, Germany

IIQAB - Department of Environmental Chemistry, CID-CSIC, Barcelona, Spain

King's College London, United Kingdom

Environmental Institute, Kos, Slovak Republic

DVGW-Technologiezentrum Wasser, Karlsruhe, Germany

Optoelectronics Research Centre, University of Southampton, United Kingdom

Siemens, Erlangen, Germany

Central Research Laboratories, Hayes, United Kingdom

Water Research Institute, Bratislava, Slovak Republic

The project coordinator is *Prof. Dr. Günter Gauglitz*, University of Tübingen, Germany; e-mail: guenter.gauglitz@ipc.uni-tuebingen.de. The public presentation of the AWACSS project results will take place in Tuebingen on 26th March 2004 from 10:30 a.m. - 4:00 p.m. at the Institute of Physical and Theoretical Chemistry (IPTC). For information on how to get to the institute see http://www.uni-tuebingen.de/Chemie/index_plaene.html. In case you intend to attend the presentation, please, send the information to e-mail: jens.tschmelak@ipc.uni-tuebingen.de.

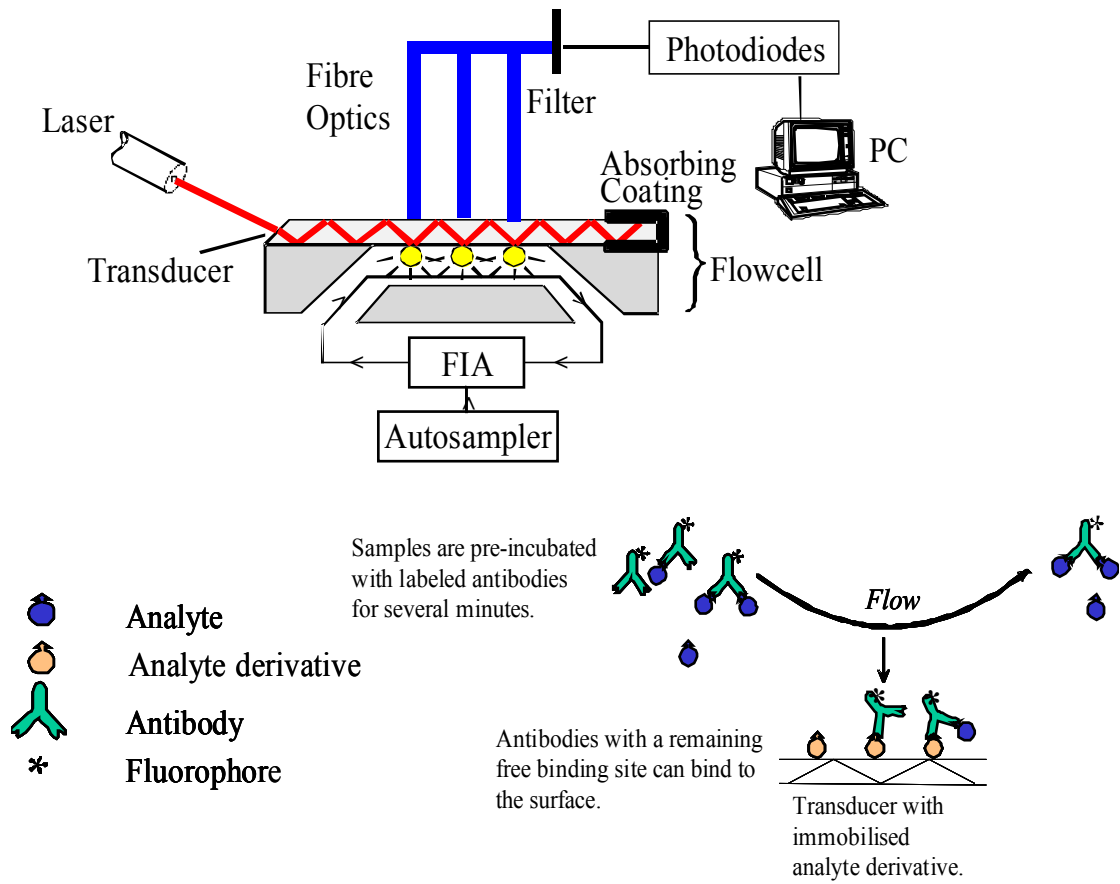


Figure 1: TIRF experimental set-up and the immunoassay principle.

AWACSS Device

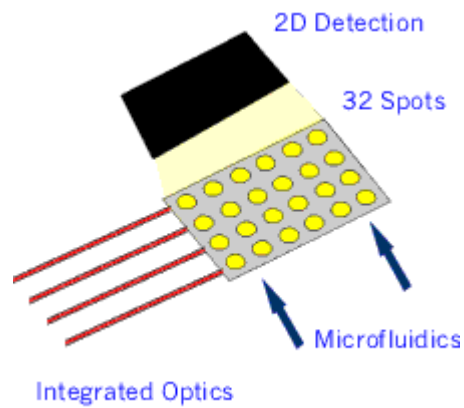


Figure 2: The design of AWACSS allows for the fast simultaneous measurement of up to 30 different analytes in a single analysis.

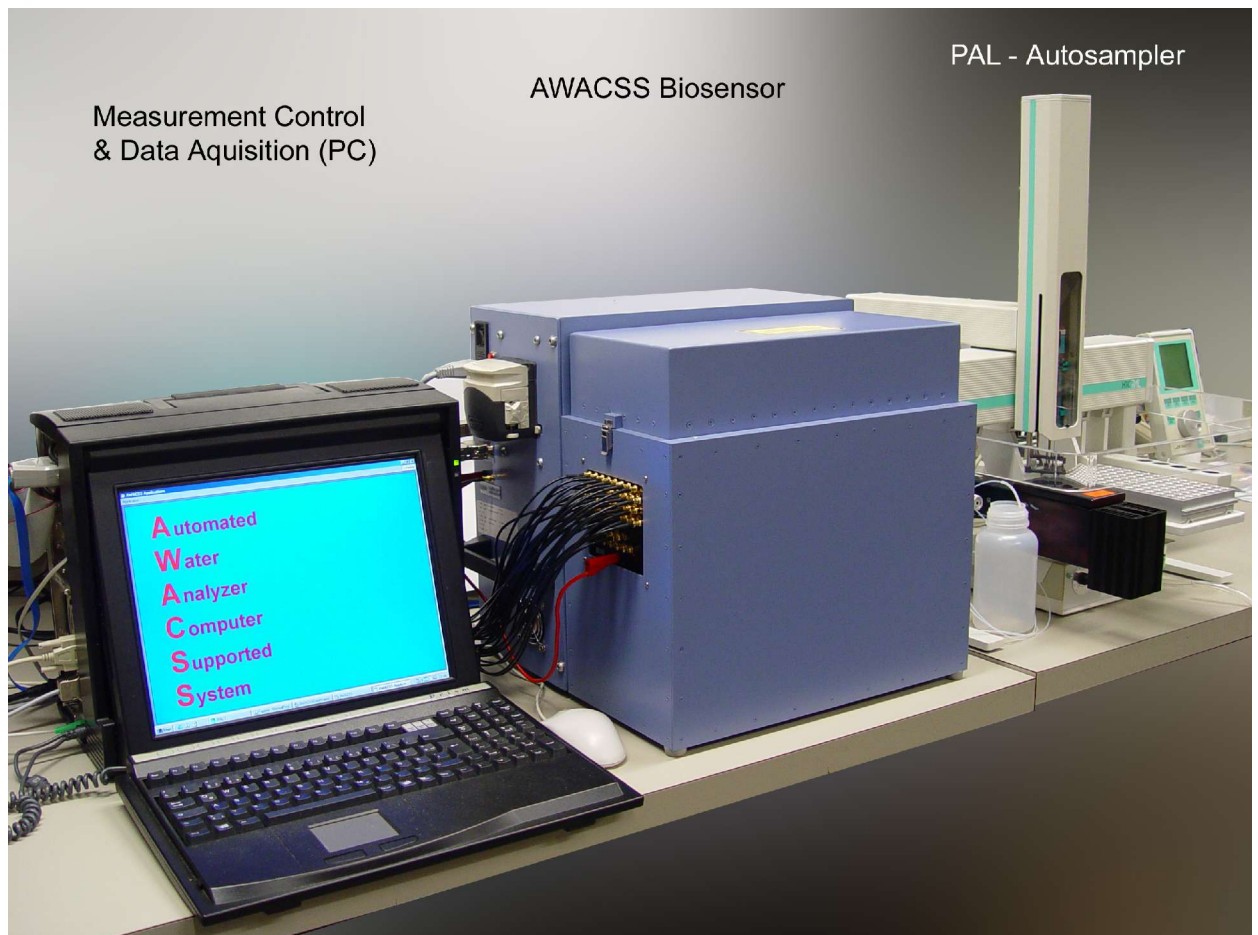


Figure 3: AWACSS biosensor is equipped with software for measurement control and data acquisition, remote control and data storage via dedicated AWACSS Database.