Organic Process Research & Development **2000**, *4,* 102–106

On-Line Monitoring of Process HPLC by Sensors

Thomas Wessa,*,† Stephan Küppers,† Gregor Mann,† Michael Rapp,‡ and Jürgen Reibel§

Schering AG, Chemical Development, D-13342 Berlin, Germany, Forschungszentrum Karlsruhe, IFIA B341, P.O. Box 3640, D-76021 Karlsruhe, Germany, and Bürkert GmbH&CoKG, Christian-Bürkert-Str. 2, D-01900 Grossröhrsdorf, Germany

Abstract:

On-line process control with a SAW (surface acoustic wave) sensor-based gas-sensor microsystem (SAGAS PC = surface acoustic wave based aroma and gas analyzing system) for industrial applications is presented. The monitoring of the gradient former of a preparative HPLC system is a useful tool for the on-line process control of pilot plant production. All applications deal with the detection and subsequent qualification as well as quantitation of volatile organic compounds (VOCs), for example, organic solvents or even water, by investigating their vapor with the SAGAS PC-system. To demonstrate the suitability of the system for the above-mentioned purpose different examples of typical binary solvent mixtures for normal phase (NP) HPLC have been chosen. The accuracy, precision, and robustness of the applied method are determined, and the results meet the specification requirements. Furthermore, on-line measurements in a pilot plant environment are presented.

Introduction

Over the past decade sensor application has gained entry into analytical departments, and the methods developed have continued to gain more and more ground in industrial processes. Additionally, sensors fulfill many of the features demanded of on-line process control. Commonly, physical sensors (pressure, temperature, ultrasonic, pH, etc.) are used for this purpose because of the quick and independent response allied to low production costs and high robustness. Even chemical sensors which typically consist of a sensitive recognition element (usually polymer layers) and a special transducer (converts the chemical information into an electrical signal) have become more reliable. Different types of transducer principles have already been realized. Sensor systems based on semiconductors, mass-sensitive devices, optical or electrical signal-transduction are commercially available.

On the other hand, on-line monitoring as well as control of chemical reactions and production procedures in industrial processes is one of the most promising fields of process optimization. Usually on-line analytical procedures are the process-time determining step in chemical plants. Therefore, combining the advantages of sensor systems for routine analytical investigations is an obvious move: a fast and reliable result, which can be obtained without an analytical expert, by the pilot plant staff will shorten the above-mentioned time-consuming step. Furthermore, the result can be used directly for process control.

This paper deals with an application of on-line monitoring in industrial processes. As a suitable example we have chosen the control of a gradient former in preparative process HPLC. A mass-sensitive sensor-system—surface acoustic wave (SAW) devices are integrated in this system—is used for on-line measurements in the pilot plant at Schering AG, Berlin.

In preparative HPLC systems for process research and development the separation of mixtures containing drug substance intermediates and different by-products is controlled exclusively by, for example, an in-line UV detector. Therefore, changes in the mobile phase composition cannot be observed by the collector, and consequently, if the composition of the mobile phase departs from the set value, the separation is no longer optimal. A tool that will monitor and, in addition, control the eluent mixing step is obviously a desirable goal. An instrument capable of fulfilling this task has to facilitate the following:

- the time between an “out-of-control” situation and subsequent adjustment must be as short as possible to keep the quality of the product always in the specification range,
- the results obtained must be suitable for use in adjusting the actual mobile phase composition to the desired value immediately,
- the user interface of the tool has to be suitable for use by qualified plant personnel and not only by analytical chemists,
- the instrument has to be entirely reliable for routine use, and
- it should be commercially available at moderate cost.

The complete set up needs to be automated including sampling, analysis, and data evaluation.

The elutions will usually be performed isocratically with different mobile phase compositions, and binary solvent mixtures will be commonly used. Table 1 summarizes the most typically applied solvent mixtures for normal phase (NP)- and reversed phase (RP)-HPLC applications.

The concentration range of one component in the mixtures varies typically from 5 to 95% (by volume). As a consequence this application demands an analytical procedure which shows high precision and accuracy over a wide working range.

For NP-HPLC investigations the deviation from accuracy has to be less than 1 vol % and for RP-HPLC this value

---

* Corresponding author. Telephone: 030/46811968. E-mail: thomas.wessa@schering.de

† Schering AG.

‡ Forschungszentrum Karlsruhe.

§ Bürkert GmbH&CoKG.

10.1021/op990080v CCC: $19.00

Table 1. **Most Commonly Used Binary Solvent Mixtures in Industrial HPLC**[a]

NP-HPLC	RP-HPLC
hexane/ethyl acetate	methanol/water
hexane/acetone	acetonitrile/water
dichloromethane/acetone	
dichloromethane/ethyl acetate	
less common:	
hexane/*tert*-butylmethyl ether	

[a] On the basis of our experience, these combinations of mobile phase compositions enable more than 90% of all HPLC separations

should not exceed 2 vol %. The precision for both application fields should be less than 0.5 vol % (absolute standard deviation).

A possible solution for the described on-line application is the use of a sensor-based (gas-) analysis system. The detection of volatile organic compounds (VOCs) in particular can be achieved by the use of polymer-coated surface acoustic wave (SAW) devices such as chemosensors. Many applications of these sensors for use in laboratories have already been described[1−5] but only a few industrial-applications for the quantitative determination of solvents or mixtures thereof are known.[6]

The SAW-resonators are coated with polymers which are responsible for the sensitivity and selectivity of the devices. The resulting chemosensors are incorporated in an oscillator circuit, and the resonance frequencies, which are mainly correlated to mass changes in the polymer matrix, are recorded.[7] A high selectivity can be achieved by combining several mass-sensitive devices to a sensor array. This approach is realized in a commercially available instrument: the SAGAS PC-system. This sensor system delivers data patterns which can be easily analyzed by chemometric methods such as multi-component analysis and artificial neural networks.[8] This paper describes the application of neural networks based on feed-forward nets.[9]

This approach—commonly called "electronic nose"—is one of the most intensively investigated fields in sensor development.

For the application of the SAGAS PC instrument in process HPLC (PHPLC), the vapor of the solvent mixtures has to be investigated and thus a special permeation cell had to be constructed.

In this study the results for quantitative analysis of the following normal phase mixtures are presented:

- hexane/ethyl acetate
- hexane/acetone
- toluene/isopropyl alcohol/ethyl acetate

Experimental Section

The SAGAS PC-system was developed at the Forschungszentrum Karlsruhe and can be purchased from Bürkert GmbH&CoKG, and even a modification suitable for ex-areas is available. The latter was used for all on-line measurements in this study. The system consists of a liquid-sampling system which takes a sample of the actual mobile phase and stores it in the permeation cell. After a defined equilibration time the gas phase in the permeation cell is pumped into the SAW-sensor unit where the composition of the gas is determined. Afterwards, the SAW-sensors and the permeation cell are flushed with nitrogen. The calibration of the system is performed by the analysis of mixtures with known compositions.[6] The resulting data patterns are used for net-training purposes, and the neural network, so calculated, is subsequently applied for all on-line determinations.

The fluidic system is completely automated and directed by the sensor software in parallel. The solvent fluidics facilitate the continuous transport of actual mobile phase mixtures into the measuring chamber. This task is realized in a kind of a "stopped-flow-process" performed by a combination of three ex-valves (Bürkert) which are controlled by a sequential program inside an SPS (stored program system) module. The SPS module (SPS S-200, Siemens AG, Germany) controlling the solvent fluidics is located inside the explosion protection unit. The SPS internal sequential program, which determines the steps of the stopped-flow-process and the step times, can be easily changed at any time. For on-line control purposes, four consecutive steps are necessary: filling of the measuring chamber with the particular mobile phase, keeping this sample for a certain time, removing the sample from the measuring chamber, and drying the chamber by a nitrogen flow. This synchronization of the gas and the solvent fluidics is guaranteed by trigger signals sent from the sensor system to the SPS module. The scheme of all components described is shown in Figure 1. This complete system is coupled to the PHPLC-apparatus in the pilot plant by connecting the measurement cell with the eluent flow of the PHPLC-system. The connection is placed after the gradient former and before the HPLC-column. Therefore, only pure mobile phases without any sample content will be analyzed.

To verify the correct composition of the calibration and test solutions, reference analyses were performed using headspace gas chromatography (sampler: Perkin-Elmer HS40, gas-chromatograph: Hewlett-Packard HP5890 Series II, DB624 column).

Artificial Neural Network. The sensor system is controlled exclusively by the "Analyzer" software.[10,11] This

---

(1) Wessa, T.; Göpel, W. *Fresenius J. Anal. Chem.* **1998**, *361*, 3, 239.

(2) Dickert, F.; Zenkel, M.; Bulst, W.; Fischerauer, G.; Knauer, U. *Fresenius J. Anal. Chem.* **1997**, *357*, 27.

(3) Rapp, M.; Böss, B.; Voigt, A.; Gemmeke, H.; Ache, H. J. *Fresenius J. Anal. Chem.* **1995**, *352*, 699.

(4) Bodenhöfer, K.; Hierlemann, A.; Seemann, J.; Gauglitz, G.; Koppenhöfer, B.; Göpel, W. *Nature* **1997**, *387*, 577.

(5) Bodenhöfer, K.; Hierlemann, A.; Juza, M.; Schurig, V.; Göpel, W. *Anal. Chem.* **1997**, *69*, 4017.

(6) Wessa, T.; Küppers, S.; Rapp, M.; Reibel, J.; Stahl, U. *Fresenius J. Anal. Chem.* **1999,** *363* (8), 744−748.

(7) Auld, B. *Acoustic Fields and Waves in Solids*; Krieger Publishing Company: Malabar, Florida, 1990.

(8) Hierlemann, A.; Schweizer-Berberich, M.; Weimar, U.; Kraus, G.; Pfau, A.; Göpel, W. Pattern Recognition and Multicomponent Analysis. In *Sensors Update: Sensor Technology-Applications-Markets*; Baltes, H., Göpel, W, Eds.; VCH: Weinheim, 1996; Vol. 2, p 119.

(9) Rapp, M.; Reibel, J.; Stier, S.; Voigt, A.; Bahlo, J. *Proc. IEEE Freq. Control Symp.* **1997**, 129.

(10) Eppler, W.; Hartmann, V.; Gemmecke, H. Conference Proceedings, *Transducers 99*, Japan, 1999.

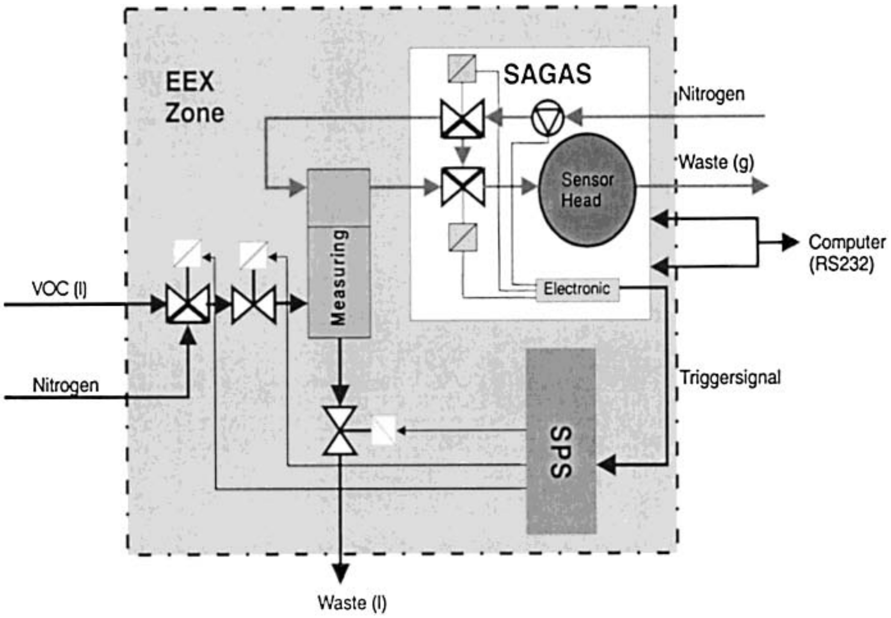

Figure 1. **Scheme of the different fluidic components. All gas flows are directed by valves which are controlled by the SAGAS system. The valves used for liquid handling are controlled by the SPS module. The complete system is ex-shielded and therefore suitable for a pilot plant environment.**

program is able to collect the sensor data from the 8 SAW devices and the recorded patterns can be analyzed easily with modern methods of pattern recognition like principal component analysis or neural networks. The latter application uses the intelligence of the program to create and to train different kinds of artificial neural networks, which can subsequently be used for on-line classification of the sensor data obtained. The example presented here deals with two different neural networks: feed forward (FF)-nets and for discrimination of unknown analytes radial basis function (RBF)-nets.

For the quantitative analysis of the recorded sensor data, a FF-net is necessary (consisting of eight input neurons, one hidden layer with six hidden neurons, two output neurons, training pattern: two measurement cycles for each concentration—mean of 20 patterns; learning algorithm: Rprop, 5000 learning steps).

Because of the complexity of the complete software a special user interface module was created, which is suitable for routine procedures in the pilot plant. This additional module of the software enables the user to record and save the different frequency values and, furthermore, generates the results of the neural network, which can be simultaneously shown on the monitor and saved automatically to a file.

Results

After initial experiments in the laboratory the ex-shielded sensor-system was qualified and a typical procedure was validated. The results were obtained using typical preparative HPLC systems (30-cm inner diameter (i.d.) columns) running in the laboratory. Afterwards, the sensor-system was integrated in the HPLC system running in the pilot plant at Schering. Depending on the routine work in the pilot plant, first investigations were performed in the laboratory with the following NP-HPLC applications only:

- hexane/ethyl acetate
- hexane/acetone
- toluene/isopropyl alcohol/ethyl acetate

The sensor-system had to run in parallel to the HPLC separation. It took 25 mL of the actual mobile phase every 2 min and analyzed the mixture quantitatively (60 s measuring time + 90 s purging time of the sensors).

Hexane/Ethyl Acetate. The binary mobile phase system, hexane/ethyl acetate, was chosen to demonstrate that the instrument can be validated for GMP purposes. The results of these experiments are described in detail elsewhere,[12] and the following analytical performance parameters were determined. The regression coefficients of the calibration curves (*linearity*) were found to be greater than 0.999. The *limit of detection* was calculated for hexane (1.09 vol %) and for ethyl acetate (0.67 vol %). The *limit of quantitation* for the solvents was determined for hexane (3.64 vol %) and ethyl acetate (2.2 vol %). Both analytes can be detected with an *accuracy* error lower than 1 vol %. The *precision* (*repeatability*, *reproducibility*, *intermediate precision*), the *robustness*, and the specificity were checked and meet the limits of the validation plan.

For all measurements, the temperature of the liquid and its volume in the measurement cell have to be monitored simultaneously. The temperature of the cell was kept constant at 19 °C, and the SPS was programmed such that 25 mL of the liquid was directed automatically to the cell. This volume was adjusted with a 50/50 mixture of the particular solvent mixture and monitored manually during the on-line measurements.

---

(11) Hartmann, V.; Wessa, T. *GIT Fachz. Labor.* **1999**, *4*, 332−335.

(12) Wessa, T.; Küppers, S.; Rapp, M.; Reibel, J. *Sens. Actuators B*, manuscript accepted for publication, 2000.

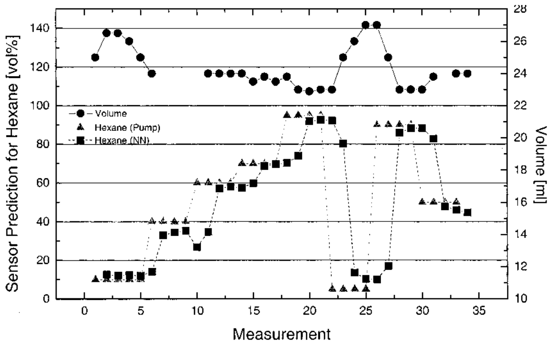

Figure 2. **Predicted (NN, means neural network) and adjusted values (pump) of the hexane concentration (left axis) during an on-line measurement in the laboratory are shown. In parallel, the corresponding sample volume in the permeation cell was plotted (right axis).**

The on-line results of the hexane/ethyl acetate system are given. Figure 2 shows the sensor prediction for different hexane/ethyl acetate compositions which are mixed by the gradient former of the HPLC-system (left axis). Furthermore, the determined cell volumes are plotted (right axis). This figure depicts the following features:

- A quick and reliable sensor prediction is recorded for the complete concentration range. This demonstrates the suitability of the sensor system for the on-line control of the HPLC system.
- The accuracy of the measurement decreases a little when compared with the off-line calibration measurements (i.e., GC). The latter shows an accuracy significantly lower than 1 vol %,[12] the on-line measurement shows deviations of 3−4 vol %.
- The biggest difference between the concentration adjusted by pump and the sensor prediction is found at low hexane concentrations. This correlates with differences in liquid volume for these concentrations. In both cases (5 and 10 vol % hexane, respectively) the solvent volume increases significantly by 10%.
- A time shift is monitored between pump adjustment and sensor prediction. The sensor normally predicts the correct concentration, already one cycle after adjustment, which correlates with a time-shift of 2−3 min.

Hexane/Acetone. Another sample mixture (hexane/acetone) was investigated and the results are shown in Figure 3. All features found in the case of hexane/ethyl acetate hold for hexane/acetone, too. The suitability of the sensor system is demonstrated again, the accuracy increases compared to the above-mentioned example (difference of 1−2 vol %), and the time-shift is obtained once more.

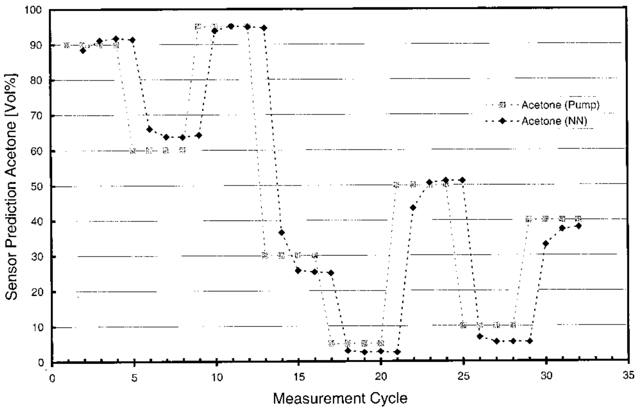

Figure 3. **Predicted (NN, means neural network) and adjusted values (pump) of the acetone concentration during an on-line measurement in the laboratory are shown.**

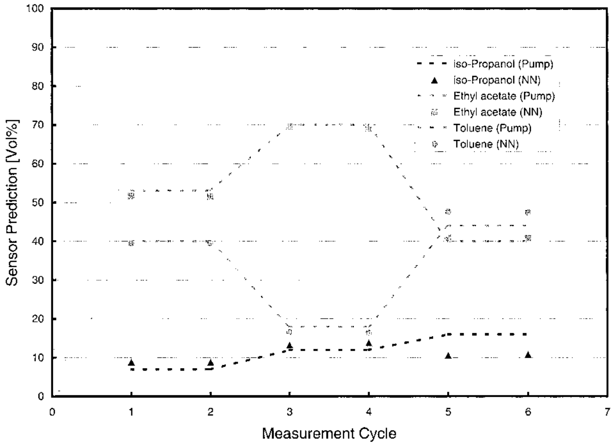

Figure 4. **The sensor prediction for a ternary mixture. Predicted (NN, means neural network) and adjusted values (pump) of the different solvent concentration (left axis) during an on-line measurement in the laboratory are shown. One can find a very good prediction for ethyl acetate and a significant misfit for low isopropyl alcohol concentrations.**

Ternary Mixture of Toluene/Ethyl Acetate/Isopropyl Alcohol. In a preliminary experiment, a ternary mixture of toluene, ethyl acetate, and isopropyl alcohol was investigated to demonstrate the suitability of the sensor system for this case. Figure 4 summarizes the different predictions of the sensor system for all three analytes. It can be seen that the accuracy for ethyl acetate was outstanding and completely met the user's requirements. A problem arose when analyzing the isopropyl alcohol content of the mixture with increasing alcohol concentration. In consequence, the toluene prediction became even worse because the sum of all three components has to be 100 vol %. Nevertheless, it was exclusively a problem of the HPLC-pump used in the laboratory, which was not suitable for use with solvents of high viscosity such as isopropyl alcohol. It is important to make clear that only 15 calibration points (different mixtures for net training) were necessary to obtain the above-mentioned results. The inevitable calibration procedure had to be increased for only a few experiments—in comparison to the calibration procedure necessary for binary mixtures—and takes 3−4 h. The 15 different calibration points were chosen intuitively.

Conclusions

SAW sensors are a useful tool for the fast and reliable on-line monitoring of the mobile phase used for HPLC even if a gradient is used. The results obtained demonstrate the suitability of the SAGAS PC-system for on-line HPLC control. After a short testing period in the laboratory, the ex-shielded sensor system was established in the pilot plant and subsequently used for routine monitoring of the gradient former in preparative process HPLC.

Mixtures of hexane/ethyl acetate and hexane/acetone (NP-HPLC) can be analyzed with a high accuracy.

The advantages of integrating a sensor system into PHPLC are obvious:

- the SAW-instrument is a fast tool for solvent composition determination,
- any changes in the mobile phase composition or the gradient are directly observed and can immediately be corrected,
- the system was found to be reliable (proved in 30-cm i.d. columns) and can be operated in pilot plant environments (ex),
- the developed analytical procedures can be validated according to the ICH (International Conference on Harmonization) guidelines,
- the software for routine measurements is very easy to handle and also includes the fluidic control, and
- the recorded sensor data can be evaluated without an analytical expert

Hence, the sensor-system described represents a useful tool for further on-line applications in industrial processes. Another typical example in this field is the identification of raw materials. The suitability of the instrument for this purpose has already been shown. Further investigations, for example, the implementation of the sensor system in distillation columns, will be performed.

Acknowledgment

The help of the following persons is gratefully acknowledged: Nico Harwardt (Schering AG) for all the sensor measurements, the group of Gregor Mann (Schering AG) for all their support in PHPLC, Dr. Peter Müller (Bürkert GmbH&CoKG) for support during development of the ex-shielded sensor system, Volker Hartmann (FZK) for the great support with all *"Analyzer"* software problems, Achim Voigt (FZK) for all his support concerning the electronics.

Received for review September 13, 1999.

OP990080V