

## Multi – Way Biosensors: Development and Commercialization

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### Introduction

The main milestones in biosensor history till 2000 include, e.g.,

- 1962 – 1<sup>st</sup> Description of an (amperometric glucose) biosensor
- 1969 – 1<sup>st</sup> Potentiometric biosensor (for urea)
- 1972/75 – 1<sup>st</sup> Commercial biosensor (YSI, Ohio, USA)
- 1982/1986 – 1<sup>st</sup> Commercial biosensor in Europa ( CSE Berlin, Germany / PGW Medingen / ENH Hamburg, Germany)
- 1987 – 1<sup>st</sup> Commercial biosensor for Blood Glucose Home Monitoring (MediSense/ExacTec)
- 1990 – Launch of SPR based BIAcore (Pharmacia/Sweden)

In 1997 the IUPAC committee did agree on the following definition of a **biosensor**:

*An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with an electrochemical transduction element. Because of their ability to be repeatedly calibrated, we recommend that a biosensor should be clearly distinguished from a bioanalytical system, which requires additional processing steps, such as reagent addition. A device that is both disposable after one measurement, i.e., single use, and unable to monitor the analyte concentration continuously or after rapid and reproducible regeneration should be designated as a single-use biosensor.*

From the beginning in 1975 the Berlin biosensor group did deal **NOT** with “single-use biosensors”, but with “multi-use” or “multi-way biosensors”. In comparison to single-use biosensors multi-way biosensors can be used for more than one analysis, at least twice.

The advantages of multi – way biosensors in principle are i) the possibility of calibration causing a high analytical performance and ii) the possibility to achieve cost-effective analytical tools.

### Multi-way biosensors for laboratory use

Today, multi-way biosensors for glucose and lactate are state of the art in clinical chemistry. In most clinical laboratories of Europa stand alone glucose analyzers are used for diabetes diagnosis and therapy control. The YSI Model 23 Glucose Analyzer was introduced by Yellow Spring Instruments of Yellow Springs, Ohio in 1974 in 1972. This model and later YSI Glucose Analyzers served historically as gold standards with which the accuracy of other glucose analyzers was compared. The YSI glucose analyzer was followed by various families of instruments launched by Eppendorf-Netheler-Hinz (ENH) in 1986, 1987, 1988 and 1990. All the ENH instruments did work with hemolysed blood samples and

the detection is based on BST Bio Sensor Technology membrane type multi-way biosensors. The 50fold dilution of blood samples with a suitable buffer solution is causing the complete disruption of the erythrocytes within seconds. Because of the interruption of glycolysis the resulting samples are stable for 24 h. That is very reliable for usage in big hospital laboratories.

The BST membrane type glucose biosensors are designed to provide selectivity for glucose, rapid response, low drift and long operational life in highly diluted hemolyzed samples. These analytical performance has been approved by Multicentre Evaluations.

Later in 2001 BST introduced and manufactures now a biosensor based on an electrochemical thickfilm structure on a ceramic substrate combined directly with an enzyme immobilized in the stable BST matrix. The even more reliable glucose thick film biosensor can be exchanged in an easy way after a shelf life of month. These thick film type biosensors are on the market for glucose and lactate and used in various laboratory analyzer measuring hemolyzed blood samples again.

The analytical performance is even better as compared to the membrane type biosensor, the reproducibility is more defined and the trash is reduced:

- i) The run in time of any dry stored biosensor inside an analyzer is below 15 min
- ii) After 60 days the sensitivity is still around 70 % of that of day one and still more than enough for the usage in the analyzer.
- iii) The serial imprecision with  $n = 10$  samples of hemolyzed blood containing 5.5 mM glucose is better than 1.5 %
- iv) Over more than 1 month the relative sensitivity between glucose, ascorbic acid and acetaminophen is much better than 1:1:1:1. Thus, potential influence of reducing substances on the original glucose signal can be disregarded.
- v) The correlation of hemolyzed blood sample glucose values measured by membrane type and thick film type glucose biosensor as well, is excellent. One example with  $n = 955$  hospital samples resulted in

$$Y = (1,0077 x + 0,0146) \text{ mM } (r = 0,99572).$$

### **Multi-way biosensors for Point of Care Testing**

Today glucose monitoring is the most important tool to diagnose and monitor diabetes. Diabetes itself is an increasing problem of health care worldwide. In 2025 the World Health Organization is expecting more than 300 million diagnosed diabetes patients worldwide.

State of the art of glucose analysis today is characterized by two main kinds of analysis. In clinical laboratories high quality analysis is performed by lab analyzers described above. Investment is relatively high, sample price is quite low, and results are available for patients or medical doctors not immediately. The alternative way is a patient near detection using single use strips or single use biosensors. These portable tools are simple to use without any investment, results are available immediately after sample withdrawal. However, the quality is limited (the single use biosensor can not be controlled or calibrated) and prices are different, in some regions quite high, in some areas very compatible to lab analysis.

In 2004 we succeeded to place our multi-way biosensor technology into the field of POCT. It is worldwide the first and only mobile glucose measuring device for decentralized locations based on a multi-way biosensor. After first placements in Germany the first generation is on the international market since 2005. The Glukometer 3000 is combining the advantages of the current well known technical fields, the lab analyzer and the single use biosensor as well – allowing a portable glucose measuring device. Due to the requirement of the Clinical Laboratory Improvement Amendments of 1988 (January 30, 2008) this instrument is characterised by simple handling, high quality and low price and the option to be connected with a central information system is given.

To realize a successful POCT instrumentation the procedure has to be as simple as possible. The most important features are:

- i) No preanalytics
- ii) Low sample volume
- iii) Procedure not volume dependent
- iv) Portable instrumentation (low weight and small size)
- v) Connectivity to Laboratory and Hospital Information System

### ***The instrument***



Figure 1: Glukometer 3000 – A POCT glucose measuring instrument based on a BST multi-way biosensor.

The Glukometer 3000 (see figure 1) is working based on a BST multi-way glucose biosensor. Each biosensor is delivered and stored dry in a blister package. The storage stability in dry state at +4°C is 1 year and the sensor inside the instrument is so stable that it requires replacement only after 30 days of use or after assay of as many as 1000 samples. Following assay of a whole blood sample, the fluidics of the Glukometer 3000 allows it's cleaning with as little as a few microliters of rinsing solution

The management of lifetime is internally controlled.

The exchange of the biosensor is simple and software guided. A newly placed biosensor needs a run in time of 60 minutes. After a successful calibration the system can be used again for 1000 patient samples or 30 days. It is powered by an internal accumulator which is recharged after a period of time. Environmental temperature for application is 15 – 40 °C; relative humidity can be between 20 – 95 %. It is CE certificated and distributed in Germany, Europe and Asia. The system is used in wards of hospitals, emergency rooms and physician's offices, filling, at low cost, the gap between large central laboratory glucose analyzers and single-use biosensors

### ***The BST multi-way glucose biosensor***

The multi-way glucose biosensor is the combination of a thick film electrode on ceramic and glucose oxidase immobilized in special polymers. The size is about 6 x 24 x 0,2 mm. The procedure is working with undiluted whole blood without any pre-treatment. The concentration range from 0.5 mM to 33.0 mM has been achieved by optimal design of the diffusion layer causing a 50fold glucose dilution inside the biosensor matrix.

Signal identification is based on H<sub>2</sub>O<sub>2</sub> detection and selectivity for glucose is provided by the special immobilization of the glucose oxidase (see table 1), thus, H<sub>2</sub>O<sub>2</sub> selective membrane is not necessary.

The **imprecision** of the described glucose biosensors is less than 5 %. Figure 2 shows one example over a period of 35 days.

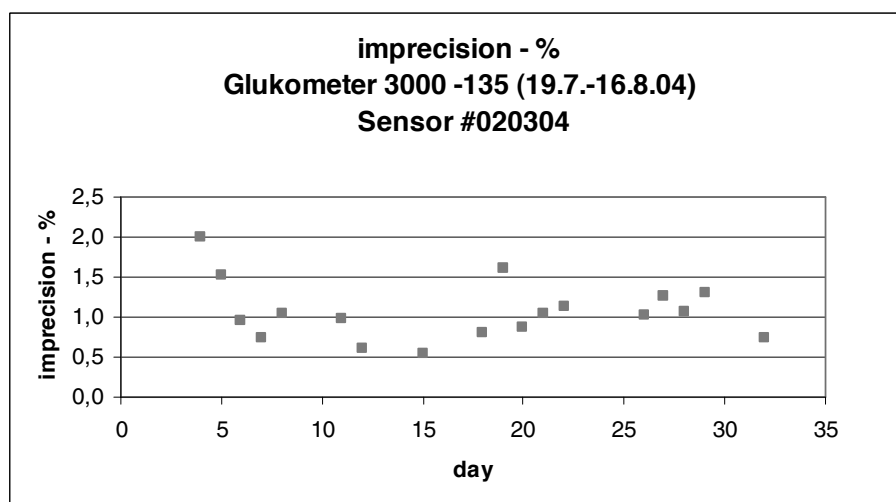


Figure 2: Imprecision of BST multi-way glucose sensor over 35 days for whole blood without pre-treatment

Table 1 is demonstrating the **selectivity** of the multi-way glucose biosensor. Because of the optimized designed polymer matrix, no reducing substances are influencing the original glucose signal significantly.

Table 1: Substances und concentrations with no influence on the original glucose signal

substance	concentration
Paracetamol	100 µg/ml
Salicylic acid	500 µg/ml
Urea	500 mg/dl
Creatinine	30 mg/dl
Uric acid	20 mg/dl
Dopamine	50 µg/ml
Galactose	150 mg/dl
Maltose	110 mg/dl
Sucrose	50 mg/dl
Xylose	100 mg/dl
Ibuprofene	400 µg/ml
Glutathione	1 mg/dl
Ascorbic acid	17,5 mg/dl
Lactic acid	100 mg/dl
Heparine	1000 I.E./L
Potassium oxalate	800 mg/dl
Sodium fluoride	200 mg/dl
EDTA	200 mg/dl
Chlorpropamide	750 µg/ml
Na-citrate-dihydrate	1000 mg/dl

As recommended in literature (D'Orazio et al 2005) in connection with **accuracy** and the correlation to comparable methods it is very important to consider the material used for analysis.

The multi-way biosensor used in the Glukometer 3000 has been developed and optimized for application to whole blood. This material is recommended by the manufacturer and it is the only material of sense for a POCT instrument. It can be used unprocessed and immediately. The instrument is calibrated to capillary whole blood.

If the results of measurements with the Glukometer 3000 shall be correlated with a plasma measuring analyzer, the general difference in glucose concentration of whole blood and plasma samples has to be taken into account.

The concentration of water in plasma is 0, 93 % in average and is 0, 84 % in whole blood. Glucose is only found in the water compartment of any sample. Therefore, in average and applying the normal values of hematocrit of 0, 43 and reference values of electrolytes as well a difference in glucose concentration of 11% is found between plasma and whole blood samples (D'Orazio et al. 2005).

Figure 3 demonstrates the results of a correlation between Glukometer 3000 (using whole blood) and DIMENSION (DADE-BEHRING, using plasma) after correction of plasma values. A very good correlation is found with 135 samples.

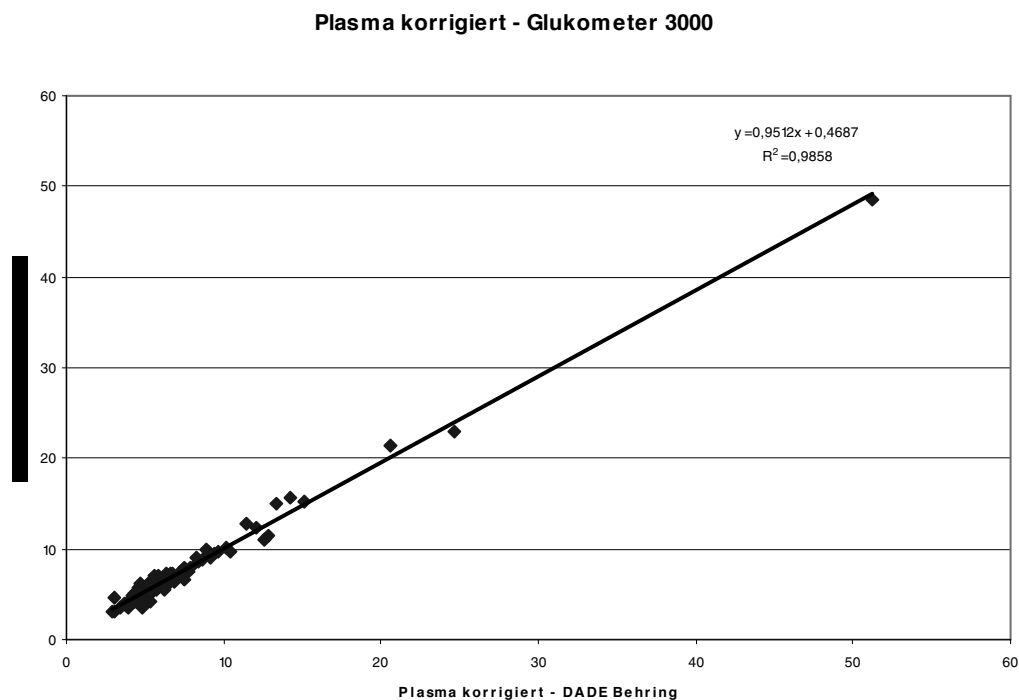


Figure 3: Correlation of glucose concentrations measured by Glukometer 3000 (whole blood) and DIMENSION (DADE Behring, Plasma)

## References

P D'Orazio, RW Burnett, N Fogh-Andersen et al. "Approved IFCC Recommendation on Reporting Results for Blood Glucose" Clin. Chem. 51:9 (2005): 1573-1576