

Metrological Aspects of Activity Measurements

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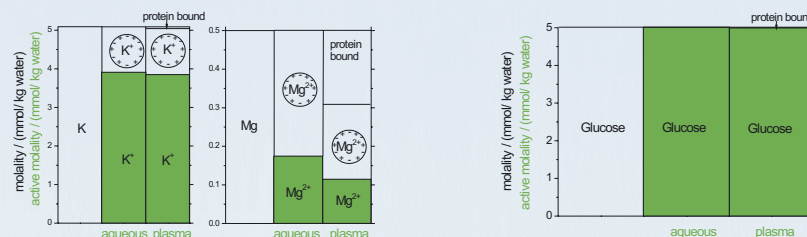
Introduction

The national regulations and European directive for in-vitro diagnostics (98/79/EG from 27. October 1998) require that traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order. METAS' contribution is to provide traceable reference values for chemical activity measurements of ions in complex electrolytes and of glucose in solutions. These two types of analyte measurements are most common tasks of a clinical chemical laboratory and of high relevance to the physicians.

Amount of substance content versus chemical activity

The chemical activity of dissolved ions and of glucose in physiological liquids corresponds to the freely available part of these components of a mixture. It determines their biochemical effects. Potentiometric and chronoamperometric measurements using ion-selective electrodes and biosensors, respectively, allow to directly determine chemical activity. However, many measurement instruments and methods are currently in use which do not clearly differentiate between total substance concentration and its biologically active component.

Figure 1 and 2: In physiological solutions, such as blood plasma, chemical activity clearly differs from ion or substrate concentration. Figure 1 depicts the pronounced differences between molality and active molality (green bars) for the monovalent ion potassium and the divalent ion magnesium. For glucose in the physiological range chemical activity (green bars) differs only slightly from molality as shown in Figure 2.



Potentiometric measurements with ion-selective electrodes

At equilibrium, the membrane potential of an ion-selective membrane is a direct measure for the chemical activity for a particular electrolyte ion.

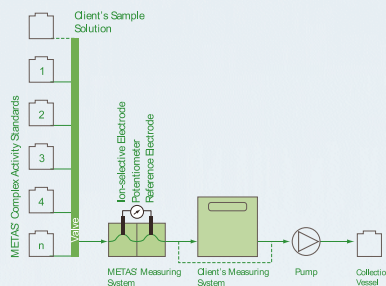


Figure 3: The METAS measuring system comprises a cell with two electrodes - an ion-selective electrode and a reference electrode - and a potentiometer. Potentiometric measurements are a measure of the activity of the given solution. METAS offers two services: the determination of the chemical activity of complex electrolytes and the calibration of measuring instruments containing ion-selective electrodes.

A complex activity standard for the physiologically important ions of sodium, potassium, calcium, magnesium and chloride is an aqueous mixture of these ions. The traceability of these standards to national standards involves a number of stages. Firstly, the purity of the water and of the salts of sodium, potassium, calcium and magnesium chloride is determined. Secondly, the reference solutions are gravimetrically produced by adding the salts to water. Finally, the chemical activity of the ions in the complex mixture is calculated using Pitzer's theory based on the component fractions of the ions identified.

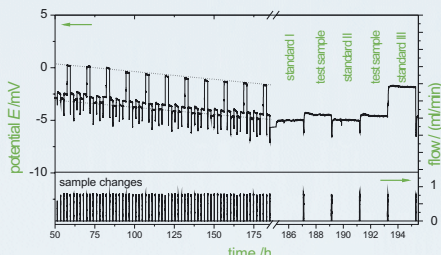


Figure 4: The full line shows the potentiometric response signal of a K⁺-sensitive electrode as a function of time. The linear signal drift of the standards I and III are depicted as dotted lines. After a sample change the signal stabilises in approx. 30 minutes. The mean potential of the last minute is used for the subsequent analysis.

From the gravimetric values, corrected for salt purity [$\mu = (0.05 \text{ to } 0.5) \% \text{ rel.}$], and the potentiometric response signals [$\mu = (0.3 \text{ to } 0.8) \text{ mV}$] of the standards the chemical activity of the test sample is determined using the bracketing technique with a two sided linear regression according to ISO standard 6143. The uncertainty of the activity is dominated by the uncertainty of the diffusion potential. In the physiological range the expanded uncertainty equals to max. 4 % rel. for the monovalent ions of sodium, potassium and chloride, whereas for divalent ions calcium and magnesium the uncertainty doubles.

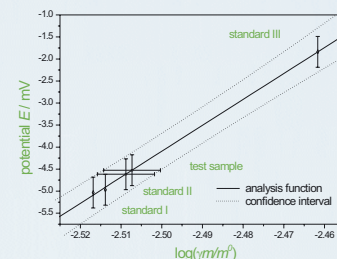


Figure 5: Analysis function of the potentiometric response signal using three bracketing standards is shown as a full line. The confidence interval is displayed by the two dotted parabolas. The uncertainty u_x for the analyte ion in the test sample is 1.6 % rel.

Amperometric measurements with biosensors

Glucose oxidase is often used to determine glucose enzymatically, a process during which the chemically active form of α -D-glucose is transformed highly selectively into 5-gluconolactone. Two electrons are released for each converted glucose molecule. This electron current is the evaluable phenomenon in chronoamperometry.

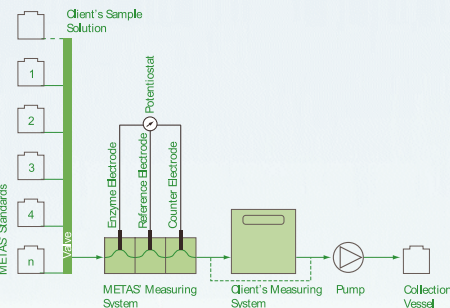


Figure 6: The METAS measuring system consists of a cell with three electrodes - an enzyme electrode, a reference electrode, a counter electrode - and a potentiostat. The chronoamperometric signals are a measure of the glucose content of a given solution. METAS offers two services: determination of glucose contents in aqueous solutions and calibration of glucose measuring instruments.

A standard for glucose measurements is a buffered aqueous glucose solution of a particular ionic strength. The traceability of these standards to national standards involves two stages. First, determining the purity of the water and glucose is extremely important. Second, the reference solutions are produced by gravimetrically mixing glucose in water.

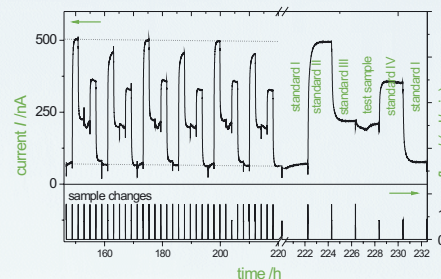


Figure 7: The full line shows the chronoamperometric response signal of a glucose-sensitive electrode as a function of time. The very small linear signal drift of the standards I and II is depicted as a dotted line. After a sample change the signal stabilises in (30 to 90) minutes, depending on the ionic strength of the sample. The mean current of the last 5 minutes is used for the subsequent analysis.

From the gravimetric values, corrected for glucose purity [$\mu = (0.5 \text{ to } 1.0) \% \text{ rel.}$], and the chronoamperometric response signals [$\mu = (0.2 \text{ to } 1) \text{ nA}$] of the standards the chemical activity of the test sample is determined using the bracketing technique with a two sided linear regression according to ISO standard 6143. The uncertainty of this chemical activity determination is dominated by the uncertainty of the amount of substance content in the gravimetrically prepared reference solutions. For aqueous glucose solutions in the physiological range (3 to 10) mmol/kg water the expanded uncertainty is (4 to 2) % rel.

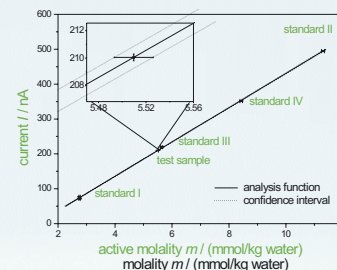


Figure 8: Analysis function of the chronoamperometric response signal using four bracketing standards is shown as a full line. The confidence interval is displayed by the two dotted parabolas. The inset highlights the resulting value and uncertainty u_x for the test sample.

Conclusions

The traceability of chemical activity measurement results of electrolytes (except pH) and glucose has not yet been established. Standardisation bodies link the chemical activity results using conventionally fixed factors to amount of substance content results. The traceability chain for chemical activity measurements has only been partly solved for pH-measurements. We propose to expand this approach of primary activity standards to the traceability of other physiologically relevant ions. Thus, a much clearer and universal standardisation becomes feasible. This traceability ensures international comparability and avoids laboratory dependent measurement results.