

## Electrochemical Methods with a Focus on Potentiometry

### Chapter Overview

In this chapter, we will look at electrochemical methods, important tools used in clinical biochemistry. These methods connect chemical reactions with electrical signals and make it possible to measure the concentration of ions and other substances in body fluids very precisely. Our main focus will be on potentiometry, since it is one of the core techniques built into modern biochemical analyzers.

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# 1 Introduction to Electroanalytical (Electrochemical) Methods

Imagine we want to find out the concentration of a certain substance in a solution – for example, sodium in blood plasma or the pH of urine. One option is to use optical methods (measuring absorbance or fluorescence), but sometimes substances have no optical properties, or we need a more accurate and direct way of measuring. In such cases, **electrochemical methods** are the tool of choice.

These methods are based on the fact that some **chemical reactions are inseparably linked to the movement of electrons** and thus to electrical phenomena. If we place electrodes into a solution and close an electric circuit, we can observe how the chemical composition of the solution is reflected in electrical quantities – such as **potential (voltage)**, **current**, or **conductivity**. In other words, electrochemical methods can “translate” chemical information (how many and what types of ions are in the sample) into an electrical signal, which can be measured easily and precisely.

## 1.1 Why Are Electrochemical Methods Important in Medicine?

Electrochemistry plays a role at almost every step in the clinical laboratory. For example:

- **Measuring blood pH** is essential for assessing acid–base balance.
- **Determining ion concentrations** ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ) is key in diagnosing disorders of body fluids.
- **Blood glucose** monitoring with a small device (the glucometer) is a typical example of electrochemical methods in practice.
- Even more complex parameters, such as **plasma osmolality**, are determined electrochemically.

The big advantages of these methods are their **speed, sensitivity, and ability to be automated** – all of which are indispensable in laboratory diagnostics.

## 1.2 Basic Principle: Electrodes and Electrical Units

Every electrochemical measurement takes place between two or more electrodes immersed in an electrolyte (a solution containing ions). Electrodes act as “mediators” between the solution and the electrical circuit.

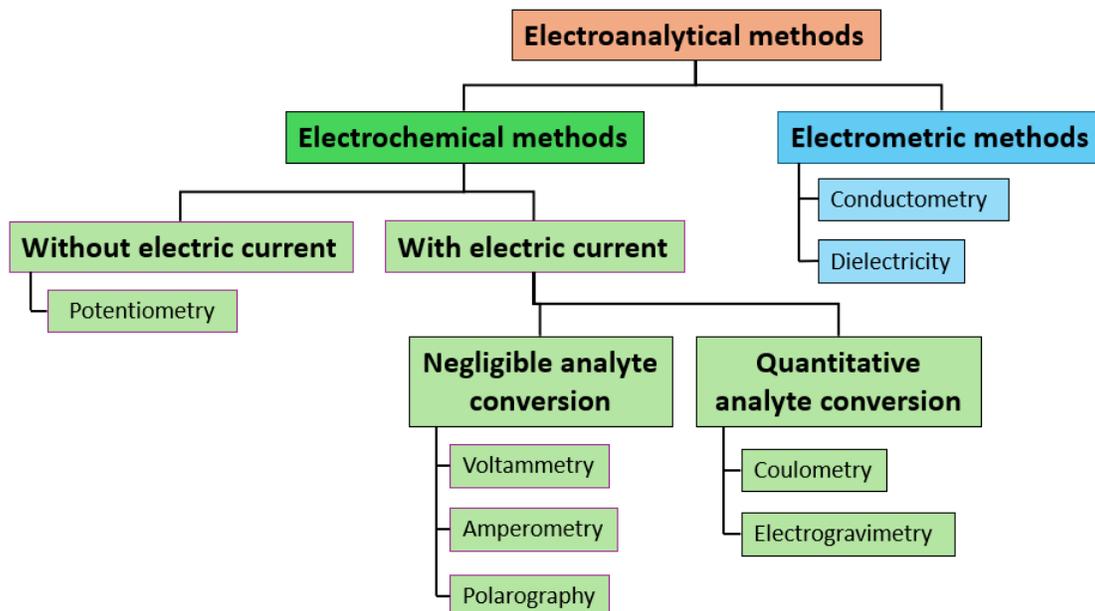
- **Indicator electrode** – the “sensor” for a specific substance. Its surface is designed to respond to a particular ion or analyte. For example, the glass electrode is sensitive to protons ( $\text{H}^+$ ) and is used to measure pH, while ion-selective electrodes can measure  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Ca}^{2+}$ .

- **Reference electrode** – provides a stable, unchanging potential, against which the signal of the indicator electrode can be compared. Without it, the measurement would not be reliable. Common examples include the Ag/AgCl electrode or the calomel electrode.
- **Auxiliary electrode** – in some methods, a third electrode is used to allow current to flow through the system, preventing the reference electrode from being “worn out.”

The measurement itself can focus on different electrical quantities:

- **Potential (voltage):** the difference in electrical potential between electrodes. A typical example is pH measurement.
- **Current:** the flow of electrons during a reaction. This is used, for instance, in amperometry (e.g., glucometers).
- **Conductivity:** the ability of a solution to conduct electric current, which depends on the total amount of ions in the solution. This is applied, for example, in measuring osmolality.

### 1.3 Electrochemical methods – general overview



## 2 Potentiometry

**Potentiometry** is one of the basic electrochemical methods. Its principle is the measurement of voltage between two electrodes immersed in a solution. To really understand potentiometry, we first need to explain what is meant by *electric potential* and why the potential difference between electrodes can provide information about the chemical composition of a sample.

### 2.1 What Is Electric Potential?

You can think of electric **potential as an “energy level” for electrons**. It is similar to water in a tank: the higher the water level, the greater the potential energy to flow downward. Electrons behave in the same way – if they can move, they will “flow” from a point with higher potential to one with lower potential, just like water flows downhill.

When we compare two points in an electric field, the **difference in their potentials** is perceived as voltage. This is the quantity that can be measured with a voltmeter.

### 2.2 Potential in Electrochemistry

In electrochemistry, this potential arises at the **interface between the electrode and the solution**. On the electrode surface, the following processes occur:

- **Redox reactions** (transfer of electrons), or
- **Ion exchange** (ions in the solution interacting with the electrode surface).

These processes lead to the arrangement of charges at the interface, creating an electrical voltage.

### 2.3 Principle of Potentiometry

The principle of potentiometry is that chemical information about ion concentration in the sample is reflected in an electrical voltage, which can be easily measured. When we immerse a pair of electrodes – an indicator and a reference – into the solution, an **electromotive voltage** is created between them. The indicator electrode changes its potential depending on the concentration of a specific ion, while the reference electrode keeps a stable potential. The difference between them is therefore a direct indicator of the solution’s composition.

To describe this quantitatively, the Nernst equation is used. It shows how the electrode potential depends on the activity (effective concentration) of the ion:

$$E = E^0 + \frac{RT}{zF} \ln a$$

where:

- $E$  – measured potential,
- $E^0$  – standard electrode potential,
- $R$  – gas constant,
- $T$  – absolute temperature,
- $z$  – ion charge,
- $F$  – Faraday constant,
- $a$  – activity (effective concentration) of the ion.

From the equation it is clear that changes in ion concentration lead to logarithmic changes in potential. In practice, this means that after electrode calibration, the concentration of an analyte can be calculated directly from the measured voltage.

## 2.4 Electrodes Used in Potentiometry

Every potentiometric measurement is based on a system of two electrodes – an **indicator** and a **reference** electrode. Together, they form a bridge between the chemical environment of the solution and the electrical circuit. For reliable measurements, one electrode must respond to changes in the sample, while the other provides a stable reference point. Modern instruments often use **combined electrodes**, which integrate both the indicator and the reference electrode into a single unit. These electrodes simplify handling, reduce the required sample volume, and improve measurement reliability.

### 2.4.1 Reference Electrode

The role of the reference electrode is to provide a **stable and well-defined potential** that does not change during the measurement, regardless of the solution's composition. This ensures that any measured potential difference is due only to the indicator electrode.

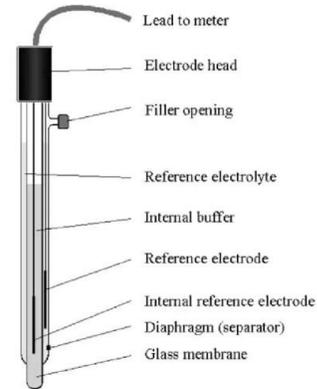
The most common types are:

- **Ag/AgCl electrode** (silver/silver chloride): simple, stable, widely used in clinical practice.
- **Calomel electrode** (Hg/Hg<sub>2</sub>Cl<sub>2</sub>): historically important but used less today due to mercury content.
- **Standard hydrogen electrode** (SHE): defined as 0 V and serves as an international reference point but is too complex for routine use.

### 2.4.2 Indicator Electrode

The indicator electrode is the part of the system sensitive to the specific analyte. Its potential changes depending on the concentration of a given ion or substance in the solution.

- **Glass electrode** – The most common type of ion-selective electrode. Its glass membrane is sensitive specifically to hydrogen ions ( $H^+$ ), allowing precise pH measurement. The voltage is recorded between the glass electrode and a stable reference electrode. Samples can include blood, urine, cerebrospinal fluid, or gastric juice, measured using pH meters or modern blood gas analyzers. Clinically, pH measurement is crucial for evaluating acid–base balance, assessing gastric acidity, and in 24-hour reflux monitoring.



(Source: <https://microbeonline.com/ph-meter-parts-principle-and-applications/>)

- **Ion-selective electrodes (ISE):** These use membranes with ionophores that selectively bind a chosen ion. This enables specific measurement of sodium ( $Na^+$ ), potassium ( $K^+$ ), chloride ( $Cl^-$ ), or calcium ( $Ca^{2+}$ ). ISEs are widely integrated into modern electrolyte analyzers and are essential in diagnosing fluid and electrolyte disorders, hydration status, and acid–base imbalances.



(Source: [https://ach.upol.cz/ucebnice2/memb\\_eldy.htm](https://ach.upol.cz/ucebnice2/memb_eldy.htm))

- **Enzyme-based biosensors:** Special electrodes that combine an electrochemical sensor with an enzyme. They indirectly measure metabolite concentrations through the products of enzymatic reactions. A typical example is the glucose electrode, which uses glucose oxidase immobilized on the sensor surface; the enzyme catalyzes glucose oxidation, and the resulting product is electrochemically detected. This is the principle behind everyday glucometers.

### 2.4.3 Automation and Biochemical Analyzers

Ion-selective electrodes (ISE) are now a standard part of biochemical analyzers used in clinical biochemistry laboratories. These analyzers combine potentiometry (ISE) with other biochemical methods, such as photometry, enzymatic assays, or immunoassays, to provide comprehensive quantification and monitoring of a wide range of analytes in biological samples.

Biochemical analyzers equipped with ISE can perform many types of tests. In addition to measuring ions and parameters of acid–base balance, ISE-based biosensors can also be applied for the determination of glucose/lactate, creatinine, or urea.

*Example of the size of electrodes used in biochemical analyzers:*



(Source: [https://is.muni.cz/el/med/podzim2019/KBOMII/um/lontove\\_selektivni\\_elektrody\\_-ISE.pdf](https://is.muni.cz/el/med/podzim2019/KBOMII/um/lontove_selektivni_elektrody_-ISE.pdf))

## 2.5 Medical Applications of Potentiometry

Potentiometry is one of the fundamental electrochemical methods with broad use in clinical practice. It is most often applied in pH measurement and in determining the levels of ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ) in blood, plasma, serum, or urine. These parameters are key for evaluating acid–base balance (ABB) and for diagnosing disorders such as acidosis or alkalosis. Different types of electrodes are used: the glass electrode for pH, the Severinghaus electrode for  $\text{pCO}_2$ , and the Clark electrode for  $\text{pO}_2$ . Modern analyzers combine multiple methods and also measure electrolytes using ion-selective electrodes (ISE).

### pH measurement

Blood pH is a basic indicator in intensive care medicine—its deviations point to metabolic or respiratory disorders. Urine pH is monitored in nephrology and urology, for example in infections, urolithiasis, or targeted alkalization therapy.

### Electrolyte determination

Measuring sodium, potassium, or chloride concentrations in plasma is part of routine laboratory testing. Sodium reflects hydration status, potassium is essential for cardiac and neural function, and chloride together with bicarbonate plays a key role in maintaining acid–base balance.

### Biosensors and instruments

Practical use of potentiometry includes biosensors, such as glucose meters with enzymatic electrodes for glucose measurement. Blood gas and electrolyte analyzers, which measure pH,  $\text{pCO}_2$ , ions, and other parameters, are also based on potentiometry. Modern POCT (Point-of-Care Testing) devices enable rapid diagnostics **directly at the patient's bedside**—critical for emergency medicine.

## 2.6 Electrodiagnostics – Other Applications of Electroanalytical Principles

Beyond laboratory potentiometry, measuring electrical potentials also has major applications in clinical practice through the recording of bioelectrical activity of tissues. Electrodes here function on the same principle as in potentiometric systems—measuring the potential difference between two points—but they capture naturally occurring signals of living cells and tissues.

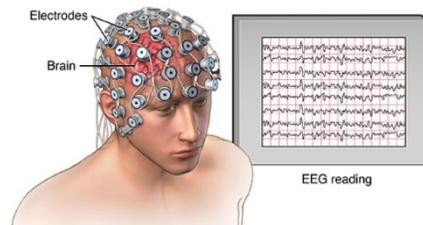
### Electrocardiography (ECG)

ECG records the electrical activity of the heart using electrodes placed on the skin. They detect potential differences arising during depolarization and repolarization of the myocardium in each cardiac cycle. The ECG trace provides information on heart rate, rhythm, and pathological conditions such as myocardial ischemia or conduction disorders. (Source: <https://www.szo.cz/cs/lekarske-pristroje/?ekg-pristroj-edan-se-601c-ekgedan601>)



### Electroencephalography (EEG)

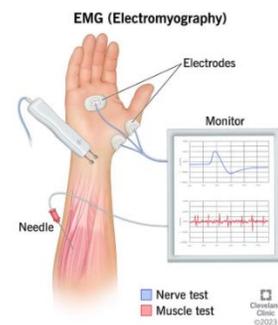
EEG records the electrical activity of the cerebral cortex with surface electrodes on the scalp. It measures the summation of postsynaptic potentials of neurons, producing characteristic rhythms and wave patterns. Clinically, EEG is used in diagnosing epilepsy, sleep disorders, encephalopathies, and for monitoring brain activity during anesthesia.



(Source: <https://somatosphere.com/2019/staging-seizure-the-chronic-contingency-of-epilepsy-diagnosis.html/>)

### Electromyography (EMG)

EMG evaluates the electrical activity of skeletal muscles using surface or needle electrodes. These detect action potentials of muscle fibers at rest and during contraction. EMG is essential in diagnosing neuromuscular diseases, peripheral neuropathies, or myopathies and helps assess the function of neuromuscular transmission.



(Source: <https://my.clevelandclinic.org/health/diagnostics/4825-emg-electromyography>)

### Other related methods

**Electrooculography (EOG):** measures the potential difference between cornea and retina, used to analyze eye movements.

**Electroretinography (ERG):** records the retina's electrical response to light stimulation, applied in ophthalmology.

**Evoked Potentials (EP):** evaluate brain electrical activity triggered by sensory stimuli (visual, auditory, somatosensory).

**Galvanic Skin Response (GSR):** tracks changes in skin conductance caused by sweat gland activity, used in psychophysiology.

## 3 Amperometry

Amperometry is an electrochemical method in which the electric current flowing between electrodes is measured at a constant voltage. On the working electrode, an oxidation or reduction reaction of the target substance takes place, and the number of electrons transferred is directly proportional to its concentration. The resulting current therefore provides direct information about the amount of analyte in the sample.

Thanks to its high sensitivity and speed, amperometry is well suited for the analysis of biological fluids such as blood, plasma, or urine. In clinical practice, it is mainly applied in the form of biosensors:

- **Glucose meters (glucometers):** These devices use the enzyme glucose oxidase, which catalyzes the oxidation of glucose. The generated current is proportional to the glucose concentration in the blood, enabling fast and reliable measurement in both home and hospital care.
- **Blood gas analyzers:** These instruments use the so-called **Clark electrode**, which measures the partial pressure of oxygen ( $pO_2$ ) based on its reduction at the working electrode.

The ability to be miniaturized and integrated into modern instruments makes amperometry one of the most widespread electrochemical methods in clinical diagnostics.



**Interesting fact:** *In intensive care, acid–base balance is monitored several times a day. For example, in a ventilated patient, even a small change in  $pCO_2$  may require adjusting the ventilation settings. Potentiometry (for pH,  $pCO_2$ ) and amperometry (for  $pO_2$ ) are therefore literally used in life-saving ways.*

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## 4 Voltammetry and Polarography

Voltammetry is an electrochemical method in which the current flowing between electrodes is monitored as a function of the applied voltage. When the voltage at the working electrode is gradually increased or decreased, oxidation or reduction reactions of dissolved substances start to occur at its surface. Once the applied voltage reaches the threshold required for a particular reaction, a characteristic change in current is recorded. The result is a voltammogram—a graph of current versus voltage—which provides information about which substances are present in the sample (qualitative analysis) and in what amounts (quantitative analysis).

A classical form of voltammetry is polarography, which employed the dropping mercury electrode. Its main advantage was the constantly renewing mercury surface, ensuring high sensitivity and reproducibility. In modern practice, mercury is rarely used for safety reasons and is replaced by carbon, platinum, or gold electrodes, often as part of specialized electrochemical analyzers.

In biology and medicine, voltametric methods are used more in research than in routine diagnostics. Applications include:

- Determination of trace metals (lead, cadmium) in biological fluids,
- Measurement of vitamins (e.g., ascorbic acid),
- Studying redox processes in cells and tissues, providing valuable insights into metabolism and drug effects.

## 5 Coulometry

Coulometry is an electrochemical method that does not monitor instantaneous current (as in amperometry or voltammetry) but instead measures the **total electric charge** that passes through a cell during the complete reaction of the analyte. The reaction continues until all of the analyte in the sample has been transformed. According to Faraday's law of electrolysis, the total charge is directly proportional to the amount of substance that has been electrochemically converted. This allows analyte concentrations to be determined with very high accuracy.

In medicine, coulometry is used primarily for the determination of chloride in sweat, which is a key diagnostic test for cystic fibrosis. In patients with this genetic disorder, chloride levels in sweat are significantly elevated, and coulometry provides reliable measurement.

Specialized coulometers, often integrated into analyzers, are used for this purpose. The method is valued for its precision and reproducibility, though its clinical use is mostly limited to specific tests rather than routine analysis.



**Interesting fact:** Diagnosis of cystic fibrosis is based on coulometric measurement of sweat chloride. If  $\text{Cl}^-$  concentration exceeds **60 mmol/L**, the diagnosis is almost certain. This simple electrochemical test remains the gold standard despite the genetic basis of the disease.

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## 6 Conductometry

Conductometry is an electrochemical method based on measuring the **electrical conductivity** of a solution, which depends on the concentration and mobility of dissolved ions. Two electrodes are placed into the solution, and an alternating current is applied between them. The use of alternating voltage prevents electrolysis products from forming on the electrode surfaces, which could otherwise distort results. The principle is simple: the more ions present in the solution, the easier current flows, and the higher the conductivity. The measured value thus provides information about the total ionic content.

In clinical practice, conductometry is mainly applied in:

- **Measuring urine and serum osmolality**, important for assessing hydration status, kidney function, or monitoring certain metabolic disorders,
- **Monitoring ionic balance**, where changes in conductivity can indicate pathological conditions.

Because of its simplicity and versatility, conductometry is a method that can be applied to almost all aqueous biological fluids.

## 7 Electrochemical Method in Practice (Potentiometric Titration)

As part of the practical sessions, you will become familiar with the electrochemical method of **potentiometry**, specifically in the form of **potentiometric titration**. The following chapters will focus on this topic.

### 7.1 What Is Titration?

Titration is a classical quantitative analytical method used to determine the concentration of an analyte (the substance being measured) with the help of a precisely known volume of a standard solution of a titrant (the titrating solution). The principle is the gradual addition of the titrant into the sample solution until the equivalence point is reached—that is, the moment when the analyte in the sample has been exactly stoichiometrically consumed.

This point is usually detected using an appropriate indicator (color change) or instrumentally (e.g., potentiometrically, conductometrically).

## 7.2 Combining Potentiometry and Titration

Potentiometric titration is the most common objective method for identifying the **equivalence point**—when the amount of titrant added is chemically equivalent to the amount of analyte present.

During the titration, the **electromotive force (EMF)** of a cell formed by an indicator and a reference electrode is recorded alongside the volume of titrant added.

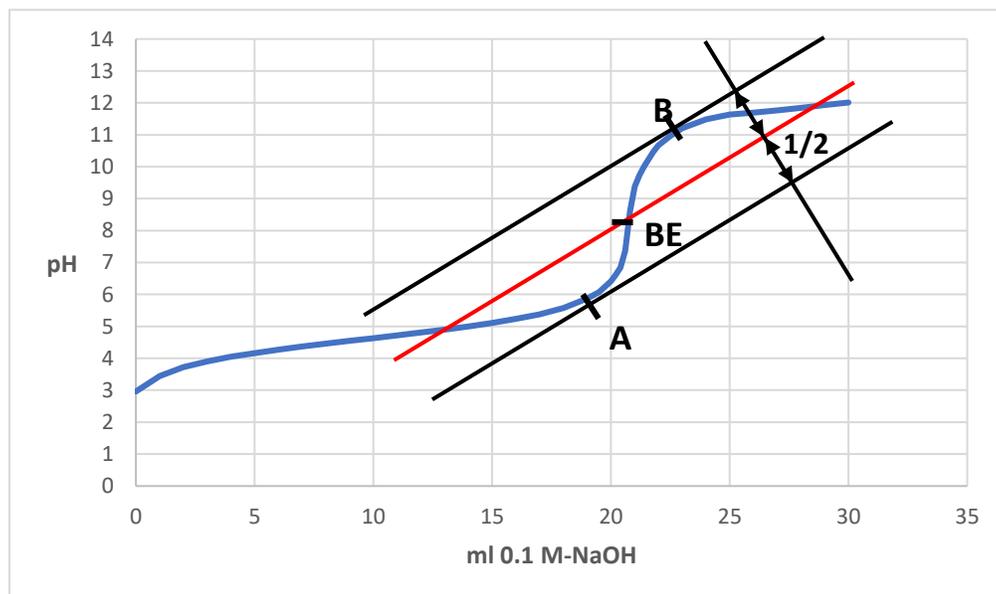
### Advantages:

- High accuracy,
- Applicable to colored or turbid solutions,
- Possibility to determine multiple ions in solution simultaneously,
- Provides deeper insights into reaction mechanisms,
- Allows the determination of constants (e.g., dissociation constants of acids and bases, stability constants of complexes).
- Fully automated titrations are possible with modern **automatic titrators**.

Potentiometric titration is performed similarly to classical visual titration. Intensive stirring of the solution is ensured by a **magnetic stirrer**. The titrant is added in small increments, and after each addition and stabilization of the potential, the consumed volume of titrant and the corresponding electromotive potential or pH are recorded.

With automatic titrators, the system directly plots the **titration curve** or calculates the concentration of the analyte.

The titration curves have an S-shaped profile, either increasing or decreasing, with a more or less steep central part referred to as the **potential jump** (the region with the largest change in potential). The **inflection point** on this steep section defines the **equivalence point (BE)**, see Fig. 1).



As mentioned above, the equivalence point corresponds to the inflection point on the steep part of the titration curve. The equivalence point can be determined using precise mathematical methods, or more simply—though less accurately—graphically. In graphical determination, a tangent is drawn at the midpoint of the lower curved section (point A), and a parallel tangent is drawn touching the upper curved section (point B). A third line, drawn parallel between the two tangents and passing through the midpoint of their distance, intersects the titration curve at the equivalence point (Fig. 1).



**Note:** At the inflection point, the slope of the tangent to the titration curve changes from increasing to decreasing. This means that at the inflection point the tangent to the curve is steepest. Mathematically, this corresponds to the **first derivative** of the titration curve having its maximum value, while the **second derivative** equals zero.

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## 7.3 Potentiometric Titration – Measurement Conditions and Possible Sources of Error

### 7.3.1 Electrode Calibration

Calibration of electrodes is an essential step before every measurement, as it ensures correct and reliable electrode response according to the Nernst equation. Calibration is carried out using standard solutions of known concentration with precisely defined values. For pH electrodes, buffers with pH 4, 7, and 10 are typically used, covering the normal working range of measurement. Proper calibration makes it possible to convert the measured potential into the corresponding ion concentration and minimizes systematic errors that would otherwise lead to incorrect interpretation of results.

### 7.3.2 Cleanliness of Environment and Instruments

Any contamination on the electrode surface poses a risk of incorrect results. Residues of sample, proteins, or salts can coat the electrode membrane and prevent proper contact with the solution. This leads to slow response or unstable potential. For this reason, electrodes must be rinsed with distilled water after each measurement and regularly cleaned with special solutions according to the manufacturer's recommendations. Proper storage is also critical—for example, pH electrodes must be stored in KCl solution, which prevents the membrane from drying out and maintains the appropriate ionic environment. If the electrode dries out, its function is often irreversibly damaged.

### 7.3.3 Effect of Temperature

Temperature is a significant factor influencing potentiometric measurements. It alters electrochemical equilibria and directly appears in the Nernst equation as the variable  $T$  (absolute temperature). Fluctuations in temperature can therefore cause shifts in measured values and lead to misinterpretation of results.

### 7.3.4 Possible Interferences and Sample Errors

Interferences and sample-related errors are among the most common causes of inaccuracy in potentiometric measurements. They may arise from different sources and can be grouped as follows:

- **Biological interferences** – originate from changes within the sample itself. A typical example is hemolysis, where the release of intracellular potassium from erythrocytes leads to falsely elevated  $K^+$  concentrations.
- **Chemical interferences** – caused by foreign substances in the sample. For instance, contamination of urine with disinfectant may distort measured pH, or the presence of drugs and their metabolites may affect electrode response.
- **Physical interferences** – related to the properties of the environment. Variations in ionic strength of the solution can affect the potential at the electrode–solution interface, while air bubbles can physically prevent electrode contact with the sample, resulting in unstable readings.

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## Summary

Electrochemical methods connect chemical reactions with electrical signals, allowing the determination of ion concentrations and other analytes in biological fluids. The most important method is **potentiometry**, based on measuring the voltage between an indicator and a reference electrode. A special case is **pH measurement**, which is essential for evaluating acid–base balance.

In practice, potentiometry is mainly used for determining electrolytes ( $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $Ca^{2+}$ ) and pH, and in combination with other methods also for measuring blood gases ( $pO_2$ ,  $pCO_2$ ). **Coulometry** is applied in the diagnosis of cystic fibrosis, **amperometry** in glucose meters, and **potentiometric titration** enables precise determination of the equivalence point.

Correct interpretation of results requires awareness of possible sources of error (hemolysis, temperature fluctuations, sample contamination, improper calibration). These methods form the basis of routine laboratory diagnostics and are indispensable, particularly in intensive care.

## Control Questions

1. What is the difference between an indicator electrode and a reference electrode?
2. How does a glass electrode work in pH measurement?
3. What is the difference between potentiometry and amperometry?
4. Which electrochemical methods are used for measuring pH,  $pCO_2$ , and  $pO_2$  in blood?
5. Why is it important to measure plasma concentrations of  $Na^+$  and  $K^+$ ?
6. What advantages does potentiometric titration have compared to classical titration with an indicator?
7. What are the most common errors and interferences in potentiometric measurements?