pH-Stat
Theory and Practice

Radiometer analytical

- when you need to be sure...

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pH-Stat in Theory

Introduction

The study of the kinetics of a chemical reaction over time is one of the simplest and quickest ways of measuring the efficiency of active ingredients in drugs for the pharmaceutical industry.

The most common examples are the measurement of enzyme kinetics and the activity of antacid products. However, there are many other examples of chemical reactions versus time in organic and mineral chemistry such as the measurement of the dissolution rate of fertilisers or the solubility of an additive to cattle feed to test its digestibility.

The vast majority of reaction kinetics concern chemical reactions that free or consume H_3O^+ or OH^- ions. Obviously, their speed of formation depends on operating conditions, in particular the pH of the reactive media. To study this, it is therefore important to keep the pH of the reactive media stationary. This is what is known as pH-Stat.

A pH-Stat study takes place as follows:

- determining an optimum value for the pH of the studied reaction,
- keeping the pH constant by adding a reagent to neutralise H^+ or H_3O^+ ions,
- calculating the kinetics of the studied reaction based on the consumption of reagent required to keep the pH constant over time.

This technique was first used by Knaff-Lenz in 1923 to study an esterase. At that time, the reagent was added manually by the operator who had to keep watching and adjusting the pH of the substrate.

Although always called pH-Stat, this technique also applies to other ions, cations or anions, whenever an electrochemical sensor is present. In this case, the pH electrode is replaced by an ion-selective or metal electrode.

During the 1950s, Radiometer A/S introduced a system for controlling pH automatically in collaboration with the Carlsberg brewery in Denmark. This system consisted of a burette to add titrant and a chart recorder to plot the reagent consumption curve. The reagent was added at a fixed speed on an "all or nothing" basis and calculations were performed manually.

The pH-Stat systems which arrived on the market between 1965 and 1975 used either mini peristaltic pumps or burettes for titrant addition. The addition speed was controlled according to the proportional band (as used in preset end-point titration) and a long or infinite time-delay.

In 1986, a microprocessor-controlled instrument joined Radiometer A/S's TitraLab range. It included dedicated embedded software for reaction kinetics which, in addition to a practical calculation module, controlled the reagent addition burette by Proportional Integral and Derivative (or PID) algorithms.

Towards the end of the 90s, Radiometer Analytical SA launched the PHM290 pH-Stat Controller. This new system is capable of controlling a pump or a burette and is suitable for use in all media, however strongly buffered, irrespective of the titrant concentration. It offered two different titrant addition methods: PID and AAA (Adaptive Addition Algorithm).

Radiometer Analytical SAS has put its 60 years' experience in titration to good use to develop its latest highly innovative pH-Stat Titration Workstations, the TitraLab 854 and TitraLab 856, which are ideal for use both in research laboratories and pilot plants.

TitraLab 854 pH Stat Titration Workstation

Fields of Application

In the laboratory

Here, the aim is usually to measure the activity of the product by quantifying its reaction speed.

The instrument needs to be able to:

- monitor the kinetics by using small volumes of titrant,
- store the reagent consumption curve versus time in order to make the necessary calculations.

pH-Stat is frequently used for analytical control especially in the pharmaceutical and biomedical industries:

- measuring the activity of certain effervescent drugs (e.g. antacids),
- monitoring the activity of enzymes such as lipase, cholinesterase or trypsin,
- studying the efficiency of certain toothpastes by measuring the precipitation kinetics of dicalcium phosphate.

Other fields of application include geochemistry:

- study of the complexing power of various organic constituents of soil,
- the dissolution or precipitation kinetics of various minerals,

and the food and beverage industry:

- monitoring the digestibility of various milk proteins,
- measuring the activity of yeast.

In the pilot plant (preparatory chemistry)

Here it is necessary to work with a regulation system that can:

- monitor large volumes of titrant over long periods (over 12 hours). This can be achieved either by using peristaltic pumps or by a dual-burette system working alternately (second burette operational during refill of first) allowing permanent control of the process,
- use two competing reagents (in separate burettes) in order to be able to monitor a process liable to evolve in different directions versus time.

Some examples:

- balanced reactions depending on the pH; for this type of reaction the yield will depend on the working pH which needs to be kept stable.
- manufacture by precipitation of hydroxyl mineral salts (e.g. calcium or aluminium phosphates or various combinations of hydroxides) where the solubility product depends on the pH, requiring operation at a controlled pH.

Various Types of Reactions

A pH-Stat reaction kinetics system can control any reaction in which a species formed or consumed during the reaction can be measured using an indicator electrode and a reference electrode together. The most common reactions in particular in enzyme kinetics, generate hydrogen or hydroxide ions. It is also possible to use an ion-selective electrode to monitor the appearance or disappearance of a cation or anion or a platinum electrode for reactions involving redox changes.

Constant speed reactions

If the speed of a reaction remains constant over time, the titrant consumption curve will be represented by a straight line after a start-up period. (Diagram 1).

This type of reaction is common in biochemistry and the measurement of enzyme activity is based on this principle.

Enzyme activity is defined as the speed of conversion of a substrate. This activity is measured in the presence of an excess of the substrate and remains constant throughout the experiment as shown in Diagram 1.

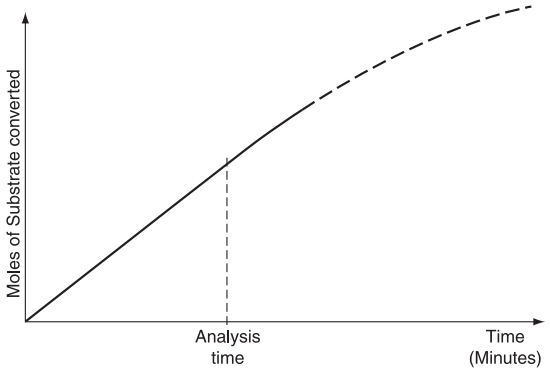


Diagram 1

In this case it is possible to write

Reaction rate = $V = \frac{d(H^{+})}{dt} = K$ (enzyme concentration)

In other words, this is a zero-order reaction.

Non constant speed reactions

Kinetic studies of reactions in solution do not always produce titrant consumption curves versus time that are straight lines.

In most cases, one of the participants in the reaction (usually the substrate) is at a low concentration. As this concentration decreases during the experiment, the reaction speed, which is dependent on concentration, will decrease in turn.

The study of antacids and many dissolution kinetics follow this principle, shown in Diagram 2.

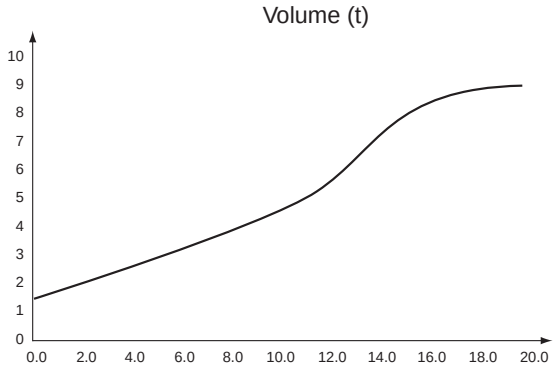


Diagram 2

Titrant reagent addition

Whatever the type of reaction studied, whether or not it is at constant speed, a titrant is added so as to keep the pH (or another parameter) constant over time. This speed of reaction of this reagent is a representation of the speed of the reaction studied.

The experimental value used by the system to regulate the titrant reaction speed is ΔpH, i.e. the difference between the measured pH value of the solution and the set pH value.

The reagent addition speed also depends on its concentration, on the buffer capacity and the volume of the solution. This can be expressed as follows:

$$DV_t = k * ß * (V_0 + V_t) * \Delta pH / C \quad (1)$$

DV_t: Volume of titrant to be added at time t

K: Constant

ß: Buffer capacity of the solution

ΔpH: Deviation from the set value in pH

V_0: Initial volume of the solution

V_t: Volume of titrant already added at time t

C: Titrant concentration

By referring to (1), it can be seen that, for a given ΔpH, the volume of titrant to be added is:

- directly proportional to the buffer capacity of the solution,
- inversely proportional to the titrant concentration.

The buffer capacity of a solution, generally expressed as ß, can be said to equal the amount of strong base or strong acid required to increase or decrease the pH of the solution by one pH unit:

$$ß = (dC_b)/(dpH) = (dC_a)/(dpH)$$

By adding a strong acid or base of concentration C of volume v to a solution of volume V, it is possible to calculate the buffer capacity of the solution in question by measuring the pH change.

$$ß = C * v / V * d(pH)$$

Using the above formula, the buffer capacity of a solution can be expressed in moles or millimoles of strong acid or base in volume units and pH units.

This buffer capacity is expressed without a unit in many publications.

An efficient pH-Stat system can be expected to use the formulas and algorithms necessary to adapt to a wide range of working conditions without the operator being obliged to modify regulation data.

pH-Stat in Practice

pH-Stat Titration Station Regulation Modes

The pH regulation of an experiment involves the following steps:

- bringing the pH of the solution to the set pH as quickly as possible without significant overshooting,
- maintaining the set pH by continually adding titrant.

A proportional control (as used in simple end-point titration) based on the difference in pH at the set value of the titrant addition speed is not sufficient to solve this problem. In theory, it is impossible to reach the set point as the titrant addition speed will be calculated as zero at the set value. A minimum titrant addition speed has to be introduced. The reagent is then added on an "all or nothing" basis. The introduction of this new parameter minimises the problem but does not solve it completely as there is often a pumping effect around the set point.

In addition to proportional control, the following are necessary:

- a derivative function allowing the system to react more quickly to a change in the pH of the sample,
- an integral function avoiding the titrant speed becoming zero even though the pH is close to the set point.

The titrant speed will then be the result of the algebraic sum of the three functions at time t:

- a proportional function:

  $K_1 * \Delta pH$

- a derivative function:

  $K_2 * d(\Delta pH) / dt$

- an integral function:

  $K_3 * \Sigma d(\Delta pH)*dt + K_4 * \Sigma \Delta pH$

Working in PID mode

In PID (Proportional, Integral and Derivative) mode, the operator has two parameters allowing the response to the reactive medium of the regulatory system to be adapted: the GAIN and the TIME CONSTANT.

Constant K_1 only depends on the GAIN, constants K_2 and K_3 and K_4 depend on both the GAIN and the TIME CONSTANT.

Working in AAA mode

The improvement obtained by working in AAA (Adaptive Addition Algorithm) mode is mainly in the expression of K_1.

$$K_1 = f(\Delta V/\Delta pH * 1/H)$$

In this mode, K_1 is no longer a constant but a variable depending on the variation in buffer capacity of the reactive medium. K_1 is adjusted by the operator introducing a parameter H called HORIZON which is expressed in seconds.

This modification in the expression of the proportional function of the titrant addition speed is optimised in the embedded software of the pH-Stat Titration Workstation and allows response to any change in the buffer capacity of the solution during the experiment without any intervention from the operator.

The TIME CONSTANT is also available in AAA mode and plays an identical role as in PID.

Choosing PID or AAA Regulation

Although it is difficult to lay down strict guidelines, it is recommended to use PID regulation for solutions with a low buffer capacity (less than 10^{-6} moles/l.pH).

AAA is more suitable in the following cases:

- study of solutions with a high buffer capacity ß, i.e. over 10^{-5} moles/l.pH,
- study of systems where the reaction speed varies with time.

For an identical regulation in terms of quality, AAA mode allows the set pH to be reached more quickly.

"Gain" in PID *regulation*

It is difficult to give standard figures for this parameter although the most common values are in the vicinity of 0.1 to 0.2.

However, it can be noted that:

- too high a GAIN at the beginning of the experiment will lead to overshooting of the set pH and the pH values measured during the experiment may oscillate around the set point by as much as several tenths of a pH unit. This will

lead to irregularities in the addition speed of the titrant and may even cause all or nothing addition,

- too low a GAIN will make it difficult or even impossible for the system to reach the programmed set value.

The influence of the gain on the regulation is illustrated in Diagram 3.

Influence of the gain

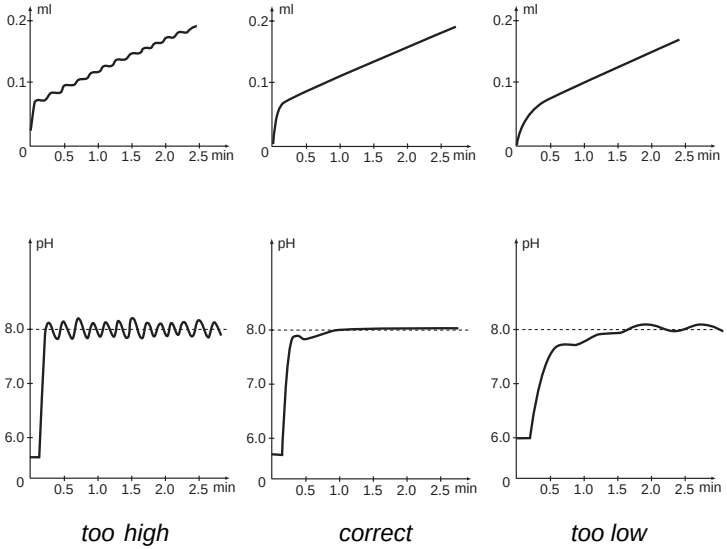


Diagram 3

If the buffer capacity of the solution to be studied is known, it is possible to calculate a practical gain value taking into account the algorithms used by applying the following formula:

$$\text{Gain} \approx 10 * \sqrt{ß}$$

"Horizon" in AAA regulation

The HORIZON is the time the AAA algorithm requires to reach the set point without overshooting it. It is expressed in seconds.

A short HORIZON or low value will lead to overshooting of the set pH value and may cause oscillations.

A long HORIZON or high value will lead to difficulties in reaching the set value (similar for too low a GAIN in PID regulation).

The influence on the HORIZON is shown in Diagram 4.

Influence of the horizon

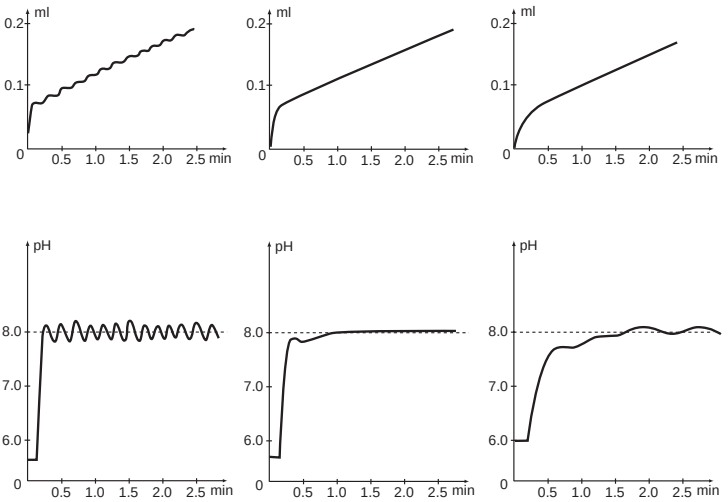


Diagram 4

The HORIZON value, taking into account the reaction kinetics, may need to be increased if the pH change is great. For a highly buffered solution, the value will need to be decreased.

An average value is around 30 to 40 s.

The HORIZON can be expressed as follows in relation to the buffer capacity (ß) of the solution:

$$\text{Horizon} \approx 1.16/\sqrt{ß}$$

This is established by performing a series of tests in a pH 7.000 buffer. (Radiometer Analytical part no. S11M020) at various dilutions and using the neutralisation by HCl 0.1M of a NaOH 0.1M solution added to the titration recipient at constant speed. The results are as follows:

ß	Horizon	1/√ß
0.025	10	6.32
0.005	15	14.14
0.0025	25	20
0.00125	35	28.28
0.0005	55	44.72

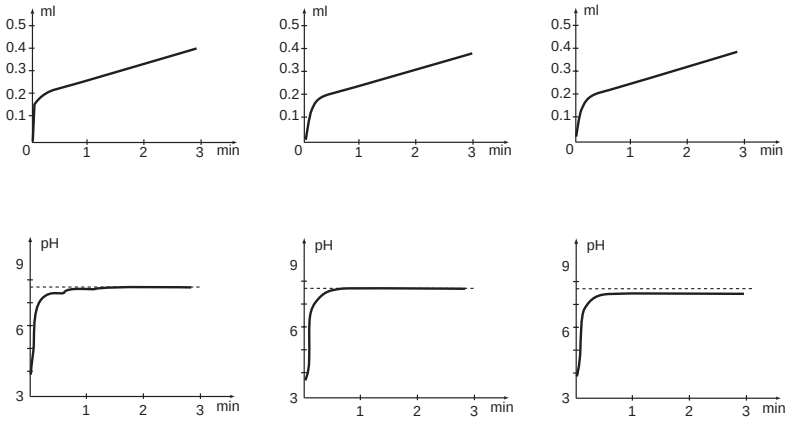
Time constant in PID or AAA regulation

Irrespective of the regulation mode, the TIME CONSTANT allows the working algorithms to be adapted. By adjusting the time constant it is possible to take into account the response of the electrode and the hydrodynamic conditions of the experiment (volume and stirring).

As the average value of this constant is around 2 s, it will need to be increased if, for example, solution volumes greater than 150 ml or electrodes with long response times are used.

Too great a TIME CONSTANT will lead to difficulties in reaching the set point even if the GAIN or HORIZON value is right. This is shown in Diagram 5.

Influence of the time constant



too high *correct* *too low*

Diagram 5

In order to develop a pH-Stat method quickly, the titration workstation allows the reagent addition parameters to be modified during operation, in particular:

GAIN and TIME CONSTANT (PID regulation)
HORIZON and TIME CONSTANT (AAA regulation)
SET POINT
DURATION OF THE EXPERIMENT

It is also possible to modify the data leading to the results while the experiment is in progress.

Optimising Experimental Conditions

In addition to parameters managing titrant addition (gain and time constant in PID mode and horizon and time constant in AAA mode), other factors need to be taken into account to obtain accurate and reproducible results.

Electrode condition

The measurement of reaction kinetics controlled by pH naturally depends on the regulation pH. This means that the single or combined electrodes need to be perfectly calibrated. It is therefore recommended to calibrate the electrodes just before starting the experiment. The operator should also check the response time and the stability of the *asymmetrical* potential of the electrodes used.

Changes in the response time of the electrodes can be checked by noting the time taken for the signal to become stable during calibration. 20 seconds is an optimum time for a stability criterium of 15 mpH/min. This time corresponds to the stability of the measured signal (for 99% of the measured signal). It should not be confused with response (approx. 2 seconds) indicated in the PID or AAA data of the pH-Stat Titration workstation which corresponds to the time required by the electrode to detect a variation in the measured pH.

The asymmetrical potential of the electrode system used which reflects the state of the electrolytic junction of the reference electrode cannot be easily measured by the operator. However, the state of the liquid junction can be determined by a simple measurement. A pH measurement made WITH and WITHOUT stirring gives two values, the difference between which should be no greater than 5 mV. In the event of a result greater than this value, it is necessary either to clean the liquid junction of the reference electrode or to replace the reference or combined electrode.

As kinetic studies often use small quantities of sample, it is essential to use dedicated electrodes. Radiometer Analytical SAS offers glass/reference combined microelectrodes with low response times to meet this need.

Electrode and delivery tip position

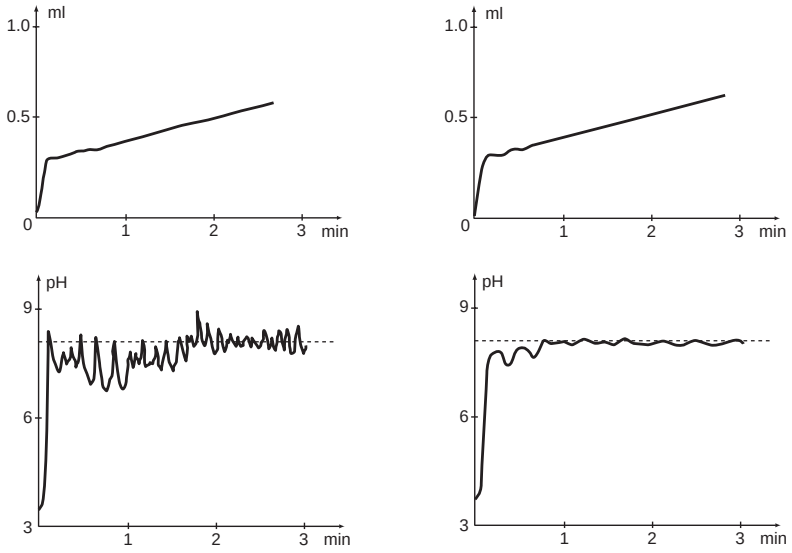
To ensure the best possible stability of the measured signal, the electrolytic junction of the reference electrode needs to be located in the most stable position possible from a hydrodynamic point of view. It should therefore not be placed in the middle of the vortex caused by stirring.

The titrant should be delivered in such a way as to ensure that the solution mixes as quickly as possible.

In order to avoid "pumping" and obtain better regulation and therefore better stability of the set value; the indicator electrode and the titrant addition tip can be placed opposite each other. Taking into account the stirring direction, it is then possible to ensure that the titrant covers the largest distance possible before reaching the indicator electrode.

The influence of the electrode and delivery tip position is shown in Diagram 6.

Influence of the titrant delivery tip position



Delivery tip too close

Electrode and delivery tip correctly positioned

Diagram 6

Stirring speed

Efficient stirring is essential. The size of the stirring bar needs to be suitable for the volume of the solution and the speed as fast as possible without introducing air bubbles. An average stirring speed is around 600 rpm.

Working temperature

Many kinetic reactions (in particular enzymatic ones) require a perfectly controlled temperature. To simplify this, Radiometer Analytical SAS offers thermostated cells for volumes from 2 to more than 100 ml.

Titrant burette capacity

When adding small titrant volumes, it is important to choose a burette with a capacity suitable for the total volume of titrant to be used.

In the case of a maximum titrant volume of less than 1 ml, a 1 ml burette should be chosen rather than a 10 or 5 ml one. The correspondence between the burette capacity and titrant volume has a significant effect on the efficiency of the regulation of pH-Stat titration workstations.

For reactions using large volumes of titrant, it is recommended to choose either a peristaltic pump or a dual-burette system such as the TitraLab 856 to avoid the experiment being uncontrolled during burette refilling.

Setting up an analysis

When setting up a pH or potential regulation analysis, it is important to check the following parameters:

- condition of the indicator electrode and its response time,
- correct capacity of the burette for the titrant volume,
- correct position of the indicator electrode in relation to titrant delivery,
- stirring speed,
- the GAIN or HORIZON setting (depending on the regulation mode),
- the TIME CONSTANT setting.

Starting an analysis

A regulation experiment should ideally take place in two steps. First, the set pH should be reached as quickly as possible without overshooting. Then, the actual analysis takes place with the titrant addition maintaining as regular a speed as

possible to ensure an optimum stability of the pH of the solution. A stability of the set value of ±2 to ±8 mpH depending on the concentration of the titrant and the buffer capacity of the reactive medium is an excellent result.

In the event of a kinetic study, in particular of enzymes, the experiment is carried out in three stages:

- bringing the substrate medium to the set pH value (preset end-point titration),
- preparation of the titrator (choice of pH-Stat method to be used, introduction of sample data and start-up of the method),
- addition of the enzyme to be studied.

This enables the titrator to react instantly to the introduction of the sample, thereby reducing the time required to reach the set pH.

pH-Stat Titration Station Calculations

In reaction kinetics, in particular enzymatic kinetics, the results are expressed in different units depending on what is customary in various industries or parts of the world.

In order to be able to satisfy all requirements, pH-Stat titration workstations calculate four types of results.

Two are calculated from the slope of the titrant consumption curve versus time and correspond to a formula like:

$$R = C_t * S * F / Smp \quad (2)$$

C_t = Titrant concentration

S = Slope of the titrant consumption curve (in ml/min)

This slope can be an average or a maximum slope for a given period.

Smp = sample quantity

F = Multiplication factor available to the operator

For a result based on the maximum slope, the period set by the operator is divided into ten equal intervals; the slope of the curve is calculated for each interval and then the maximum slope is used.

A third type of result is calculated from a titrant volume added at a time set by the operator and corresponds to the following type of formula:

$$R = C_t * V_t * F / Smp \quad (3)$$

V_t = Titrant volume delivered at a set time

The last type of result represents the time taken by the system to deliver a preset volume of titrant. It is as follows:

$$R = T (d/h/min/s) \quad (4)$$

6 results based on formulas (2), (3) and (4)

and

2 equations defined by the operator

plus a system of "global variables" which allow the results to be transferred from one method to another. This means that the TitraLab 854/856 pH-Stat Titration Workstations can be customised to meet the needs of all users.

Thanks to their multitasking function**, the TitraLab 854 and 856 pH-Stat Titration Workstations** allow all the calculation parameters to be modified **in SUPERVISOR mode** while the experiment is still under way, in particular the intervals used to calculate the titrant consumption curve versus time**.**

pH-Stat Titration Workstation Features

Radiometer Analytical's pH-Stat titration workstations offer the same measuring possibilities as all the systems in the TitraLab range. For pH measurement or zero or imposed current potentiometry, regulation takes place in the PID or AAA modes described above.

As they can perform preset end point titration, these systems can link the conditioning of a substrate and enzymatic kinetics in one method.

For some applications it is possible to use temperature as set parameter to control the delivery of a titrant on an all or nothing basis while measuring an electrode pH or potential.

A single-burette instrument like the TitraLab 854 is ideally suited for studying monodirectional reaction kinetics in the laboratory.

A biburette instrument like the TitraLab 856 also allows:

- bidirectional regulation using two competing reagents (in this case, a hysteresis or dead zone avoids any pumping of the reagents),
- regulation over a long period without losing control of the kinetics studied; in this case; two burettes contain the same reagent and work alternately, one filling while the other is operational,
- the delivery of a second reagent which is necessary but does not affect the regulation process. This can be achieved with a dual-compartment cell separated by a semipermeable membrane; one burette is used to regulate one of the compartments while the second delivers a different reagent in the second compartment at the same speed, ensuring a permanent balance of the ionic force of the solutions on either side of the membrane.

Conclusion

It is often essential to keep a constant pH or potential in a reactive medium in analytical as well as in manufacturing processes.

Dedicated pH-Stat systems are suitable for use for analytical control in the laboratory as well as in manufacturing processes in industry. Single-burette or single-pump systems are particularly suitable for the laboratory whereas biburette systems are ideal for pilot plants.

Data subject to change without notice.

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