Instruction Manual for Using the BI-ZTU

The Autotitrator for Use with Brookhaven's Zeta Potential Instruments

Brookhaven Instruments Corporation
Brookhaven Corporate Park
750 Blue Point Road
Holtsville, New York 11742-1832
USA

Telephone : +1(631)758-3200
FAX : +1(631)758-3255
E-mail : info@brookhaveninstruments.com
Web : www.brookhaveninstruments.com

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Please Read

This is your instruction manual for using your Brookhaven BI-ZTU, the autotitrator and associated software. Please read it carefully before making measurements. The "How To" section describes how to install the software and what the manual covers. You may familiarize yourself with some of the features of this software by reloading sample data files (**Titrator/Review pH Titrations**). If you have any questions or suggestions, please contact Brookhaven Instruments.

The BI-ZTU is an option for use with Brookhaven's zeta potential instruments. The software is not sold separately. Software is never really finished, because there are always additions and changes. As these become available, they will be added to the back of this manual as appendices. Please look at the appendices if you cannot find the answer to your questions in the main part.

Please contact the factory at info@brookhaveninstruments.com if you have questions. When you do contact the factory, mention the instrument model number (currently BI-ZTU), the instrument serial number, and date of manufacture as indicated on the identification sticker affixed to the rear of the instrument. Also, mention the software version number found by clicking Help on the main menu bar. Finally, indicate from whom the instrument was purchased.

Remember the old saying: "When in doubt, read the instruction manual." Sometimes the solution to your problem has already been addressed. You just need to find it. Thanks for purchasing a Brookhaven.

The software requires Windows 98 or higher and at least 8 Mb of RAM.

Important Warning

If the autotitrator and flow cell are not going to be used for extended periods, always flush the complete system with water.

If the instrument was left with water and bacterial growth is suspected, refer to Appendix A of this manual.

If the instrument was left for more than 90 days, dried salts may prevent the pumps from priming properly. In this case, follow instructions in "If the pumps don't self prime" on page IV-9 of this manual.

Software License Agreement

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Section I: Introduction

About the BI-ZTU

The BI-ZTU is a four-pump, automatic titrator that can be used with Brookhaven's ZetaPlus, ZetaPALS or the corresponding options on the 90Plus, the BI-Zeta and BI-PALS. The BI-ZTU is used to automatically determine zeta potential as a function of pH, added surfactant, or added salt. For surfaces that change with the addition of acids or bases, the most common use is to map out the zeta potential vs. pH curve. The most interesting feature of this curve is the isoelectric point, the pH at which the zeta potential is zero. This represents the pH at which the two-phase suspension is unstable and aggregation followed by sedimentation will eventually occur. Finding the optimal concentration of added surfactant is another use. Here the absolute value of the zeta potential should increase if the surfactant is adsorbed onto the colloid's surface. Once there is no more surface area on the particle for the surfactant to cover, the absolute value of the zeta potential should reach a maximum and further addition does not enhance electrostatic stability. That is, a plateau in zeta potential vs. added surfactant is reached. The concentration where the plateau begins is the optimum concentration under the particular set of suspension conditions. Finally, since the absolute value of the zeta potential decreases in aqueous suspensions with the addition of salt, one can use the BI-ZTU to quantify the relationship for a particular suspension.

Before using the BI-ZTU, please read the ZetaPlus or ZetaPALS manuals. Make sure those instruments are operating properly in batch mode for the manual measurement of samples before launching into an automatic series of measurements using the BI-ZTU.

Measurements are saved in the same folders and files described in the respective ZetaPlus or ZetaPALS manuals. Each measurement at a different pH or added surfactant or salt concentration is saved as a separate file. Therefore, to plot zeta potential or electrophoretic mobility vs. pH or added reagent concentration, use the **Titrator/Review pH Titration Measurements** selection from the main menubar.

References on polymers, colloids, acids and bases

The concept of acids and bases as well as the concept of neutralization, which is analogous to the isoelectric point where the zeta potential is zero, is reviewed in any general chemistry textbook. The complexity of measuring and maintaining a sample around pH 7, due to dissolved CO_2, is not addressed in simpler texts. More information on surfaces and colloids than is available in undergraduate textbooks is found in Section II: Theory. Below are a few general references that may be of use.

1. Allcock and Lampe, Contemporary Polymer Chemistry, Prentice-Hall publisher, 1981.

2. Hiemenz and Rajagopalan, Principles of Colloid and Surface Chemistry, Third Edition, Revised and Expanded, Marcel Dekker publisher, 1997.

3. Levine, Physical Chemistry, McGraw-Hill publisher, 1983.

This list of references is by no means complete.

Section II: Theory

How Do Surfaces Become Charged?

In general, surfaces are contaminated at a number of different levels. The exceptions are freshly cleaned surfaces, where the cleaning process is exceptionally vigorous, including, depending on the thermal stability of the surface, high temperatures and vacuum. Figure II-1 shows a metal surface contaminated by a hierarchy of possible contaminates. The purpose of this figure is to acquaint the user with a variety of possible situations. Note that this is not representative of any particular surface and that most such surfaces do not include all these layers. Metals can oxidize or contain sulfides or other salts at the surface. The salts and sulfides may arise during manufacturing and dry onto the surface. The oxide is developed as a result of oxidation, often brought on at higher temperatures; however, over long periods of time, dry surfaces may oxidize from contact with air. Water vapor in the air likes the polar oxide, sulfides and salts. After all, water is

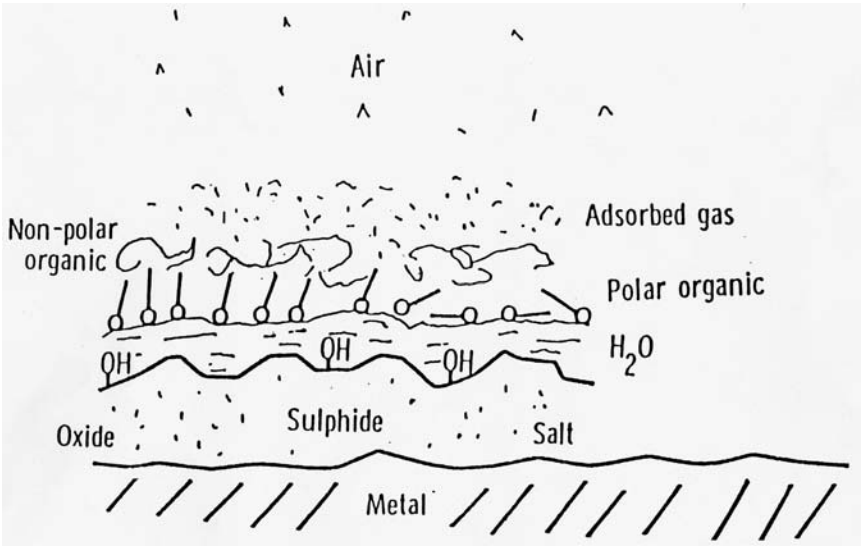

Figure II-1: Hierarchy of spontaneously adsorbed layers on a metal surface.

a polar liquid and dissolves many sulfides and salts. Polar organics, shown as a surfactant-like molecule with a round, polar head pointing towards the polar water and a long, non-polar tail, pointing towards a possible non-polar layer is next. Finally, non-polar, adsorbed gases followed by air are next. Again, this example is all-inclusive and unlikely to happen in practice. Yet, almost all dry powders, metals or not, have one or more of these layers that give rise to charged and polar species.

Figures II-2, II-3 and II-4 show a variety of ways that solid surfaces in water can become charged. The possibilities include:

- Differential solubility (ionic solids in common-ion electrolyte solution)
- Direct ionization of surface groups (acid/base groups covalently bonded to surface)
- Specific ion absorption (such as added wetting agent, surfactant, or stabilizing agents)

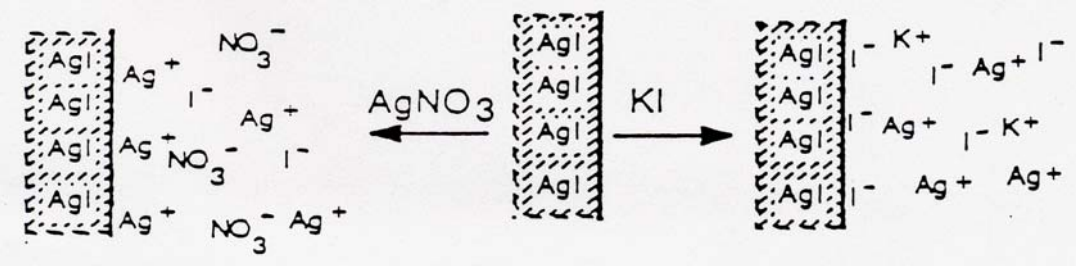

Figure II-2: Differential ion solubility can lead to net positive charge (Ag^+ in $AgNO_3$) shown on left or net negative charge (I^- in KI) shown on right.

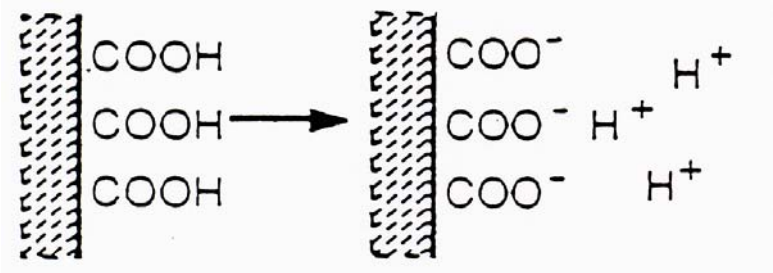

Figure II-3: Direct ionization of surface groups, here a carboxylic group to produce, at the particular pH of the suspension, a net negative surface charge.

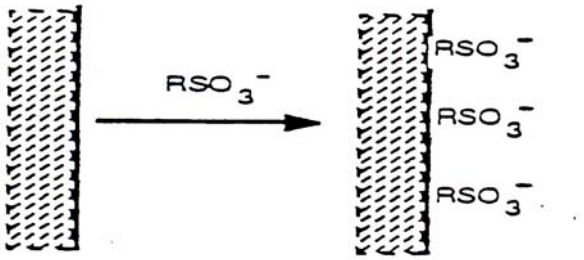

Figure II-4: Specific ion adsorption such as an *added* anionic surfactant or wetting agent.

In nonpolar solvents, charging is more complicated and may arise from swollen (water) reverse micelles adsorbed onto the surface with ions dissolved in the water, or trace water attached to the surface. Some mostly nonionic surfactants and polymers, attached to the surface, may also carry charge.

The main point is that for colloidal suspension of particles in water and often in nonpolar liquids too, charged surfaces are the rule not the exception. So the question is then what effect does the surface charge have on the suspension?

Surface charge causes electrostatic repulsion between particles. If it were not for such repulsion, the ever-present attraction (van der Waals) would result in small particles coming together, aggregating, and eventually settling (higher density than liquid) or rising (lower density than liquid). Two separate phases would exist instead of a finely divided particle phase known as a stable colloid. Thus, surface charge is a primary mode for producing stability in colloidal dispersions, especially in water and other polar liquids. A secondary method known as steric hindrance arises from nonpolar surfactants and polymers attached to surfaces. For pure steric hindrance, no charge is involved and no zeta potential is measured.From here on in this section, it is assumed there is at least some charge that gives rise to zeta potential.

How Is Zeta Potential Defined Theoretically?

When there are charges covalently or otherwise bound to the surface of a particle in a liquid, counterions are close by. In addition, any dissolved salts or other free ions of opposite charge to the surface charge, are found at higher concentration (than out in liquid) closer to the surface. Likewise, free ions of the same sign as the surface charge ions are pushed away from the region near the surface. Far from the surface, the positive and negative ions are randomly and equally found in any region of space that is large enough to encompass a large number of ions. Electroneutrality still holds overall. The situation is shown in its very simplest form in Figure II-5. While there are many more advanced pictorials, this simple one is sufficient for most purposes.

In this cartoon, the surface charge density is shown on the positive Y-axis as negative. It is also shown as equally spaced. This is rarely the case, but simplifies the drawing. The counterions, here positive, are close by. Additional layers are identified in advanced theories. Here the Stern layer is shown. Note: Sometimes layers are called

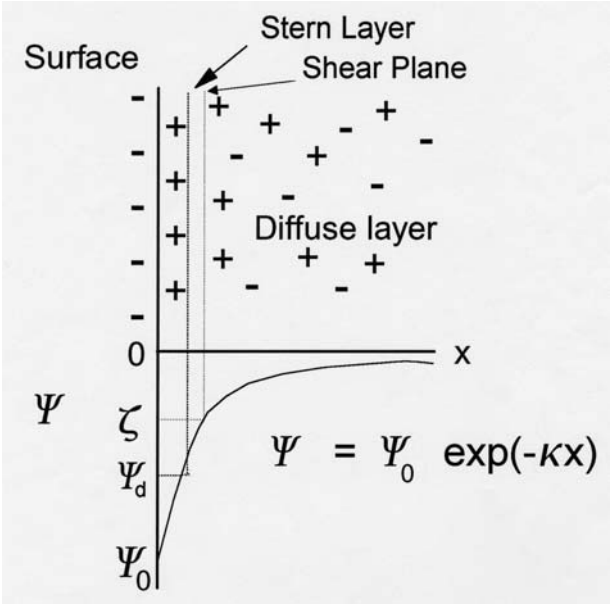

Figure II-5: Electrical double layer, zeta potential, and double layer thickness, 1/κ.

planes and planes are called layers. They mean the same thing in most descriptions of colloidal behavior.

The distance from the surface of any particular layer is not constant. It varies over the surface of an irregular particle and as a result of the non-uniform, surface charge density. Pictures like this are averages over colloids that are rotating very fast compared to the time it takes to make macroscopic measurements of their motion between electrodes to which a voltage has been applied. Such measurements will lead to electrophoretic velocity, from which is calculated the electrophoretic mobility and ultimately the zeta potential.

There is a special plane called the shear plane or layer. It is imagined, in the simplest theory, that inside the shear plane, the liquid and charges move with the colloidal particle, as if they were strongly attached to the surface; outside the shear plane, the liquid and dissolved ions or other, dissolved, neutral molecules do not move with the colloidal particle. Thus, shear occurs at the aptly named shear plane when, for example, the charged particle is attracted to an electrode of opposite charge such as is the case in a zeta potential determination.

In Figure II-5, the negative half of the Y-axis is used to represent the electrostatic potential differences between two points. Electrostatic potential differences, Ψ, between two points are measured in volts. For example, the surface potential, Ψ_o, is the potential difference between a point right at the surface and a point far out (large X-axis value) in the liquid equally surrounded by positive and negative ions.

If we could use a voltmeter to directly measure Ψ_o, there would be no need for zeta potential determinations. Since it is impossible to find probes small enough to attach to a colloidal surface, and since colloidal particles are not standing still in a liquid long enough to "glue" them in place, it is impossible at the present time to measure Ψ_o directly. Instead, as learned from the zeta potential manuals, we measure electrophoretic velocity, from which electrophoretic mobility is calculated as is zeta potential. From zeta potential we infer what we need to know about colloidal stability from charge.

The zeta potential is defined as the electrostatic potential difference, ζ, between an average (rotationally averaged) point on the shear plane and one far out in the liquid. Its absolute value is less than that of Ψ_o. Sometimes, it even has an opposite sign to that of Ψ_o. But mostly it has the same sign. Though strictly incorrect, the experimentally determined zeta potential is sometimes referred to as the surface potential. Not only are the absolute magnitudes of these two potentials different, as shown in Figure II-6, it can happen that they have a different sign, demonstrating that the sign of the charge at the surface is sometimes opposite to the sign of the charge at the shear plane.

Figure II-6 shows a case where the surface potential is +80 mV. With nothing physically adsorbed onto the surface that affects the charge density around the particle, the zeta potential is approximately + 60 mV. Now assume that an ionic surfactant is adsorbed. This can happen when the positive charge density at the surface is low, allowing for hydrophobic regions where the nonpolar tail of the anionic surfactant specifically ad-

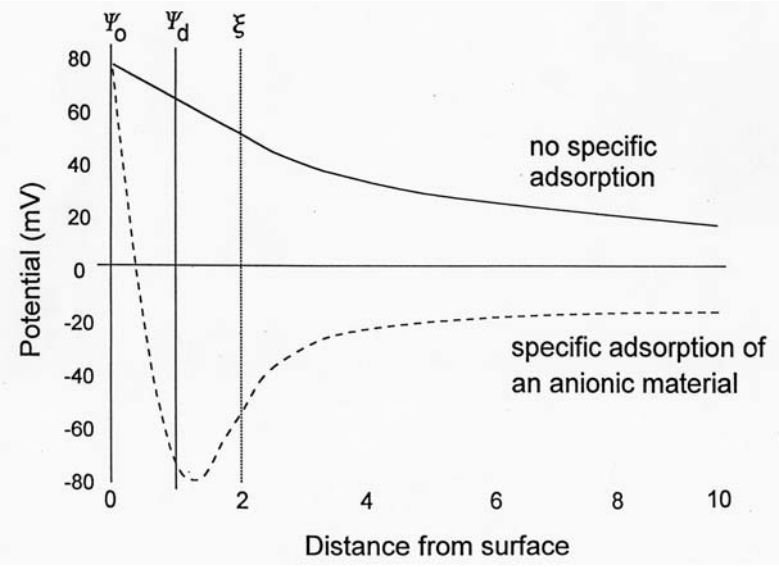

Figure II-6: Example of specific adsorption that results in the reversal of sign between the surface charge and that at the shear plane.

sorbs. The free energy of this process is more favorable than the process of the negative head of the anionic surfactant neutralizing the positive charges on the surface. Or, initial addition of the anionic surfactant neutralizes the positive surface charge and subsequent addition yields a negative charge density at the shear plane. In this situation, the zeta potential is approximately – 60 mV and any inference about the surface charge density from a single zeta potential measurement with the anionic surfactant adsorbed would lead to the wrong conclusion. A measurement with and without the surfactant is needed to reach the right conclusion. NOTE: In Figure II-6, there is only one shear plane shown for simplicity, although such a model is not entirely correct. The shear plane position in the case of no adsorption is closer to the surface, and with the adsorption of a surfactant chain, the shear plane is undoubtedly shifted further out along the X-axis.

Debye Double Layer Thickness, 1/ κ

The situation depicted in Figure II-5 can be simplified by considering the idea that charges of opposite sign, one at the particle surface, and one spaced a small distance away (counterions), give rise to a capacitor whose electrical characteristics are described as a double layer of charge. Thus, the idea of a double layer was born. But this picture ignored the diffusive motion of charge in the liquid, and so it was replaced by the concept of a diffuse double layer associated with Peter Debye who helped to characterize it theoretically.

In this simple theory, the electrostatic potential falls away exponentially as shown in Figure II-5. The decay constant, κ, with units of inverse length, determines how rapidly the potential drops. The inverse of this decay constant, with units of length (often nanometers) is called the Debye double layer thickness. When it is large (low salt in water or nonpolar liquid), the surface and zeta potentials are relatively similar in magnitude and the electrostatic repulsion between charged colloidal particles is felt over relatively

long distances. When it is small (high salt in water), the surface and zeta potentials are very different in magnitude and the electrostatic repulsion between charged colloidal particles is felt over only relatively short distances. At sufficiently high salt, one can *collapse* the double layer, meaning a very low zeta potential, allowing particles to come together and perhaps aggregate, sediment, and separate into two phases rather than a dispersion of fine colloidal particles in a liquid. This is an unstable colloid.

Interestingly, even in more advanced double layer theories κ is used as a scale factor along with particle size to determine length scales over which electrostatic effects are important. The equation defining κ is given in the appendix of your zeta potential manual. It is related to the dielectric constant of the liquid and the ionic strength (calculated from the concentration of free ions from added salt, acids or bases). Here, we only wish to point out graphically what you can expect when measuring at two different salt concentrations.

In Figure II-7, a positive surface is depicted with a zeta potential $\zeta_1 > \zeta_2$ because $c_1 < c_2$. Here c_1 and c_2 refer to the concentration of free salt ions. In other words, the higher the free salt ion concentration, the lower the absolute value of zeta potential. At high enough salt concentration, zeta potential can be driven so close to zero that electrostatic repulsion of the particles is negligible. Suggestion: If the chemistry does not require measurements in high salt, use 1 millimolar of $NaNO_3$ or KNO_3. Why measure in much higher salt that will drive down the magnitude of the thing you want to determine? It isn't necessary.

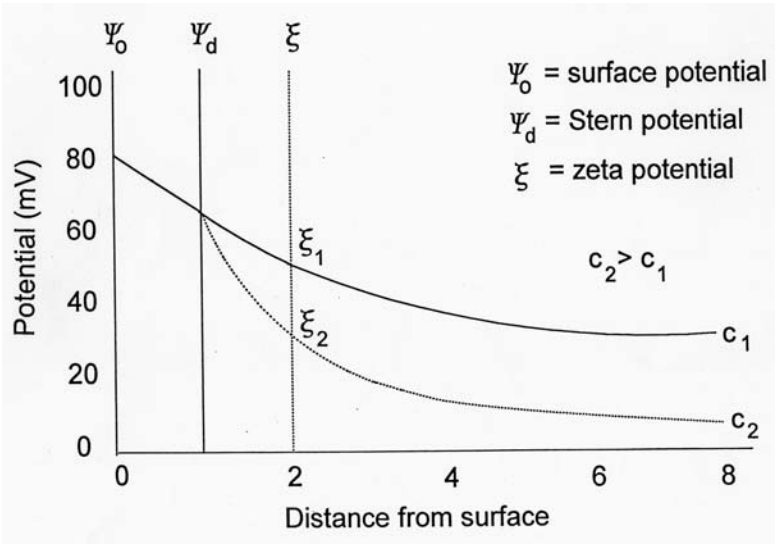

Figure II-7: Effect of salt addition on the zeta potential.

How Is Zeta Potential Defined From Experimental Measurements?

For the same reasons that it is impossible to directly measure surface potential, it is impossible to measure zeta potential directly. So when you hear of a zeta potential

measurement, what is really meant is that it is calculated or determined from electrophoretic mobility. The electrophoretic mobility is defined as the electrophoretic velocity divided by the strength of the electric field, which is in turn calculated from the applied voltage and electrode geometry.

Before laser-based zeta potential instruments, electrophoretic mobility was determined by dividing the distance, as observed with a microscope or CCD camera, individual particles moved under the influence of an electric field by the time. So it was a direct, though tedious and often biased measurement. With lasers, one can measure a Doppler frequency shift (ELS, ZetaPlus mode of operation) that is proportional to the electrophoretic velocity. Or one can also measure a phase shift (PALS, ZetaPALS mode of operation) that is proportional to the electrophoretic velocity. Though these are indirect methods, they are far less tedious, and are less prone to operator bias than direct methods. In addition, they can be applied to particles that are too small to be seen with a microscope.

What Does the Zeta Potential Depend On?

As already discussed, the zeta potential is a function of two parameters: the charge density at the shear plane (related normally to the charge density at the particle surface unless specific adsorption has occurred to reverse the normal situation as in Figure II-6, dotted curve); and the concentration of free salt ions. So the zeta potential depends on

- Charge: surface sites, how many, what type; or adsorbed, charged species, and
- Solution conditions: pH in water, electrolyte concentration, i.e. free salt ion concentration.

Without specifying solution conditions, specifying a zeta potential has little meaning.

What Is The Zeta Potential Used For?

The square of the zeta potential is proportional to the electrostatic force of repulsion between two charged particles. In practice, the force is never calculated. Instead, the magnitude and sign of the zeta potential are used as guidelines for colloidal stability or as a measure of how saturated the surface is with adsorbed species (wetting agents, surfactants, stabilizing agents).

For example, as a rule-of-thumb, for zeta potentials greater than approximately + 25 mV or more negative than approximately - 25 mV, repulsion is thought to be sufficient and the dispersion is likely to be stable unless additives force a change. For zeta potentials less than + 25 mV and greater than - 25 mV, the possibility of an unstable dispersion is likely. Without steric hindrance to keep particles apart, as the zeta potential approaches zero, an unstable dispersion is very likely. Obviously, the +/- 25 mV values are an approximation.

The sign and magnitude of the zeta potential is also used as a guide when mixtures of colloidal particles are present. This is discussed after introducing the concept of the isoelectric point, the IEP.

Definition of IEP and Acidic and Basic Surfaces

Imagine that an acid like a carboxyl group or a base like an amino group is responsible for the electrostatic stabilization. It is then easy to imagine, by addition of acid or base, that one can change the charge of the functional group from positive to negative, going through a special pH at which the group is neutralized. It is neither positive nor negative, but neutral. This pH is called the isoelectric point, the IEP. If the charged group at or near the surface (shear plane) is capable of rapid and reversible addition of H^+ or OH^- ions, then one can start at either end—acidic or basic—and approach the IEP from either side. Some surfaces are like this but some are more rapidly equilibrated starting at one or the other side of the IEP. See Figure II-8.

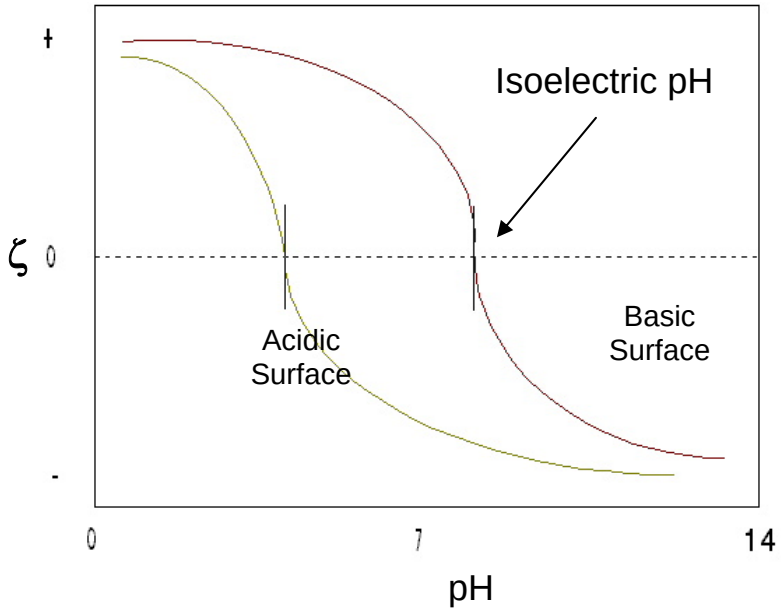

Figure II-8: Definition of the IEP.

The zeta potential is determined as a function of pH and plotted. The pH at which it crosses the zero axis is the IEP. Two are shown here. When the IEP is greater than 7, the surface is called a basic surface. When the IEP is less than 7, the surface is called an acidic surface.

Remember: At the IEP, the zeta potential is zero by definition, there is no electrostatic repulsion, and when the particles collide, they should aggregate. This is the pH of maximum instability if pH is what controls the magnitude of the charge responsible for repulsion.

Several IEP values, rounded to the nearest whole pH value, are listed in Table II-1. Silicon dioxide is the most acidic; magnesium oxide is the most basic. Due to dissolved CO_2 in water, so-called neutral water is slightly acidic with a pH around 5.8. Thus, if a very clean titanium dioxide surface is in water and there is nothing on its surface to shift the IEP, it would be very unstable. This is the reason that DuPont and other manufacturers add surfactants, stabilizers or a thin layer of other oxides (SiO_2 or Al_2O_3) to prevent aggregation when this type of titanium dioxide is used as a pigment.

Note: The values in Table II-1 are for illustrative purposes only. This collection of literature and textbook values refers only to super clean surfaces. Referring to Figure II-1, it should not be surprising that normal surfaces are contaminated either by accident or on purpose. Thus, by putting aluminum oxide onto the surface of titanium dioxide, one can shift the IEP towards 9. Alternatively, by putting silicon dioxide onto the surface of titanium dioxide, one can shift the IEP towards 2.

In fact, this is common practice: Attach surface groups to change the stability such that under the conditions of use the dispersion is stable while still maintaining the bulk properties of the underlying colloid. Titanium dioxide (the rutile crystalline form) is used as a white pigment in paint and a large number of other dispersions. It is white because its bulk refractive index is 2.66 (zero absorption in the visible) and so it scatters a lot of visible light. However, to make an appropriate dispersion, it is almost always coated with some other agent depending on, in water, whether an acidic or basic suspension is subsequently required.

Oxide	pH at IEP
Silicon Dioxide	2
Manganese Dioxide	3
Zirconium Dioxide	4
Titanium Dioxide	6
Chromium Oxide	7
Iron Oxide	8
Aluminum Oxide	9
Lead Oxide	10
Cadmium Oxide	11
Magnesium Oxide	12

Table II-1: Common IEP values for super clean surfaces of some metal oxides.

If it were not for the fact that surface chemistries are generally not the same as bulk chemistries, or if super clean surfaces were the norm, then zeta potential determinations would not be required. Looking up literature values would be all that is required. This is almost never the case and so measurement is required. Remember: a trace amount of impurity adsorbed onto the surface controls the zeta potential and colloidal stability.

Examples of Real IEP Curves and Some Observations

Four IEP curves are shown in Figure II-9:

1. Illite, a form of SiO_2, a very acidic surface with an IEP around pH 1.5.
2. Rutile, a form of TiO_2, a nearly neutral surface with an IEP around pH 6.6.
3. Alumina, Al_2O_3, a common basic surface with an IEP around pH 9.2.
4. Calcium carbonate, $Ca(CO_3)$, a very basic surface with an IEP around pH 11.0.

The $Ca(CO_3)$ example is a good one because it emphasizes that one cannot forget about bulk chemistry in these types of measurements. When the acidity of the liquid is too high, $Ca(CO_3)$ chemically reacts to form CO_2, water, and the calcium salt of the acid added to lower the pH. The $Ca(CO_3)$ particle will no longer exist. Understand that over the range you want to determine zeta potential, the particle must still maintain its integrity; it is assumed that only the surface changes.

Suppose you want a stable suspension of these particular TiO_2 and Al_2O_3. At what pH should you work? It cannot be between pH 6.6 and 9.2, for then the Al_2O_3 particles are positive and the TiO_2 particles are negative and will stick together, not forming a stable suspension. You must work either below pH 6.6 or above pH 9.2 where both particles are positive or negative, respectively. To avoid the possibility of drifting into the mutually exclusive pH region when other components are added the pH should be least 1 pH

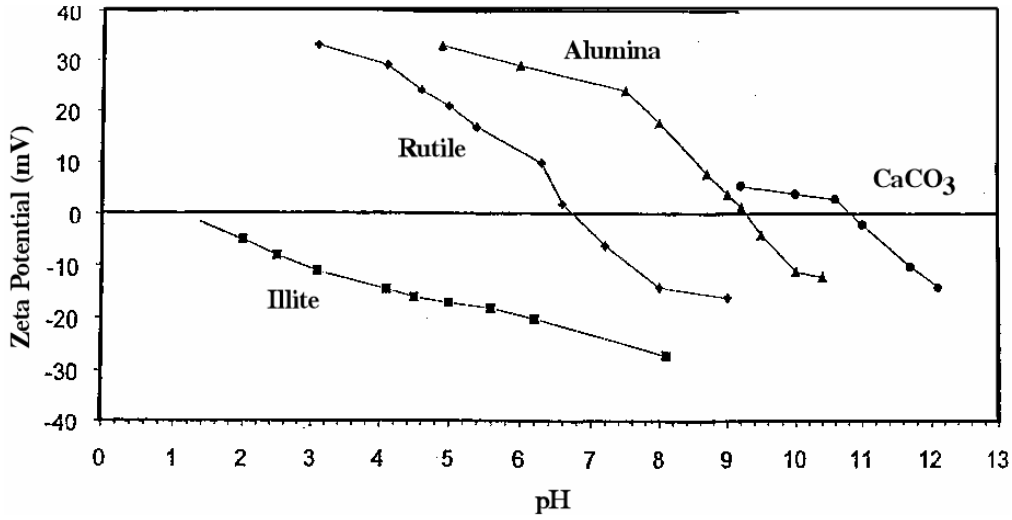

Figure II-9: Several zeta potential vs pH curves.

unit lower than 6.6 or higher than 9.2. The pH you pick depends on the rest of the chemistry, but the IEP curves give you a starting point.

Alternatively, suppose you have small-sized TiO_2 particles and large-sized Al_2O_3 particles. Your goal is to coat the Al_2O_3 with a lot of small TiO_2 particles, creating an alumina core with a titania surface. What would you do for these particular samples? Add the alumina to the suspension first, making sure the pH is between 6.6 and 9.2, say pH 8

so the alumina is positively charged. Now add the titania at the same pH. It will be negatively charged and stick onto the alumina surface.

There are endless possibilities to this exercise. It should be recognized that the order of addition of charged particles can affect the final result.

Not all IEP values are pH's.

Because oxide surfaces are common, because carboxyl and amino surface groups are also common, it is assumed that the definition of the isoelectric point is the pH at which the surface is neutral. This is not the most general definition. The most general definition is the concentration of any additive at which the zeta potential is zero. If a surface can be neutralized by addition of any ion, then its concentration, often expressed a pX, is the IEP value. Table II-2 shows some examples.

Hydroxyapatite [$Ca_5(PO_4)_3OH$]	pH 6
Calcite ($CaCO_3$)	pH 9
Fluorite (CaF_2)	pCa 3
Barite ($BaSO_4$)	pBa 7
Silver Iodide (AgI)	pAg 5.5
Silver Sulfide (Ag_2S)	pAg 10

Table II-2: Isoelectric points of some ionic solids dispersed in a colloidal suspension.

For the particular fluorite referred to in this table, if soluble calcium nitrate is added at 1 mM, then the calcium ion concentration is 10^{-3}, the pCa is by definition 3, and the fluorite should eventually aggregate since the IEP is reached.

What does "eventually" imply kinetically?

In dilute suspensions, if the IEP is reached by the addition of reagents (acid, base, or some other potential determining ion), it does not mean the particles will immediately aggregate and eventually grow large enough to sediment or be seen by eye. It takes time for the diffusing particles to collide and then stick together. They can be helped by mechanical mixing. So it is quite possible to pass through the IEP without seeing precipitate in dilute suspensions. This is a good thing; otherwise, it would be difficult to measure across the IEP.

In concentrates, where collisions are much more frequent, reaching the IEP will induce precipitation much more quickly. The inter-particle collision rate is proportional to concentration squared.

Particle kinetics also plays a much more subtle role especially with the attainment of surface equilibria in metal oxides. Starting with a dry oxide, for example, zirconia (also called zirconium oxide, though it is a dioxide, ZrO_2), you get very different IEP curves if you start around pH 2 or if you start around pH 12. Starting around pH 12 normally yields the classic curve as shown in Figure II-8, with an IEP in the range of pH 4 to

5, depending on what has absorbed at the particle surface. However, starting at pH 2 can result in apparently more than one IEP, most likely due to measurements made long before the various complex ions have obtained equilibria. If you waited long enough, the same IEP curves would obtain. The lesson here is that for many surfaces, metal oxides in particular, you can expect hysteresis in the IEP curves.

Optimizing the Concentration of Additives Using the Titrator

The BI-ZTU can be used for more than IEP determination. It can be used to determine the concentration of an added potential determining ion (surfactant, dispersing agent, for example) above which the surface is saturated. This represents the optimal concentration of additive for the particular total surface area of suspended particles. If there is no more surface on which to adsorb, the zeta potential cannot change (except for an increase in free ion concentration driving zeta potential towards zero).

Figure II-10 shows an example of zeta potential vs. additive concentration. Here the concentration is given as wt/vol percent (1% wt/vol corresponds to 10 mg/mL or 10 g/L). Notice that while the particle has a positive zeta potential at zero additive concentration, the surface is made more positive, more stable, by addition of the additive. This effect is useful until approximately 2% wt/vol of this particular surfactant. Adding more does not increase stability. Adding less decreases stability.

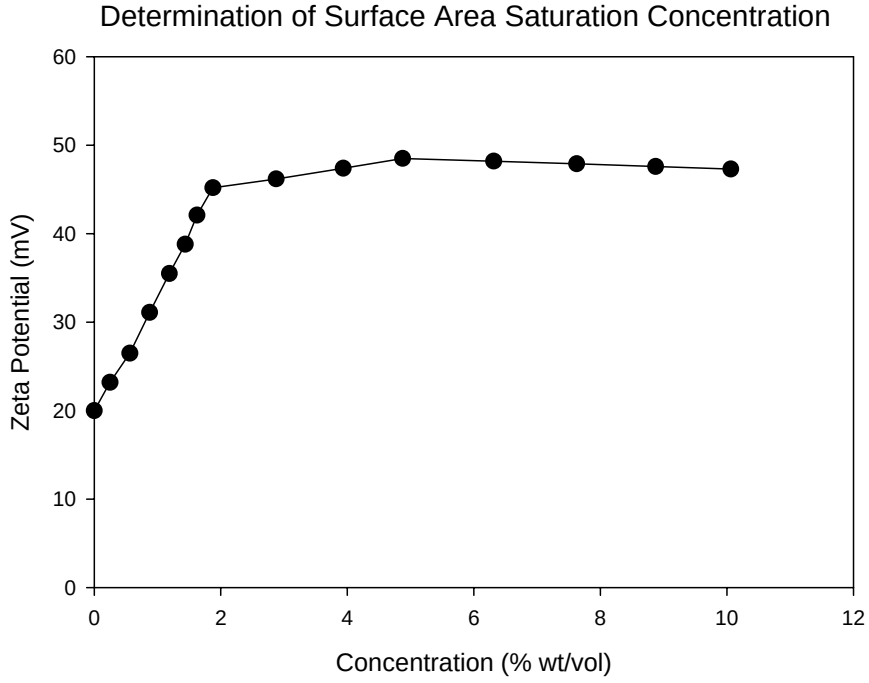

Figure II-10: Using the BI-ZTU to determine the optimum additive concentration.

Please note that 2% wt/vol concentration is the optimum in this particular case. It is not a generally true that for all stabilizing additives one should use 2% wt/vol. It depends on the particular sample (e.g., particle concentration and total surface area) and ad-

ditive. However, it is generally true that such concentrations vary from about 0.1% wt/vol to about 2% wt/vol and that too often additives are found at too high a concentration. Besides wasting chemicals, possibly increasing environmental hazards, possibly shifting other properties such as surface tension and viscosity, adding too much surfactant can also cause depletion flocculation, an effect that, rather than promoting stability, causes instability. A more complete description of this effect and others is found in more advanced colloid texts.

Section III: Selecting Acids, Bases, Salts, and Surfactants

Acids and Bases

Which Ones to Use: HNO_3 and KOH.

For changing the pH of a solution, we recommend using nitric acid, HNO_3, and potassium hydroxide, KOH. The nitrate ion, $(NO_3)^{-1}$, and the potassium ion, K^{+1}, are not known to strongly absorb at the surface of particles except at extreme concentrations. Whereas, the chloride ion, Cl^{-1}, the phosphate ion, $(PO_4)^{-3}$, the sulphate ion, $(SO_4)^{-2}$, and other anions may specifically absorb onto various colloidal particle surfaces, thus increasing the surface charge density and changing the native electrophoretic mobility and zeta potential. For example, TSPP, tetrasodium pyrophosphate and NaHMP, sodium hexametaphosphate, in water, yield various phosphate ion species that do specifically adsorb onto many metal oxide particles. Thus, do not use HCl, H_3PO_4, or H_2SO_4 to decrease the pH. Use HNO_3.

Similarly, the base NH_4OH should be avoided since the ammonium ion, $(NH_4)^{+1}$ may specifically adsorb onto some colloidal surfaces. Specific adsorption is the reason that buffers, like the pH 4, 7, and 10 buffers commonly used to calibrate pH probes, or the tablet buffers available throughout the range of 1 to 14, should be avoided as they are based on the equilibrium constants between acids and bases and the salts of acids and bases. The counterions present in these buffers may adsorb onto the particle surface changing the native surface charge density. In addition, the very action of buffering makes it very difficult to easily shift the pH if a study of zeta potential vs. pH is required.

Concentrations: 0.1 N and 0.001 N for each acid and base to cover pH 2 to 12

If you intend to cover the full pH range allowed by the BI-ZTU autotitrator, pH 2 to pH 12, you will need 0.1 N and 0.001 N HNO_3 and 0.1 N and 0.001 N KOH. In general, to reach a certain pH, it is convenient to have an acid or base that is 10 times more concentrated. For example, to reach pH 2 that corresponds to a hydrogen ion concentration of 0.01 M, one of the pumps of the titrator should be pumping 0.1 N HNO_3. Note: With monoprotic acids and monobasic bases, 1 N equals 1 M.

If your goal is to cover a smaller range in pH, for example pH 3 to 8, you could use 0.01 N HNO_3 and 0.001 N KOH. This would leave two of the four pumps free for use for adding surfactant or salt or nothing at all.

The simplest procedure is to purchase 0.10 N bottles of HNO_3 and KOH. Add 1.0 mL of either one to 99.0 mL of DI water to produce 0.001 N concentrations of each. While you can save money and space by purchasing a 1.0 N bottle of HNO_3 and KOH, you have to use two dilutions to reach the desired amount of 0.1 N and 0.001 N concentrations of each. As long as you are careful to dilute with errors no greater than 10% in total, dilutions are acceptable.

Remember: pH is a log scale. Thus, you can make a 5% error in concentration and the pH is not much affected. In fact, the table below shows the error is 0.022 pH units across the entire range if you make a 5% error in concentration. In studies involving the IEP, or the zeta potential vs. pH on either side of the IEP, errors of 0.1 to 0.25 pH units are often considered insignificant. To maintain stability, one wants the pH to be at least an entire pH unit away from the IEP. Thus, an error of 0.022 pH units is insignificant.

Concentration	pH
0.100 N HNO_3	1.000
0.095 N HNO_3	1.022
0.00100 HNO_3	3.000
0.00095 HNO_3	3.022
0.00100 KOH	11.000
0.00095 KOH	10.978
0.100 KOH	13.000
0.095 KOH	12.978

Table III-1: pH for various concentrations of acid and base.

Quantities: What volume of acids and bases to use?

The BI-ZTU comes as standard with four, 100 mL, GL-45 capped bottles. If you find that you are filling the bottles too frequently, you have a couple of choices. First, you can purchase 1 L bottles that take GL-45 caps and use these in place of the 100 mL bottles supplied as standard. Contact Brookhaven for longer tubing to accommodate the larger bottles. Alternatively, though more expensive, you can purchase the 0.1 N acid and bases with GL-45 caps from some chemical supply houses and use them directly instead of the 100 mL bottles supplied with the BI-ZTU. However, you will have to dilute 0.1 N acid and base to obtain the recommended 0.001 N acid and base to achieve good results in the middle pH ranges.

In an extreme case, you use about 10 mL each of 0.1 N acid, 0.001 N base, and 0.1 N base when starting at neutral pH, moving to pH 2, and titrating all the way to pH 12. Likewise, if you start at neutral pH, move to pH 12, and titrate all the way to pH 2, you use about 10 mL each of 0.1 N base, 0.001 N and 0.1 N acid. Thus, in the extreme case, you might use up all 100 mL in each bottle with 10 titrations. Consider this when

you decide what quantities of acids and bases to purchase. More likely, you will use much less because you will not cover the entire 2 to 12 range.

SAFETY WARNING

Strong acids and strong bases, carry warning label for a reason. Use protective clothing, gloves, and goggles when handling them. Avoid any liquid contact with eyes, skin or clothing. Do not breathe their vapors. Keep containers closed when not in use. Use with adequate ventilation. Wash hands thoroughly after handling them.

Be sure to read and follow the warning labels that come on the bottles.

Salts

As shown in the theory section of this manual, salt ions, those not specifically adsorbed onto the particle, but free to roam around in the solution, also have an effect on the zeta potential. As free salt ion concentrations increase, they cause a decrease in the zeta potential by decreasing the thickness of the double layer surrounding the particle. Indeed, at high enough concentrations, the double layer collapses, and particles, when they collide, can aggregate. Thus, it is clear that too high a salt concentration makes it harder to distinguish between a zeta potential determined by surface charge density (really, charge density at the shear plane) and one determined by free salt ion concentration.

Yet, stripping out all free ion concentration is usually not practical either. When titrating with an acid, the associated anion, NO_3^{-1} for example, or when titrating with a base, the associated cation, K^+ for example, are also free salt ions that will contribute to the change in the zeta potential through their effect on the double layer thickness.

Therefore, if your goal is to separate these two effects –the effect of specific adsorption (potential determining ions or PDI's) and the effect of changes in double layer thickness (non-potential determining ions or non-PDI's)—then add some salt such that for most of the pH range of interest, the total concentration of free ions is relatively constant. Experimentally, this will result in a more or less constant conductivity across the pH range.

For example, if the pH range is 3 to 11, then starting with 1 mM of KNO_3 will keep the conductivity reasonably constant except at the extremes of the pH range or if you cycle back and forth across the pH range. As you cycle back and forth over the pH range, you are always adding to the free salt ion concentration. You can avoid this, if it is important, by starting with fresh sample each time.

For a particular case, you may need to use a particular salt such as NaCl. However, if no such constraints exist, we recommend KNO_3 since chloride ions specifically adsorb onto some particles. You can, of course, check if they do by noting a larger than expected decrease in zeta potential with the addition of chloride ions. And, as stated above, we recommend 1 mM for the concentration. Why collapse the double layer with

100 mM KNO_3, making it harder to determine changes in zeta potential, if such a high concentration of salt is not required? Stay with 1 to 10 mM of KNO_3 whenever possible.

Wetting, Dispersing, and Stabilizing Agents: Surfactants

Strictly speaking, these different categories of additives have distinct purposes: wetting agents are used to spread a liquid completely over a solid surface; dispersing agents are used to add charge or nonionic species onto surfaces to keep them apart once they have been separated (sometimes mechanical energy is also required); and stabilizing agents are used to keep particles stabilized (apart) for periods longer than dispersing agents, longer than is required for initial characterization, long enough for the material to fulfill its intended purpose (years if long shelf life required).

While surfactants can act as wetting and dispersing agents, they are generally not long-term stabilizing agents. Such agents need to be strongly anchored to the particle surface. Thus, long-term stabilizing agents are often long-chain macromolecules. Some additives can act as wetting/dispersing or dispersing/stabilizing agents, but no single agent can act as all three.

Surfactants are often classified as shown in Table III-2:

Class	Charge	Examples
Anionic	Negative	Alkyl(benzene) sulphonates
Nonionic	None	Polyoxyethylenes
Cationic	Positive	Trimethylammonium halides
Amphoteric	pH dependent	Propionates

Table III-2: Surfactant classifications

A wetting agent absorbs at the air-liquid interface. Its purpose is to cause a liquid to spread over any solid surfaces such as that presented by colloidal particles. Two factors control the efficiency of a wetting agent: the contact angle and air-liquid interfacial tension. The interfacial tension also plays a role in the penetration of liquid into pores. Thus, always pick a wetting agent that reduces the contact angle towards zero (better spreading) but does not change significantly the interfacial tension.

To choose a good wetting agent, you need to know whether a surface is lyophobic (solvent hating) or lyophilic (solvent loving). Do the following, simple test to determine the character of a dry powder. Sprinkle a very small amount on the surface of the liquid of interest. If the particles sit on the surface, unable to break the surface tension, they are lyophobic (hydrophobic, if the liquid is water). If the particles break through the surface, they are lyophilic (hydrophilic, if the liquid is water). There are a few exceptions. Some-

times particles are so dense, or so aggregated, that they break the surface because the gravitational force is larger than the force from the surface tension. Typically, such particles sink very fast, often carry tiny but visible bubbles, and do not disperse with gentle swirling. Lyophobic particles will require a wetting agent.

Common lyophobic surfaces include metals and inorganic crystals. Common lyophilic surfaces include proteins, polyelectrolytes, and other ionic surface that dissolve in the water.

Dispersing agents adsorb at the solid-solution interface, adding charge or neutral molecules to the surface in order to increase the repulsion between particles to keep them apart. Often, but not always, wetting agents also add charge to the particle surface, acting as dispersing agents once the loosely-held agglomerates (secondary and higher order aggregates) are broken up with the minimum amount of mechanical energy. Dispersing agents, typically but not always surfactants—whose primary job is to decrease the liquid-air surface tension and then at higher concentrations enter the liquid to either form micelles or adsorb onto colloidal surfaces—are used to keep particles apart long enough to be measured. For simple particle size and zeta potential determinations, one does not need a long-term stabilizing agent whose purpose is to keep particles apart for times that are measured in days, months, or even years.

For oxides (clays, some inorganic pigments, and many ceramics) in water, polyphosphates are good wetting and dispersing agents. For example, use sodium hexametaphosphate, NaHMP, with zinc oxide, ZnO, and tetrasodium pyrophosphate, TSPP, with titanium dioxide, TiO_2. Use polysilicates with silica, SiO_2, quartz (another form of silica), and many clays. Polyacrylates and polysaccharides also work well as dispersing agents for many aqueous systems.

For non-aqueous systems try phosphate esters, block copolymers (EO/PO polymers, also known as Pluronics), phospholipids (fats), polyhydroxystearic acid, and silicone phosphates (silicones) as dispersing agents.

Any wetting, dispersing, and stabilizing agents, if they add charge to the particle surface, will dramatically affect the zeta potential. That is their nature: to add repulsion, often through charge, to help the dispersion process. If they are mostly nonionic, but can anchor to the surface, and protrude further out past the initial charged layer, they will reduce the absolute zeta potential. Thus, when using such agents, as you often must in order to obtain good dispersions, remember that it is the effect of these agents that you are measuring when the zeta potential changes with increasing additive concentration. One simple experiment is to determine the increase in the absolute zeta potential as a function of ionic additive concentration, thereby finding the concentration beyond which no significant increase occurs, that is the saturation concentration. It is that concentration that defines the optimum for that particular additive.

The list of wetting and dispersing agents, including surfactants, is mind-bogglingly long. Consult a classic industrial listing like McCutcheon's. Volume 1 lists emulsifiers and detergents (specialized surfactants). Volume 2 lists functional materials

such as dispersing aids, surfactants and much more. These two-volume sets come in North American and International Editions.

For more information, check this website: http://www.gomc.com/. Click on "McCutcheon's Publications." As these volumes are expensive, consult a library.

Section IV: Setting Up the Instrument

The BI-ZTU is an automated liquid handling device for adjusting sample conditions prior to measurement. Care is required to ensure that it is correctly hooked up. If tubing from the metering pumps is not inserted into the correct bottles or if concentration and reagent types are not entered correctly, the instrument will fail to work properly. This is similar to a manual titration; dispensing acid or base of unknown concentration would also lead to failure. Fortunately, with an automated system, the setup need only be done once. In a manual titration, equal care is required for each titration. Follow the steps below carefully to ensure correct operation of the BI-ZTU.

Preparing for Installation

1. Check the packing containers for signs of external damage. Register with the shipping company any signs of external damage.
2. Check the received parts against the packing list.
   a. Main titrator unit
   b. Power brick
   c. Flow cell (BI-ZELF)
   d. pH 4 buffer packet
   e. pH 7 buffer packet
   f. pH 10 buffer packet
   g. Metric hex keys, (2.5 mm, 3 mm)
   h. Luer-tip syringe
   i. Patch tube for syringe
   j. USB cable
   k. Titration cups
   l. Test sample
   m. 4 reagent bottles with lids
   n. Holder for reagent bottles
   o. Installation CD
   p. pH probe
3. In addition, for setup and continued operation of the BI-ZTU, assemble the following consumable reagents and supplies:
   a. DI water in a wash bottle for rinsing the BI-ZTU.
   b. 0.1 M Nitric acid. Purchase commercially prepared 0.1 N Nitric acid volumetric standard. 50 to 100 mL of a known concentration (+/- 10%).
   c. 0.001 M aqueous monoprotic acid. Dilute the above acid 100:1 by adding 1 mL of 0.1 N Nitric acid to 99 mL of DI water.
   d. 0.1 M Potassium hydroxide. Purchase commercially prepared 0.1 N KOH volumetric standard. 50 to 100 mL of a known concentration (+/- 10%).
   e. 0.001 M Potassium hydroxide. Dilute the above base 100:1 by adding 1 mL of 0.1 N KOH to 99 mL of DI water.
   f. pH 4, pH 7, and pH 10 buffer for calibration of the pH probe.
   g. pH probe storage solution (or pH 4 buffer) for storing the pH probe.
   h. Facility power for the BI-ZTU unit (one outlet, 110/115/220/240 VAC, 50/60 Hz, 25 Watts).

i. Beaker for collecting waste while rinsing.

Attaching the flow cell

1. Place the BI-ZTU to the left of your Brookhaven Zeta instrument.
2. Gently place the flow cell on top of the Brookhaven Zeta instrument, placing it safely to ensure that it will not roll or drop.
3. Thread the red connector on the flow cell tube into the red bulkhead union on the BI-ZTU. The nut should turn easily.
4. Thread the black connector on the flow cell tube into the black bulkhead union on the BI-ZTU. The nut should turn easily.
5. Tighten both the red and black connectors until they are finger tight.
6. Visually confirm that nothing is blocking tan bulkhead union on the side of the BI-ZTU is open to allow air to enter the system.

Installing the software

1. Turn on your Brookhaven zeta instrument, but do not start the zeta software.
2. If software was installed at the factory, please skip steps 3, 7, and 8.
3. Run the installation program from the installation CD to update your zeta software for use with the BI-ZTU. Leave the CD inside the computer.
4. Plug the power brick into your facility power. Plug the 12 volt output into the back of the BI-ZTU.
5. Plug the USB cable into the USB port on the BI-ZTU. Plug the other end of the cable into a USB port on the back of your zeta instrument.
6. Turn on the BI-ZTU with the switch on top of the unit.
7. A Windows dialog will appear asking you to install a USB driver. Choose to search locally for the driver. Then, choose the CD ROM drive as the location of the USB driver.
8. Two USB drivers will be installed, one for the port, the other for the instrument. If Windows does not do so automatically, repeat step 7 .

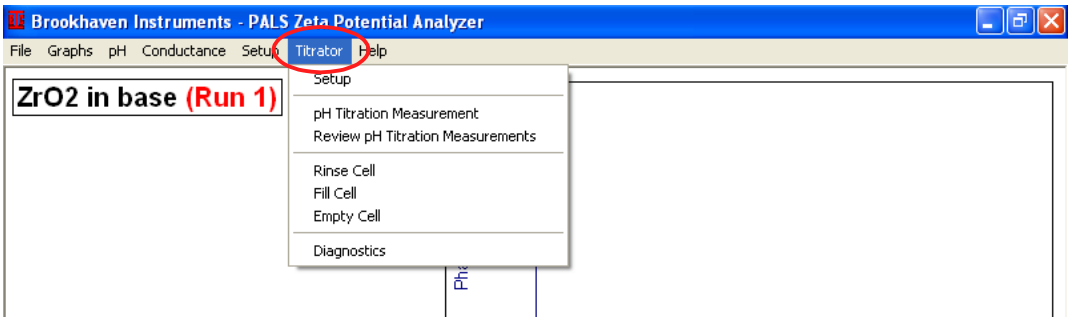

Figure IV- 1: Location of Titrator menu item in ZetaPALS software.

9. Start the zeta software. A menu item will appear called **Titrator** as shown in Figure IV- 1. Select this menu item. If the option **Empty cell**, located second from the bottom, is grayed out, check to make sure that the BI-ZTU is plugged in, turned on (the switch at the top of the unit will be red and the fan will turn), and the USB cable is connected. If you had to correct any of these, you will have to close the zeta software and start it again.

Calibrating the pH probe

1. Plug the BNC connector on the pH probe into the back of the BI-ZTU unit (not the zeta instrument), but leave the protective bottle on the electrode.
2. On the **Titrator** menu, click **Setup**. The top line of the resulting dialog box will show current pH and the last calibration date as shown in Figure IV- 2. On a new installation the calibration corresponds to an ideal pH probe.
3. Click **Recalibrate pH Probe** as shown in Figure IV- 2. A dialog, as shown in Figure

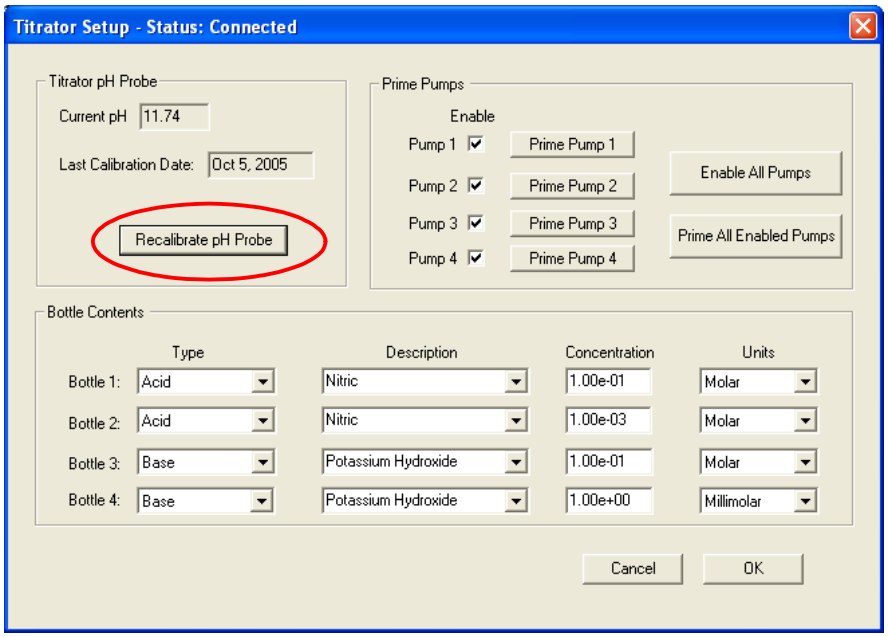

Figure IV- 2: Setup Dialog showing calibrate pH probe button.

IV- 3, will appear asking if you wish to do a two-point or three-point calibration. Use the three-point calibration.

4. Next, you will be asked to fill in the pH values of the buffers used for calibration. Calibrate with pH 4, 7, and 10 buffers with pH values known to 0.01 pH unit. Read these values off of the packages for the buffers you are using; the values differ for different suppliers. Enter these values and then click **OK**.

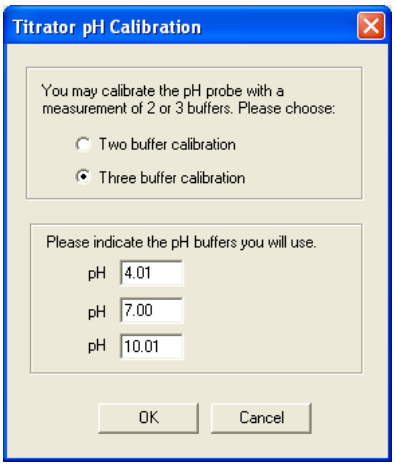

Figure IV- 3: pH calibration start dialog. Enter actual calibration buffer pH values here.

5. Remove the pH probe from the protective bottle and retain this bottle and its contents for probe storage. Rinse the probe with DI water. Place the pH probe into your first buffer (e.g., pH 4 buffer) and click **OK**. A dialog will appear to indicate that the instrument is waiting for the pH probe to achieve equilibrium. The next dialog will indicate that the instrument is waiting for a stable signal from the pH probe. Finally, you will be prompted for the next pH buffer.
6. Repeat step 5 for the remaining buffers, taking special care to rinse the pH probe with DI water between buffers.
7. After you are done, you will be returned to the Titrator Setup dialog.

WARNING: It is important that the pH probe remains wet at all times. Fill a sample cup with 20 mL of liquid and place on titrator arm to prevent drying of the pH probe tip. Note that while DI water is adequate for short periods of less than 6 hours, pH probe storage solution or pH 4 buffer is necessary for overnight or longer storage. The pH probe is a consumable item and is not covered by the BIC warranty. However, with proper care, it will last a long time. The pH probe will fail if it dries.

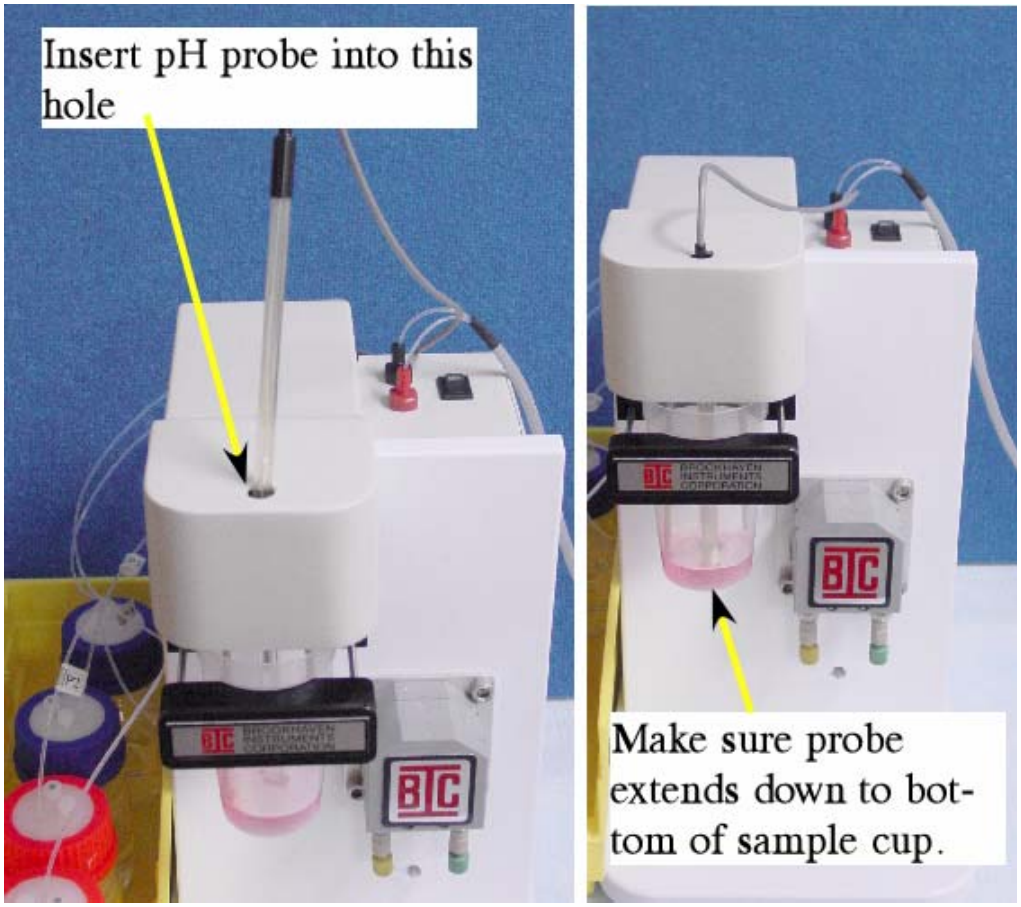

Figure IV- 4: Insertion of pH probe into BI-ZTU.

8. After calibration, gently push the pH probe all the way down into the hole at the top of the BI-ZTU main unit arm as shown in Figure IV- 4.

Filling the reagent bottles and setting up the bottle contents in the software

1. After pH probe calibration is completed, you will return to the Setup dialog. Alternatively, choose the **Titrator** menu option from the main menu bar of the zeta software and then click on **Setup**.

WARNING: Acids and bases are corrosive and caustic materials. All users of the titrator must be trained in working with these acids and bases. Your local lab safety officer and chemical supplier can provide you with specific advice including the types of gloves and goggles that are to be worn when handling these materials.

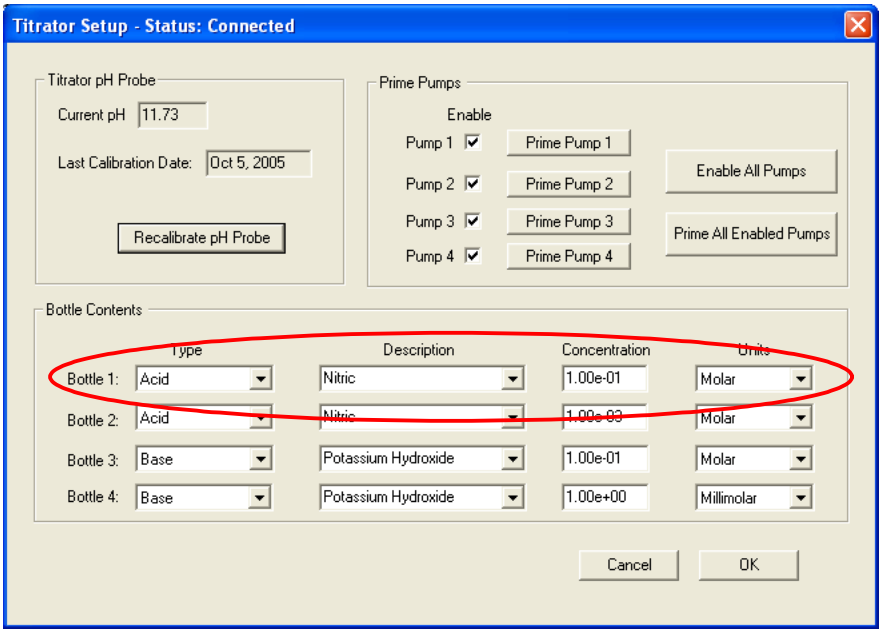

Figure IV- 5: Setup dialog showing region with bottle information.

2. There are four metering pumps, numbered from one to four that dispense reagents (here acids and bases) from four bottles, correspondingly numbered from one to four. The region for entering bottle contents into the software is shown in Figure IV- 5.
3. Confirm that the reagent bottle contents are assigned as follows:
   a. Pump/Bottle 1: Acid, Nitric, 0.1 Molar
   b. Pump/Bottle 2: Acid, Nitric, 0.001 Molar
   c. Pump/Bottle 3: Base, Potassium Hydroxide, 0.1 Molar
   d. Pump/Bottle 4: Base, Potassium Hydroxide, 0.001 Molar
4. Insert tube 1 into the bottle with the red cap labeled 1. Remove the cap from the bottle. Push the tube into the open hole of the cap, then grab and pull from the other side so that about 100 mm of tube protrudes from the bottom of the cap.
5. Screw cap back onto bottle 1 and make sure the tube reaches the bottom of the bottle.
6. Remove the cap from the bottle and fill the bottle with 50~80 mL of 0.1 M Nitric acid.

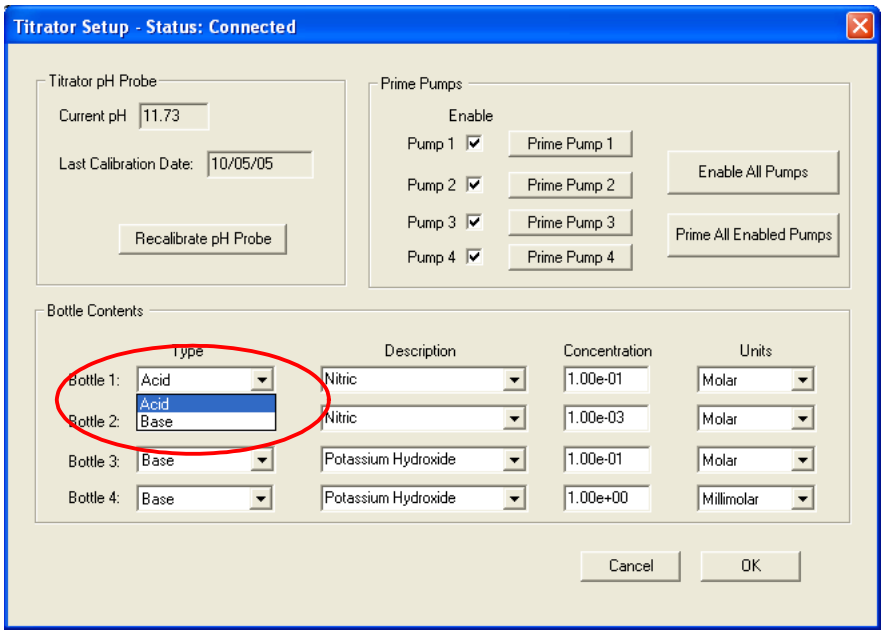

Figure IV- 6: Setup dialog showing choice of acid and base for bottle type.

7. In the Titrator Setup dialog, for bottle 1, pick a *Type* of Acid (Figure IV- 6), a *Description* of Nitric (Figure IV- 7), and enter the *Concentration* in Molar (Figure IV-8). This will be a value close to 0.1 (or 1.00e-01).

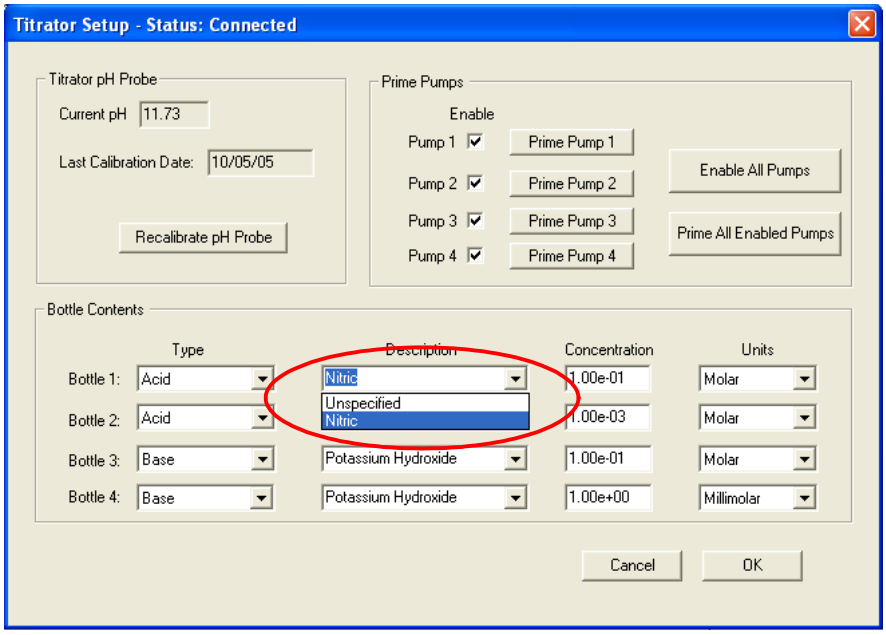

Figure IV- 7: Setup dialog showing description field. If you wish to type in your own description, choose unspecified and type the name into the field.

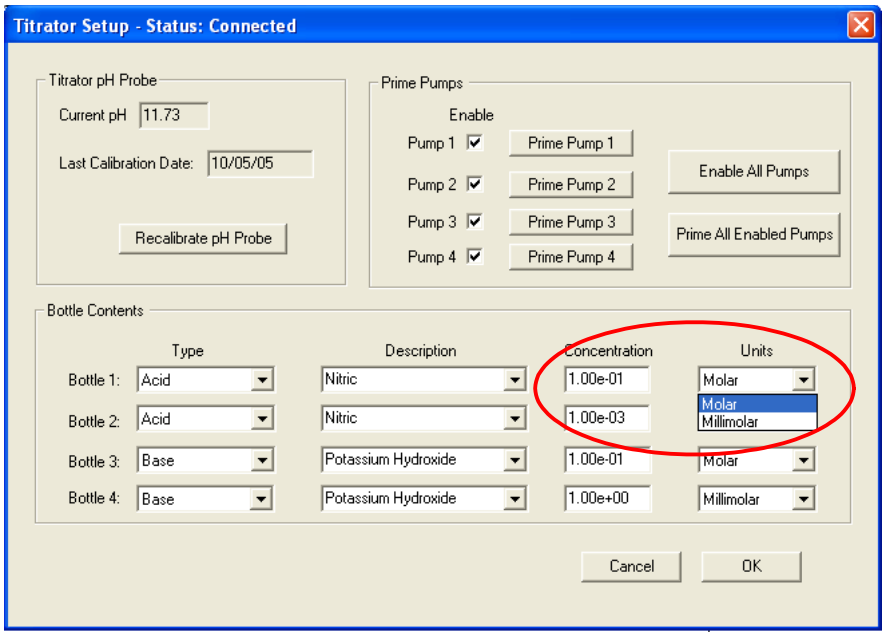

Figure IV- 8: Setup dialog showing concentration and units fields.

8. Repeat steps 4 through 7 for bottles 2 through 4. For bottle 2, pick a *Type* of acid, a *Description* of Nitric, and enter the *Concentration* in Molar. This will be a value close to 0.001 (or 1.00e-03). For bottle 3, pick a *Type* of base, a *Description* of Potassium Hydroxide, and enter the *Concentration* in Molar. This will be a value close to 0.1 (or 1.00e-01). For bottle 4, pick a *Type* of Base, a *Description* of Potassium Hydroxide, and enter the *Concentration* in Molar. This will be a value close to 0.001 (or 1.00E-03). Fill each bottle with the corresponding reagent.

Priming the metering pumps and filling the reagent tubing.

1. Make sure a disposable sample cup is inserted into to the arm of the titrator. In order to insert the sample cup, grasp the cup on the BI-ZTU with one hand. Maneuver the cup so that it is immediately below the pH probe, impeller, and siphons. Gently raise the cup making sure that the pH probe, impeller, and siphons are inside the cup. Use your other hand to pull the black handle towards you and gently push the cup all the way up into the resulting hole as far as it will go. Release the handle. The lip of the cup should be above the cup holder. Tug the cup gently to ensure that it is secure. In this step, the metering pumps will be sending drops of acid and base to the cup.

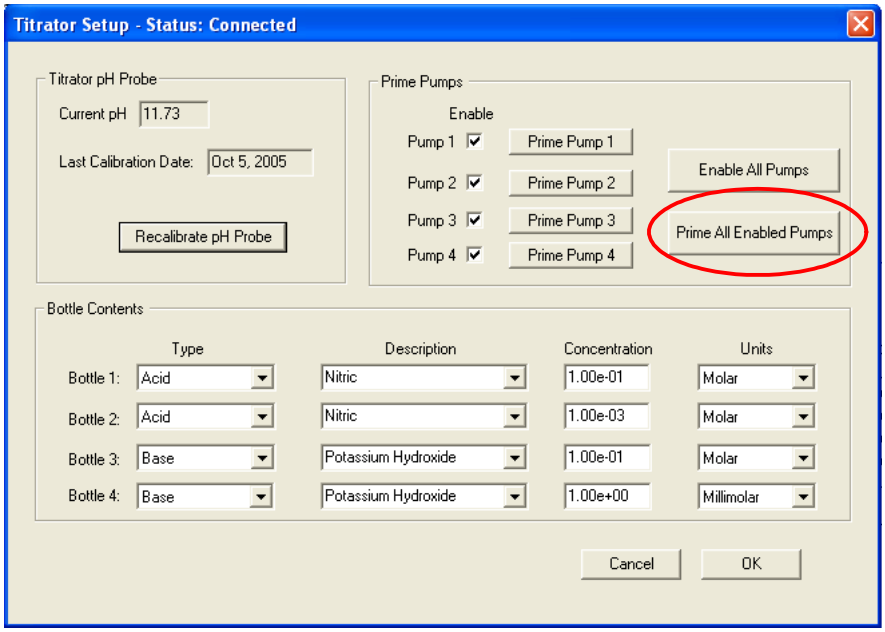

Figure IV- 9: Setup dialog showing Prime All Enabled Pumps button

2. In the Titrator Setup dialog, click on the **Prime All Enabled Pumps** button as shown in Figure IV- 9. All four metering pumps should begin pulsing and you should hear clicks from the titrator arm. If all four metering pumps are not enabled, then click on the **Enable All Pumps** button.
3. Observe the tube from each bottle and make sure that liquid is being drawn up each tube. Initially, liquid will move up the tubes slowly.
4. If the liquid is not moving at all, you will need to force the check valves in the metering pumps to open. This is described in the next section.
5. Confirm the proper operation of each individual pump. To do so, click on the **Prime Pump 1** button and observe the cup to ensure that drops of acid are falling into the cup from this pump. Once you observe drops falling, then you can click cancel on the interim dialog box to stop the pump.
6. Repeat step 5 for pumps 2 through 4, using the buttons corresponding to each pump in order to ensure that each pump is delivering reagents properly.
7. Click **OK** to close the Titrator Setup dialog.
8. Remove the sample cup and rinse the pH probe, impeller, and siphons with DI water.
9. Place a sample cup with 40 mL of DI water on the Titrator arm. Choose **Titrator** from the main menu bar and then click on **Fill Cell** and check that the sample cell fills. Then, choose **Titrator** from the main menu bar and then click on **Empty Cell** and check that the sample cell empties completely.

If the pumps don't self prime

1. There are two plugs on the cap of reagent bottle. Remove the plug with the metal frit (not the solid plastic plug). See Figure IV- 10.

2. Fill the provided Luer-tip syringe with air.
3. Insert the syringe tip into the hole in the cap (which is a Luer size).
4. Push down on the syringe plunger to force air into the bottle until liquid is forced up the tube.
5. Continue to push down on the plunger until the pump stops pumping (the pumping dialog box on the zeta instrument screen will close).
6. After the pump has stopped pumping, remove the syringe and replace the plug with the metal frit.
7. Press the prime button corresponding to the pump that doesn't prime and make sure that liquid continues to move up the tube.
8. Repeat this for each pump that is not operating properly.
9. Click on the **Prime All Pumps** button again and observe the sample cup. Drops should be falling into the liquid.

Figure IV- 10: Injecting air into a sample bottle to start a stuck pump

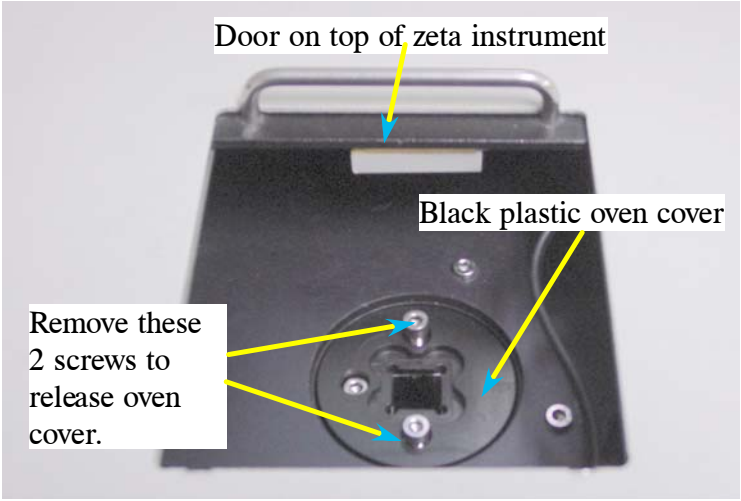

Figure IV- 11: Removing oven cover from before inserting flow cell.

Placing flow cell into the zeta instrument

1. Use a 3 mm hex key to remove the two screws holding down the plastic oven cover as shown in Figure IV- 11. Retain these screws.
2. Lift the black plastic oven cover straight up and retain.
3. Insert flow cell into the zeta instrument. The flow cell incorporates a plastic oven cover. Use the two screws from the oven cover to secure flow cell inside of instrument.
4. Note that if you remove the flow cell to perform measurements with the standard zeta electrodes or to perform sizing measurements, the oven cover should be replaced to ensure optimal temperature control.

Figure IV- 12: Flow cell inserted into zeta instrument.

Section V: A Quick Tour of the BI-ZTU Software

The BI-ZTU software is integrated into the Brookhaven Instruments zeta potential software. All BI-ZTU features are accessed from the **Titrator** menu item in the main menu bar of your zeta potential software, as shown in Figure V- 1. Note that the **pH** menu item in the zeta potential software accesses the pH probe connected to the back of the main instrument, not the pH probe used by the BI-ZTU.

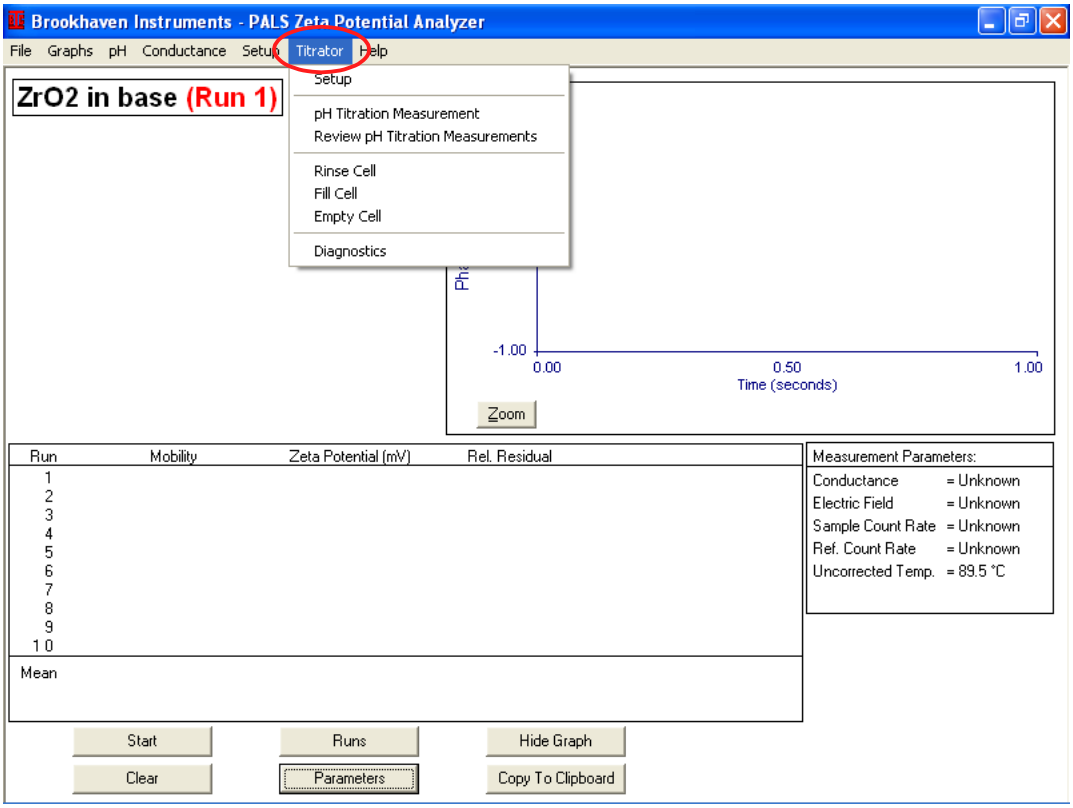

Figure V- 1: Main screen of zeta software showing the BI-ZTU menu options.

Setup Menu Item

The **Setup** menu item opens the Titrator Setup dialog, shown in Figure V- 2. This dialog is for the purpose of entering experimental parameters related to the titration and readying the titrator for a measurement. Use of this dialog is covered more fully in Section IV of this manual.

In the Titrator pH Probe section, the real-time pH reading along with the last calibration date is shown. There is also a button for recalibrating the pH probe.

In the Prime Pumps section, there are check boxes for enabling and disabling pumps, along with buttons for priming each pump, or all pumps. Pumps need to be primed when the tubing from the bottle to the pump and the pump to the sample cup is empty. Otherwise, the BI-ZTU will spend a great deal of time pumping air. Furthermore, the algorithms for adjusting pH can fail if nothing is dispensed for a long time. If you do not wish to use a pump, clear the Enable Pump checkbox.

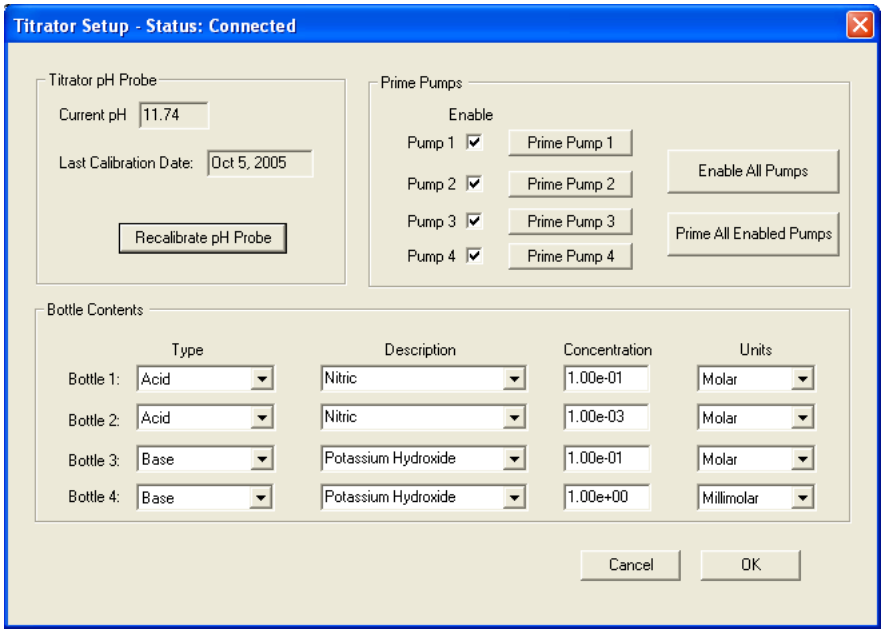

Figure V- 2: Titrator Setup dialog accessed by choosing Titrator, then Setup from the main menu of the zeta software.

In the Bottle Contents Section are edit boxes for entering the contents of each bottle (which correspond to each pump). The pH adjustment algorithm in the software uses the entered concentration and reagent type (acid or base) data to determine from which bottle to dispense reagents during a titration. Therefore, these parameters should be entered with care. The description field will be copied to your results page. It is generally good practice to make sure this information is accurate in order to correctly describe and reproduce experimental results.

Clicking **OK** will close this dialog box and save the changes. Clicking **Cancel** will close this dialog box and clear any changes.

pH Titration Measurement Menu Item

The **pH Titration Measurement** menu item is for setting up and initiating an automated titration measurement once the BI-ZTU is set up as described in Section IV of this manual. The dialog in Figure V- 3 will appear. Use of this menu item is described in Section VI of this manual. The dialog shows the Current pH which is the real-time pH reading of the pH probe on the BI-ZTU. Either enter an initial pH, or choose to start a measurement at the initial pH of the sample. If Use Initial Sample pH is chosen by enabling the checkbox, the BI-ZTU will immediately determine the zeta potential of the sample before adjusting pH. Otherwise, the BI-ZTU will adjust the pH to the entered initial value, and then determine pH. Enter the final pH for the measurement. Enter the number of steps for the measurement. The Initial Volume is the volume of the sample when the sample cup is first inserted into the BI-ZTU. We recommend an initial volume of 20 to 25 mL. The number should be correct to within 10% for pH titrations. Since the pH is measured, precision calculation of concentration is not necessary for pH titration measurements. Choose a pH adjustment resolution, which is the target precision of the

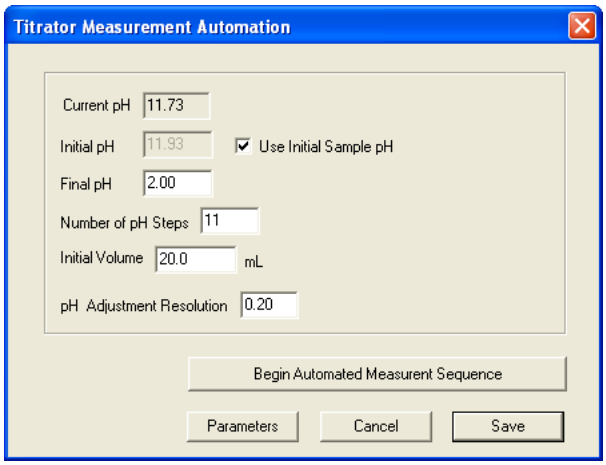

Figure V- 3: Beginning of autotitration measurement. This dialog is accessed by clicking Titrator from the main menu and clicking on pH Titration Measurement.

pH adjustment. We recommend a value of 0.2. Smaller values will lengthen the duration of the measurement.

The **Begin Automated Measurement Sequence** button starts the autotitration measurement. The zeta potential determinations will be made according to the parameters (such as sample name, number of runs, number of cycles, etc.) set in the zeta potential software parameters page. The software will ask if you wish to rinse the cell first. Click OK, and proceed through the rinse sequence, described in this Section under the **Rinse Cell** option. Then, once the sample is loaded, the software will check and attempt to predict if any critical errors will occur such as pH values that cannot be accessed, or a sample cup overflow. Next, the software will adjust the pH if needed, rinse the cell, fill the cell, and determine zeta potential. After the zeta potential determination, the cell will be emptied and pH adjusted again. This process will repeat until the measurement is completed.

The **Parameters** button allows access to the zeta software Parameters window. Please see the zeta software manual for a full description of this window.

The **Cancel** button will close the dialog box without saving any changes.

The **Save** button will close the dialog box after saving the changes that were entered.

Review pH Titration Measurement Menu Item

The **Review pH** Titration Measurement menu item presents data collected by the BI-ZTU. In addition to saving zeta potential data to the database during a pH titration, information about the experimental conditions is saved. Choosing this option will open a window similar to the Database window described in the zeta manual. However, instead of presenting individual zeta measurements, the window will show groups of measure-

ments from a single titration. Results from the titration will then be plotted when a group of measurements is chosen. This option is described more fully in Section VII of this manual.

Rinse Cell Menu Item

The **Rinse Cell** menu item cleans the sample cell in the zeta instrument. Choosing this option will lead to a guided sequence of steps for rinsing the flow cell. Note that even after the flow cell is emptied, a small amount (<1 mL) of material remains. Rinsing will mix this small amount of material with the contents of the sample cup. We recommend running two rinses with fresh rinse liquid when changing samples. The first rinse should be immediately at the end of a measurement, and the second immediately before a new measurement.

Fill Cell Menu Item

The **Fill Cell** menu item fills the sample cell with the contents of the sample cup. Please insert the sample cup before filling the cell.

Empty Cell Menu Item

The **Empty Cell** menu item empties the sample cell into the sample cup. Please insert the sample cup before emptying the cell. Otherwise, the contents of the cell will be emptied onto your lab bench.

Diagnostics Menu Item

The **Diagnostics** option allows low level control of the BI-ZTU. This feature is generally for factory trained personnel and trouble shooting. Choosing this option will cause the Diagnostics dialog to appear, as shown in Figure V- 4.

The Current pH reading is the real-time pH measured by the pH probe attached to the BI-ZTU and is updated approximately twice a second. Note that the pH probe, if any, attached to the rear of the zeta instrument is controlled by the **pH** main menu item.

The Pumps section shows a drop volume field for each pump. These values allow one to enter the volume of each drop, a mechanical value for each pump. The *Drop Volume* fields do not change the pump behavior. In order to activate a single pump, enter the number of desired drops (pulses) into the *Drops* field of the corresponding pump. Then, click the **Dispense** command button to start the pump.

The Valves section allows low level control of the valves. Clicking the radio button from low to high will energize the corresponding valve. Clicking the radio button from high to low will de-energize the corresponding valve.

The Sample Handling section allows low level control of the circulator pump and the stir motor. In order to operate the circulation pump (peristaltic pump), enter a time in the Circulator Time field, then click the **Circulate** command button. Enabling the *Stirrer*

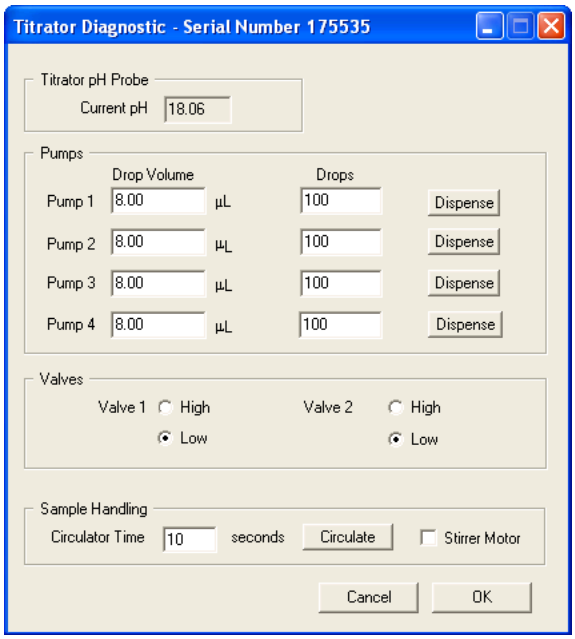

Figure V- 4: Titrator Diagnostic dialog for low level control of the BI-ZTU.

Motor check box will turn on the stirrer motor. Clearing the check box will turn off the stirrer motor.

To close this dialog and cancel any changes, click **Cancel**. To close this dialog and save any changes, click **OK**.

Section VI: Making a Measurement

Set up the BI-ZTU as discussed in section IV. Titration measurements are not single point zeta potential determinations. Rather a titration measurement is a sequence of single point zeta potential determinations coupled with a sequence of additions of reagent to modify the sample suspension. Therefore, the titration lasts much longer than a single zeta potential measurement. Since this is the case, single point zeta potential measurement conditions (concentration, diluent, runs, cycles, etc.) should be developed before making titration measurements on a sample.

To initiate the measurement sequence, select the **Titrator** menu then click **pH Titration Measurement** and the Titrator Measurement Automation dialog will appear as shown in Figure VI- 1. Enter a starting pH (Initial pH) or choose to start with the initial pH of the sample. In the former case, the BI-ZTU will adjust the pH of the sample before making the first zeta potential determination. In the latter case, the BI-ZTU will first determine the zeta potential of the sample without adding any reagents, then proceed to adjust pH. Enter the number of steps. For example, with an initial pH of 11 and a final pH of 3, enter 9 steps for data that is spaced approximately 1 pH unit apart. Note that since an autotitration will take a long time, try to choose only the range of pH values of interest and the minimum number of steps. Enter the initial sample volume (e.g., 20 mL). A good starting volume is 20 to 25 mL. Finally, enter a pH adjustment resolution. Try a value of 0.2. Smaller values will lengthen the measurement as the BI-ZTU tries to hit the pH values more accurately. Now, the autotitration parameters are set up for your measurement.

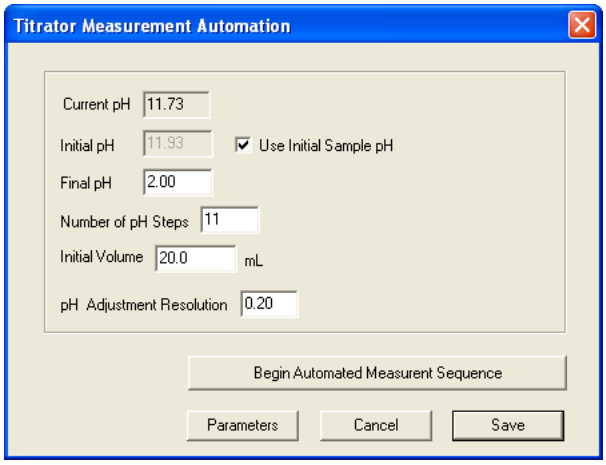

Figure VI- 1: Titrator Measurement Automation Dialog.

Next, set up the details of the zeta potential measurement. Click the **Parameters** command button in the Titrator Measurement Automation dialog. The zeta parameters dialog box will appear. Details about this dialog box are in your zeta instrument manual. Enter your sample name, operator ID, and sample notes. Choose the number of runs and number of cycles (for example, 10 runs of 5 cycles). Enter a temperature that is close to your lab temperature (generally about 25 ºC). Enable the Auto Save Results check box.

Click **OK** to close the parameters dialog box. The software will then return to the Titrator Measurement Automation dialog box.

There are several choices once the experimental conditions are entered. Clicking **Save** will cause the pH Measurement Automation dialog to disappear and the experimental conditions will be stored. Then, selecting **Titrator** on the main menu bar followed by clicking **pH Titration Measurements** will recall the pH measurement dialog. Clicking Cancel will cause the pH Measurement Automation dialog box to disappear, but the changes in experimental conditions will not be stored. Alternatively, click **Begin Automated Measurement Sequence** to begin a titration measurement.

Figure VI- 2: Inserting and removing the sample cup.

In the next step, you will insert and remove the sample cup. This procedure is shown in Figure VI- 2. In order to insert the sample cup, grasp the cup on the BI-ZTU with one hand. Maneuver the cup so that it is centered below the pH probe, impeller, and siphons. Gently raise the cup making sure that the pH probe, impeller, and siphons are inside the cup. Use your other hand to pull the black handle towards you and gently push the cup all the way up into the resulting hole as far as it will go. Release the handle. The lip of the cup should be above the cup holder. Tug the cup gently to ensure that it is secure.

Once a titration measurement begins, a dialog box will appear asking if you wish to rinse your sample cell. Rinse liquids and additives should be chosen to effectively disperse or dissolve any previous samples. Even after the zeta flow cell is empty, some (< 1 mL) of liquid remains. Therefore, a rinse with diluent (or even new sample) will prevent cross-contamination of samples. Click **Yes** in the dialog box that appeared. When the dialog asks you to do so, remove the cup from the titrator arm, rinse the pH probe, impeller, and siphons with DI water and insert a cup with 40 mL of fresh rinse liquid. Then click **OK** in the dialog box that appeared. The BI-ZTU will then fill and empty the sample cell in the zeta instrument to clean it with the rinse liquid. When the dialog asks if you wish to rinse again, click **No** to proceed to the titration measurement.

Now the BI-ZTU is ready to receive your sample. When the dialog asks you to insert the sample cup, remove rinse cup from the arm and insert a cup filled with sample into the BI-ZTU and click **OK**. Wait until the first measurement has begun to check that there are no warnings that stop the automated titration. The BI-ZTU will rinse the cell with the sample, fill the cell, and proceed to determine zeta potential. After the first measurement, the titrator will add acid or base to adjust the pH to the next target pH, generally taking several iterations to do so. Then it will rinse the cell, fill the cell, measure the pH, and determine zeta potential. A graph of zeta potential as a function of pH will appear to show the progress of the measurement. During a titration measurement, the window sizes and locations are only accessible during the zeta potential determination, not while the BI-ZTU is dosing the sample with acid or base.

Once the titration measurement is completed, the BI-ZTU will empty the flow cell back into the sample cup. Clear the results by clicking on the **Clear** button on the zeta screen. Even after the flow cell is emptied, there will still be a small (<1 mL) amount of sample left in the flow cell. Therefore, in order to ensure that your previous sample does not corrupt subsequent measurements, rinse the titrator flow cell after making a measurement. The cell should be rinsed immediately with a good dispersing agent for your sample. For example, if the previous sample was ZrO_2 and the measurement ended at pH 2 with a large (magnitude greater than 25 mV) zeta potential, pH 2 acid would be a good dispersing agent for rinsing the flow cell. Since the isoelectric point of ZrO_2 is close to that of clean water, using DI water could lead to aggregation and settling of particles rather than cleaning. The rinse procedure is detailed below:

a. Choose **Titrator** from the main menu bar and click **Rinse Cell**.

b. When the dialog box appears, remove the sample cup from the titrator arm.

c. Rinse the pH probe, impeller, and siphons with water. Press **OK**.

d. Insert a cup of rinse liquid (e.g., pH 2 acid for ZrO_2) and press **OK**.

e. The titrator will then use this acid to rinse the flow cell.

f. You can then perform a second rinse with either DI water or acid.

Finally, it is critical to ensure that the pH probe is immersed in pH probe storage solution or pH 4 buffer before leaving the instrument. Even one night of dry storage will ruin a pH probe. See the warning in the Setup section of this manual.

For short periods of inactivity, less than 2 months, the titrator is best left with the pH probe in buffer storage solution and the reagent bottles full of liquid (acid or base). For longer periods of inactivity, or for shipping the titrator, empty the reagent bottles and fill with water. Then, press prime all pumps two times so that the water will flush the acid and base from the tubes and pumps. Press prime all pumps two times so that the water will fill the pumps. Finally, repeat with the tubes from the reagent bottles in air to empty the pumps of water and fill with air.

Section VII: Data Analysis and Interpretation

Data interpretation depends on the goal of the measurements. In order to rapidly summarize data from a series of measurements, the software automatically presents a plot of zeta potential as a function of pH. The pH at which the zeta potential is zero is known as the isoelectric point (IEP). For systems where colloidal stability is required, such as for long shelf life, dispersions should be prepared under conditions far from the isoelectric point to enhance electrostatic stabilization. For systems where flocculation is desirable, such as for settling out solids, dispersions should be prepared under conditions close to the isoelectric point to eliminate electrostatic stabilization.

Results can be accessed by selecting **Titrator** from the main menu bar of the zeta potential instrument software followed by clicking **Review pH Titration Measurements**. A dialog, shown in Figure VII- 1 and similar to the Database dialog in the zeta software will appear. However, in this case, only titration measurements are displayed. Single point zeta potential determinations are not shown. Choose a titration measurement and click **OK** to obtain a graph of zeta potential vs. pH.

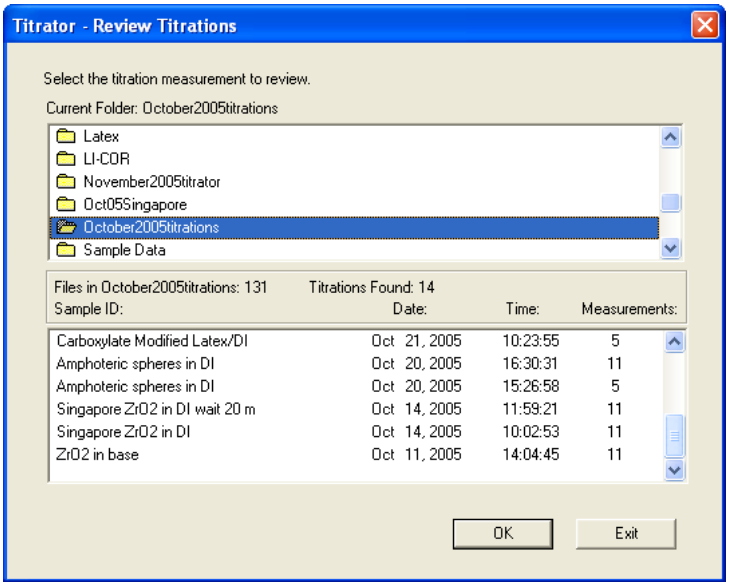

Figure VII- 1: Dialog for choosing an autotration measurement to review. This dialog can be accessed by choosing Titrator followed by clicking on Review pH Titration Measurements.

The graph of zeta potential vs. pH is shown in Figure VII- 2. On the left side, pH and combined zeta potential values are tabulated. Below is a button, **Copy to Clipboard**, which will copy the graph to the clipboard as a bitmap for export to other programs such as Paint. The **Copy for Spreadsheet** button will copy the tabulated data (comma delimited) to the clipboard for export to other programs such as Notepad. Above the plot, information on the sample is shown. The sample name, operator ID, and notes are from the zeta potential software parameters page. Next are the details of the reagents entered in the Setup dialog. The line immediately above the graph shows the coordinates of the

crosshairs that can moved with the mouse (click inside the graph area). The displayed IEP is the linearly interpolated value of the IEP using two points. The first point corresponds to the last positive zeta potential before (on the low pH side) the IEP, and the second corresponds to the first negative zeta potential after the I.E.P. If there are multiple points where the data crosses zero zeta potential, the software uses the leftmost (lowest pH value) zero crossing.

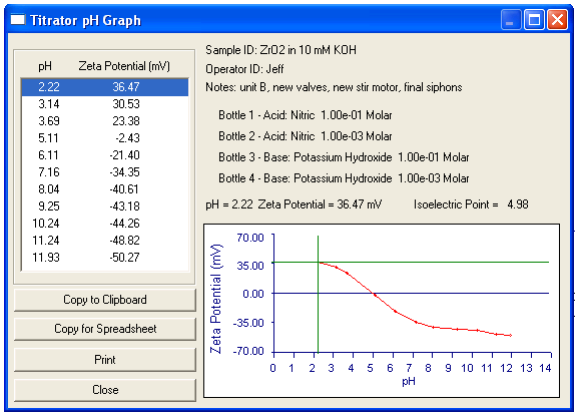

Figure VII- 2: Zeta potential vs. pH graph generated by the autotitration software.

Appendix A: Clearing Blockages & Removing Bacterial Growth

If liquid with acid, base, salt, surfactant or polymer solutions dry inside the cell, the pumps, or the tubing, the titrator will not perform properly and the zeta potential results will suffer. The signal will drift continuously as the dried material is slowly desorbed off the cell/pump/tubing walls and back into solution/suspension. In addition, dried material in the pumps or lines leading into them will change the drop volume per pulse until air is expelled and they are properly primed again.

The first line of defense is never to let any solution dry in the flow cell or tubing. If you will not use the titrator for more than 8 weeks, always flush all four reagent pumps with DI water.

If something has dried in the cell and/or peristaltic tubing, do the following. Remove the mixing cup. Use a squirt bottle with rinse liquid to wash down the pH probe, impeller, and siphons. Rinse with water the cup and replace it filled with 50 mL of water.

If something has dried in one or more of the pumps or tubes leading to one of the metering pumps for dispensing reagents, the pump will no longer dispense liquids. When that happens, assume that all four pumps and sets of tubing need the following rinsing procedure. Add 50 mL of DI water to each of the four 100 mL screw-cap bottles supplied with the instrument. Then, follow the instructions in the section “Priming the metering pumps and filling the reagent tubing.” on page IV-8.

If the cell interior is dirty and cannot be cleaned by rinsing, then disassemble the cell by removing the four screws on the bottom of the cell collar as shown in Figure A- 1. The glass exterior and collar will then slide free of the electrodes as a single unit as

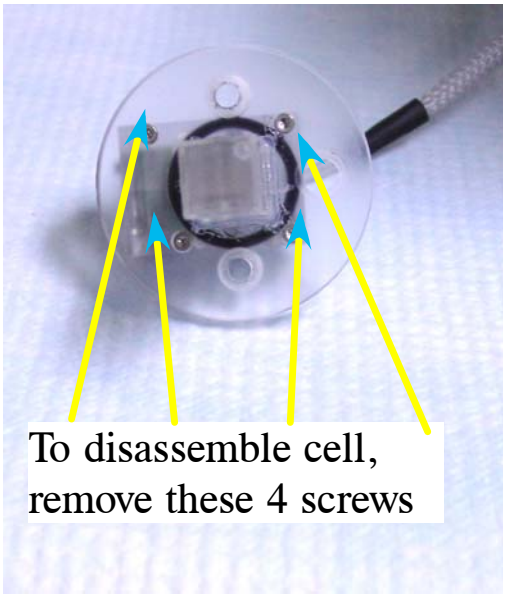

Figure A- 1: Cell disassembly for cleaning.

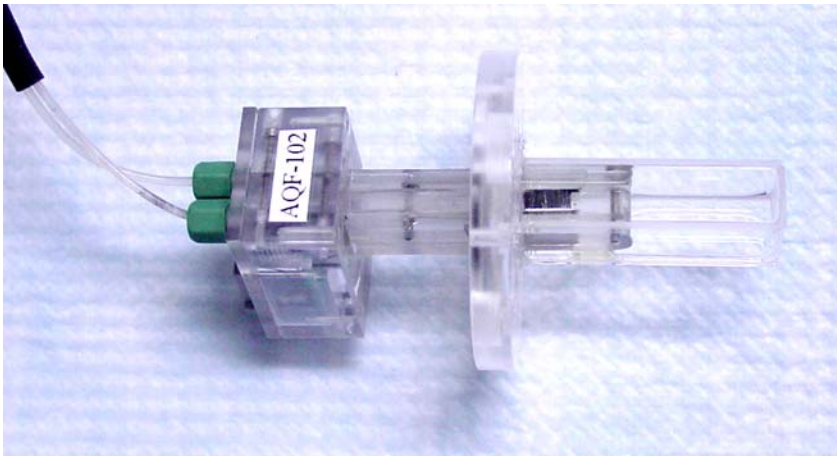

Figure A- 2: Removing bottom of the flow cell from the electrode assembly.

shown in Figure A- 2. Clean the cell, then rinse with DI water and reassemble. Be sure to tighten the screws in an X pattern to ensure the gasket is evenly compressed. If the cell fails to fill after reassembly, then loosen and reattach the collar to eliminate leaks.

If the tubing is blocked with dried salt then soak the tubing in DI water for an extended period.

Appendix B: Solvent Compatibility and Flushing

All these surfaces are resistant to HNO_3 at pH 1 or greater and KOH at pH 13 or less, beyond the extreme cases of acid and base recommended for use, and the surfactant and polymer solutions used as dispersants and stabilizing agents. Do not use stronger acids or bases or chemicals known to be corrosive to the materials listed below such as hydrofluoric acid, which etches glass, or solvents that will attach acrylic.

The BI-ZTU flow cell exterior is glass. The electrode holders are acrylic. The tubing from the solution bottles, to the pumps, valves, and into the sample mixing cup is PTFE. The peristaltic tubing is Santoprene. The siphons are PEEK. The internal parts of the pumps and valves are PTFE and EPDM. The fittings are PEEK or Tefzel.

Flushing

Whether you are rinsing or changing solvents, remember that there are five independent flow circuits. The four circuits from the four, 100 mL solution bottles, to the four pumps, and ending with the mixing cup are called the pump-to-mixing-cup (PMC) circuits. The circuit from the mixing cup through the peristaltic pump to the cell and back is called the mixing-cup-to-sample-cell (MCSC) circuit.

Never allow any liquid to dry inside the cell, tubing, pumps, or valves. Always flush any aqueous solutions first with water to prevent precipitation. If you are using a non-aqueous solution (typically with dissolved dispersant), flush with the pure solvent to prevent precipitation. Then flush with an intermediate solvent as explained below.

Flush a PMC circuit by filling the 100 mL, screw-cap bottle with about 10 mL of solvent. When dealing with acids/bases, salts and surfactants dissolved in water, the solvent is water. When working with surfactants or polymers dissolved in non-aqueous solvents, the solvent is the non-aqueous solvent. Use the same solvent for the initial rinse to prevent the possible precipitation of any acids, bases, and especially salts and surfactants. Select **Titrator** in the main menubar, then choose **Setup**. Click on the choice labeled **Prime All Enabled Pumps**. This will initiate a 200 pulse sequence, lasting about 200 seconds (3 minutes and 20 seconds). If a pump is delivering 8 μL/pulse, 1.6 mL will be delivered. After the pumping sequence is completed, click the **Prime All Enabled Pumps** button again. At the end of the second sequence, the lines will be completely full with the solvent.

Flush the MCSC circuit by following several steps; these directions are incorporated into the software rinse procedure. Click the **Titrator** command button in the main menu bar. Click on the choice labeled **Rinse Cell**. The contents of the cell will be emptied into the sample cup. Then, use a squirt bottle with rinse liquid to wash down the pH probe, impeller, and siphons. Clean or replace the mixing cup with one filled with 50 mL of clean solvent. Now click **OK**. The cell will be rinsed with the contents of the cup. The few drops of solution still in the cell are now diluted with 50 mL of clean solvent.