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The Preparation of Buffers and Other Solutions: A Chemist's Perspective

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Editor's note: Many, perhaps most, molecular biology procedures don't require perfection in the handling of reagents and solution preparation. When procedures fail and logical thinking produces a dead end, it might be worthwhile to carefully review your experimental reagents and their preparation. The author of this discussion is an extremely meticulous analytical chemist, not a molecular biologist. He describes the most frequent mistakes and misconceptions observed during two decades of experimentation that requires excruciating accuracy and reproducibility in reagent preparation.

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BUFFERS

Why Buffer?

The primary purpose of a buffer is to control the pH of the solution. Buffers can also play secondary roles in a system, such as controlling ionic strength or solvating species, perhaps even affecting protein or nucleic acid structure or activity. Buffers are used to stabilize nucleic acids, nucleic acid–protein complexes, proteins, and biochemical reactions (whose products might be used in subsequent biochemical reactions). Complex buffer systems are used in electrophoretic systems to control pH or establish pH gradients.

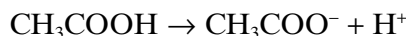
Can You Substitute One Buffer for Another?

It is rarely a good idea to change the buffer type—that is, an amine-type buffer (e.g., Tris) for an acid-type buffer (e.g., phosphate). Generally, this invites complications due to secondary effects of the buffer on the biomolecules in the system. If the purpose of the buffer is simply pH control, there is more latitude to substitute one buffer for another than if the buffer plays other important roles in the assay.

How Does a Buffer Control the pH of a Solution?

Buffers are solutions that contain mixtures of weak acids and bases that make them relatively resistant to pH change. Conceptually buffers provide a ready source of both acid and base to either provide additional H⁺ if a reaction (process) consumes H⁺, or combine with excess H⁺ if a reaction generates acid.

The most common types of buffers are mixtures of weak acids and salts of their conjugate bases, for example, acetic acid/sodium acetate. In this system the dissociation of acetic acid can be written as



where the acid dissociation constant is defined as $K_a = \frac{[\text{H}^+][\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$.

Rearranging and taking the negative logarithm gives the more familiar form of the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK} + \log \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COOH}]}$$

Inspection of this equation provides several insights as to the functioning of a buffer.

When the concentrations of acid and conjugate base are equal, $\log(1) = 0$ and the pH of the resulting solution will be equal to the pK_a of the acid. The ratio of the concentrations of acid and conjugate base can differ by a factor of 10 in either direction, and the resulting pH will only change by 1 unit. This is how a buffer maintains pH stability in the solution.

To a first approximation, the pH of a buffer solution is independent of the absolute concentration of the buffer; the pH depends only on the ratio of the acid and conjugate base present. However, concentration of the buffer is important to buffer capacity, and is considered later in this chapter.

When Is a Buffer Not a Buffer?

Simply having a weak acid and the salt of its conjugate base present in a solution doesn't ensure that the buffer will act as a buffer. Buffers are most effective within ± 1 pH unit of their pK_a . Outside of that range the concentration of either the acid or its salt is so low as to provide little or no capacity for pH control. Common mistakes are to select buffers without regard to the pK_a of the buffer. Examples of this would be to try to use $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ ($\text{pK}_a = 6.7$) to buffer a solution at pH 4, or to use acetic acid ($\text{pK}_a = 4.7$) to buffer near neutral pH.

What Are the Criteria to Consider When Selecting a Buffer?

Target pH

Of primary concern is the target pH of the solution. This narrows the possible choices to those buffers with pK_a values within 1 pH unit of the target pH.

Concentration or Buffer Capacity

Choosing the appropriate buffer concentration can be a little tricky depending on whether pH control is the only role of the buffer, or if ionic strength or other considerations also are important. When determining the appropriate concentration for pH control, the following rule of thumb can be used to estimate a reasonable starting concentration.

1. If the process or reaction in the system being buffered does not actively produce or consume protons (H^+), then choose a moderate buffer concentration of 50 to 100 mM.
2. If the process or reaction actively produces or consumes protons (H^+), then estimate the number of millimoles of H^+ that are involved in the process (if possible) and divide by the solution volume. Choose a buffer concentration at least 20× higher than the result of the estimation above.

The rationale behind these two steps is that a properly chosen buffer will have a 50:50 ratio of acid to base at the target pH, therefore you will have 10× the available capacity to consume or supply protons as needed. A 10% loss of acid (and corresponding increase in base species), and vice versa, results in a 20% change in the ratio ($[CH_3COO^-]/[CH_3COOH]$ from the Henderson-Hasselbalch example above) resulting in less than a 0.1 pH unit change, which is probably tolerable in the system. While most biomolecules can withstand the level of hydrolysis that might accompany such a change (especially near neutral pH), it is possible that the secondary and tertiary structures of bioactive molecules might be affected.

Chemical Compatibility

It is important to anticipate (or be able to diagnose) problems due to interaction of your buffer components with other solution components. Certain inorganic ions can form insoluble complexes with buffer components; for example, the presence of calcium will cause phosphate to precipitate as the insoluble calcium phosphate, and amines are known to strongly bind copper. The presence of significant levels of organic solvents can limit solubility of some inorganic buffers. Potassium phosphate, for example, is more readily soluble in some organic solutions than the corresponding sodium phosphate salt.

One classic example of a buffer precipitation problem occurred when a researcher was trying to prepare a sodium phosphate buffer for use with a tryptic digest, only to have the Ca^{2+} (a nec-

essary enzyme cofactor) precipitate as $\text{Ca}_3(\text{PO}_4)_2$. Incompatibilities can also arise when a buffer component interacts with a surface. One example is the binding of amine-type buffers (i.e., Tris) to a silica-based chromatography packing.

Biochemical Compatibility

Is the buffer applied at an early stage of a research project compatible with a downstream step? A protein isolated in a buffer containing 10mM Mg^{2+} appears innocuous, but this cation concentration could significantly affect the interaction between a regulatory protein and its target DNA as monitored by band-shift assay (Hennighausen and Lubon, 1987; *BandShift Kit Instruction Manual*, Amersham Pharmacia Biotech, 1994). Incompatible salts can be removed by dialysis or chromatography, but each manipulation adds time, cost, and usually reduces yield. Better to avoid a problem than to eliminate it downstream.

What Can Generate an Incorrect or Unreliable Buffer?

Buffer Salts

All buffer salts are not created equal. Care must be exercised when selecting a salt to prepare a buffer. If the protocol calls for an anhydrous salt, and the hydrated salt is used instead, the buffer concentration will be too low by the fraction of water present in the salt. This will reduce your buffer capacity, ionic strength, and can lead to unreliable results.

Most buffer salts are anhydrous, but many are hygroscopic—they will pick up water from the atmosphere from repeated opening of the container. Poorly stored anhydrous salts also will produce lower than expected buffer concentrations and reduced buffering capacity. It is always wise to record the lot number of the salts used to prepare a buffer, so the offending bottle can be tracked down if an error is suspected.

If a major pH adjustment is needed to obtain the correct pH of your buffer, check that the correct buffer salts were used, the ratios of the two salts weren't switched, and finally verify the calculations of the proper buffer salt ratios by applying the Henderson-Hasselbalch equation. If both the acid and base components of the buffer are solids, you can use the Henderson-Hasselbalch equation to determine the proper mass ratios to blend and give your target pH and concentration. When this ratio is actually prepared, your pH will usually need some minor adjustment, which should be very minor compared to the overall concentration of the buffer.

pH Adjustment

Ionic strength differences can arise from the buffer preparation procedure. For example, when preparing a 0.1 M acetate buffer of pH 4.2, was 0.1 mole of sodium acetate added to 900 ml of water, and then titrated to pH 4.2 with acetic acid before bringing to 1 L volume? If so, the acetate concentration will be significantly higher than 0.1 M. Or, was the pH overshoot, necessitating the addition of dilute NaOH to bring the pH back to target, increasing the ionic strength due to excess sodium? The 0.1 M acetate buffer might have been prepared by dissolving 0.1 mole sodium acetate in 1 liter of water, and the pH adjusted to 4.2 with acetic acid. Under these circumstances the final acetate concentration is anyone's guess but it will be different from the first example above.

The best way to avoid altering the ionic concentration of a buffer is to prepare the buffer by blending the acid and conjugate base in molar proportions based on Henderson-Hasselbalch calculations such that the pH will be very near the target pH. This solution will then require only minimal pH adjustment. Dilute to within 5% to 10% of final volume, make any final pH adjustment, then bring to volume.

Generally, select a strong acid containing a counter-ion already present in the system (e.g., Cl^- , PO_4^{3+} , and OAc^-) to adjust a basic buffer. The strength (concentration) of the acid should be chosen so that a minimum (but easily and reproducibly delivered) volume is used to accomplish the pH adjustment. If overshooting the pH target is a problem, reduce the concentration of the acid being used. Likewise, choose a base that contains the cations already present or known to be innocuous in the assay (Na^+ , K^+ , etc.)

Solutions of strong acids and bases used for final pH adjustment usually are stable for long periods of time, but not forever. Was the NaOH used for pH adjustment prepared during the last ice age? Was it stored properly to exclude atmospheric CO_2 , whose presence can slowly neutralize the base, producing sodium bicarbonate (NaHCO_3) which further alters the buffer properties and ionic strength of the solution?

Buffers from Stock Solutions

Stock solutions can be a quick and accurate way to store "buffer precursors." Preparing 10× to 100× concentrated buffer salts can simplify buffer preparation, and these concentrated solutions can also retard or prevent bacterial growth, extending almost indefinitely the shelf stability of the solutions.

The pH of the stock solutions should not be adjusted prior to dilution; the pH is the negative log of the H^+ ion concentration, so dilution by definition will result in a pH change. Always adjust the pH at the final buffer concentrations unless the procedure explicitly indicates that the diluted buffer is at an acceptable pH and ionic concentration, as in the case with some hybridization and electrophoresis buffers (Gallagher, 1999).

Filtration

In many applications a buffer salt solution is filtered prior to mixing with the other buffer components. An inappropriate filter can alter your solution if it binds with high affinity to one of the solution components. This is usually not as problematic with polar buffer salts as it can be with cofactors, vitamins, and the like. This effect is very clearly demonstrated when a solution is prepared with low levels of riboflavin. After filtering through a PTFE filter, the filter becomes bright yellow and the riboflavin disappears from the solution.

Incomplete Procedural Information

If you ask one hundred chemists to write down how to adjust the pH of a buffer, you'll probably receive one hundred answers, and only two that you can reproduce. It is simply tedious to describe in detail exactly how buffer solutions are prepared. When reading procedures, read them with an eye for detail: Are all details of the procedure spelled out, or are important aspects left out? The poor soul who tries to follow in the footsteps of those who have gone before too often finds the footsteps lead to a cliff. Recognizing the cliff before one plunges headlong over it is a learned art. A few prototypical signposts that can alert you of an impending large first step follow:

- Which salts were used to prepare the “pH 4 acetate buffer”? Sodium or potassium? What was the final concentration?
- Was pH adjustment done before or after the solution was brought to final volume?
- If the solution was filtered, what type of filter was used?
- What grade of water was used? What was the pH of the starting water source?

What Is the Storage Lifetime of a Buffer?

A stable buffer has the desired pH and buffer capacity intended when it was made. The most common causes of buffer failure are

pH changes due to absorption of basic (or acidic) materials in the storage environment, and bacterial growth. Commercially prepared buffers should be stored in their original containers. The storage of individually prepared buffers is discussed below. The importance of adequate labeling, including preparation date, composition, pH, the preparer's name, and ideally a notebook number or other reference to the exact procedure used for the preparation, cannot be overemphasized.

Absorption of Bases

The most common base absorbed by acidic buffers is ammonia. Most acidic buffers should be stored in glass vessels. The common indicator of buffer being neutralized by base is failure to achieve the target pH. In acidic buffers the pH would end up too high.

Absorption of Acids

Basic buffers can readily absorb CO₂ from the atmosphere, forming bicarbonate, resulting in neutralization of the base. This is very common with strong bases (NaOH, KOH), but often the effect will be negligible unless the system is sensitive to the presence of bicarbonate (as are some ion chromatography systems) or the base is very old. If high concentrations of acids (e.g., acetic acid) are present in the local environment, basic buffers can be neutralized by these as well. A similar common problem is improper storage of a basic solution in glass. Since silicic materials are acidic and will be attacked and dissolved by bases, long-term storage of basic buffers in glass can lead to etching of the glass and neutralization of the base.

Microbial Contamination

Buffers in the near-neutral pH range can often readily support microbial growth. This is particularly true for phosphate-containing buffers. Common indicators of bacterial contamination are cloudiness of the solution and contamination of assays or plates.

Strategies for avoiding microbial contamination include sterilizing buffers, manipulating them using sterile technique, refrigerated storage, and maintaining stock solutions of sufficiently high ionic concentration. A concentration of 0.5 M works well for phosphate buffers. For analytical chemistry procedures, phosphate buffers in target concentration ranges (typically 0.1–0.5 M) should be refrigerated and kept no more than one week. Other buffers could often be stored longer, but usually not more than two weeks.

REAGENTS

Which Grade of Reagent Does Your Experiment Require?

Does your application require top-of-the-line quality, or will technical grade suffice? A good rule of thumb is that it is safer to substitute a higher grade of reagent for a lower grade, rather than vice versa. If you want to apply a lower grade reagent, test the substitution against the validated grade in parallel experiments.

Should You Question the Purity of Your Reagents?

A certain level of paranoia and skepticism is a good thing in a scientist. But where to draw the line?

New from the Manufacturer

The major chemical manufacturers can usually be trusted when providing reagents as labeled in new, unopened bottles. Mistakes do happen, so if a carefully controlled procedure fails, and you eliminate all other sources of error, then consider the reagents as a possible source of the problem.

Opened Container

Here's where the fun begins. Once the bottle is opened, the manufacturer is not responsible for the purity or integrity of the chemical. The user must store the reagent properly, and use it correctly to avoid contamination, oxidation, hydration, or a host of other ills that can befall a stored reagent. How many times have you been tempted to use that reagent in the bottle with the faded label that is somewhere over 40 years old? A good rule of thumb is if the experiment is critical, use a new or nearly new bottle for which the history is known. If an experiment is easily repeated should a reagent turn out to be contaminated, then use your judgment when considering the use of an older reagent.

How can you maintain a reagent in nearly new condition? Respect the manufacturer's instructions. Storage conditions (freezer, refrigerator, dessicator, inert atmosphere, etc.) are often provided on the label or in the catalog. Improper handling is more likely than poor storage to lead to contamination of the reagent. It is rarely a good idea to pipette a liquid reagent directly from the original bottle; this invites contamination. Instead, pour a portion into a second container from which the pipetting will be done. Solids are less likely to be contaminated by removing them directly from the bottle, but that is not always the case. It's usually satisfactory to transfer buffer salts from a bottle, for instance, but use greater care handling a critical enzyme cofactor.

Reagents Prepared by Others

Never blindly trust a reagent prepared by someone other than yourself, especially for critical assays. It's a lot like packing your own parachute—it's your responsibility to prepare your important solutions. If you want to trust the outcome of an important experiment to something someone else may have prepared while thinking about an upcoming vacation, it's up to you. Prepare critical solutions yourself until you have a solid working relationship with whomever you plan to share solutions with. Even then, don't get offended if they don't trust your solutions!

Reagents Previously Prepared by You

How reliable are your solutions? Your solutions are probably fine to use if:

- Your labeling and record-keeping are contemporary and accurate.
- You don't share solutions with anyone who could have mishandled and contaminated them.
- Your material is within its expected shelf life.

What Are Your Options for Storing Reagents?

Storage is half the battle (handling is the other half) in keeping reagents fit for use. Follow the manufacturer's recommendations.

Shelf (Room Temperature)

Solids, like buffer salts, are usually stored on the shelf in sealed bottles. Sometimes it is appropriate (e.g., for hygroscopic materials) to store them in a dessicator on a shelf. Many nonflammable liquid reagents can be also stored on a shelf. Care should be taken to store incompatible chemicals separately. For example, store acids and bases separated; store strong oxidizers away from other organics.

Vented Flammables Cabinet

Flammables or reagents with harmful vapors (e.g., methylene chloride) should be stored in ventilated cabinets designed for chemical storage. These cabinets are designed to minimize the chance of fire from flammable vapors; they often are designed to contain minor leaks, preventing wider contamination and possible fire. It is a good practice to use secondary spill containers (e.g., polypropylene or TeflonTM trays) in the flammables cabinet if they are not already built into the design.

Refrigerators

Many reagents require refrigeration for storage stability. Working buffers, particularly phosphates, will usually last a little longer if refrigerated between uses. Refrigerators used for storing chemicals must not be used to store foodstuffs.

Freezer

Check the label; many standards require freezer temperatures for long-term stability. Check that the freezer is functioning properly.

Are All Refrigerators Created Equal?

Household Refrigerator

It is cheap, stays cold, and is often perfectly fine for storing aqueous samples. It can have serious problems storing flammable organics, however, since the thermostat controls are usually located inside the refrigerator, which can spark and ignite flammable vapors.

Flammable Storage Refrigerator

The thermostat controls have been moved outside the cooled compartment. Unless a refrigerator is specifically labeled “Flammable Storage” by the manufacturer, don’t assume it is appropriate for storing flammables.

Explosion-Proof Refrigerator

These units meet specific requirements regarding potential spark sources and can be used in hazardous environments. They are usually extremely expensive.

Safe and Unsafe Storage in Refrigerators

Volumetric Flasks and Graduated Cylinders

How tempting to prepare a fresh solution in a volumetric flask and store it in the refrigerator. Then, an hour later, you reach into the refrigerator to grab a sample prepared the previous week, and accidentally knock over the flask. Tall narrow vessels like volumetric flasks and graduated cylinders are unstable, especially if they sit on wire refrigerator shelves. Solutions should be transferred to a more stable bottle or flask before storing in the refrigerator.

The Shelf in the Door

A long time ago in a basement laboratory, reagents were stored within a shelf in a refrigerator door. The refrigerator was opened, the shelf broke, and bottles spilled onto the floor, breaking two of them. One was dimethyl sulfate, a strong alkylating reagent, and the other was hydrazine, which is pyrophoric. Upon exposure to the air, the hydrazine burst into flame, vaporizing the dimethyl sulfate. It was several days before it was clear that the people exposed to the vapors wouldn't die from pulmonary edema. It may be 20 years before they know whether they have been compromised in terms of lung cancer potential.

Hazardous reagents should not be stored on shelves in refrigerator doors.

Poorly Labeled Bottles

A heavily used, shared refrigerator quickly begins to resemble a dinosaur graveyard. Rummage around in back, and you find a jumble of old, poorly or unlabeled bottles for which nobody assumes responsibility. Ultimately someone gets assigned the task of sorting out and discarding the chemicals. It is much simpler to put strict refrigerator policies in place to avoid this situation, and conduct regular refrigerator purges, so no ancient chemicals accumulate.

What Grades of Water Are Commonly Available in the Lab?

Tap Water

Tap water is usually of uncontrolled quality, may have seasonal variations such as level of suspended sediment depending on the source (municipal reservoir, river, well), may contain other chemicals purposely added to drinking water (chlorine, fluoride), and is generally unsuitable for use in important experiments. Tap water is fine for washing glassware but should always be followed by a rinse with a higher-grade water (distilled, deionized, etc.).

Distilled Water

Distillation generally eliminates much of the inorganic contamination and particularly sediments present in tap water feedstock. It will also help reduce the level of some organic contaminants in the water. Double distilling simply gives a slightly higher grade distilled water, but cannot eliminate either inorganic or organic contaminants.

Distilled water is often produced in large stills that serve an entire department, or building. The quality of the water is depen-

dent on how well the equipment is maintained. A significant stir occurred within a large university's biochemistry department when the first mention of a problem with the house distilled water was a memo that came out from the maintenance department that stated: "We would like to inform you that the repairs have been made to the still serving the department. There is no longer any radium in the water." The next day, a follow-up memo was issued that stated: "Correction—there is no longer any sodium in the distilled water."

Deionized Water

Deionized water can vary greatly in quality depending on the type and efficiency of the deionizing cartridges used. Ion exchange beds used in home systems, for instance, are used primarily to reduce the "hardness" of the water usually due to high levels of divalent cations such as magnesium and calcium. The resin bed consists of a cation exchanger, usually in the sodium form, which releases sodium into the water in exchange for removing the divalent ions. (Remember that when you attempt to reduce your sodium intake!) These beds therefore do not reduce the ionic content of the water but rather exchange one type of ion for another.

Laboratory deionizing cartridges are usually mixed-bed cartridges designed to eliminate both anions and cations from the water. This is accomplished by preparing the anion-exchange bed in the hydroxide (OH^-) form and the cation-exchange resin in the acid (H^+) form. Anions or cations in the water (including monovalent) are exchanged for OH^- or H^+ , respectively, which combine to form neutral water. Any imbalance in the removal of the ions can result in a pH change of the water. Typically water from deionizing beds is slightly acidic, often between pH 5.5 to 6.5.

The deionizing resins can themselves increase the organic contaminant level in the water by leaching of resin contaminants, monomer, and so on, and should always be followed by a bed of activated carbon to eliminate the organics so introduced.

18 M Ω Water (Reverse Osmosis/MilliQTM)

The highest grade of water available is generally referred to as 18 M Ω water. This is because when the inorganic ions are completely removed, the ability of the water to conduct electric current decreases dramatically, giving a resistance of 18 M Ω . Commercial systems that produce this grade of water usually apply a multiple-step cleanup process including reverse osmosis, mixed-

bed ion exchangers, carbon beds, and filter disks for particulates. Some may include filters that exclude microorganisms, resulting in a sterile water stream. High-grade 18MΩ water tends to be fairly acidic—near pH 5. Necessary pH adjustments of dilute buffer solutions prepared using 18MΩ water could cause discrepancies in the final ionic concentration of the buffer salts relative to buffers prepared using other water sources.

When Is 18MΩ Water Not 18MΩ Water?

Suppose that your research requires 18MΩ water, and you purchased the system that produces 500 ml/min instead of the 2 L/min version. If your research doesn't require a constant flow of water, you can connect a 20L carboy to your system to store your pristine water. Bad Move.

18MΩ is not the most inert solvent; in practice, it is very aggressive. Water prefers the presence of some ions so as your 18mΩ water enters the plastic carboy, it starts leaching anything it can out of the plastic, contaminating the quality of the water. The same thing happens if you try to store the water in glass. 18mΩ water loves to attack glass, leaching silicates and other ions from the container. If you need the highest purity water, it's best not to store large quantities, but rather prepare it fresh.

For the same reason, the tubing used to transfer your high-grade water should always be the most inert available, typically Teflon™ or similar materials. Never use highly plasticized flexible plastic tubing. Absolutely avoid metals such as copper or stainless steel, as these almost always guarantee some level of contaminants in your water.

What Is the Initial pH of the Water?

As mentioned above, the initial pH of typical laboratory-grade distilled and deionized water is often between 5.5 and 6.5. Check your water supply from time to time, particularly when deionizing beds are changed to ensure that no major change in pH has occurred because of seasonal variation or improperly conditioned resin beds.

Although the initial pH of laboratory water may be slightly acidic, the good news is deionized water should have little or no buffer capacity, so your normal pH adjustment procedures should not be affected much. Pay particular attention if your buffer concentrations are very low (<10mM) resulting in low buffer capacity.

What Organics Can Be Present in the Water?

The answer to this important question depends on the upstream processing of the water and the initial water source. Municipal water drawn from lakes or streams can have a whole host of organics in them to start with, ranging from petroleum products to pesticides to humic substances from decaying plant material to chlorinated species like chloroform resulting from the chlorination process. Well water may have lower levels of these contaminants (since the water has been filtered through lots of soil and rock, but even groundwater may contain pesticides and chlorinated species like trichloroethylene depending on land use near the aquifer.

Municipal processing will remove many organic contaminants from the tap water, but your in-lab water purifier is responsible for polishing the water to a grade fit for experimental use. Most commercial systems do a good job of that, but as mentioned previously, care must be taken to not introduce contaminants after the water has been polished. Plasticizers from tubing or plastic storage tanks, monomer or resin components from deionizer beds, and surfactants or lubricants on filters or other system components are the most common type of organic to be found in a newly installed system.

Another common, yet often overlooked source, is microbial contamination. In one case, a high-grade water purifier mounted on a wall near a window suddenly started showing evidence of organic background. Changing the carbon cartridge did not help the situation. Close inspection of the system showed the translucent plastic tubing connecting the reverse osmosis holding tank to the deionizer beds, and ultimately the lines that delivered the polished water to the spigot, had been contaminated by microbial growth. It was surmised that the intense sunlight during part of the day was providing a more hospitable environment for microorganisms to gain a foothold in the system. The clear tubing was replaced with opaque tubing and the problem disappeared.

In a second instance, a facility changed its water source from wells to a river draw-off. This drastically changed the stability of the incoming water quality. During periods of heavy rain, silt levels in the incoming water increased dramatically, quickly destroying expensive reverse osmosis cartridges in the water purifier system. The solution was to install two pre-filters of decreasing porosity in line ahead of the reverse osmosis unit. The first

filter needed replacing monthly, but the second filter was good for three to six months. The system functioned properly for a while, but then problems reappeared in the reverse osmosis unit. Inspection showed heavy microbial contamination in the second pre-filter which had a clear housing, admitting sunlight. After cleaning and sterilizing the filter unit, the outside of the housing was covered with black electrical tape, and the microbial contamination problem never returned.

As discussed in Chapter 12, dispensing hoses from water reservoirs resting in sinks can also lead to microbial contamination.

What Other Problems Occur in Water Systems?

Leaks

Leaks are sometimes one of the most serious problems that can occur with in-lab water purification systems. Leaks come in three kinds, typically. Leaks of the first kind start as slow drips, and can be spotted and corrected before developing into big unfriendly leaks.

Leaks of the second kind are generally caused by a catastrophic failure of a system component (tubing, valve, automatic shutoff switch, or backflush drain). Although highly uncommon, they usually occur around midnight on Fridays so as to maximize the amount of water that can escape from the system, therefore maximizing the resulting flooding in the lab. The likelihood of a leak of the second kind seems to increase exponentially with the cost of instrumentation in laboratories on floors directly below the lab with the water purifier system.

Leaks of the third kind result when a person places a relatively large vessel beneath the water system, begins filling, and walks away to tend to a few minor tasks or is otherwise distracted. The vessel overflows, flooding the lab with the extent of the flood depending on the duration of the distraction.

Leaks of the third kind are by far the most common type of leak, and are also the most preventable. Locating the water purification system immediately above a sink, so that any vessel being filled can be placed in the sink, usually prevents this type of catastrophe. If placement above a sink is not possible, locating the water purification system in a (relatively) high-traffic or well-used location in the lab can also minimize or eliminate the possibility of major spills, since someone is likely to notice a spill or leak.

Leaks of the first or second type are highly uncommon, but do occur. The best prevention is to have the system periodically inspected and maintained by qualified personnel, and never have

major servicing done on a Friday. Problems seem to be most likely after the system has been poked and prodded, so best to do that early in the week. Then the system can be closely watched for a few days afterward before leaving it unattended.

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