

Most scientific disciplines claim that they stand upon the shoulders of giants as the practitioners modestly describe their additions to the knowledge base of the science. In the wastewater analysis field it is perhaps more appropriate to say that we sit upon the shoulders of giants. We would like to think that progress is being made in the wastewater analysis field, however, what is most commonly seen is a rapid degradation in the knowledge base. Sometimes when surveying the lack of progress that has been made over the last 30 years I tend to feel that we actually sit at the feet of giants, waiting for them to tell us stories of chemical amazement.

A majority of people who are employed in the wastewater business are under the impression that laboratories are black boxes. You collect a sample and send it to the lab. The black box of the lab does something to the sample and sends you back a report with results. Then you use the results to make decisions – sometimes decisions that are quite costly or have significant public health ramifications. A further illusion is that all laboratory black boxes generate identical products (information) just like boxes of Tide® detergent found at different supermarkets. Each box of Tide® is identical and the only differentiating factor is cost. The different supermarkets charge different prices based on economy of scale, where the market is located, how much the employees are paid, the size and upkeep of the building, etc.

Nothing could be further from the truth. Each laboratory is different. The equipment they use is different. The methods used vary from one lab to the next. The level of implementation of the methods varies from cursory to complete. The range of technical ability of the laboratory workers varies widely from totally untrained to highly competent, degreed, and licensed analysts. The results on the final analytical report could represent anything from a slam-bang, take-it or leave-it single attempt at the test on the sample (including a dry-lab result) to a technically detailed and verified examination.

The major difference between just any laboratory and a competent laboratory is the level of training of the analysts. And it is not simply the number of BS, MS, or PhD degrees that are employed in the lab. The degree-granting educational programs only prepare a person to learn. The actual presentation/ acquisition of relevant facts and melding them together to make knowledge is the responsibility of the laboratory training officer.

These lectures are the written version of a course I have been teaching for several years at Analytical Services, Inc. and the Georgia Water and Wastewater Institute. I flatter myself by thinking that these are the facts that are needed to make a capable benchlevel analytical chemist in our industry. Well, at least they have proven many times over to be sufficient preparation for passing the ABC Analyst Certification exams.

R.-K. Smith, February, 1999

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Lecture 1

Regulatory Framework

The vast majority of wastewater analyses that are performed in the United States are under the direction of one or more Federal and state regulations. The first law enacted at the Federal level that directly addressed wastewater was the *Federal Water Pollution Control Act* (FWPCA) of 1948. This Act was updated in 1965. In 1970 the *Environmental Protection Agency* (EPA) was formed to enforce this and other Acts of Congress relating to protection of the environment. The FWPCA was replaced by the *Clean Water Act* (CWA) in 1972, which was itself subsequently amended in 1987. Many states have enacted laws that mirror or extend the requirements of the Federal Acts.

The CWA was designed to prevent pollution of the surface waters of the United States. Where waters were already polluted, the Act intends to return them to a “fishable and swimmable” condition by reducing the pollutant loading and allowing them, through natural attenuation, to recover. In general, the CWA is based on the following concepts:

1. No one has the right to pollute the navigable waters of the United States.
2. Permits shall limit the composition of a discharge and the concentrations of pollutants in it.
3. Some permit conditions require the best controls technology can produce, regardless of the receiving water’s ability to purify itself naturally.
4. Any limits or control higher than the minimum federal requirements must be based on receiving water quality.

Section 301(a)(1) of the CWA lists regulated pollutants. Three classes are recognized: 1) Conventional pollutants, 2) Non-conventional pollutants, and 3) Toxic pollutants. These are listed in Table 1-1.

Lecture 2

Quality Assurance

Within the environmental industry there seems to be a great deal of confusion between the terms quality assurance and quality control. Quality assurance (QA) is an umbrella term that is correctly applied to everything that the laboratory does to assure product reliability. As the product of a laboratory is information, anything that is done to improve the reliability of the generated information falls under quality assurance. Quality controls (QC) are single procedures that are performed in conjunction with the analysis to help assess in a quantitative manner the success of the individual analysis. Examples of quality controls are blanks, calibration, calibration verification, surrogate additions, matrix spikes, laboratory control samples, performance evaluation samples, determination of detection limits, etc. The success of the quality control is evaluated against an acceptance limit. The actual generation of the acceptance limit is a function of quality assurance; it would not be termed a quality control.

Quality assurance includes all the quality controls, the generation of expectations (acceptance limits) from the quality controls, plus a great number of other activities. A few examples of these other activities include analyst training and certification, data review and evaluation, preparation of final reports of analysis, information given to clients about what tests are needed to fulfill regulatory requirements, use of the appropriate tests in the laboratory, obtaining and maintaining laboratory certifications/accreditations, conducting internal and external audits, preparing responses to the audit results, the receipt, storage, and tracking of samples, and how - and from where - standards and reagents are purchased. The performance of QC is just one small aspect of the QA program.

The functions of quality assurance are embodied in the terms “analytically valid” and “legally defensible.” Analytically valid means that the target analyte has been:

- Correctly identified
- Quantified using fully calibrated tests

and

- The sensitivity of the test (method detection limit) has been established.
- Analysts have demonstrated that they are capable of performing the test.
- Accuracy and precision of the test on the particular sample has been determined.
- The possibility of false positive and false negative results has been evaluated through performance of blanks and other test-specific interference procedures.

Lecture 3

Metals

Most people are aware that atoms are composed of protons, neutrons and electrons. The protons and neutrons are located in the nucleus of the atom, and the electrons orbit around the nucleus. The number of protons in the atom defines the element, and the number of neutrons identifies the various isotopes of the element. The number of electrons around the atom defines the chemistry of the element.

Quantum theory states that the electrons around each atom exist in only allowed energy states. The transition of electrons between different energy states is accompanied by the release or absorption of energy exactly equal to the difference between the allowed energy states. When we consider the transition of an electron between just two allowed energy states, the amount of energy absorbed by the atom for the electron to move from the lower energy state to the higher energy state is exactly equal to the amount of energy emitted from the atom when the electron moves from the higher energy state to the lower state.

The energy loss or gain can have several physical forms; however, the form that is most useful in laboratory analysis is light. Light is related to energy through the equation:

$$E = hv$$

where: E is the energy of the electron transition
 h is Planck's constant
 v is the frequency of the light.

Measurement of the frequency (or more commonly its inverse, wavelength) of light absorbed or emitted from an atom can help identify the atom, while measurement of the intensity of the light absorbed or emitted generates information on how many atoms of the particular element are present. The major instruments used for metals analysis are designed to measure the frequency and intensity of light.

Atomic Absorption (AA) spectrophotometers pass light through a sample and measure how much light of a specific wavelength is absorbed by the sample. The vital parts of the AA spectrophotometer consist of the light source, a sample holder, a monochromator (diffraction grating) and a photomultiplier tube (Figure 3-1). Other parts include power sources and data processors.

Lecture 4

Organics by Gas Chromatography

A gas chromatograph (GC) is used to separate organic compounds in a mixture. The basic components of a GC system include a flowing, inert carrier gas, an injector, a column, an oven, a detector, and a data system. These are illustrated in Figure 4-1.

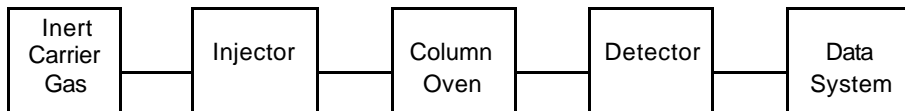


Figure 4-1. Basic components of a gas chromatograph

The column performs the separation. The column contains a thin layer of a liquid phase, generally a high molecular weight silicone gum on an inert support. In capillary columns the support is the inside wall of the column. In packed columns the liquid phase is coated onto a powder of a ceramic-like material, then the coated support is used to fill the column. As the vast majority of wastewater analyses are performed with capillary columns, when the word “column” is used in this lecture it will mean “capillary column”.

In order to separate the substances in the sample on the column, they must be in the gaseous state. The molecules of a substance inside the GC column are either dissolved in the liquid phase or they are floating free in the gas state. Individual molecules are constantly moving between the two states, they are not static. If there is no overall flow of gas through the column, the molecules of the substance tend to spread in both directions by dispersion, and no separation occurs between different substances (Figure 4-2).

Lecture 5

Organics by Gas Chromatography-Mass Spectrometry

In the previous lecture extracting chemical information from eluting peaks on a gas chromatograph (GC) was discussed. The two-dimensional detectors are characterized as translating a portion of the chemical information present in the peak into a response from time of injection. The ECD responds to halogens, nitro groups, and a few other functionalized organic molecules. The FID burns compounds containing the C-H bond to produce positive charged ions that generate a current in a potential applied across the flame. However these chemical characteristics of the compound(s) in the peak are only a small fraction of the chemical information that can be extracted. Other detectors, such as the mass spectrometer, infrared spectrometer, and ultraviolet-visible spectrometer, are capable of presenting substantial portions of the available chemical information in an interpretable form that can result in a unique identification of the eluting compound. These types of detectors are termed “three dimensional” because the chromatogram has three coordinate axes of information: response, retention time, and either mass or wavelength (Figure 5-1).

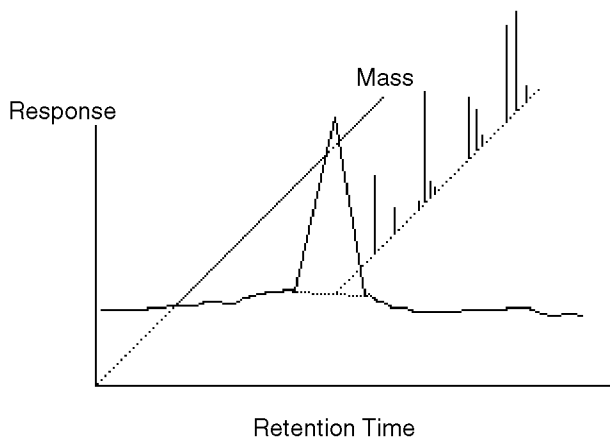


Figure 5-1. Three dimensions of information from the GC-MS

Attaching a mass spectrometer as a detector to a gas chromatograph creates an instrument with a very powerful ability to conclusively identify and quantitate analytes as they elute from the GC. Keep in mind that the GC-MS is not simply a mass spectrometer with a fancy sample introduction device. Just as the success of the GC analysis is critically dependent upon sample preparation, the success and utility of the mass spectrometer analysis is critically dependent upon maximizing

Lecture 6

Demands

A fundamental operational consideration for treatment plant operators is the waste content of the plant influent since it relates to how much waste must be treated. Wastewater that arrives at the municipal treatment plant from the collection system (sewers) contains domestic waste, industrial waste, and combined runoff from rain events. The majority of at least domestic waste is organic in nature: human waste products and residues from food processing. The amount of waste to be treated can be calculated by multiplying the flow into the plant by the concentration of waste in the flow.

Most wastewater treatment plants use aerobic biological treatment in the form of ponds, activated sludge basins, or trickling filters to reduce the organic loading of the wastewater. Of the many possible approaches to determination of the waste concentration, one that mimics the biological operation of the treatment plant would be most relevant to treatment decisions. So the starting point in developing a test procedure is that the waste is going to be added to a population of organisms that will degrade the waste. The common properties of all the biological organisms used in treatment is that they require oxygen as they eat and assimilate the food in the wastewater, and that they increase biomass through growth and reproduction. Rather than attempting to measure some growth of biomass property, a simple solution is to measure the oxygen consumption of the organisms as they eat the food in a portion of the wastewater.

Conceptually then, the test for determining organic waste determination consists of adding a known amount of the wastewater to a representative population of microorganisms, measuring the oxygen content of the solution at time zero, then at some future defined time, again measuring the oxygen content. If the test is conducted in a closed bottle, then the assumption is that the difference in oxygen measurements is the amount of oxygen used by the microorganisms as they eat the food in the wastewater. More food requires more oxygen. And that is the theory and idea behind biochemical oxygen demand (BOD).

The biological organisms that are used to reduce the waste loading in the treatment plant are aerobic; they use oxygen to metabolize the organic materials in an oxidative process. There are also chemical means to determine the oxidative requirements of the waste. Various chemical oxidants that could potentially be used include dichromate, permanganate, periodate, perchlorate, peroxide, and a host of others. A known amount of a defined chemical oxidant can be added to a known amount of the waste, and the consumption of oxidant can then be determined. This type of test is called chemical oxygen demand (COD).

A characteristic of the COD result is that the strength of non-organic contents in the waste is determined in addition to the organic loading. This can be a big

Lecture 7

Solids

The major function of the wastewater treatment plant is to reduce the organic loading of domestic wastewater so that it can be safely discharged to a receiving stream. Essential unit processes are biological treatment and sedimentation. The monitoring of the effluent for demands, covered in Lecture 6, is a measure of the effectiveness of the biological treatment process. The effectiveness of the sedimentation process is monitored through the total suspended solids (TSS) parameter. Suspended solids from the treatment plant are mostly organic in nature and can serve as refuges for harmful bacteria and other microorganisms, besides being unsightly. TSS is a monitoring parameter included on almost all NPDES permits as a conventional pollutant.

Besides TSS there are a variety of solids that are of interest in wastewater analysis. There are the solids that are dissolved in the water, termed total dissolved solids (TDS). Then there are all the solids associated with the sample, termed total solids (TS). Other solids of interest are listed in Table 7-1.

Table 7-1. Solids fractions

<i>SM</i> name	EPA name	EPA Method	<i>SM</i> Number	Drying Temp. °C
Total solids (TS)	Total residue	160.3	2540 B	104
Total dissolved solids (TDS)	Filterable residue	160.1	2540 C	180
Total suspended solids (TSS)	Non-filterable residue	160.2	2540 D	104
Total volatile solids (TVS)	Volatile residue	160.4	2540 E	550
Settleable solids	Settleable matter	160.5	2540 F	N/A

Conceptually, these are simple terms, and TS should be the sum of TSS and TDS. However, operationally it's a little more complex. Just considering the total solids, the way the test is performed is to take a portion of a sample, normally 100 mL, and remove the water until the sample is dry. The resulting weight of the dry residue is equal to the number of milligrams of solids in the 100 mL of sample. The TSS and TDS fraction determinations are similar with the exception that a filter is used to separate the two. The questions that arise are, how to remove the water, how much water to remove, and what is the definition of dry?

To achieve drying, or more correctly evaporation, the transition of a substance from a liquid state to a gaseous state, sufficient energy has to be added to the substance to overcome the attraction of the individual molecules to each other. Although this phenomenon occurs all the time at room temperature, the rate of evaporation is subject to the degree of saturation of the atmosphere with water

Lecture 8

Nitrogen

Nitrogen is element number seven in the periodic chart, with an average mass of 14.007 g/mol. The most common isotope is ^{14}N . ^{15}N is the other naturally occurring isotope having an abundance of 0.37%. All other isotopes of nitrogen are radioactive, having half-lives ranging from milliseconds to seconds for the most stable. The largest reservoir of nitrogen on Earth is in the atmosphere which is approximately 80% diatomic nitrogen (N_2) by volume. Although the original name for the element was *azote*¹, meaning *without life*, due to the inertness of nitrogen gas, nitrogen is an essential part of the molecules that are found in living organisms.

Besides nitrogen gas, with a nominal valence of zero, nitrogen is capable of combining with a variety of other elements and exhibiting a wide range of formal valences. These range from ammonia, NH_3 , where nitrogen is in the -3 valence state, to the complex anion nitrate, NO_3^- , where nitrogen exhibits a +5 valence. Organic nitrogen can also exhibit a range of valences, with individual nitrogen atoms within a single compound having different valences. See Table 8-1.

Table 8-1. Valences of nitrogen compounds

Substance	Formula	Nitrogen valence
Organic nitrogen		
Amino acids	$\text{RCH}(\text{COOH})\text{NH}_2$	-3
Trimethylamine	$(\text{CH}_3)_3\text{N}$	-3
N-nitrosodimethylamine	$(\text{CH}_3)_2\text{NNO}$	-2 and +2
Nitromethane	CH_3NO_2	+3
Nitroglycerine	$\text{C}_3\text{H}_5\text{N}_3\text{O}_9$	+5
Inorganic nitrogen		
Ammonia	NH_3	-3
Nitrogen	N_2	0
Nitrous oxide	N_2O	0 and +2
Nitric oxide	NO	+2

¹ Lide, D. R. (Editor-in-Chief), 1995. *Handbook of Chemistry and Physics*, 76th Edition, CRC Press, Boca Raton, FL. The prefix “azo” still lingers in many compound names to denote the presence of nitrogen.

Lecture 9

Phosphorus

Phosphorus is element number 15 in the Periodic Chart, and is placed immediately below nitrogen. Phosphorus has only one major isotope that occurs naturally, mass 30.974. The element exists as P_4 molecules in the shape of a tetrahedron at temperatures up to 800 °C. Above 800 °C, the diatomic P_2 , similar to N_2 , exists, but reforms P_4 on cooling. White phosphorus is a crystalline form of elemental phosphorus made up of P_4 molecules. It is highly reactive to oxygen in the atmosphere, generating clouds of dense white smoke and flames. However, when stored under water or in an inert atmosphere, white phosphorus is quite stable.¹ The misnamed phosphorus pentoxide (P_4O_{10}) is actually a P_4 tetrahedron with one oxygen atom bonded to each phosphorus atom at the vertices of the tetrahedron, and each of the other oxygens (6) bonded to two phosphorus atoms along the edges of the tetrahedron.

Chemically, phosphorus is found in combination with many elements, with valences of +3 and +5 predominating. Almost all bonding is covalent in nature. Representative inorganic phosphorus forms are presented in Table 9-1. Phosphorus is found in many mineral deposits, generally in the form of apatite, $Ca_3(PO_4)_2$, hydroxyapatite, $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$, and fluoroapatite, $Ca_3(PO_4)_2 \cdot CaF_2$.

The thermodynamically stable form of inorganic phosphorus is the oxyanion orthophosphate, PO_4^{3-} , with phosphorus in the formal +5 valence state. Heating phosphorous acid or hypophosphorous acid results in an oxidation-reduction reaction generating phosphine and orthophosphoric acid.



Phosphine (PH_3) is a non-water soluble, very toxic gas, commonly used as a fumigant in grain silos and other food storage facilities to control insects and rodent pests. Unlike ammonia, NH_3 , phosphine has very little tendency to accept a proton forming the phosphonium ion, PH_4^+ , which is unknown outside the research laboratory.

¹ Merck Index, 12th Edition, Monograph 7503.

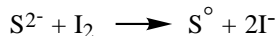
Lecture 10

Sulfur

Sulfur is element number 16, located immediately below oxygen in the Periodic Chart. It has an atomic mass of 32.06. A number of isotopes are known; four are naturally occurring: sulfur of mass 32 at 95.02% abundance, sulfur of mass 34 at 4.21% abundance, sulfur of mass 33 at 0.75% abundance and finally sulfur of mass 36 at 0.02% abundance. In mass spectrometry sulfur is classed as a M+2 element. Each sulfur-containing fragment will exhibit a main m/z signal accompanied by a peak two mass units higher of 4% relative abundance. Elemental sulfur, sulfur in the zero valence state, exists in a number of forms. Flowers of sulfur, the common material found in drug stores and pharmacies, contains the ring shaped S₈ molecule. Elemental sulfur is a non-polar material and is insoluble in water, but very soluble in oils and carbon disulfide. Elemental sulfur is mined from naturally occurring deposits, the salt domes of the Gulf Coast area of the United States are a major world source.

While molecular oxygen, O₂, is a very reactive substance and ozone, O₃, is a very powerful oxidant, sulfur is much less reactive. The S₈ molecule is relatively stable and not especially reactive. However, similar to the use of oxygen as a primary electron transfer sink in aerobic ecosystems, sulfur is used in anaerobic systems. Deep sea anaerobic ecosystems clustered around oceanic thermal vents are sulfur-based.

Unlike oxygen, which is limited to the -2 valence in almost all compounds, sulfur displays a wide variety of valence states, from -2 to +6. The -2 valence state is hydrogen sulfide, a product of reducing environments (anaerobic). Unlike oxygen, which is quite stable in the -2 state, sulfide is easily oxidized to sulfur. Sulfide rapidly reacts to reduce molecular iodine to iodide. This reaction is used to standardize sulfide solutions.



Elemental sulfur can combine directly with several metals to form the sulfide. The most common clean-up procedure for elemental mercury spills is to cover the spill with flowers of sulfur and sweep up the excess sulfur and the formed mercuric sulfide, HgS. Sulfur dissolved in organic solvents is readily removed by treatment with metallic copper to form copper sulfide. Most sulfide compounds, other than those with the alkali metals and ammonium, are insoluble in water. Many ores that are commercially useful as metal deposits are sulfides, for example cinnabar is mercuric sulfide, galena is lead sulfide, PbS, and iron pyrite is iron disulfide, FeS₂.

Sulfur in the +2 valence state is not commonly encountered outside the research laboratory. Most compounds with sulfur in this state are highly reactive.

Lecture 11

Chlorine and Chloride

Chlorine is element number 17. It has an average atomic mass of 35.45. A number of isotopes are known; however, naturally occurring chlorine consists of only two: mass 35 and 37 in a 75.8:24.2 relative abundance. Chlorine, like sulfur, is classed as a $M + 2$ element in mass spectrometry. Chlorine is a very abundant element, present in about 1.5% by weight of seawater, all in the form of the chloride ion.

The inorganic chemistry of chlorine is rich and varied. Beginning with chloride ion in the -1 valence state, chlorine atoms can exhibit formal valences up to +7. However, the most stable form of chlorine is as the chloride ion, Cl^- . All of the other forms are strong oxidizers on their way to becoming chloride. Some of the most common forms are presented in Table 11-1.

Table 11-1. Inorganic forms of chlorine

Acid form	Formula	Chlorine Valence	Anionic Form	Formula
Hydrogen chloride	HCl	-1	chloride	Cl^-
Hypochlorous acid	HOCl	+1	hypochlorite	OCl^-
Chlorous acid	HOClO	+3	chlorite	ClO_2^-
Chloric acid	HClO_3	+5	chlorate	ClO_3^-
Perchloric acid	HClO_4	+7	perchlorate	ClO_4^-
Other Forms				
Chlorine	Cl_2	0		
Dichlorine monoxide	Cl_2O	+1		
Monochloramine	H_2NCl	+1		
Dichloramine	HNCl_2	+1		
Trichloramine	NCl_3	+1		
Chlorine dioxide	ClO_2	+4		

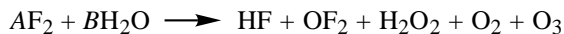
The oxidative strengths of the complex chlorine anions are in the order $\text{OCl}^- < \text{ClO}_2^- < \text{ClO}_3^- < \text{ClO}_4^-$. In other words, as the formal valence on the chlorine increases, the strength of the substance as an oxidizer increases. For example, the hypochlorite anion is an effective disinfectant agent, meaning that it can incapacitate pathogenic bacteria and other organisms, which generally are very fragile creatures. Hypochlorite is not a sterilization agent, and is ineffective

Lecture 12

Fluoride

Fluorine is element number 9 and in nature exhibits a single isotope of mass 19.0 amu. The element is a diatomic molecule that is a highly corrosive, yellow gas at room temperature. Fluorine and all soluble inorganic fluoride compounds are toxic. Fluorine is the most electronegative of all elements and in all compounds, even oxygen difluoride, OF_2 , it will be in the -1 formal valence state. Fluorine is found in nature in recoverable quantities as the minerals fluorapatite, $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaF}_2$, fluorspar (fluorite), CaF_2 , and cryolite, Na_2AlF_6 . Crystals of fluorspar have the property of doubling the images of items observed through the crystal. Fluorine is present in the earth's crust in greater abundance than chlorine.

Fluorine has many industrial uses as the element, hydrofluoric acid, and a few inorganic materials. As the element fluorine, F_2 , is one of the most reactive substances known. It reacts, commonly with ignition, with most materials, including water. The reaction with water is violent and generates oxidation products of water:



Uncontrolled mixing of fluorine gas with organic materials most commonly results in a violent explosion and fire. Under carefully controlled conditions fluorine will react directly with hydrocarbons, substituting fluoride for hydrogen. A thin skin of inert fluoropolymer is created on molded polyethylene items through treatment with fluorine gas.

Hydrogen fluoride, HF, is a very polar but covalent molecule. It is barely a gas at room temperature; the boiling point is only 19.7 °C at sea level. It is maintained in the liquid state due to a high degree of internal hydrogen-bonding of the molecules, similar to that seen in water. It is miscible with water. By itself, hydrogen fluoride will display self-ionization, $K = 10^{-10}$, greater than the water constant, 10^{-14} , and the fluoride ion behaves as a base.



In water, hydrogen fluoride is called hydrofluoric acid. There is very little ionization of the molecule when compared to the strong acids like hydrochloric, and it is a weak acid, pK_a 3.19, K_a 6.46×10^{-4} mol/L. Hydrogen fluoride forms a constant boiling mixture at 112 °C with water at 38.2% HF. Concentrated hydrofluoric acid is normally sold at 48% w/w composition.

Hydrofluoric acid and hydrogen fluoride are very reactive toward silicon-containing materials, forming the silicon hexafluoride anion, SiF_6^{2-} . Hydrogen fluoride is normally shipped and stored in steel cylinders with all metal fittings. The acid is stored in plastic bottles.

Lecture 13

Cyanide

Cyanide is an anion formed from a carbon atom triple bonded to a nitrogen atom with an overall charge of -1. Although organic chemists like to localize the charge on one or the other atom, the inorganic chemistry of the anion is best represented with a delocalized charge spread over both the carbon and nitrogen. It is normally abbreviated as CN^- . Cyanide forms simple stable salts with many cations, such as sodium cyanide, NaCN , and mercuric cyanide, $\text{Hg}(\text{CN})_2$.

Cyanide is also found as a partner in stable complex anions with certain metals, for instance, potassium ferrocyanide, $\text{K}_4[\text{Fe}(\text{CN})_6]$, and potassium ferricyanide, $\text{K}_3[\text{Fe}(\text{CN})_6]$.

Cyanide will bond with hydrogen in a covalent fashion to form hydrogen cyanide, HCN . Hydrogen cyanide is barely a gas at room temperature, BP 25.6°C , and very volatile. It mixes with water at all concentrations, but will off-gas easily. The gas has the characteristic odor of almonds, and the threshold for toxicity is about the same as the threshold for odor detection. Hydrogen cyanide is classified as an extremely weak acid; in water the ionization is insufficient to turn litmus red (pK of hydrogen cyanide is 9.0). Mild acidification of any simple cyanide salt solution will create hydrogen cyanide.

Hydrogen cyanide is an extremely poisonous substance; however, less so than hydrogen sulfide. The toxic action of cyanide is to bind to ferricytochrome oxidase preventing the iron (III) in hemoglobin from being reduced to iron (II) and thus preventing oxygen transport by hemoglobin through the blood. One of the classic symptoms of cyanide poisoning is cyanosis, a blue coloration of the beds of the fingernails and anywhere the skin is thin such as the neck and around the eyes. The color is due to the de-oxygenated hemoglobin being blue. The ability of hydrogen cyanide to dissolve in water means that it readily passes across wet membranes found in the eyes, mouth, nose, and lungs. Cyanide salts are viewed as being less toxic than hydrogen cyanide. However, ingestion of a cyanide salt easily creates hydrogen cyanide in the acidic environment of the stomach. Fish are much more sensitive to cyanide than humans and other mammals.

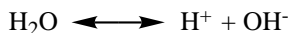
Other materials related to cyanide are the cyanates and the thiocyanates, where oxygen and sulfur respectively have been added to the molecule. These materials are significantly less toxic than cyanide, and thiocyanate is a detoxification product of cyanide. The addition of oxygen or sulfur is locationally specific; if the oxygen is attached to the carbon, $[\text{OCN}]^-$, the anion is called a cyanate. Likewise sulfur attached to the carbon, $[\text{SCN}]^-$, is called a thiocyanate. As with cyanide, the charge is delocalized over the entire anion, and it is not directional. However, when adding a hydrogen to cyanate, depending on the conditions, two distinctly different materials can be formed, the common cyanic acid, HOCN , or the much rarer isocyanic acid HNCO . Addition of hydrogen to thiocyanate gives a mixture of the tautomeric (interconverting) forms HSCN and

Lecture 14

pH, Hardness, Alkalinity, and Conductivity

pH, hardness, alkalinity, and conductivity are general measures of the ionic characteristics of water. There are several others that could be added to this group, such as redox potential and salinity; however, these four are the most important. pH is a conventional pollutant found on every single NPDES permit with limits of 5-9. Hardness, alkalinity, and conductivity are normally not NPDES compliance-monitoring parameters, but the results from these tests play a large role in the day-to-day operation of the treatment plant.

The fundamental ions in water are the hydrogen ion, H^+ , and the hydroxyl ion, OH^- . The concentrations of these two ions are always related in water. The chemical equation for the process is:



and the related equilibrium expression is:

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]}$$

where the square brackets, [], mean concentration in terms of *Molar* concentration, i.e. moles/liter.

The water constant, K_w , is derived from this equilibrium expression by allowing the molar concentration of bulk water to be 1. In actuality the bulk molar concentration of water at 4 °C is 55.56 *M*, easily calculated from the molecular weight, 18, and the density of water at 4 °C, 1.000 g/mL. However, by convention, the concentration of bulk water is defined to be 1.

$$K_w = [H^+][OH^-]$$

As with all equilibrium constants, the value of the water constant changes with temperature. At room temperature, $K_w = 1.00 \times 10^{-14}$. As the temperature rises the value of the ionization constant increases as illustrated in Figure 14-1 and Table 14-1. This makes innate sense since the ionization process itself takes energy, and if more energy is available to the system, there will be a greater degree of ionization.

The hydrogen and hydroxyl ions are not isolated charged particles floating in an inert sea of water molecules. They interact with surrounding water molecules to form solvated clusters best formulated as $(H_2O)_nH^+$ and $(H_2O)_nOH^-$. In the simplest form, H_3O^+ , the ion is called the hydronium ion, and it is impossible to specify which of the hydrogen atoms bears the charge at any one time. Most salts

Lecture 15

Oil, Grease, Surfactants, and Hydrocarbons

Lectures 4 and 5 discussed the analysis of individual organic compounds by gas chromatography and gas chromatography-mass spectrometry. Use of these methods requires a specific defined target analyte, such as 1,2-dichlorobenzene. The instrument is calibrated for the specific target analyte, and part of the analysis is the determination that the target compound is present and not being confused with another closely related compound such as 2-chlorotoluene.

There are literally millions of possible organic compounds that can potentially be present in wastewater, and it is impossible to even consider trying to analyze the sample for every compound. At the same time, the mass of these non-target organic compounds can contribute substantially to the pollution potential of the effluent.

There are test methods available that can directly or indirectly estimate the organic loading of an effluent. Biochemical oxygen demand (BOD) discussed in Lecture 6, is an indirect measure of the organic material present, as is total organic carbon, TOC. BOD is oriented toward measuring how much of the organic material is degradable, but it is effected by contributions from degradable inorganic substances. TOC is a more complete measure. However, it requires that the material be soluble in the water sample, due to the mechanics of the sample introduction. Organic substances that are not soluble in water are inefficiently measured by either TOC or BOD.

The insoluble organic materials are of concern. Aside from any specific toxic effect due to ingestion of a particular component of the mixture, insoluble organic material can present environmental problems. Most persons are familiar with the pictures of the results of oil spills on the fauna and flora in the immediate area, the oiled birds and dead sea otters, the coated rocks. Insoluble organic materials in effluent create the same problems only on a smaller scale because of the smaller quantities (mg/L to g/L). Even on this smaller scale they can cause death, coating the gill surfaces of fish, amphibians, insects, and other creatures living in water, preventing the transport of oxygen from the water into the animal and interrupting respiration.

The total amount of insoluble organic material in an effluent can be analyzed. A simple isolation of the insoluble organic material from the water portion of the sample and then determination of the weight of the isolated material is a valid direct measurement. The exact details of the isolation process depend on the composition of the organic material. In broad terms, insoluble organic material is composed of oils and greases, surfactants, petroleum hydrocarbons, and a variety of miscellaneous substances from chemical manufacturing and other industrial processes.

The term oil is meant to indicate a water insoluble organic material that is a liquid at room temperature. The term grease means a water insoluble organic

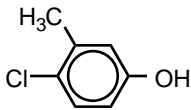
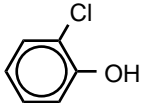
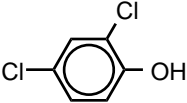
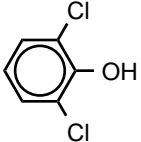
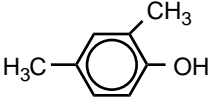
Lecture 16

Total Phenols

Phenols are organic molecules with a hydroxy group attached to an aromatic carbon. The simplest member of the group, hydroxybenzene, is known as phenol, C_6H_5OH , or by the older term carbolic acid. There are numerous other phenols, which differ from each other by a huge variety of substituents on the aromatic ring. See Table 16-1 for some of the phenols that are of environmental importance.

Addition of a methyl group, H_3C^- , to the ring creates three different compounds, called cresols. If the hydroxy and the methyl group are adjacent to each other on the ring the compound is called *ortho*-cresol. If the substituents are in a 1,3- relationship the compound is called *meta*-cresol. If the substituents are on opposite sides of the aromatic ring in a 1,4- relationship, the compound is called *para*-cresol.

Table 16-1. Commonly encountered phenols

Compound	CAS No.	Structure
4-Chloro-3-methylphenol	59-50-7	
2-Chlorophenol <i>o</i> -chlorophenol	95-57-8	
2,4-Dichlorophenol	120-83-2	
2,6-Dichlorophenol	87-65-0	
2,4-Dimethylphenol	105-67-9	

Lecture 17

Microbiological Testing

The importance of public water supplies as a transmission agent for diseases dates back to the Broad Street Study in London, conducted in the 1850s by Dr. John Snow. Testing for microorganisms in wastewater, for the most part, uses the same techniques used in the drinking water laboratory. The major difference is that in the drinking water lab the presence or absence of the organisms is the major concern, while in the wastewater laboratory, it is pretty much a given that the organisms are present, the question is how many. Thus many of the same methods are used, such as most probable number (MPN) determined by multiple tube fermentation, and discrete organism counts performed by membrane filtration. However, tests such as Presence-Absence are of little use.

Another similarity between the two regulatory programs is the use of indicator organisms. It is impossible to test every sample for every organism that is known to be dangerous to humans. It's impossible to even consider the list of major threats. When a disease outbreak has occurred, say, for instance, cholera, it is possible to test samples for one or more of the causative bacteria using specific antibody screenings. This procedure is used to trace the source of the contamination, but it requires the services of a very specialized medical microbiological laboratory. Instead, in the wastewater industry, we concentrate on the detection and quantitation of an organism that, when present, indicates that conditions are right for the existence of other harmful bacteria.

Such an indicator organism, should live in the same micro-environment as the disease causing organisms, the intestinal tract of mammals, particularly humans. The reason for choosing the intestinal tract is that most water-borne diseases are taken into the body through the mouth and dispersed from the body in the excrement from the intestinal tract. The diseases manifest themselves most commonly as gastrointestinal problems (diarrhea, cramps, vomiting), although other, more severe, symptoms can soon follow as the disease progresses. Formerly common water-borne bacterial diseases include typhus, typhoid fever, dysentery, and cholera. Virus diseases such as polio and hepatitis can also be water-borne. Although now rare in the United States and other developed countries, these diseases are still seen in outbreaks in under-developed third world countries. The diarrhea that is the first symptom of the infection can contaminate water supplies and serve to disperse the causative organisms to other victims. Thus the organism that is used as an indicator should prefer an environment that is dark, very wet, temperature controlled (around body heat of 98.6 °F), abundant food, and limited amounts of oxygen, i.e. similar to the mammalian intestine. Further, the organism should be part of the normal flora of the mammalian intestinal tract in order to serve as an indication that fecal contamination of the water has occurred.

The organism that fits the bill and was selected many years ago, reputedly in 1894 by F. Schardinger, is *Escherichia coli*, commonly referred to as *E. coli*. It is

Lecture 18

WET Testing

There are two different approaches to evaluating environmental samples for contamination. The first approach is to determine *a priori* what chemical substances are dangerous to the environment, then test each sample for this list of analytes. Most of us in the industry are familiar with this approach, as embodied in monthly analysis of effluent for zinc, chromium, lead, 1,2-dichloroethane, and all the other substances on our permit. The advantages to this type of testing are that the laboratory knows what to look for in each sample, they can use identification techniques specific to the analytes of interest, and a multi-point calibration can be prepared for each analyte for reliable quantitation. The drawback is that regardless of how many analytes you test for, there is always the sneaking suspicion that you have overlooked something in the sample that is really harmful to the environment.

The alternate approach is to treat a living test organism with a portion of the sample and see if the creature exhibits any untoward effects, such as dying. This approach is formalized in **Whole Effluent Toxicity** (WET) tests. The advantages are that a lot of samples can be screened for toxicity in a short period of time. The disadvantages are that the actual results of a test on a particular effluent are somewhat dependent upon the creature selected for testing. Further, assuming that toxicity in the sample is found, there is no indication of what exactly in the sample is responsible for the toxicity. However, the ability to segregate effluents that exhibit toxicity from those that don't, means that efforts and resources to maintain water quality can be concentrated upon the real problem areas. An alternate approach, particularly useful in diagnosing problems in treatment plants, is to perform a sludge respiration inhibition test. This test is discussed at the end of this Lecture.

Terms and Definitions Used in WET Testing

WET testing is full of new terms and technical jargon that are unique to the procedures and interpretation of the results. Some of the more common terms are as follows:

- Toxicant (poison) - A substance that is harmful to living organisms due to detrimental effects on tissue, organs, or biological processes.
- Toxicology - The study of poisons and their effects on organisms.
- Dose Response curves - Dose is the amount of toxicant (mg/kg of body weight, or dilution factor) the organism is challenged with, response is the biological effect on the organism, most commonly death. The curves are a plot of % response vs. log dose. One standard deviation on the curve runs

Lecture 19

Biosolids

Biosolid is the modern term given to the solid by-products of wastewater treatment plants, traditionally known as sludge or sewage sludge. The solids (trash) that are removed in the preliminary treatment with bar screens or grit chambers are not included in the definition of biosolids. Biosolids are the result of biological treatment of the wastewater. The mainly organic domestic waste entering the plant is consumed as food by the organisms that comprise the biological treatment units. The treatment organisms grow and multiply, and what was originally waste product is converted into biomass. Most organic materials that are found in the plant influent are radically altered by the metabolism of the treatment organisms, assuming of course that the organisms are able to use the material as a food/energy source. Dissolved metals entering the plant may be incorporated by the organisms, or they may be simply adsorbed on the surface of the cells that make-up the biomass.

Historically human solid waste and animal manure have been used as a valued agricultural soil amendment in the raising of crops. Oriental farmers for thousands of years have collected and used “night soil” to maintain the essential micro-nutrient levels in their gardens. Although the nutrient value of raw human waste or the derived biosolids is low, as measured in the American fertilizer standard units of nitrogen-phosphorus-potash, there are trace elements vital for plant growth that are present in biosolids in readily absorbable forms. Addition of biosolids as nutrient supplements to the fields used in the modern American mono-culture farming techniques has been demonstrated to be a valuable way to extend the productivity of the fields for years, especially when combined with regular crop-rotation.

The traditional means of disposal of biosolids from treatment plants has been incineration or shipment to the local municipal landfill. Both of these methods result in loss of a valuable resource. The EPA has been promoting re-use of biosolids by application to land used for pasture or agriculture (land application) or by general surface disposal.

A major concern in the re-use of biosolids is the potential for the introduction of pollutants back into the environment. The EPA has promulgated rules and regulations that establish standards for the disposal of sewage sludge in 40 CFR 503. The standards consist of regulatory limits for specified pollutants and analytes, and specific management/operational practices that allow classification of the biosolid as suitable for re-use in land application, surface disposal, or other purposes. Additionally, information about the nutrient content of the biosolids is desirable.

Biosolids are not a defined constant matrix. Depending on the design of the plant processing units, the material that is produced for disposal can range from a very fluid suspension (watery, 1% solids), to a thick, mobile slurry (pea soup-

Lecture 20

Process Control Calculations

Many different calculations are used in laboratory work. There are calculations of the concentration of reagent and standard solutions. There are calculations of results based on the sample preparation and the response from the instrument. These type calculations have been covered elsewhere in these Lectures. The subject of this Lecture is the use of the numbers derived in the laboratory to predict the results of treatment operations in the plant. The operations that we will look at are: effluent discharge and efficiency, activated sludge monitoring, and treatment chemical dosages.

Most wastewater treatment plants are operated in a flow-through mode (Figure 20-1). For every gallon of water that enters the plant, a gallon leaves. No wastes or water are stored at the plant. A reasonable way to deal with the calculations is in terms of flow rather than in terms of volumes. The basic flow unit is millions of gallons per day (MGD), and plants are characterized by the flow. For example a 2.4 MGD plant would serve a medium-size town of less than 50,000 population, while large cities with over 1,000,000 population probably have several large 25 MGD plants.

Laboratory results normally are reported in a Metric unit such as parts per million (ppm) or mg/L, while the plant operates in the US units of pounds and gallons. A standard practice in the treatment plant is to convert the mg/L laboratory result to the corresponding US unit, pounds per million gallons (lb/MG). Most operators will tell you that this is done by first equating the ppm units of mg/L and gal/MG, then making the apparent assumption that all materials have the same density as water, 8.34 lb/gal.

$$X \frac{\text{lb}}{\text{MG}} = X \frac{\text{mg}}{\text{L}} \times 8.34$$

In actuality the factor of 8.34 is correct; no assumptions about density are necessary. It is derived from the units conversion of mg to pounds (453.59 g/lb) and liters to million gallons (3.785 L/gal), and is exactly 8.3445. It's half as much effort to memorize 8.34 as to memorize 3.785 and 453.49.

Many NPDES pollutant discharge limits are written in units of pounds per day. To convert the lb/MG result of the laboratory analysis to the lb/day units, all that is necessary is to multiply the result by the daily flow, which is expressed as MG/day.

Lecture 21

Data Interpretation

Is it possible to discuss environmental testing without also mentioning both quality assurance and data interpretation? The answer is no. In fact you have to do more than mention the topics. They are integral to the testing process. In previous Lectures, where the chemistry and instrumentation of the various methods were presented, each presentation was accompanied by a discussion of the quality controls that are part of the method. The discussion focused on how the quality controls generate information about the success of the test on the sample. That is part of data interpretation: using the results of the quality controls to measure the analytical validity of the test. However, this is only one side of data interpretation. The other side of data interpretation is rooted in the necessity to assure compliance with the regulatory and legal framework within which monitoring analysis is performed.

Let's define two terms, evaluation and validation. Evaluation, based on the dictionary¹ definition, is, "to determine or fix the value of." Validation is "to make legally valid, to grant official sanction." In the context of data interpretation then, evaluation is the process through which analytical validity is determined; the legal acceptability of the data is assessed through validation. With reference to Figure 21-1, validation defines the shape and contents of the Laboratory Black Box while evaluation examines the passage of the individual sample through the Box.

Validation is a process that should take place before the sample is tested. It is verifying that the laboratory is using the approved test procedure. It should be one of the major functions of the quality assurance program. Examining data after it is generated and discovering that the laboratory used a method from SW-846² rather than a method approved in Table I, 40 CFR 136.3, is a lot like closing the barn door after the horses have escaped. The samples have already been collected, tested, properly disposed, and the invoice paid, when you discover that the data are useless. Or more frequently, the discovery is made by the regulatory agency, and you find out about the lack of data validity when you receive a certified letter from the regulator stating that the data are not compliant with the regulations and are being rejected.

¹ Webster's Seventh New Collegiate Dictionary, G. & C. Merriam Company, Springfield, MA, 1967.

² USEPA, Office of Solid Waste and Emergency Response. *Test Methods for Evaluating Solid Waste Physical/Chemical Methods*, Third Edition, Update III, December, 1996.

Lecture 22

PBMS

Approved Federal methods for wastewater analysis are listed in 40 CFR 136. These approved methods were written over the last 25 to 30 years as quite prescriptive procedures, i.e. using the specified instrument, do step 1, then do step 2,... and so forth. The legal interpretation, in both the federal and state courts, has consistently been that the methods must be followed exactly as written or the data will be unacceptable for compliance monitoring purposes. This has resulted in several scientific *non-sequiturs*, for instance the requirement in EPA Method 365.2 for continuing calibration to be within 2% of the initial calibration. The published 2% is probably a typographical error and should be 20%; however, the written law is the law. I know one certification officer who prides himself on the knowledge of this quirk and uses it to grant each lab he visits a deficiency.

Although the methods, if followed exactly, in general worked and provided for similar data being generated by many different laboratories, major complaints about the methods include that they are prescriptive, that they are based upon older, out-dated analytical techniques and instruments, and that the methods are inconsistent in their quality control requirements. The methods have a little bit of flexibility for slight modifications in case of intractable samples. However, to enact a major change to the procedure it is necessary to obtain written EPA approval (under the provision in 40 CFR 136.5) as an alternate testing procedure. In short, the approved methods system has served to keep marginal quality analytical techniques (pre-packaged test kits used with pre-calibrated colorimeters) out of compliance monitoring, which is a good objective, while at the same time preventing the introduction of superior quality state-of-the-art analytical instruments and methodologies.

In the 28 March, 1997 *Federal Register* (Vol. 62, No. 60 pp. 14975-15049) the EPA Office of Water published a Proposed Rule that completely redefines the approved methods structure and how newer methods can be brought into the system. The proposed rule is called *Flexibility in Existing Test Procedures and Streamlined Proposal of New Test Procedures* and is referred to as “Streamlining.” Streamlining is the Office of Water’s version of Performance Based Measurement Systems (PBMS), which the EPA is planning to implement on an agency-wide basis (*Federal Register*, Vol. 62, No. 193, Monday, 6 October, 1997, pp. 52098-52100).

PBMS is part of an overall environmental management system that stresses examination of what needs to be done on the site through pre-planning. This is a major change from the current system where the project is managed by first using all the available analytical tools to gather data, then attempting to sort through the data and determine what needs to be done. The pre-planning is achieved through the Data Quality Objective (DQO) process. This is a step-wise guide to planning. The first step in the process is forming a written statement of the

Lecture 23

Laboratory and Analyst Certification

The environmental laboratory industry is one of the most complex areas of analysis. It is complicated not only by the difficulty of actual hands-on analysis of samples, ranging from relatively clean water to maple syrup-like industrial discharges, but also by the many rules and regulations that provide the framework for the industry. The EPA is, in general, regarded as the source of most rules and regulations, but the responsibility for enforcing those same rules is held, in most cases, by the individual states.

In many states there are laboratory certification requirements. Generally these certifications are granted by regulatory area such as drinking water, wastewater, hazardous waste characterization, etc. The certification requirements for any particular regulatory area can vary widely from state to state; however, most entail an examination to one degree or another of the Quality Assurance program, Standard Operating Procedures, laboratory facilities, equipment, methods implementation, quality control procedures, sample Chain-of-Custody, data handling procedures, and Performance Evaluation sample results, along with some exchange of fees.

Laboratories that are certified in more than one state for wastewater find that there is an extraordinary lack of uniformity among the states in the emphasis placed on the individual aspects of laboratory operation and interpretation of the methods and method requirements. What is considered to be standard operating procedure in one state may be specifically disallowed in another.

One basic fact that is common to all state certification programs is that they establish and grant certification according to minimum standards. Another basic fact is that the different states have different ideas as to what minimum standards to implement. One state looks only at Standard Operating Procedures (SOP), while another concentrates upon the Quality Assurance Manual, and still another weights their decisions on Performance Evaluation (PE) sample results. The possession of a state certification is only an official recognition that the laboratory has at some time in the past met those minimum standards. Further, it is no guarantee that the laboratory maintains the minimum standards. Decertification by a state regulatory authority is not an easy process. Particularly when attorneys become involved, the process can drag out for years, and all the while, the laboratory is still in operation, churning out results and perhaps not performing at even a minimum level of expectations.

Beginning in 1991, there has been an effort led by the EPA to develop a nationwide environmental certification. This has evolved into the National Environmental Laboratory Accreditation Program (NELAP). NELAP is overseen by EPA, but implemented by the states. A small group of states are certified through an audit and inspection program as NELAP accreditors. Individual laboratories then contract with one of these designated states to become NELAP

Appendix A

Code of Federal Regulations

TITLE 40--PROTECTION OF ENVIRONMENT

CHAPTER I--ENVIRONMENTAL PROTECTION
AGENCY (CONTINUED)

PART 136--GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE
ANALYSIS OF POLLUTANTS--Table of Contents

Sec. 136.3 Identification of test procedures.

Notes

- (a) Parameters or pollutants, for which methods are approved, are listed together with test procedure descriptions and references in tables IA, IB, IC, ID, and IE. The full text of the referenced test procedures are incorporated by reference into tables IA, IB, IC, ID, and IE. The references and the sources from which they are available are given in paragraph (b) of this section. These test procedures are incorporated as they exist on the day of approval and a notice of any change in these test procedures will be published in the Federal Register. The discharge parameter values for which reports are required must be determined by one of the standard analytical test procedures incorporated by reference and described in tables IA, IB, IC, ID, and IE, or by any alternate test procedure which has been approved by the Administrator under the provisions of paragraph (d) of this section and Secs. 136.4 and 136.5 of this part 136. Under certain circumstances (Sec. 136.3 (b) or (c) or 40 CFR 401.13) other test procedures may be used that may be more advantageous when such other test procedures have been previously approved by the Regional Administrator of the Region in which the discharge will occur, and providing the Director of the State in which such discharge will occur does not object to the use of such alternate test procedure.
- (b) The full texts of the methods from the following references which are cited in Tables IA, IB, IC, ID, and IE are incorporated by reference into this regulation and may be obtained from the sources identified. All costs cited are subject to change and must be verified from the indicated sources. The full texts of all the test procedures cited are available for inspection at the Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 26 West Martin Luther King Dr., Cincinnati, OH 45268 and the Office of the Federal Register, Room 8301, 1110 L Street, NW., Washington, DC 20408.

Appendix B

Table II. Required Containers, Preservation Techniques, and Holding Times

Parameter No./name	Container 1	Preservation 2,3	Maximum holding time 4
Bacteria Tests			
1-4 Coliform, fecal and total	P,G	Cool, 4 °C, 0.008% Na ₂ S ₂ O ₃ ⁵	6 hours
5 Fecal streptococci	P,G	Cool, 4 °C, 0.008% Na ₂ S ₂ O ₃ ⁵	6 hours
Aquatic Toxicity Tests			
6-10 Toxicity, acute and chronic	P,G	Cool, 4 °C 16	36 hours 17
Inorganic Tests			
1. Acidity	P, G	Cool, 4 °C	14 days
2. Alkalinity	P, G	Cool, 4 °C	14 days
4. Ammonia	P, G	Cool, 4 °C, H ₂ SO ₄ to pH <2	28 days
9. Biochemical oxygen demand	P, G	Cool, 4 °C	48 hours
10. Boron	P, PFTE, or Quartz.	HNO ₃ to pH 2	6 months
11. Bromide	P, G	None required	28 days
14. Biochemical oxygen demand, carbonaceous	P, G	Cool, 4 °C	48 hours
15. Chemical oxygen demand	P, G	Cool, 4 °C, H ₂ SO ₄ to pH <2	28 days
16. Chloride	P, G	None required	28 days
17. Chlorine, total residual	P, G	None required	Analyze immediately
21. Color	P, G	Cool, 4 °C	48 hours
23-24. Cyanide, total and amenable to chlorination	P, G	Cool, 4 °C, NaOH to pH >12, 0.6g ascorbic acid ⁵	14 days ⁶
25. Fluoride	P	None required	28 days
27. Hardness	P, G	HNO ₃ to pH <2, H ₂ SO ₄ to pH <2	6 months
28. Hydrogen ion (pH)	P, G	None required	Analyze immediately
31, 43. Kjeldahl and organic nitrogen	P, G	Cool, 4 °C, H ₂ SO ₄ to pH <2	28 days
Metals:⁷			
18. Chromium VI	P, G	Cool, 4 °C	24 hours

Appendix C

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Appendix D

Glossary/Acronyms

4-AAP. 4-Antiaminopyrinbe.

AA. Atomic absorption.

Accuracy. The ability of a test to give the true amount of target analyte.

Acetonitrile partition. A technique for removing fat and oil interference from organic extracts.

Acid digestion. Method for obtaining metal analytes in solution for analysis.

Acid extractables. Organic analytes that are removed from acidified water with methylene chloride.

Acid-base partition. Clean-up technique for organic analysis.

Activated carbon. Carbon heated to 900 °C in the absence of oxygen.

Activated charcoal. Charcoal heated to 900 °C in the absence of oxygen.

Acute. Immediate effects.

ADP. Adenosine diphosphate.

AES. Atomic emission spectrometry.

AFCEE. Air Force Center for Environmental Excellences.

AFD. Alkali flame detector.

AIA. Alkaline-iodide-azide.

Alkalinity. A measure of the acid-neutralizing ability of the sample.

Ames Test. A common screening test for mutagenic properties.

Analyte-free water. Water that has been treated to remove impurities of interest.

Analytical balance. Electronic balance capable of accurate weighings to 0.1 mg.

Analytically valid. Term used to indicate a procedure has been performed with sufficient controls to assure a high degree of confidence in the result.

Areal composite sample. Samples taken over an area then mixed to give an overall analysis of the site.

Areal domain. Samples taken at a variety of points within a larger sampling site.

Ascarite. A sodium hydroxide treated asbestos.