Purified water is the most common reagent found in the laboratory, used in experimental protocols in virtually every type of application. Despite its ubiquity in the research setting, the contribution of water to the success of experimental outcomes is often overlooked. Used as blanks, for dissolution and dilution of samples, dilution of standards, preparation of mobile phases and for media and buffer preparation, water is central to experimental success

Water purity is critical for a wide range of analytical and biological applications. Impurities in water can interfere with HPLC, LC-MS, PCR, microarrays and immunoassays and can impact cell culture and proteomics studies.

Awareness of what may be in the water used for experiments and the possible impact of contamination can help scientists more quickly identify the source of spurious results and put in place best practices to avoid this problem in the future.

"The emergence of new contaminants and the ability to detect existing ones in water at extremely low levels present an ongoing challenge to both scientists and developers of water purification systems"

Author Details:

Estelle Riche,
PhD, Application Scientist at Merck Millipore
Estelle_riche@millipore.com

Maricar Tarun,
PhD, Application Scientist at Merck Millipore
Maricar_tarun@millipore.com

Know Your H_2O

What's in Your Water?

Purified water in laboratories comes from tap water, which commonly contains substances that may be classified as particles, ions/inorganic compounds, organic molecules, microorganisms, and dissolved gases. Depending on how the water is used in the laboratory, some of these contaminants, if not removed effectively by the laboratory's water purification system, may have an influence on experimental results.

Chromatography

Few factors affect high-performance liquid chromatography analyses more than contaminants in the water used for the mobile phase. Contamination of water used in reversed-phase HPLC by organic molecules is known to cause baseline noise and may lead to the appearance of extraneous peaks that can interfere with the spectral identification and quantitation of low-level analytes.

In a study [1], commercially available HPLC-grade bottled water without total oxidisable carbon (TOC) specifications was compared with freshly delivered ultrapure water with a TOC level below 5 ppb. (TOC is a measure of organic contamination in water.) When bottled water was used in HPLC separations, baseline variability and poor chromatographic performance were observed, as compared to the results observed when ultrapure water was used.

Ion chromatography also requires water free of any contaminants. The presence of ions may result in noisy baselines and higher background, translating into poor sensitivity. Trace analysis of ions is carried out in many laboratories, and this requires water that is free of ions that could compromise the quality of data obtained. In addition, the presence of organic contaminants in water used for eluent preparation could interact with the analytical column, reducing its separation efficiency.

Other water contaminants, such as particles and microorganisms, also may interfere with high-pressure liquid chromatography systems. They may damage pump heads, plug frits, increase system back pressure and damage detectors.

Genomics and Gene Expression

The presence of bacteria or bacterial by-products such as nucleases can also have a significant impact on genomic experiments which require nuclease-free water in order to prevent sample degradation and maintain the integrity of RNA and DNA. Nucleases in a laboratory's water can interfere with experiments employing PCR and other methods that measure or manipulate these nucleic acids.

DNA microarrays are now a ubiquitous tool for measuring the variation or expression levels of thousands of genes in a single experiment. The presence of organic compounds and ions in the water can adversely affect hybridisation and interfere with the detection and measurement of genes using fluorescence.

Proteomics

High purity water is essential for proteomics studies. It is required during sample preparation to prevent degradation or contamination of the sample and to successfully separate and identify proteins using electrophoresis or chromatography. High purity water is also needed for protein or peptide analyses that use mass spectrometry to avoid contamination of the ionization chambers and minimise interferences in mass analysis.

Immunoassays

Alkaline phosphatase conjugated to antibodies is often used in immunoassays. These assays are notably sensitive to experimental conditions and can be impacted by bacterial alkaline phosphatase resulting from bacterial contamination [2].

Cell culture

A number of compounds found in tap water can impact cell cultures, such as heavy metals (mercury, lead, zinc, nickel, chromium, cadmium), which are known to be toxic to various cells, including glial and neural cells. Organic molecules are commonly present in raw water supplies (humic acids, tannins, pesticides, endocrine disrupters) and may remain in tap water. These substances are known to affect the development of cells and should therefore be removed by suitable processes during the production of laboratory grade water. Magnesium inhibits glucose-6-phosphate dehydrogenase and DNA nuclease II. Manganese causes DNA polymerase to incorporate ribose instead of deoxyribose into nascent DNA chains.

In addition, endotoxins in a laboratory water supply can disrupt cell growth and function in cell-based assays.

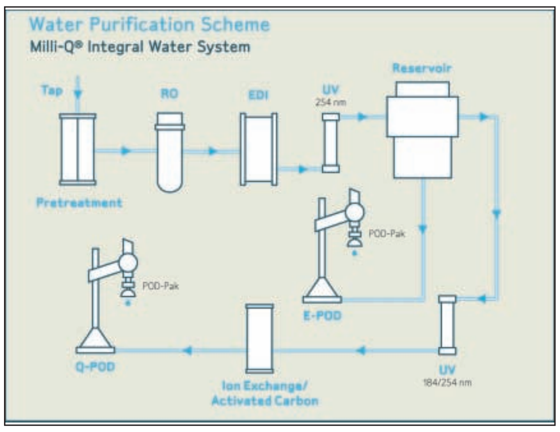

Figure1. The Milli-Q® Integral Water System removes a wide variety of contaminants by combining purification technologies such as reverse osmosis (RO), electrodeionisation (EDI), ultraviolet radiation (UV), and activated carbon, ion-exchange resins.

Water is accessed via independent Point-Of-Delivery units (PODs); different PODs (E-POD® and Q-POD®) are available for delivery of pure or ultrapure water. Final polishers (application packs) can be attached to the outlet of individual PODs to eliminate specific contaminants that might interfere with certain applications.

Emerging Contaminants

In addition to the more common and widely recognised contaminants that can be found in water, 'new' contaminants continue to be identified. These 'emerging contaminants' are considered to be substances characterised by a real or perceived threat to human health or the environment, for which there is no published health standard or a standard is currently being developed. These substances can include pharmaceuticals, personal care products, endocrine disrupting compounds, and chemicals used in products and packaging.

In the EU, the study of emerging contaminants is being carried out by a number of organisations including the NORMAN network. The network initiated activities in 2005 with the financial support of the European Commission and is now established as a self-sustaining network of reference laboratories, research centers and related organisations focused on the monitoring of emerging environmental pollutants.

The identification of emerging contaminants is closely related to the increasing sensitivity of analytical capabilities. The increased sensitivity of mass spectrometry for example, has allowed detection of virtually any new and potentially harmful contaminant at extremely low levels. As such, a number of new or previously ignored or unrecognised contaminants recently found in water are now more closely scrutinised. Recent studies have shown the presence of a wide range of pharmaceuticals including analgesics, antibiotics, barbituates, beta-blockers, psychiatric drugs, and lipid regulators in a number of European rivers.[3][4]

While such contaminants may be found in ground, surface and even drinking water, should they be a concern for researchers? Are these contaminants making their way from the tap into the high purity water used in the laboratory?

Analytical laboratories monitoring the presence of such emerging contaminants must ensure their laboratory water is purified to the highest degree possible, so that even minute amounts of contaminant in the purified water do not interfere with trace level analyses.

Other laboratories that require a similar standard of purity for water are those that develop sensitive methods for the detection of emerging contaminants and their metabolites in various matrices and those that focus on toxicity testing.

Emerging contaminants may also have an impact on cell-based or other biological assays. Effects of these contaminants on experimental outcomes may be so subtle, however, that the cause of unexpected results might not be immediately traced back to the water, leading to wasted time and effort in tracking down the culprit.

Ensuring High Purity Lab Water

Merck Millipore water purification systems remove a wide variety of contaminants by combining various purification technologies such as reverse osmosis, electrodeionisation, activated carbon, ion-exchange resins and ultra-violet radiation. Point-of-use (POU) disposable cartridges have been designed to answer the needs of scientists requiring water free of specific contaminants. The company routinely monitors reports of emerging contaminants and evaluates whether they are effectively removed via water purification systems or perhaps require additional purification steps. Two recent examples include perchlorate and endocrine disrupter chemicals (EDCs).

Perchlorate salts are used in explosives, solid rocket fuel, matches, and air bags. Perchlorate is chemically inert under most conditions and until recently was not considered to be a hazardous substance; as such, the compound was commonly disposed of in wastewater systems. In the late 1990s, development of a sensitive method for perchlorate detection in ground and surface water showed widespread contamination in the US. A 2006 study [5] found the widespread presence of perchlorate in food and beverage samples from around the world. In the UK, the National Centre for Environmental Toxicology, run by the WRc Group, was recently awarded a contract with the Drinking Water Inspectorate/Department for Environmental, Food and Rural Affairs (Defra) to monitor English and Welsh drinking water sources for perchlorate [6].

The growing number of environmental laboratories monitoring perchlorate in water and food require perchlorate-free analytical grade water. To support this need, Merck Millipore developed an ion chromatography method to analyse perchlorate at the nanogram-per-litre level in high purity water and assessed the removal efficiency of various combinations of water purification techniques [7]. Because perchlorate was not present in the tap water used to feed the water purification systems, it was added to assess removal efficiencies. A company study showed that reverse osmosis alone removed 97% of the added perchlorate while ion exchange resins and electrodeionisation removed all remaining traces. A water purification system that combines these technologies ensures high-purity water used in the laboratory is free of perchlorate.

EDCs [8] are natural and synthetic substances that alter the function of the endocrine system and consequently cause adverse effects in an organism or its progeny. Substances suspected of being EDCs include organohalogens (chloroform, dioxins), chemicals used in pesticides (DDT), and plastics (bisphenol A, phthalates). Research groups are actively designing sensitive analytical methods to identify and quantify EDCs for which high purity water is a requirement. In addition to EDCs entering the laboratory water from the tap, the materials used in the water purification system itself may contaminate water. Plastic materials that can leach EDCs include filtration membranes, resin housings, and plastic piping.

Merck Millipore developed a point-of-use cartridge optimised for EDC removal [9]. The cartridge, which contains a specific type of activated carbon, is made of materials selected to prevent recontamination of the purified water. GC-MS analysis showed that water purified using a combination of technologies commonly used in Merck Millipore water purification systems with the addition of the specific carbonaceous POU cartridge contained no measurable amounts of EDCs such as phthalates and dioxins.

The Possible Impact

Awareness of all possible water contaminants – both those that are well known and those that are emerging – must remain high for both developers of laboratory water purification systems and researchers. New contaminants will certainly be identified and may require novel purification techniques, while concentrations of known contaminants that currently do not pose a concern for most laboratories may rise to levels that have a widespread impact on analyses and experimentation.

As the sensitivity of analytical techniques keeps improving and new contaminants emerge, there is a constant need for laboratory water to reach new levels of purity.

The emergence of new contaminants and the ability to detect existing ones in water at extremely low levels present an ongoing challenge to both scientists and developers of water purification systems. The scientific community must remain mindful of how these contaminants might impact laboratory analyses and the concentration levels that may be cause for concern. Scientists and water system manufacturers must work together to ensure existing purification technologies are effectively removing contaminants that can affect laboratory results and design new strategies as needed.

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Particle Characterisation System Supports Charge Optimisation in Water Treatment Plants

An advanced particle characterisation system from **Malvern Instruments** is being used to support water treatment plant operators in their optimisation of treatment processes, and is removing the need to wait for external laboratory results. Saving valuable resources, the Zetasizer Nano enables operators to easily measure and track the zeta potential (charge) of inflowing waters on site - hourly, daily, or across cycles, according to seasonal needs. These parameters are critical to final water quality and treatment plant efficiency.

Gregory Dehmlow, of Dehmlow Optimisation in the US, is a water treatment consultant specialising in plant optimisation. Mr Dehmlow said: "The Malvern Zetasizer Nano allows operators to perform essential analyses in house, rather than having to rely on external laboratories. It is user friendly and treatment operators are comfortable with the functionality and reliability of the unit."

"In the past, instruments measuring zeta potential have been rather difficult to work with outside of a laboratory setting. Following Malvern's specified procedure and because of the ease with which the unit performs time and time again, operators readily accept this as part of their daily laboratory analysis. It is part of their routine in optimising and producing the highest water quality for their customers."

The zeta potential of influent waters received by the treatment facility affects the formation of floc following the rapid mix or flocculation step during the treatment process. Different chemicals are added to disinfect the raw water, change the pH, and reduce the inherent charge to optimise coagulation. Samples are taken following each chemical application in order to analyse the water clarification process and track changes in parameters and optimise flocculation conditions over time.

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