PURIFIED WATER: AN ESSENTIAL COMPONENT FOR DRUG QUALITY

Mark Bosley, Development Manager for water purification specialists Purite, explains the importance of high quality water for biopharmaceutical applications.

Highly purified water is an essential commodity in the biopharmaceutical sector, being used both as an ingredient material in a wide range of research procedures and drug formulations, and for general process duties, including cleaning and rinsing. In each case, the water source must be processed and treated to eliminate microbial, chemical and endotoxin contaminants that would otherwise compromise the quality, safety, efficacy, strength or purity of the finished product or in the case of research, compromise results.

The required level of water purity will vary from task to task, with the highest levels typically being required in cell culture, media preparation, DNA sequencing and genome research. Water purity for these applications is normally measured in terms of either resistivity (expressed as Megohms centimetre or Megohm.cm) or conductivity (measured in microSiemens per centimetre or µS/cm), as the concentration of ionic contaminants will determine how electricity is conducted; the purer the water the higher the resistivity, as lower concentrations of ions are less able to conduct current.

Regulating Water Quality

There are three commonly used grades of water - purified water (PW), water for injection (WFI), and highly purified water (HPW) - with water quality being regulated by various national and international bodies. For example, Europe, Japan and the United States each publish official documents listing drugs and giving directions to achieve specific quality attributes.

These publications are known as pharmacopoeia; they typically refer to water for injection and purified water as compendial waters, and include details of water grades, specific water quality parameters and test procedures. For example, the latest United States Pharmacopoeia, USP26, specifies both conductivity and total organic carbon (TOC) in addition to bacteria levels as the appropriate measures for WFI and HPW, with WFI also being required to have an endotoxin level of less than 0.25 EU/ml. WFI can only be produced through distillation, with the feedwater to the still being generated by a PW plant.

The various pharmacopoeias specify parameters and measuring criteria for water to be used for certain applications, however they do not necessarily prescribe the design or maintenance regimes required to repeatedly achieve these requirements. This falls within the remit of a number of validating bodies, such as the US Food and Drug Administration (FDA), which aim to ensure compliance with standards through ensuring that system design, installation, monitoring and maintenance procedures are in place to facilitate consistent and traceable performance to standards.

In addition, documents such as the Current Good Manufacturing Practice (CGMP) and the ISPE Water and Steam Guide also provide assistance with water usage and processing.

It should be noted that the need to design compendial water systems to meet legally enforceable standards can have far reaching consequences as system designers, engineers and operators tend to concentrate on solutions that have a proven validatory track record. However, the standards are regularly updated to ensure that the benefits from new technological advances are incorporated in system design after an appropriate period of validation and documentation.

System Design

One of the main design and operational criteria for biopharmaceutical companies is that the water purification system, from generation through to the distribution network and storage vessels, can be successfully validated and consistently produces water that meets the appropriate standards, such as USP26.

Pure water generation capacity can vary enormously from a few litres per hour for primary research up to thousands of litres per hour for a production facility. It may be that for laboratory research full FDA-type validation is not required and that local standalone wall or

bench mounted small systems are the preferred pure water generation system, however it is likely that these will utilise the same water purification processes as a plant supplying a complete production facility.

Treatment Processes

The design of any water treatment system is generally based on a number of variables including the quality of the source water, the required output quality, and other considerations such as operating costs. A PW system is generally fed from a potable water source, while a WFI system is usually fed by a PW system with a resistivity of between 0.5 and 5.0 Megohm.cm. By contrast, a HPW system is normally fed by a primary loop with a resistivity of between 16 and 18 Megohm.cm and a TOC of 30 ppb or less. Highly purified water will, therefore, require more treatment stages than a WFI or PW system.

Mains water in Europe varies enormously in both chemical composition and the concentration of ionic contamination, so the exact method used to produce purified water from mains water may vary geographically and seasonally. However, the core technologies used for removing ions from water are generally reverse osmosis (RO) and ion exchange, or usually a combination of both where RO removes the bulk of the contaminants, followed by further purification stages utilising ion exchange.

Most systems designed to generate purified water comprise a number of process stages:
1. Pre-treatment
2. Reverse Osmosis (Primary deionisation)
3. Secondary deionisation
4. HPW would add a Polishing deionisation stage to take the water up to maximum theoretical purity.

Pre-treatment

Pre-treatment has two main functions: to remove particulate matter from the mains water, and to remove any contaminants that would impair the RO stage of the process.

Particulate matter can be removed in a variety of ways including sand or multimedia filtration, or by finer cartridge-type filters. It is normal to utilise a number of filters in series, with each filtration stage taking out smaller particles than the previous stage. Ultrafiltration, using membrane technologies, has become increasingly popular where the raw water quality demands.

For an RO plant to operate effectively and efficiently the feed water should be softened to prevent the build up of calcium deposits on the RO membranes. This is normally achieved using softening resins that remove the hardness by exchanging calcium ions, (the main contributor to hardness), for sodium ions. The process is reversible, which allows the softener to be automatically regenerated in situ by passing a brine solution over the exhausted softener resin. It is usual to use a twin softener comprising two vessels containing softener resin; this provides 100% redundancy. A favoured configuration is to have two vessels with the capacity to treat 100% of the flow each operating in parallel.

Under normal conditions each vessel would treat 50% of the duty flow, however when one vessel goes into regeneration then the other will take full flow until the regeneration cycle is complete. This has the benefit over traditional duplex softeners, where one vessel is the duty vessel with the other in standby mode, in that neither vessel contains static water under normal conditions. Regeneration occurs on either a time elapsed or volume of water treated basis.

In addition to softening, carbon filtration is normally used in the pre-treatment stage. This has the dual function of removing any free chlorine, frequently present as a biocide in drinking water, and reducing the level of dissolved organics. Both of these are important for RO treatment as chlorine can degrade the RO membranes and organic matter can lead to fouling. Where raw water is particularly rich in organic matter, then a further process utilising organic scavenging ion-exchange resins is used to remove the organic contaminants. This is a resin-based technology, similar in operation to softening.

Reverse Osmosis

RO is a process that removes ions from water using membrane technology. Water under pressure is applied to a specialised membrane; as a result purified water passes through the membrane with up to 98% of the minerals/salts being rejected. The purified water is termed

permeate, while the water containing the rejected ionic salts is called concentrate and is usually run to drain or reclaimed if there is an appropriate use for it.

Typically, RO membranes can operate at an efficiency of up to 75% recovery, dependant on feed water quality. In addition to demineralising the water, the RO membrane will remove over 99% of micro-organisms. RO water would then enter the first pure water storage tank.
In the case of a PW system supplying a still, it is common for double pass RO to be utilised without additional deionising.

Secondary Deionisation
Having removed 98% of the ionic contaminants, the next stage of the process is to target the remaining ions. Traditionally, this involved the use of ion exchange resins. These resins are cation or anion specific, meaning that they selectively exchange either positive or negatively charged ionic species for hydrogen or hydroxyl ions. When exhausted, these resins are regenerated using acid and caustic soda.
A more recent variation on this theme is electrodeionisation (EDi), which combines ion exchange resins sandwiched between charged membranes. Under an applied voltage the potential difference set up across the membranes attracts the charged ionic contaminants out of the water and towards their respective electrodes where they are rejected in a waste stream. The process is also self-regenerating due to the production, in situ, of small amounts of hydrogen and hydroxyl ions, which help to maintain the ion exchange resins in a regenerated state. At this point, the water has a resistivity in the range 10-18 Megohm.cm and is normally recirculated around the pure water storage tank to maintain purity. The removal of dissolved Carbon Dioxide, prior to the EDi cell, will further enhance the system performance at this stage and can be achieved either by traditional chemical dosing or more recently with the use of specialised membrane degassers.

Polishing Deionisation
For the production of HPW further process stages are required. After the first stages of deionisation, most of the ions will have been removed from the water; there are, however, still trace contaminants remaining. In order to remove these, water from the secondary loop is taken into a second pure water storage tank and then further treated by passing it through another set of deionising resins before being irradiated by ultra-violet (UV) light and further polishing deionisation.

The UV commonly used in this process is designed to provide a high dose of short wavelength UV light at 185 nm, which has the effect of breaking down the remaining contaminants and converting them into charged ionic species (Photo-oxidation). These can then be passed through a final polishing deionising resin bed to remove them. The resin used in the polishing part of the process should be of the highest purity, so as not to reintroduce contaminants into the water, and would not be regenerable in situ. At this point the water is highly purified. The HPW is typically distributed around a ring main and returned to the second pure water storage tank.

How is quality maintained?
Purified and HPW in particular is extremely difficult to store and any system should be designed to minimise the length of pipework between the source of HPW generation and the points of use. In the part of the system where the purified water is generated, careful consideration must be given to the material of all wetted parts, as contaminants can leach out of most metals and plastics.
It is normal to use sanitary welded 316L stainless steel piping with equivalent grade pumps, heat exchangers and components for the storage and distribution of PW and ABS can be

used for the pre-treatment stage.

Where HPW is being produced the pipe-work and HPW storage tank should be fabricated of PVDF and the tank should include nitrogen blanketing to prevent the ingress of CO_2 that would result in a marked reduction in water resistivity, and a shortening of the life of the deionising resins (as the removal of CO_2 will exhaust the resins prematurely).

How is microbiological control achieved?
Even within HPW systems, where theoretically there is little to support micro-organisms, biofilm growth on pipe-work remains an issue. There are a number of important design considerations that must be considered to minimise microbial contamination.

The first line of defence is to take regular steps throughout the system to reduce the number of micro-organisms. The first of these is the RO membrane that will remove >99% of micro-organisms. UV light at 254 nm is commonly used at various stages of the process to disinfect the water, with an appropriately sized system capable of reducing the number of viable micro-organisms by more than 99.9%. Filtration in the range 0.2 microns is commonly used to remove inactivated bacteria, post UV, however the use of filters in the distribution loop must be carefully considered.

Effective system design is also fundamental to maintain microbiological purity. For example, good hydraulic design, with turbulent flow regimes and the exclusion of dead legs and static water areas in pipe runs to inhibit the formation of biofilm, plus the use of elevated operating temperatures (normally 60° C to 85° C) will all help to minimise microbial growth.

Although the distribution of water at elevated temperatures is a generally acceptable microbial control measure, the technique can increase capital and operational costs. As a result many biopharmaceutical companies operate with ambient (cold) storage and distribution loops.
These loops are susceptible to contamination from oligotrophic bacteria; typically Pseudomonas types and Gram negative bacteria suited to low levels of nutrients. These organisms contribute to development of biofilm on piping surfaces and may also be planktonic; i.e. forming within the process water. Strict adherence to sanitization schedules and methods is, therefore, required to control microbial contaminants.

Ambient or cold loops can be sanitised using heat, with water regularly taken to above 80° C for the requisite period of time to achieve disinfection. Similarly, disinfection using steam can be employed A more recent solution, that eliminates costly heating energy and interruptions to loop operation, is the use of ozone. Although ozone is a toxic substance in the atmosphere and must be removed prior to water take-off it is an extremely effective sanitant and will destroy most bacteria in seconds by lysis of the cell wall; ozone has cell destruction kinetics that are considerably greater than that for chemicals such as chlorine and once sanitisation is achieved, ozone can be relatively easily removed.

Conclusion
Purified water is an essential part of most biopharmaceutical processes. Although generating a consistent supply of high quality water for ingredient material or process and cleaning duties requires careful planning, implementation and plant operation, it can be achieved to the extremely high standards demanded by the regulatory authorities. By utilising the latest purification technology and techniques, biopharmaceutical companies can make considerable improvements in water quality and delivery while potentially reducing both capital and operating costs.