A DISSERTATION ON

STUDY OF WATER FOR PHARMACEUTICAL AND BIOPHARMACEUTICAL PURPOSE

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF B. TECH-M. TECH DUAL DEGREE
IN BIOTECHNOLOGY

BY
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DECLARATION FORM

I, **Samiya Feroze Siddiqui**, a student of **Dual Degree B.Tech-M.Tech Biotechnology,** V Year/X Semester, Integral University have completed my six months dissertation work entitled **" STUDY OF WATER FOR PHARMACEUTICAL AND BIOPHARMACEUTICAL PURPOSE "** successfully from **Mankind Research Centre, Manesar, Haryana** under the able guidance of **Mrs. Deepmala Kumari** I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Samiya Feroze Siddiqui

Date

Dr.Reena Vishwakarma

Date

Mankind
Research Centre

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Date: 31st July, 2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Ms. Samiya Feroze Siddiqui** has completed training under the guidance of **Dr. C. Muthulingam (Sr.VP) – F&D** from period of **1st Feb, 2023 to 31st July, 2023.**

Thanking you,

Yours faithfully,

For **Mankind Research Centre.**

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I wish her good luck and bright future.

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I wish her good luck and bright future.

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Abbreviations

ABBREVIATIONS	NAME
Q A	Quality Assurance
A R	Analytical Reference
SOP	Standard Operating Procedure
IPA	Isopropyl Alcohol
mL	Milli Liter
Mg	Milli Gram
°C	Degree Celsius
µg	Micro Gram
NA	Not Applicable
NLT	Not Less Than
NMT	Not More Than
SS	Stainless Steel
USP	United State Pharmacopeia
v/v	Volume by Volume
FW	Feed Water
PW	Purified Water
RW	Raw Water
PT	Pre-Treated Water
POW	PotableWater
TOC	Total Organic Carbon
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
CTL	Contract Testing Laboratory
CFU	Colony forming unit
BSCP	Buffered Sodium Chloride peptone
RVSEB	Rappaport Sodium Chloride peptone
MCA	MacConkey Agar
MCB	MacConkey Broth
TVC	Total Viable Count
MSA	Mannitol salt agar
CT	Cetrimide agar
OOS	Out of specification
SCDM	Soyabean Casein Digest Medium
GNB	Gram Negative Broth
R2A	Reasoner’s 2A agar
XLDA	Xylose Lysine Deoxycholate agar
HSRO	Heat-sanitizable reverse osmosis

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1. INTRODUCTION

Water serves as a raw material, ingredient, and solvent in the manufacturing, formulation, and processing of pharmaceutical products, as well as in the creation of active pharmaceutical ingredients (APIs) and intermediates, compendia articles, and analytical reagents. (Rieckermann, J., & Joss, A. 2010) This comprehensive information section offers additional insights into water, its quality characteristics that aren't covered in a water monograph, techniques for enhancing water quality through processing, and a description of the minimal standards for water quality that should be taken into account when choosing a water source. This section is not meant to substitute existing regulations or guidelines that already address Good Manufacturing Practices (GMP) concerns at the national (such as FDA, EPA) or international level (such as ICH or WHO), engineering references, or other regulatory instructions for water usage. (M. Florjanič, 2008) The content aids users in gaining a better grasp of pharmaceutical water-related matters and some of the unique microbiological and chemical issues associated with water. However, this chapter isn't an exhaustive discourse on pharmaceutical waters. It presents fundamental points for consideration when dealing with the processing, storage, and utilization of water, as and when suitable. Users are responsible for ensuring that pharmaceutical water and its production adhere to relevant governmental rules, recommendations, and compendial specifications for different types of water used in compendial articles. The chemical purity control of these waters is paramount and constitutes the core purpose of the monographs within this compendium (Michele Totaro, 2019).

Unlike other official articles, the monographs for bulk water such as Purified Water and Water for Injection also specify the allowable production methods, grounded on the understanding that the nature and effectiveness of the purification process directly impacts the resulting purity (Wiley, 2019). The chemical attributes listed in these monographs are to be seen as the minimum requirements. Some applications might necessitate more stringent criteria to guarantee their suitability for specific uses. Fundamental guidance regarding the appropriate applications of these waters is found within the monographs themselves and is further elaborated upon in this section. (Jason N., 2008). Managing the microbiological quality of water is crucial for a multitude of applications. All packaged forms of water that are subject to monograph standards must be sterile, as certain intended uses mandate this feature for health and safety considerations. USP has concluded that imposing a microbial specification on the bulk monographed waters is impractical and hence hasn't been integrated into the monographs for these waters. These waters can be employed across a range of applications, some demanding rigorous microbiological

control while others require none. The necessary microbial specification for a given bulk water hinges on its intended use. Imposing a single specification for this challenging-to-control attribute would burden water users with irrelevant standards and testing requirements. Nonetheless, certain applications might call for even stricter microbial management to prevent the proliferation of water's inherent microorganisms during purification, storage, and distribution. In relation to the continuous supply nature of this raw material, a microbial specification would also be unsuitable (Ch. Rajyalakshmi, 2013). Typically, microbial specifications are assessed using methods that take 48 to 72 hours to yield results. Given that pharmaceutical waters are generally generated through continuous processes and swiftly utilized in products and manufacturing procedures, the water is often used prior to definitive test outcomes being available. In situations where a compendial specification isn't met, an investigation would be necessary to assess the impact and make a pass/fail decision for all product batches between the last satisfactory test result and a subsequent one. The technical and logistical complications stemming from delays in such analyses don't negate the requirement for microbial specifications by users (Business Media LLC, 2018). Hence, such water systems need to be managed and maintained in a controlled manner, necessitating validation of the system to ensure operational stability and quantitative monitoring of its microbial characteristics against predetermined alert and action levels that offer early indications of system control. The matters of water system validation, alert/action levels, and specifications are encompassed within this section.

Objectives of study

The aim of Purified Water System in the pharmaceutical and biopharmaceutical industry is to ensure product quality, meet regulatory requirements, assess and mitigate risks, and maintain a robust quality management system.

- Physio-chemical analysis of collected samples
- Microbial analysis of collected samples

2. REVIEW AND LITERATURE

To ensure compliance with specific minimal standards for chemical and microbiological quality, water employed in drug substance production or as source or feed water for preparing various purified waters must meet the criteria laid out by the National Primary Drinking Water Regulations (NPDWR) (40 CFR 141) issued by the U.S. Environmental Protection Agency (EPA), or the drinking water regulations of the European Union, Japan, or the WHO's drinking water guidelines. The limitations placed on certain organic and inorganic contaminants are aimed at ensuring that the water contains only safe and negligible amounts of potentially undesirable chemical components (Business Media LLC, 2022). As a result, water pretreatment systems are tasked with removing only small quantities of these potentially challenging-to-eliminate chemicals. Additionally, managing objectionable chemical contaminants at the source-water stage obviates the need for specific testing after further purification for some of these contaminants (e.g., trihalomethanes and heavy metals). The microbiological requisites for drinking water guarantee the absence of coliforms, which, if originating from feces, could indicate the potential presence of other potentially harmful microorganisms and fecal-origin viruses (C. Rambabu, 2013) . Satisfying these microbiological requirements doesn't necessarily exclude the presence of other microorganisms that might be considered undesirable if found in a drug substance or formulated product. For microbial control, disinfectants are added to drinking water by Municipal Water Authorities (Fabricio Roosevelt Melo da Silva, 2019). Chlorine-based and other oxidizing substances have been utilized for decades for this purpose and have generally been considered safe for human consumption. However, these oxidants can react with naturally occurring organic matter to form disinfection by-products (DBPs) like trihalomethanes (THMs, including chloroform, bromodichloromethane, and dibromochloromethane) and halo acetic acids (HAAs, including dichloroacetic acid and tri chloroacetic acid). The levels of DBPs generated vary based on the disinfectant type and level used and the quantity and types of organic materials present in the water, which can fluctuate seasonally (Elmer Rolando Llanos Villarreal et al, 2019). Given that high DBP levels can pose health risks in drinking water, Drinking Water Regulations mandate controlling them to generally accepted nonhazardous levels (Werbet Luiz Almeida da Silva, 2019). However, depending on the unit operations employed for further water purification, a small portion of the starting water's DBPs might carry over to the finished water. Hence, maintaining minimal DBP

levels in the starting water while achieving effective disinfection is crucial (Steven P. Djordjevic, 2008). DBP levels in drinking water can be minimized by using disinfectants like ozone, chloramines, or chlorine dioxide. Similar to chlorine, their oxidative properties can damage certain pretreatment unit operations and must be removed early in the pretreatment process. Completely eliminating some of these disinfectants can pose challenges (Mark J., 2008). For instance, chloramines might degrade during disinfection or during pretreatment removal, releasing ammonia, which could in turn carry over to the finished water. Pretreatment unit operations must be designed and operated to sufficiently eliminate the disinfectant, drinking water DBPs, and undesirable disinfectant degradation products. A significant issue can arise if unit operations designed to remove chlorine were suddenly exposed to chloramine-containing drinking water from a municipality that was required to switch from chlorine disinfection due to stricter EPA Drinking Water THM specifications. The dechlorination process might inadequately remove the chloramine, causing irreversible damage to downstream unit operations. Moreover, ammonia released during this process could persist through pretreatment, preventing the finished water from meeting compendial conductivity specifications (Orlando Vaselli, 2019). When the drinking water disinfectant changes, the purification process must be reevaluated, underscoring the importance of a strong working relationship between pharmaceutical water manufacturers and drinking water suppliers.

Given water's pivotal role in pharmaceutical and biopharmaceutical manufacturing, maintaining high-quality water is paramount to ensuring the effectiveness, safety, and quality of pharmaceutical products. Establishing robust water purification systems, conducting regular water monitoring and testing, and adhering to regulatory requirements are essential to upholding industry standards and best practices (Barbara Nisi, 2019). In less developed countries, much of the poor health outcomes are attributed to inadequate access to safe drinking water. A state of positive community health and well-being cannot be achieved without a reliable supply of safe water. Sanitation improvements in waterworks, for instance, led to significant reductions in cholera rates, dysentery rates, typhoid fever death rates, and diarrheal disease rates. Therefore, investing in quality water supply yields substantial dividends in improved health. Hence, ensuring a safe community water supply stands as one of the most effective and enduring health interventions (Lorenzo Frendo et al., 2019). Water meant for human consumption should not only be "safe" but also "wholesome." WHO defines safe and wholesome water as water that is free from pathogenic agents, harmful chemical substances, pleasant to taste, and suitable for domestic use. Water becomes contaminated when it contains infectious and parasitic agents, toxic chemicals, industrial or other waste, or sewage (Wiley, 2019.) The

provision of 150 to 200 liters of water per person per day is essential for public health. As water quality is crucial for human survival, efforts are made to consume uncontaminated water. There are three primary sources of water supply: rain, surface water (such as reservoirs, rivers, streams, tanks, ponds, and lakes), and groundwater (wells and springs). In countries like India, public water supply mainly comes from rivers or reservoirs and partly from wells. Given the higher likelihood of contamination in these water resources, analyzing their quality and preventing contamination before consumption is imperative. Reports on water contamination by infectious microorganisms are numerous (Wiley, 2019). Water can harbor microorganisms like viruses, bacteria, algae, fungi, yeast, protozoa, rotifers, crustaceans, and minute worms. Potable water could potentially contain various infectious microorganisms that could be contracted through drinking, inhaling droplet aerosols, or dermal exposure. Commonly used as an indicator of potable water quality, coliforms are microorganisms like *Escherichia coli* and related Gram-negative species of fecal origin within the Enterobacteriaceae family. Other enteric bacilli like Klebsiella and Enterobacter, as well as Citrobacter, Hafnia, and Serratia, are also termed 'coliforms.' Coliform enumeration serves as a convenient standard of sanitary significance, indicating contamination (Clontz, 2008). Fecal streptococci like Enterococcus faecalis, E. faecium, and Edurans, as well as sulfide-reducing Clostridium perfringens, can also contaminate water. Various Gram-negative bacteria (Legionella, Yersinia, Salmonella, Shigella, Campylobacter, Vibrio), Mycobacteria, enteroviruses, and intestinal protozoans (Giardia, Cryptosporidium) are significant agents of waterborne infections. Water plays a crucial role as a raw material, ingredient, and solvent in the formulation, processing, and manufacture of pharmaceutical products, active pharmaceutical ingredients (APIs), intermediates, compendia articles, and analytical reagents. This informational chapter offers further insights into water, its quality attributes not covered in a water monograph, processing methods to enhance water quality, and the minimum water quality standards that should be considered during water source selection (Morinigo, 2022) The contents aid users in gaining a better understanding of pharmaceutical water-related matters, including unique microbiological and chemical concerns. However, this chapter does not provide an exhaustive discussion on pharmaceutical waters; instead, it contains fundamental points to consider for water processing, storage, and usage.

2.1 Types of Water

There are many different grades of water used for pharmaceutical purposes. These waters can be divided into two general types: bulk waters, which are typically produced on site where they

are used; and packaged waters, which are produced, packaged, and sterilized to preserve microbial quality throughout their packaged shelf life. There are several specialized types of packaged waters, differing in their designated applications, packaging limitations, and other quality attributes (Sulakshana Deshmukh. 2020). There are also other types of water for which there are no monographs. These are all bulk waters, with names given for descriptive purposes only (Chander, 2016). Many of these waters are used in specific analytical methods. The connected text may not specify or imply certain quality attributes or modes of preparation (Horvat, 2010) These no monographed waters may not necessarily adhere strictly to the stated or implied modes of preparation or attributes. Waters produced by other means or controlled by other test attributes may equally satisfy the intended uses for these waters. It is the user's responsibility to ensure that such waters, even if produced and controlled exactly as stated, be suitable for their intended use. Wherever the term “water” is used within this compendia without other descriptive adjectives or clauses, the intent is that water of no less purity than Purified Water be used

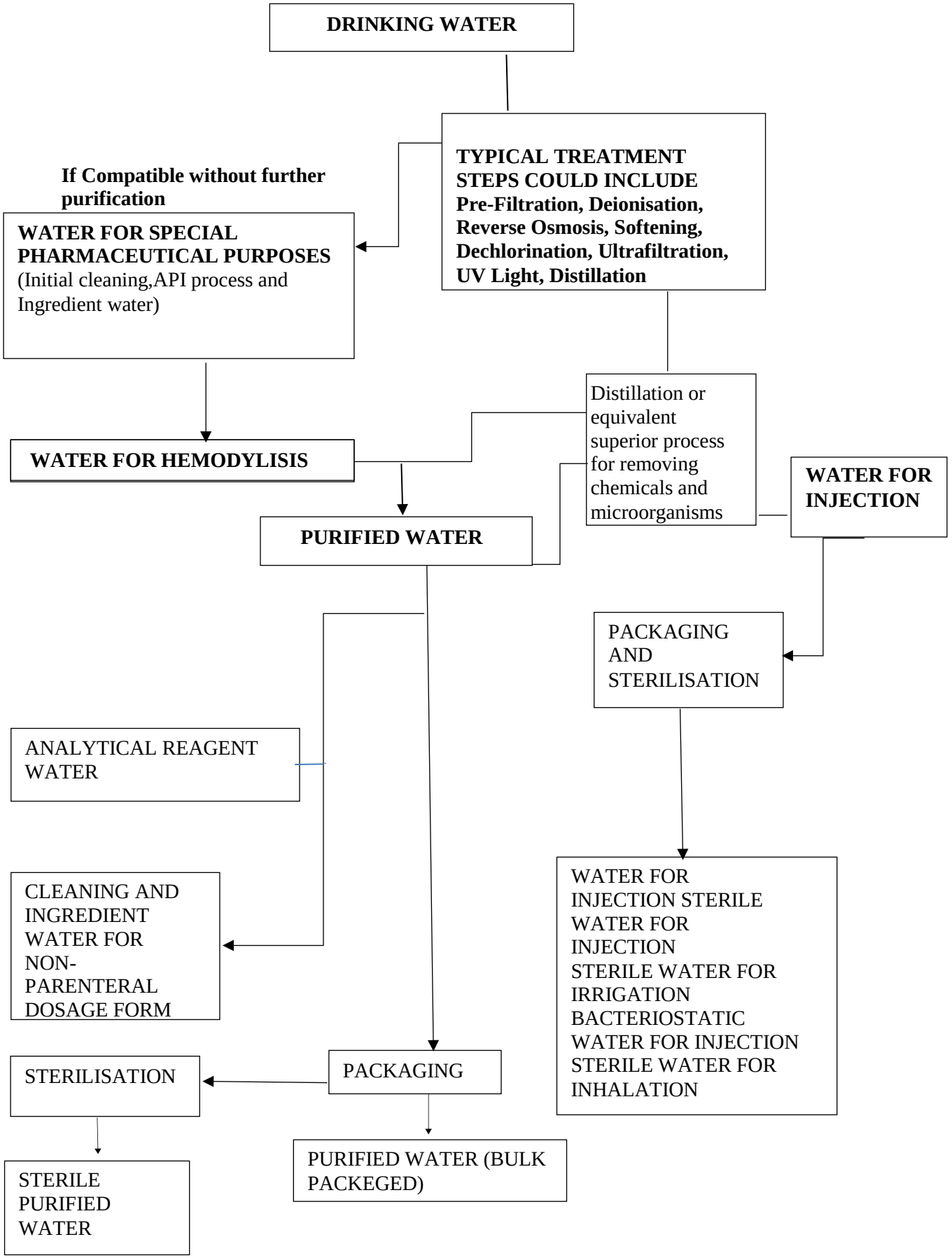


Fig.1: Water for Pharmaceutical Purpose . Source: www.pharmaguideline.com

The following waters are typically produced in large volume by a multiple-unit operation water system and distributed by a piping system for use at the same site (Damodhar, 2013). These particular pharmaceutical waters must meet the quality attributes as specified in the related monographs.

2.1.1 Purified Water

Purified Water serves as an excipient in the manufacturing of non-parenteral preparations and in various other pharmaceutical applications, including the cleaning of specific equipment and non-parenteral product-contact components. Unless explicitly specified otherwise, Purified Water is also to be employed for all tests and assays where water is required (see General Notices and Requirements). Purified Water must fulfill the criteria for ionic and organic chemical purity and must be safeguarded against microbial contamination. The minimum quality of source or feed water for producing Purified Water is Drinking Water (Vikram Reddy, 2013). This source water can undergo purification using processes like deionization, distillation, ion exchange, reverse osmosis, filtration, or other appropriate purification techniques (Schröder, 1999). Purified water systems must undergo validation to consistently generate and distribute water with acceptable chemical and microbiological quality. Purified water systems operating under ambient conditions are particularly susceptible to developing resilient biofilms of microorganisms, which can lead to undesirable levels of viable microorganisms or endotoxins in the outgoing water. These systems require frequent sanitization and microbiological monitoring to ensure adequate microbiological quality at usage points. The Purified Water monograph also permits bulk packaging for external commercial use (Das, 2012). In such cases, the required specifications match those of the packaged water Sterile Purified Water, except for Sterility and Labeling. There's a potential for microbial contamination and other quality alterations in this non-sterile bulk packaged water. Therefore, this form of Purified Water should be prepared and stored in a manner that curbs microbial growth or utilized promptly before microbial proliferation renders it unsuitable for its intended purpose (Heath, 2014). Furthermore, depending on the packaging material, there's a possibility of extractable compounds leaching into the water from the packaging. While this article may fulfill its required chemical attributes, these extractable might render the water unsuitable for certain applications. It falls upon the user to ensure the appropriateness of this packaged article for use in manufacturing, clinical, or analytical applications where the pure bulk form of the water is indicated.

Fig.2.Purified water system
Source: https://www.pharmaguideline.com/2013/08/purified-water-storage-and-distribution-system.html

2.1.2 Water for Injection

Water for Injection serves as an excipient in the production of parenteral and other preparations where control over product endotoxin content is necessary, and it's also used in other pharmaceutical applications, including the cleaning of specific equipment and components that come into contact with parenteral products (Dębska, 2004). The minimum quality of source or feed water required for generating Water for Injection is Drinking Water as defined by the U.S. EPA, EU, Japan, or WHO. This source water might undergo pretreatment to make it suitable for subsequent distillation (or another validated process as per the monograph). The final water must meet all the chemical requirements for Purified Water and include an additional bacterial endotoxin specification (Gadipelly, 2014). Given that endotoxins are produced by microorganisms that can inhabit water, the equipment and procedures involved in purifying, storing, and distributing Water for Injection must be designed to minimize or prevent microbial contamination and remove incoming endotoxin from the source water. Water for Injection systems need to undergo validation to consistently produce and distribute this level of water quality. The Water for Injection monograph also allows it to be packaged in bulk for commercial use. The required specifications include the Bacterial endotoxins test and those of the packaged water Sterile Purified Water, except for Labeling. Bulk-packaged Water for Injection must be sterile, eliminating concerns about microbial contamination and quality

changes. However, packaging extractable might make this water unsuitable for certain applications. The user's responsibility lies in ensuring the suitability of this packaged form when used in manufacturing, clinical, or analytical applications where the purer bulk form of water is indicated.

2.1.3 Water for Hemodialysis

Water for Hemodialysis is employed for hemodialysis purposes, particularly for diluting hemodialysis concentrate solutions. It's produced and utilized on-site, derived from EPA Drinking Water that has undergone further purification to reduce chemical and microbiological components. It might be packaged and stored in containers that prevent bacterial entry without causing any chemical change to the water (Newby, 1991). "Unreactive containers" signifies that the container, particularly its surfaces in contact with water, remain unaffected by the water, preventing leaching of container-related compounds or any chemical reactions caused by the water. This water doesn't contain added antimicrobials and is not meant for injection. Its attributes include specifications for Water conductivity, Total organic carbon (or oxidizable substances), Microbial limits, and Bacterial endotoxins (Richardson, 2011) The water conductivity and total organic carbon attributes align with those set for Purified Water and Water for Injection; however, instead of total organic carbon, the organic content can alternatively be measured using the Oxidizable substances test. The unique Microbial limits attribute is justified by the specific application of this water, which has microbial content requirements for safe use. Similarly, the Bacterial endotoxins specification is determined in relation to its safe use (Wennmalm, 2005).

2.1.4 Pure Steam

Pure Steam is intended for steam sterilization of porous loads and equipment, as well as other processes like cleaning where condensate comes into direct contact with official articles, containers for these articles, process surfaces that then contact these articles, or materials used in analyzing such articles (Murthy, 2012). It can also be used for air humidification in controlled manufacturing areas where official articles or article-contact surfaces are exposed to the conditioned air. The primary goal of using this steam quality is to ensure that official articles or surfaces exposed to it remain uncontaminated by steam residues. Pure Steam is prepared from properly pretreated source water, vaporized with suitable mist elimination, and pressurized for distribution (Makam, 2003). Undesirable contaminants within Pure Steam can arise from entrained source water droplets, anticorrosion steam additives, or particulate matter from the

steam production and distribution system (Barcelo D., 2013). The attributes listed in the monograph are intended to prevent most contaminants from these sources. These purity attributes are measured in the condensate of the article, underscoring the importance of the cleanliness of the Pure Steam condensate generation and collection process. Other steam attributes not outlined in the monograph, like noncondensable gases or superheated states, may also be significant for applications like sterilization. A thorough phase change (condensation) is crucial for steam's sterilization efficiency (V. L., 2018). Control over these attributes, in addition to chemical purity, might also be essential for certain Pure Steam applications. However, since these additional attributes are context-specific, they're not detailed in the Pure Steam monograph. It's worth noting that less pure plant steam may be used for steam sterilization of nonporous loads, general cleaning and sterilization of non-product contact equipment and analytical materials, and humidification of air in non-manufacturing areas (Belkacem, 2008).

2.1.5 Sterile Purified Water

Sterile Purified Water refers to Purified Water that has been packaged and rendered sterile. It's used in the preparation of non-parenteral compendial dosage forms and in analytical applications that require Purified Water. This is especially relevant when access to a validated Purified Water system isn't feasible, only a small quantity is needed, sterile Purified Water is required, or bulk-packaged Purified Water isn't adequately controlled microbiologically.

2.1.6 Sterile Water for Injection

Sterile Water for Injection is Water for Injection that has been packaged and sterilized. It's used for extemporaneous prescription compounding and as a sterile diluent for parenteral products. It's also used in other scenarios where bulk Water for Injection or Purified Water is needed but access to a validated water system is impractical or a relatively small quantity is required. Sterile Water for Injection is packed in single-dose containers no larger than 1 L.

2.1.7 Bacteriostatic Water for Injection

Bacteriostatic Water for Injection is sterile Water for Injection to which one or more antimicrobial preservatives have been added. It's intended as a diluent in the preparation of parenteral products, often for multi-dose products requiring repeated withdrawals. It can be packaged in single-dose or multiple-dose containers not exceeding 30 mL in size.

2.1.8 Sterile Water for Inhalation

Sterile Water for Inhalation is Water for Injection that has been packaged and rendered sterile. It's intended for use in inhalators and for preparing inhalation solutions. It has a less stringent specification for bacterial endotoxins compared to Sterile Water for Injection and is therefore unsuitable for parenteral applications.

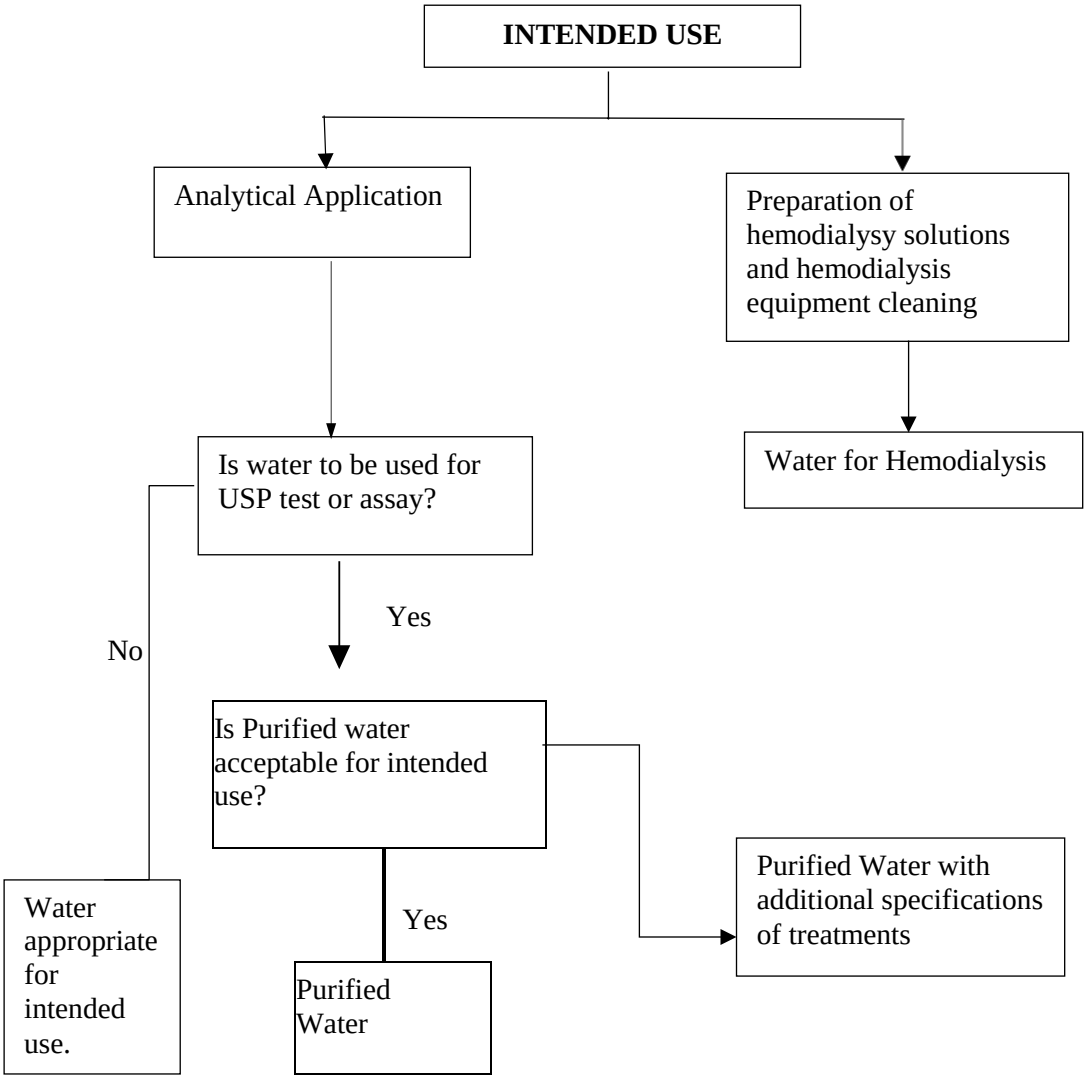


Fig.3 Selection of water for pharmaceutical purpose. Source: www.pharmaguideline.com

2.2 Unit Operation Concerns

This section provides a concise overview of selected unit operations along with associated operational and validation considerations. Not all unit operations are covered, nor are all potential issues addressed. The aim is to emphasize factors that pertain to the design, installation, operation, maintenance, and monitoring parameters critical for water system validation.

2.2.1 Prefiltration:

Prefiltration, also known as initial, coarse, or depth filtration, serves to eliminate solid contaminants of sizes ranging from 7 to 10 µm from the incoming source water (Thomas, 2021). Its purpose is to safeguard downstream system components from particulate matter that could impede equipment performance and reduce their effective lifespan. This type of coarse filtration relies on sieving effects for particle capture, utilizing a filtration medium with substantial "dirt load" capacity. Various designs of filtration units are available for different applications (Trenholm, 2019). Removal efficiencies and capacities can vary significantly, from granular bed filters such as multimedia or sand used in larger water systems, to depth cartridges suitable for smaller systems. The configuration of units and systems varies widely, including filtering media types and positions within the process. While granular or cartridge prefilters are often positioned at or near the start of the water pretreatment system, prior to unit operations designed to remove source water disinfectants, it's important to note that microbial control is still necessary due to potential biofilm growth, albeit at a slower pace in the presence of disinfectants. Operational issues impacting depth filter performance encompass media channeling, blockage by silt, microbial proliferation, and media loss during improper backwashing (Vanderford, 2009). Effective control involves monitoring pressure and flow during usage and backwashing, sanitization, and replacement of filtering media. Design concerns include filter size to prevent channeling or media loss from inappropriate water flow rates, as well as correct sizing to avoid excessive backwashing or filter replacement intervals.

2.2.2 Additives

Chemical additives play a role in water systems for various purposes: controlling microorganisms using sanitants like chlorine compounds and ozone, enhancing the removal of suspended solids through flocculating agents, eliminating chlorine compounds, preventing scaling on reverse osmosis membranes, and adjusting pH for more efficient removal of

carbonate and ammonia compounds via reverse osmosis (Vanderford, 2003). These additives don't count as "added substances" as long as they are either removed in subsequent processing steps or are absent from the finished water. Maintaining effective additive concentrations and monitoring their removal should be incorporated into the system's design and included in the monitoring protocol.

2.2.3 Softeners

Water softeners can be positioned upstream or downstream of disinfectant removal units. They employ sodium-based cation-exchange resins to eliminate water-hardness ions, such as calcium and magnesium, which could foul or hinder the performance of downstream processing equipment like reverse osmosis membranes, deionization devices, and distillation units (Cabral Ruelas, 2021) Water softeners can also remove other lower-affinity cations, such as the ammonium ion, released from chloramine disinfectants commonly used in drinking water, which might otherwise carry over through other downstream unit operations. If ammonium removal is a goal, the softener must be located downstream of the disinfectant removal step, which might release ammonium from neutralized chloramine disinfectants. Regeneration of water softener resin beds is achieved using concentrated sodium chloride solution (brine). Concerns involve microbial proliferation, channeling due to biofilm aggregation of resin particles, appropriate flow rates and contact time, ion-exchange capacity, fouling by organic and particulate resins, organic leaching from new resins, resin bead fracture, resin degradation due to excessive chlorination, and contamination from the brine solution used for regeneration (Snyder, 2003). Control measures may encompass water recirculation during periods of low use, periodic resin and brine system sanitization, utilization of microbial control devices (e.g., UV light and chlorine), locating the unit upstream of disinfectant removal (if used only for softening), determining the appropriate regeneration frequency, monitoring effluent chemicals (e.g., hardness ions and possibly ammonium), and downstream filtration to remove resin fines. For a softener used to remove ammonium from chloramine-containing source water, factors like capacity, contact time, resin surface fouling, pH, and regeneration frequency are highly significant.

Fig.4 Softener Plant

2.2.4 Reverse Osmosis (RO)

Reverse Osmosis units utilize semipermeable membranes. The "pores" within RO membranes represent intersegmental spaces among polymer molecules. These spaces are large enough to allow water molecule permeation but too small for hydrated chemical ions to pass through. Nonetheless, factors such as pH, temperature, and the differential pressure across the membrane influence the permeation selectivity. By applying proper controls, RO membranes can enhance the quality of water through chemical, microbial, and endotoxin improvement (Savelski, 2010). The process streams include supply water, product water (permeate), and wastewater (reject). Depending on the source water, achieving desired performance and reliability might require pre-treatment, system configuration adjustments, and the use of chemical additives. The permeate recovery rate, a crucial factor influencing RO performance, refers to the amount of water passing through the membrane relative to the amount rejected. This rate is significantly influenced by pump pressure. Typical recoveries are around 75%, leading to a 1 to 2 log purification of most impurities. However, this is usually insufficient to meet the conductivity specifications of Purified Water for most feed waters (S., 2020). To achieve the required, permeate purity, a second pass of the permeate water through another RO stage is usually necessary. Increasing recoveries by raising pressures to reduce reject water volume can compromise permeate purity. If higher pressures are needed over time to maintain the same permeate flow, it indicates partial membrane blockage that requires correction to prevent irreparable fouling and the need for costly membrane replacement. Other concerns in RO unit design and operation encompass membrane sensitivity to sanitizing agents and fouling by particulates, chemicals, and microorganisms; membrane and seal integrity; passage of dissolved gases like carbon dioxide and ammonia; and wastewater volume, especially

where water discharge is regulated by local authorities (Kullab, 2017). Failure in membrane or seal integrity leads to product water contamination. Control methods involve appropriate influent water pretreatment, suitable membrane material selection, addressing integrity challenges, proper membrane design and heat tolerance, periodic sanitization, and monitoring differential pressures, conductivity, microbial levels, and total organic carbon (TOC). The development of RO units that can tolerate sanitizing water temperatures and operate efficiently at elevated temperatures has significantly enhanced microbial control and prevented biofouling (Martin, 2017) RO units can be used independently or combined with deionization (DI), continuous electrodeionization (CEDI) units, and ultrafiltration for quality and operational improvements.

Fig.5 RO Plant

2.2.5 Ultrafiltration:

Ultrafiltration is a technology often applied in pharmaceutical water systems to eliminate endotoxins from water streams. It utilizes semipermeable membranes, typically polysulfone ones (K'oreje, 2006). These membranes have purposely exaggerated "pores" created during their fabrication by preventing polymer molecules from reaching their smaller equilibrium proximities. The different equilibrium control during fabrication results in membranes with distinct molecular weight "cut-offs." These cut-offs determine whether molecules with molecular weights above those ratings are rejected and unable to penetrate the filtration matrix. Ceramic ultrafilters are another molecular sieving method. They are self-supporting, durable, back washable, chemically cleanable, and steam sterilisable. However, they might require higher operating pressures than membrane-based ultrafilters (Dewulf, 2012). Ultrafiltration devices work based on molecular sieving, with cutoff ratings typically ranging from 10,000 to 20,000 Da for removing endotoxins in water systems. This technology can serve as an intermediate or final purification step. Similar to RO, effective performance hinges on upstream

unit operation water pretreatment. Concerns for ultrafiltration encompass membrane material compatibility with heat and sanitizing agents, membrane integrity, fouling from particles and microorganisms, and seal integrity. Control measures include selecting appropriate filtration media, sanitization, flow design (dead end vs. tangential), addressing integrity challenges, routine cartridge replacements, elevated feed water temperatures, and monitoring TOC and differential pressure. Ultrafiltration unit arrangement flexibility exists, such as parallel or series configurations, influencing operation options (R. (Ed.)., 2006). Care should be taken to prevent stagnant water conditions promoting microorganism growth in standby units.

2.2.6 Ultraviolet Light:

The use of low-pressure UV lights emitting a 254-nm wavelength for microbial control is discussed in the Sanitization section, but UV light's application in chemical purification is also emerging. The 254-nm wavelength is effective in destroying ozone. Medium-pressure UV lights emitting intense emissions at wavelengths around 185 nm, in addition to 254 nm, prove useful in breaking down chlorine-containing disinfectants present in source water and in intermediate stages of water pre-treatment (Schaider, 2016). This 185-nm UV wavelength, alone or in combination with other oxidizing sanitants like hydrogen peroxide, can reduce total organic carbon (TOC) levels in recirculating distribution systems (Ternes, 2014). The organic substances typically transform into carbon dioxide, equilibrating to bicarbonate, and incompletely oxidized carboxylic acids. Both can be easily removed by polishing ion-exchange resins. Concerns include adequate UV intensity and residence time, UV bulb emissivity diminishing with bulb aging, formation of UV-absorbing films at water contact surfaces, incomplete photo degradation in cases of unexpected source water hyper chlorination, release of ammonia from chloramine photo degradation, undetected UV bulb failure, and conductivity deterioration in distribution systems employing 185-nm UV lights (J., 1999). Control methods encompass periodic inspection or emissivity alarms for detecting bulb failures or film occlusions, regular UV bulb sleeve cleaning, downstream chlorine detection, downstream polishing deionizers, and periodic bulb replacement, usually annually.

2.3 Sanitization

Microbial control in water systems is primarily achieved through sanitization practices. Sanitization methods encompass thermal and chemical approaches. Thermal methods involve periodic or continuous circulation of hot water and steam use. Temperatures of at least 80°C

are commonly employed, though continuous recirculation of water at around 65°C can effectively sanitize insulated stainless steel distribution systems with uniformity and proper distribution. However, these methods are limited to systems compatible with the higher temperatures required for sanitization. Thermal approaches inhibit biofilm growth either by continuous growth inhibition or, intermittently, by killing microorganisms within biofilms. However, established biofilms are not effectively removed. Killed biofilms can act as nutrient sources, leading to rapid biofilm regrowth after sanitization conditions cease. Combining routine thermal sanitization with periodic chemical sanitization might be more effective in these cases. Chemical methods, where feasible, can be applied to a wider range of construction materials. They often employ oxidizing agents like halogenated compounds, hydrogen peroxide, ozone, peracetic acid, or their combinations. Halogenated compounds are effective sanitizers but may not flush from the system easily and could leave biofilms intact. Substances such as hydrogen peroxide, ozone, and peracetic acid oxidize bacteria and biofilms by generating reactive peroxides and free radicals, especially hydroxyl radicals. Ozone's short half-life and limited achievable concentrations require continuous addition during sanitization. Hydrogen peroxide and ozone rapidly degrade to water and oxygen, while peracetic acid degrades to acetic acid in UV light's presence. Ozone's rapid degradation via 254-nm UV lights at use points enables its effective continuous use for continuous sanitization. In-line UV lights with a 254-nm wavelength can also continuously "sanitize" circulating water in the system (Prebble, 1993). However, these devices must be appropriately sized for water flow. While these devices can inactivate a high percentage of microorganisms passing through them.

2.4 Operation, Maintenance and Control

A precautionary maintenance program should be established to ensure that the water system remains in a state of control.

The program should include (1) procedures for operating the system, (2) monitoring programs for critical quality attributes and operating conditions including standardization of critical instruments, (3) schedule for periodic sanitization, (4) preventive maintenance of components, and (5) control of changes to the mechanical system and to operating conditions.

2.4.1 Operating Procedures

Procedures for operating the water system and execution routine maintenance and corrective action should be written, and they should also define the point when action is required (Van Coillie, 2007). The procedures should be well documented, detail the function of each job, assign who is responsible for performing the work, and describe how the job is to be conducted. The effectiveness of these procedures should be measured during water system validation.

2.4.2 Monitoring Program

Critical quality attributes and operating parameters should be documented and monitored. The program may include a mixture of in-line sensors or automated instruments (e.g., for TOC, conductivity, hardness, and chlorine), automated or manual documentation of operational parameters (such as flow rates or pressure drop across a carbon bed, filter, or RO unit), and laboratory tests (e.g., total microbial counts) (Thomas, 2007). The frequency of sampling, the requirement for evaluating test results, and the necessity for introducing corrective action should be included.

Sanitization— Depending on system design and the selected units of operation, routine periodic sanitization may be necessary to maintain the system in a state of microbial control. Technologies for sanitization are described above.

Preventive Maintenance— A preventive maintenance program should be in effect. The program should establish what preventive maintenance is to be performed, the frequency of maintenance work, and how the work should be documented.

Change Control— The mechanical configuration and operating conditions must be controlled. Proposed changes should be evaluated for their impact on the whole system. The need to requalify the system after changes are made should be determined. Following a decision to modify a water system, the affected drawings, manuals, and procedures should be revised.

2.4.3 Pre-Treatment and Purification System Sampling

The location and frequency of sampling from ports within the pretreatment and purification systems may be selected based on a risk analysis of unit operation purpose. The purpose of this sampling is primarily for PC, for example, to ensure maintenance of acceptable unit operation performance, to assess maintenance procedure efficacy, and to investigate the need for remedial action. Quality deviations in the early portions of the purification process can affect unit

operation efficiency but usually do not impact the finished water quality or acceptable use.

2.4.4 Purified Water Distribution System Sampling

Purified Water distribution system sampling is intended to provide continuing assurance of ongoing PC and compliance with the user's finished water chemical and microbiological requirements (Sperger, 2011). Generally, the locations for that sampling and the frequency of testing the specific attributes are a matter of process and quality control consistency, as well as risk tolerance in the event of a deviation. Depending on the water system design, the chemical attributes of a water system tend to be relatively constant and more uniformly distributed than the microbiological attributes. Therefore, less frequent sampling at only selected locations could be justified for chemical testing based on familiarity with system design and the existence of historically consistent operational data. However, with some purification system designs, the chemical quality could change dramatically in a short period of time (such as from the exhaustion of deionization beds), so frequent or even continuous in-line/on-line monitoring of the chemical attributes would be advisable to be able to recognize and correct the cause of the problem before non-compliant water is produced and used. For microbial testing, all use points and critical sample ports in a distribution system are typically sampled routinely, including those that are infrequently used by manufacturing (Munson, 2011). There is no prescribed sampling frequency for Purified Water system outlets, so typical outlet sampling frequencies vary from daily to monthly, with sampling occurring somewhere in the system at least at weekly intervals. A risk analysis is suggested for determining the sampling plan for a Purified Water system. Factors in this analysis could include (but are not limited to) the test result history for the entire water system as well as specific outlets, the criticality of specific outlets to manufacturing, the usefulness of selected sample ports as indicators of ongoing system control, and the scope of impact on products and activities should an unfavorable test result occur. For the scope of impact, the less frequent the sampling, the more products and processes will be impacted by an unfavorable test result.

2.4.5 Water for Injection Distribution System Sampling

The sampling plans for Water for Injection distribution systems (as well as any water system where some level of bacterial endotoxin control is needed) utilize the same general sampling approaches as do Purified Water systems. However, the regulatory expectations for Water for Injection distribution system sampling plans are more prescriptive because microbial control must be much more stringent as it is related to the bacterial endotoxin attribute. In general,

Purified Water System for microbial and bacterial endotoxin testing is expected to occur daily somewhere in the system, with each outlet being sampled periodically, based on a risk assessment, to characterize the quality of the water.

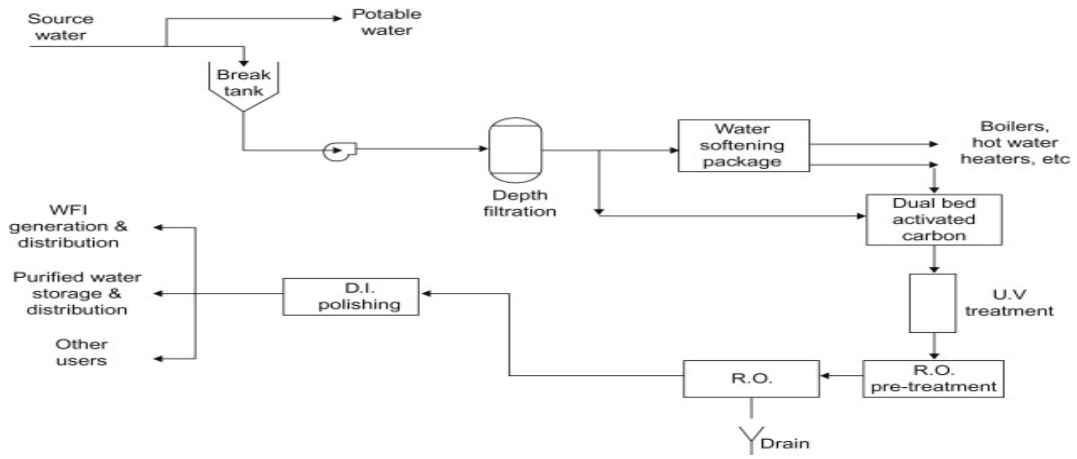


Fig.6 Operating Procedure for Purified water process in Pharmaceutical Industry
Source: https://www.semanticscholar.org/paper/Pharmaceutical-Water-System-Fundamentals.-Collentro/1bdd86f6c9c7705245228924f3bf2b8967bee2af

2.5 Considerations for Sampling

To ensure the ongoing control and production of water of acceptable quality, water systems should undergo monitoring at an appropriate frequency. Samples ought to be collected from representative points within both the processing and distribution systems. The determination of sampling frequencies should be rooted in system validation data and should encompass critical areas, including unit operation sites (V., 1986) The sampling plan should also account for the specific attributes that need to be assessed in the sampled water.For instance, when dealing with systems for Water for Injection, which have more demanding microbiological requirements, a more rigorous and frequent sampling frequency might be necessary. The analysis of water samples generally serves two main purposes: assessing in-process control and final quality control (Hilfiker, 2006). In-process control analyses primarily focus on the water's attributes within the system, while quality control concentrates on the attributes of the water supplied by the system for its various applications.

The topic of sample collection location and procedure can spark debate due to the mixed use of data generated from samples, serving both in-process and quality control purposes. In these

situations, it's advisable to consider the worst-case scenario. Consequently, samples should be collected from use points utilizing the same delivery mechanisms and procedures employed during production from those use points. In cases where direct use point sampling isn't feasible, specialized sampling ports could be utilized. Regardless of the approach, the collected sample must accurately represent the water's quality used in production (Vergeynst, 2012). Samples containing chemical sanitizing agents should undergo neutralization prior to microbial analysis. Microbiological samples should be tested immediately or stored at a suitable temperature to preserve the original microbial attributes until analysis can be initiated. While samples of flowing water provide insight into the concentration of planktonic microorganisms present in the system, it's important to note that biofilm-associated microorganisms, which are attached to water system surfaces, often have a greater presence. These biofilm microorganisms are responsible for the planktonic population recovered from grab samples. Planktonic population data is typically used as an indicator of contamination levels and forms the basis for system Alert and Action Levels. Elevated planktonic levels frequently signify advanced biofilm development, necessitating remedial action. Biofilm formation can be controlled through system management and effective sanitization practices.

Sampling for chemical analyses is also vital for in-process and quality control purposes. However, unlike microbial analyses, chemical analyses can often be performed using online instrumentation (Woldemariam, 2017). This on-line testing is primarily aimed at in-process control, as it's not conducted on water delivered by the system. Yet, chemical attributes are usually not significantly altered by hoses, making it possible to verify that attributes detected by online instrumentation are equivalent to those detected at the ends of use point hoses through verification testing. This situation creates a scenario where a defined small sampling of in-process data is used for quality control purposes.

2.5.1 Routine and Non Routine Sampling Plans

Routine sampling plans should be designed to ensure ongoing compliance with quality standards. These plans should be based on a risk assessment and should encompass various attributes based on the system's design and requirements. For instance, source water sampling ensures that the incoming water adheres to the required standards. Sampling frequency could be determined based on the risk analysis of the source water's impact on purification processes. Non-routine sampling, on the other hand, is conducted for episodic events or special situations where routine plans fall short. The purpose of the non-routine sampling dictates the procedures, attributes, and frequency of testing. Such sampling may occur from ports specifically

designated for investigational purposes and need not be part of the regular sampling plan

2.6 Chemical Considerations

In the context of Purified Water and Water for Injection, chemical attributes were historically assessed through specific and nonspecific chemistry tests, aimed at detecting chemical species indicative of incomplete purification (P., 2012) Over time, these tests have stood firm despite the evolving technology landscape. Modern analytical methods like Total Organic Carbon (TOC) and conductivity have replaced traditional chemical tests to enhance analytical accuracy without tightening quality requirements. For example, the TOC test replaces the Oxidizable substances test, which primarily targets organic contaminants. A multistage Conductivity test has replaced most inorganic chemical tests, excluding specific tests like Heavy metals. Other tests like Total solids and pH were also phased out, given their redundancy in the presence of conductivity testing.

2.7 Microbial Considerations

The primary source of microbial contamination in bulk pharmaceutical water is the feed or source water. Incoming water must comply with drinking water standards, with a particular emphasis on controlling coliform levels. A range of microorganisms, particularly Gram-negative bacteria, might be present in the incoming water, potentially compromising subsequent purification steps (Ayika, 2020). Exogenous microbial contamination can also arise from factors like unprotected vents, faulty air filters, backflow from contaminated outlets, and inadequate drain systems. Endogenous contamination can stem from unit operations, with microorganisms adsorbing to surfaces and forming biofilms. Distribution systems themselves can become sources of contamination as microorganisms colonize surfaces and create biofilms (Slater, 2010). Adequate system design, maintenance, and operational control are crucial in minimizing microbial contamination.

2.8 Storage and Hold Times for Chemical Tests

Unlike microbial impurities, the chemical purity of high-purity water samples is prone to degrade over time due to the homogeneous nature of chemical impurities in water. It's generally recommended to perform testing as soon as feasible to avoid false adverse results. Proper container selection and cleanliness are essential in ensuring accurate data from samples (Cabral

Ruelas, 2010). Container materials should be inert and not introduce contaminants into the samples. For off-line chemical tests, there are no compendia requirements for storage time and conditions, but samples should be stored cool and tested promptly to prevent potential contamination or degradation

2.8.1 Containers

When sampling water for off-line analysis, the selection and cleanliness of the container play a significant part in obtaining accurate data. For samples to be tested for chemical impurities according to Total Organic Carbon, the proper container should be one that does not contaminate the sample during the storage/hold time. For example, the use and preparation of glass containers could be very acceptable for storing samples for TOC testing, but some glass containers do leach ions over time (hours and days), and they can adversely impact a conductivity test by creating a false positive result—if the storage time is too long. Likewise, there are some polymer materials that can adversely impact the TOC chemical impurity in water. However, many polymer materials are very inert. In any case, cleanliness of the container is crucial because trace quantities of soaps and fingerprints will adversely impact the chemical purity of the water. Properly cleaned containers are acceptable because chemical impurities are easily rinsed away. Extensive chemical cleaning methods such as acid or caustic rinsing should never be needed (S. A., 2003). If they are needed, consider replacing the containers.

2.8.2 Storage Time and Conditions

There are no specific recommendations for storage of samples for water analyses. If there is some trace interaction of the container and water, then generally colder and shorter storage times are better than warmer and longer storage times. Chemical dissolution and reactivity are usually enhanced by increased temperature. Furthermore, time is always an element because the water sample can only get worse in a container, and it never gets better with time.

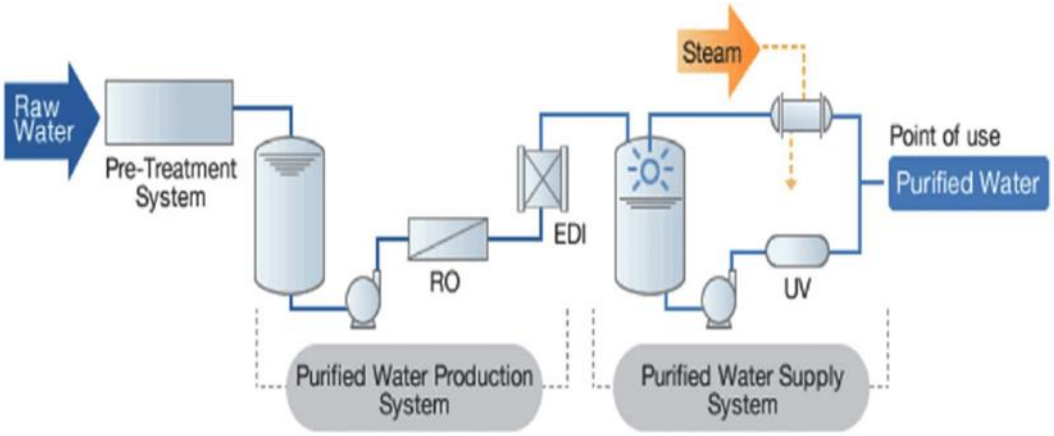


Fig 7. Water system for Pharmaceutical facilities

Source: https://www.ngk-insulators.com/en/product/cm-medical-water.html

2.9 Microorganism Sources

Exogenous microbial contamination in bulk pharmaceutical water stems from a range of potential sources, including the source water itself (Gruenhagen, 2021). At the very least, source water should adhere to microbial quality standards comparable to Drinking Water, which necessitates the absence of faecal coliforms, notably E. coli. Incoming water may also contain various other types of microorganisms, predominantly Gram-negative bacteria (Refes, 2009). If proper measures aren't taken to mitigate their presence, these microorganisms can undermine subsequent water purification procedures. Additional sources of exogenous microbial contamination encompass maintenance operations, equipment design, and the monitoring process, such as:

- Unprotected or faulty vent filters and rupture disks.
- Backflow from interconnected equipment.
- Non-sanitized openings in the distribution system during component replacements, inspections, repairs, and expansions.
- Inadequate drain air-breaks.
- Natural bioburden presents in activated carbon, ion-exchange resins, regenerate chemicals, and chlorine-neutralizing agents.
- Inadequate rinsing quality after regeneration or sanitization.
- Suboptimal sanitation of use points, hard-piped equipment connectors, and water transfer devices like hoses.
- Inefficient techniques for use, sampling, and operation.

- The external contaminants might not be typical aquatic bacteria, but rather microorganisms originating from soil, air, or even human sources. The identification of non-aquatic microorganisms may indicate contamination during sampling or testing, or even a failure in system components. To minimize microbial contamination from external sources, meticulous attention should be paid to sampling, testing, system design, and maintenance practices.

2.9.1 *Escherichia coli*

Escherichia coli, a Gram-negative bacterium often originating from faecal matter, is a common contaminant in potable water, causing diarrheal diseases. E. coli-related diarrhoea poses a significant health challenge in developing countries. Coliform monitoring serves as a global method to surveil drinking water quality. Variations in climate, particularly during wet weather, can significantly elevate E. coli levels in water systems. The presence of E. coli in food or water indicates recent faecal contamination and the potential presence of pathogenic agents.

2.9.2 *Staphylococcus aureus*

Staphylococcus aureus, a Gram-positive bacterium, naturally resides on human skin and in the nasopharynx. It can lead to various infections involving the skin, soft tissues, internal organs, and endovascular sites. S. aureus remains a substantial pathogen both in communities and hospitals, causing considerable morbidity and mortality. The bacterium can disseminate from superficial sites to internal organs through the bloodstream, potentially resulting in metastatic infections. Surgical wounds and indwelling medical devices are common infection sites in hospitals.

2.9.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa, an environmental bacterium, is responsible for opportunistic infections in humans. Its adaptability to diverse growth conditions is attributed to numerous metabolic pathways and regulatory genes. With high antibiotic resistance, numerous virulence factors, and nutritional versatility, P. aeruginosa is challenging to eradicate from infected individuals, particularly those with cystic fibrosis-related lung infections.

2.9.4 *Salmonella sp.*

The interrelationship between *Salmonella spp*. and indicator microorganisms (e.g., total coliforms, faecal coliforms, faecal streptococci, Clostridium perfringens, and coliphages) was explored in aquatic environments impacted by wastewater discharges. Correlations between microorganisms and *Salmonella spp*. depended on the source of faecal discharges. Faecal coliforms and C. perfringens exhibited strong correlations with *Salmonella spp*., with the latter yielding higher linear regression slope values. The presence of *Salmonella spp*. was detectable even with low pollution levels, indicating potential detection of pathogens independently of classical indicator microorganisms.

2.9.5 *Shigella sp.*

Shigella, Gram-negative facultative anaerobes, are primary agents of bacillary dysentery. This disease, characterized by scanty, blood- and mucus-containing stools, differs from profuse watery diarrhoea seen in choleric or enter toxigenic E. coli diarrhoea. Shigellosis is a significant contributor to acute intestinal disease, particularly among children in developing countries. The pathogenic mechanism involves invasion of *Shigella* into the colonic epithelium and subsequent inflammation, leading to colitis and ulceration of the mucosa.

2.10 Types of Water used in Water Sampling in Pharmaceutical and Biopharmaceutical Industry

2.10.1 Raw Water

Raw water denotes untreated or minimally treated water obtained from natural sources or public water supplies before undergoing purification processes. In the pharmaceutical and biopharmaceutical sector, raw water serves as the initial point for water treatment systems. Common sources of raw water used in the industry encompass:

i. Municipal Water Supply: Many facilities procure raw water from municipal sources, such as city water supplies. This water is sourced from surface water bodies or underground aquifers, treated by municipalities to meet quality standards before industrial use.

ii. Groundwater: Wells yield groundwater, another raw water source for the pharmaceutical industry. Groundwater's quality varies based on geological conditions and potential contaminants.

iii. Surface Water: Rivers, lakes, and streams also offer raw water. Surface water sources require monitoring and treatment to remove potential contaminants before industrial use.

iv. Rainwater: While rainwater may be used for non-critical applications, it's not a primary source due to contamination risks in pharmaceutical manufacturing processes.

v. It's important to note that raw water is not suitable for direct use in pharmaceutical manufacturing processes without proper treatment and purification. Raw water can contain various impurities, including suspended solids, microorganisms, organic matter, dissolved minerals, and potential contaminants. Therefore, pharmaceutical and biopharmaceutical facilities implement water treatment systems, such as filtration, disinfection, reverse osmosis, and other purification processes, to remove or reduce these impurities and ensure the production of high-quality water that meets the required standards and specifications for pharmaceutical applications.

Fig. 8: Raw Water Sampling Point

2.10.2 Feed-Water

In the pharmaceutical and biopharmaceutical industry, feed water refers to the water that has undergone some level of treatment and purification but is still in the process of being further treated to meet the specific quality requirements for pharmaceutical applications. Feed water serves as an intermediate stage between raw water and the final purified water used in manufacturing processes. Here are some common types of feed water used in the industry:

i. Potable Water: Potable water, also known as drinking water, is commonly used as a

feed water source in pharmaceutical and biopharmaceutical facilities. Potable water is sourced from municipal water supplies or private water sources and undergoes initial treatment to meet drinking water standards. This includes the removal of suspended solids, disinfection to control microbial growth, and adjustment of pH levels.

ii. Purified Water (PW): Purified water is a high-quality water type commonly used as a feed water source in the pharmaceutical industry. It is obtained by further treating potable water through processes such as reverse osmosis (RO), deionization (DI), and carbon filtration. Purified water undergoes substantial removal of impurities, including dissolved solids, minerals, organic matter, and microbial contaminants.

iii. Water for Injection (WFI): Water for injection is a highly purified water type used in critical pharmaceutical applications, such as parenteral products (injectable drugs) and certain manufacturing processes. WFI undergoes even more stringent purification processes, typically including distillation or methods like ultrafiltration, electro-deionization, or membrane filtration. WFI must meet strict standards, including low endotoxin levels and absence of particulate matter.

iv. Highly Purified Water (HPW): Highly purified water is an advanced water type used in specialized pharmaceutical manufacturing processes, particularly in the production of biopharmaceuticals. HPW undergoes additional purification steps, such as multiple-stage reverse osmosis, ultrafiltration, and advanced oxidation processes, to achieve a higher level of purity. HPW is designed to meet the stringent requirements for critical bioprocessing applications.

v. The selection of the appropriate feed water type depends on the specific requirements of the pharmaceutical manufacturing process, regulatory guidelines, and the desired level of water purity. The feed water is further processed through advanced water treatment systems, including additional filtration, disinfection, and monitoring steps, to ensure it meets the required quality standards for specific pharmaceutical.

Fig.9 Feed Water Sampling Point

2.10.3 Pre-Treated Water

Pre-treated water indicates water that has undergone initial treatment processes but has not yet undergone the comprehensive purification essential for its intended purpose. In the realm of water sampling within the pharmaceutical and biopharmaceutical sector, pre-treated water can serve certain functions. Here's how pre-treated water might be employed in water sampling scenarios:

i. Initial Sampling Phases: Pre-treated water can be employed during the initial phases of the Purified Water System, such as system flushing or purging. Flushing entails directing pre-treated water through the system to displace stagnant water, guaranteeing representative sampling. This step aids in eliminating any deposits or impurities that could have gathered within the system, ensuring that ensuing water samples captured better reflect the actual water quality.

ii. Preliminary Evaluation: Pre-treated water can be utilized for a preliminary assessment of water quality prior to the full purification process. This could involve swift tests or visual examinations to identify visible impurities or contaminants. While pre-treated water might not satisfy final purity requisites, this initial evaluation can help uncover potential issues or points of concern necessitating further scrutiny or purification.

iii. Non-Critical Applications: Pre-treated water might also suffice for non-critical applications or procedures not demanding the utmost water purity. For instance, if the Purified Water System is utilized for non-pharmaceutical objectives like cleaning validation or environmental monitoring, pre-treated water could be employed provided it adheres to specific requirements for those applications.

iv. It's pivotal to acknowledge that the particular use of pre-treated water within the Purified Water System must be assessed based on industry-specific requirements and regulations. The pre-treated water must still meet designated quality standards and specifications to ensure the collected samples accurately mirror the intended purpose. Moreover, any constraints or potential risks associated with pre-treated water use should be meticulously considered, and appropriate measures taken to uphold the precision and validity of the sampling outcomes.

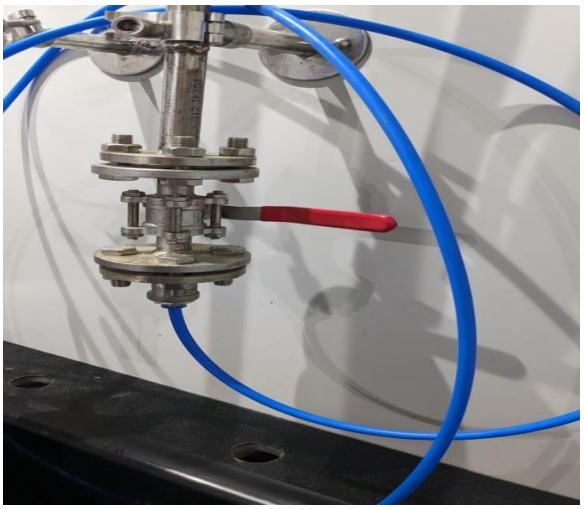


Fig:10 Pre-Treated Water Sampling Point

2.10.4 Purified Water

Purified water finds frequent application within the Purified Water System of the pharmaceutical and biopharmaceutical sector. Here's how purified water is harnessed within the Purified Water System:

i. Collection of Samples: Purified water is employed to collect samples of water that are representative of various stages in the manufacturing process, including water purification systems, storage tanks, distribution loops, and points of use. It serves as the medium for sample collection, preventing the introduction of additional impurities or contaminants that could compromise the accuracy of the sampling outcomes.

ii. Preparation of Sample Containers: Purified water is utilized to cleanse and prepare containers for water samples, such as bottles, vials, or bags, before the actual collection takes place. This step ensures that the containers are devoid of any residues or contaminants that might interfere with the sampling or subsequent analysis.

iii. Dilution of Samples: In specific instances, water samples might require dilution to bring their concentrations within the analytical range of the testing methods. Purified water is enlisted for dilution purposes, guaranteeing that the diluent does not introduce any supplementary impurities or interfere with the analysis of the targeted analytes.

iv. Preservation of Samples: Purified water can also serve for the preservation of samples, particularly when these water samples need to be transported or stored for subsequent analysis. Suitable preservatives, such as acids or chemicals tailored to the specific

analytes, can be added to the samples to uphold their stability during transportation and storage.

v. Preparation of Blanks: Purified water is harnessed for the creation of blank samples, which act as control samples to evaluate potential contamination during the process of sampling and analysis. These blank samples consist of purified water that undergoes the same processing and analysis as the actual water samples but lacks the analytes of interest.

vi. It is crucial to recognize that the quality and purity of the purified water used in the Purified Water System hold paramount importance. The purified water must align with the precise quality standards and specifications outlined for purified water in the pharmaceutical industry. This encompasses criteria for microbial counts, endotoxin levels, chemical impurities, and other pertinent parameters to safeguard the accuracy and dependability of the sampling results. Regular monitoring and validation of the purified water system are imperative to sustain its quality and suitability for the objectives of the Purified Water System

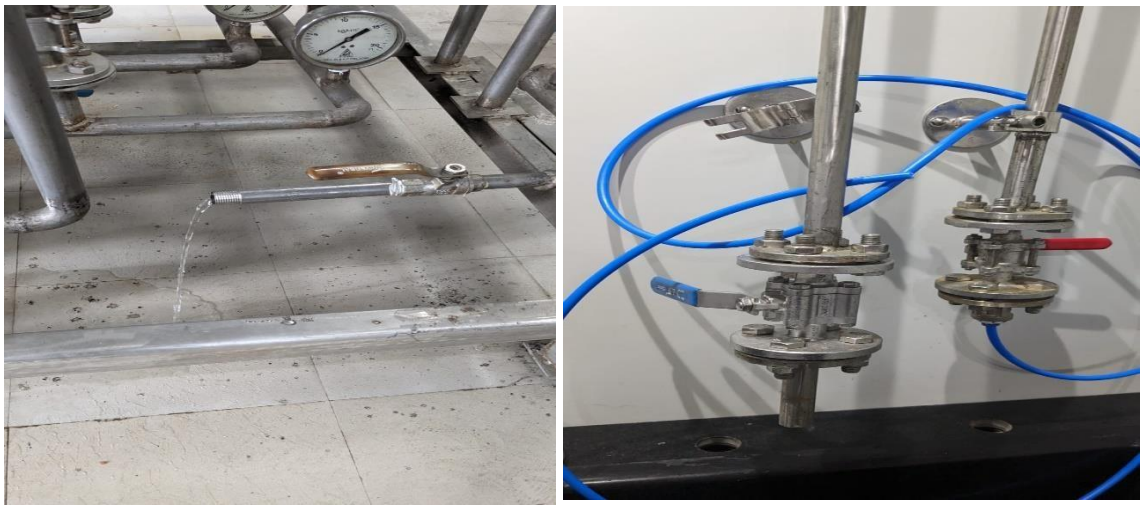


Fig.11 Purified Water Sampling Point

2.11 Water Sampling Techniques

When it comes to Purified Water System, there are several techniques that can be employed to ensure representative and accurate sample collection. The choice of sampling technique depends on the specific objectives of the study, the type of water source, and the parameters of interest. Here are some commonly used sampling techniques in Purified Water System:

2.11.1 Grab Sampling:

This is the simplest and most common technique, where water samples are collected at a specific location and time. A container is submerged into the water, and a sample is taken. Grab sampling is suitable for certain parameters that do not exhibit significant spatial or temporal variability.

2.11.2 Composite Sampling:

In this technique, multiple grab samples are collected at regular intervals over a period of time or distance. The samples are combined to create a composite sample that represents an average of the water quality during that period. Composite sampling is useful for obtaining an integrated representation of water quality over time or when there is expected variability.

2.11.3 Flow Proportional Sampling:

This technique involves collecting water samples based on the flow rate of the water source. The sample collection is synchronized with the flow rate, ensuring that the samples represent the proportion of water passing through the sampling point. Flow proportional sampling is commonly used in studies where flow-related parameters are of interest.

2.11.4 Passive Sampling:

Passive sampling involves the use of specialized devices or sorbents that are placed in the water to accumulate contaminants over a specific period. These devices absorb or adsorb contaminants from the water, allowing for the subsequent analysis of accumulated pollutants. Passive sampling can provide information on long-term exposure and is especially useful for assessing the presence of trace-level contaminants.

2.11.5 Automatic Sampling:

In this technique, automated instruments or samplers are used to collect water samples at pre-determined intervals or in response to specific triggers. These samplers can be programmed to collect samples based on time, flow rate, or other parameters. Automatic sampling is often employed in continuous monitoring or research projects where frequent sampling is required.

2.11.6 Depth Integration Sampling:

When studying water bodies with stratification or vertical variations, depth integration sampling is used. This technique involves collecting discrete samples at different depths, which are then combined in proportion to the volume of water at each depth. Depth integration sampling provides a representative average of the water quality across the vertical profile.

It's important to note that proper sample handling, storage, and preservation techniques should always be followed to maintain sample integrity and prevent contamination during Purified Water System. Additionally, regulatory guidelines and standards should be considered when selecting the appropriate sampling technique for a particular study or application.

3.MATERIALS AND METHODS

After sampling of water sample as per schedule, analysis of water was carried out and results are recorded in respective test sheets as per current applicable specifications for type of water samples. Following tests were performed during different phases of the performance qualification to qualify the PW system.

Purified Water System, Performance Qualification is planned in three phases.

- Phase I: Investigational Phase
- Phase II: Short-range control
- Phase III: Long-standing control

3.1 Phase-I: Investigational phase:

- Phase-I testing was carried out for 14 days (Two weeks).
- Samples from all the sampling points were collected.
- Testing for chemical and microbial analysis were carried out as per defined plan.
- Use and define the standard operating procedures (SOPs) for operation, preservation, purification and troubleshooting.
- Sanitization of Purified Water Generation system were done on weekly basis as per approved SOP's.
- If any abnormal results observed during or after Purified Water System analysis, the Phase-I shall be started again from first day after sanitizing the system.

3.2 Phase-II: Short-Range Control

After completion of successful Phase-I qualification of water system, Phase-II qualification of water system was carried out, this phase was carried out for 14 days (Two weeks). Water can be used for industrial purposes after fruitful completion of Phase-I and Phase-II. Phase-II approach include:

- Monitoring of system while positioning all the refined SOP's after the acceptable completion of Phase-I.
- Demonstrate consistent operation within well-known ranges.
- Demonstrate reliable production and delivery of water of the essential quality
- Define provisional alert and action limits.

3.3 Phase-III: Long-Standing Control

Phase III characteristically runs for one year after the suitable completion of phase-II Water can be used for manufacturing purposes throughout this phase which has the following objectives and features:

- Demonstrate prolonged reliable performance.
- Ensure that periodic or seasonal variations are calculated.
- The sample locations, sampling frequencies and tests shall be compact to the normal routine pattern based on conventional procedures proven during phases I and II.

Table- Parameter Acceptance Criteria for Purified Water

S. No.	**TESTS**	**SPECIFICATIONS**
1.	Description	Clear, colorless and odorless liquid.
2.	Total organic carbon or Oxidisable substances	
	Total organic carbon (mg/L) #	Not more than 0.5 (NMT 500 ppb)
	Oxidisable substances	The solution remains faintly pink
3.	Conductivity	< 1.3 micro-Siemens / cm
4.	Nitrates (ppm)	Not more than 0.2
5.	Aluminum (ppb)*	Not more than 10
6.	Heavy metals (ppm)**	Not more than 0.1
7.	Acidity or alkalinity	On addition of 0.05 ml of methyl red solution, the resulting solution is not red. On addition of 0.1 ml of bromothymol blue solution, the resulting solution is not blue.
8.	Microbial contamination	
	Specified Pathogens	
	Total viable count (cfu/mL)	Not more than 100
	(i) Escherichia coli	Should be absent per 100 mL
	(ii) Staphylococcus aureus	Should be absent per 100 mL
	(iii) Pseudomonas aeruginosa	Should be absent per 100 mL
	(iv) Salmonella sp.	Should be absent per 100 mL
	*(v) Shigella****	Should be absent per 100 mL

3.4 Objective:

To describe the procedure for sampling of Raw Water, Pre-treated Water, Feed-Water and Purified Water.

3.5 Material Requirement:

- SS container
- TOC bottles
- Cleaned water sampling bottles for chemical analysis.
- Sterile water sampling bottles for microbiological analysis
- Marker pen
- Nose mask
- Sterile gloves
- IPA 70% v/v
- 5% w/v sodium thiosulphate
- Heat Resistance/thermal gloves
- Stop Watch (if required)
- Bottle holder

3.6 Precautions

- Before water sampling ensure water is in circulation loop.
- Ensure that the sampling points are properly identified according to sampling plan.
- During sampling of water for TOC analysis don't sanitize the hand with disinfectant (70% IPA).
- After sampling of water for TOC (total organic count) analysis don't keep the vial septa in loose position

it should be in correct position adjacent to the closure.

- After sampling of water for TOC (total organic count) analysis don't keep vial closure in loose position ensure proper tightening of the closure.
- After sampling of water for TOC (total organic count) analysis ensure the correct position of septa and ensure that white surface (silicon) of the septa should always be on upper side.
- For microbiological sampling wear pre-sterilized hand gloves and sanitized the hand prior to sampling with 70% v/v IPA.
- Cleaned and pre-sterilized sampling bottle shall be used for microbiological sampling of water.

- Do not rinse the sample bottle /tubes during sampling for microbiological analysis.

3.7 Following types of water samples are to be collected

- Raw Water
- Pre-treated Water
- Feed Water
- Potable Water
- Purified Water

3.8 Sampling bottles preparation

The size of the sampling bottles shall be decided based on the test requirement.

Sampling bottles for microbiological analysis and chemical analysis use shall be cleaned. Sterilize the sampling bottles for microbiological analysis. Ready to use sterile sampling bottles shall be used for microbiological sampling of water.

Sampling bottles shall be prepared as per water sampling schedule

Ensure that all the bottles shall be properly capped and bottles are segregated as per the requirement i.e. sterilized sampling bottles for microbiological analysis, cleaned bottles for chemical analysis.

For microbiological analysis of Raw water add 0.3 ml of 5% Sodium thiosulphate solution w/ in the sampling bottles before sterilization for removal of chlorine contents.

Note: Prepare 5% w/v sodium thiosulphate, weight 5 gm of sodium thiosulphate and transfer in cleaned dry glass bottle and add 100 mL purified water.

Table 1: Collect sample quantity as per mentioned in the table.

Testing Area	Type of water	Quantity of water sample to be sampled (approx.)
		Microbiological analysis
Quality Control	Raw Water	NLT 200mL
	Pre-treated Water	NLT 200mL
	Feed Water	NLT 200mL
	Potable Water	NLT 200mL
	Purified Water	NLT 200mL

Testing Area	Type of water	Chemical Analysis	Conductivity	TOC
Quality Control	Raw Water	NLT 1000mL	NA	NA
	Pre-treated Water	NLT 900mL	NLT 100mL	NA
	Feed Water	NLT 1000mL	NA	NA
	Potable Water	NLT 1000mL	NA	NA
	Purified Water	NLT 400mL	NLT 100mL	NLT 100mL

3.9 Sampling procedure for microbiological analysis

- Cleaned and pre-sterilized sampling bottle shall be used for microbiological samples.
- Sampling container/accessories shall be carried out to the respective area.
- Wear the sterile gloves before sampling. Sanitize the hands with 70% v/v IPA and allow to dry.
- Label the sampling bottles with permanent marker as per below mention details for microbiological analysis:
- Sampling point
- Sampling date
- Sample time

- Sampled by (Name)

If nozzle/hose pipe is connected to the sampling point, collect the sample with the nozzle/hose pipe.

Note: Sampling should simulate the actual usage of water in production area. Example: Sample collected from hose pipe discharge in manufacturing activity should follow the same method used.

3.10 Water Sampling Points

Table 2: Water sampling points

SAMPLE POINT ID. NO.	SAMPLING POINT AREA NAME	SAMPLING FREQUENCY
OSD-RW-01	Before Raw Water Storage Tank (Raw Water)/Ground floor	Monthly
OSD-FW-02	After RO storage tank and before HSRO-EDI feed water tank / Second floor utility area	Monthly
OSD-PW-01	After EDI system and before Purified Water storage tank /Water room second floor utility area	Monthly Daily
OSD-PW-05	Return loop/Water room second floor utility area (Purified water)	
OSD-PW-07	Coating Room	Monthly
OSD-PW-08	Granulation and Drying Room	Monthly
OSD-PW-09	Shifting, milling and Blending Room	Monthly
OSD-PW-10	Washing area room	Monthly
OSD-PR-02	IPQA and Packing Room	Monthly

- Open the sampling/user point valve until there is a steady stream of water.
- Drain the water for 30 seconds before sampling.
- After draining adjust the valve to maintain the uniform/steady flow for collection of water sample.
- Remove the cap of sampling bottle for sample collection. Do not allow the bottle or water in the bottle to come in contact with the valve.

Note: Do not rinse sampling bottle while collecting sample for microbiological analysis Collect required quantity of water samples for analysis as per Table-I.

- Remove the bottle from the sample stream and place the cap on bottle as soon as possible, tighten the cap securely and close the valve.
- After completion of water sampling keep the water sample in SS container and transmit the sample to site analytical testing laboratory

3.11 Sampling procedure for chemical analysis

- Samples for chemical analysis shall be collected in a clean and dried bottle.
- Label the sampling bottle as per the types of water.
- Open the sampling/user point valve until there is a steady stream of water.
- Open the sampling bottle and hold it under the sampling point (at the end of nozzle/hose if present).
- The sample side of the cap should not come in connection with any surface, including the finger or hands of the individual collecting the sample.
- Rinse the container thoroughly with the water for 2-3 times and then collect the water sample quantity as per mentioned in Table-1.
- Fill the bottle and remove the bottle from the sample stream and place the cap on the bottle as soon as possible and ensure that no air bubble is captured inside the bottle.
- Do not allow the bottle or the water in the bottle to come in contact with the valve.

For TOC analysis, rinse the sampling bottle 2 to 3 time before collect the sample in the separate vials/bottles.

- Collect the sample by over flowing the vials and close with the lid/cap smoothly and immediately.
- For TOC sample should be collected in glass bottle without air gap & and bubble up to bottle neck with tight seal.
- After completion of water sampling keep all samples in sampling kit and transfer to site analytical testing laboratory.

3.12 Sampling frequency

Table 3: Water sampling shall be done as per below pre define frequency

Type of Water	Sampling Frequency	Analysis Lab
Raw Water	Once in Month	Quality Control
Feed Water	Once in Month	Quality Control
Potable Water	Once in Month	Quality Control
Purified Water	1. After storage tank and return loop daily for chemical and microbiological testing. 2. Remaining sample point once in a month on rotational basis for chemical and microbiological testing.	Quality Control

3.13 Alert and Action level

Table 4: Alert and Action level of Purified water, Raw, Feed water and Pre-treated water details are as below:

Viable count limit for water (Raw, Pre-treated, Feed and Purified Water)			
Type of Water	**Total Viable Count (CFU/mL)**		
	Alert Limit	**Action Limit**	**Specification Limit**
Raw Water	250	400	500
Pre-treated Water	250	400	500
Feed Water	250	400	500
Purified Water	50	80	100

If microbial count exceeds from the alert level/ action level / specification limit, same shall be intimated to Engineering / manufacturing / Quality assurance department for necessary corrective action.

3.14 Water Analysis

3.14.1 Purpose

To lay down the procedure for performing sampling and analysis of water i.e. Raw Water, Potable water, Purified water, Process water, Water for injection, Pure Steam, Demineralized water etc. for microbiological parameter.

3.14.2 Definitions

3.14.2.1. Alert Level:

Microbial levels, as outlined in the standard operating procedures, that surpass the specified limits should trigger an investigation to verify the continued control of the process. The alert level is customized for each facility and is determined based on a baseline established through monitoring efforts. These alert levels can be adjusted based on trend analysis conducted during the monitoring program. It's important to note that alert levels consistently remain lower than action levels.

3.14.2.2 Action Level:

Microbiological levels within the controlled environment, as defined by the standard operating procedures, that surpass the specified thresholds should initiate an inquiry and prompt corrective measures determined by the investigation's findings.

3.14.3 Procedure

3.14.3.1 Equipment Used:

- Laminar Air Flow bench
- Incubators
- Colony counter
- Membrane Filtration Assembly installed with 0.45 membrane and vacuum source
- Micropipettes
- Autoclave
- Stop watch

3.14.3.2 Precautionary measures during sampling:

- Protect the samples against contamination.
- The quality of water must not be compromised by the sampling process itself. This applies in particular to ecological contamination.
- The procedure for sampling should simulate the actual usage of water in manufacture area Example: Sampling should be collected from hose pipe release if it relates to user point.
- The bottle for sampling shall be clean, separate bottle shall be used for each sample.
- While collecting water sample for microbiological analysis leave some space.
- Do not leave the bottle uncapped unreasonably during sampling.
- Microbial analysis of the sample shall be started as soon as possible. if it is not possible to perform the analysis within 2 hrs. sample shall be held at refrigerator temperature 2- 8 °C and analyze within 24 hrs. or up to validated hold time.
- In case of sample send to outside (other unit or contract laboratory) hold time should be evaluated against the changing condition and possible impact.
- Special care should be taken for the sampling of the WFI and Pure steam sample, use heat resistance gloves or tongue to hold the bottle during sampling.
  Microbial testing of water can be carried out using pour plate technique or by using membrane filtration technique depending on the type of water.

3.14.3.3 Pour Plate Method:

i. Aseptically pipette out 1 mL of samples in duplicate in sterile petri plates of 90 mm in diameter. The sample dilution shall be prepared with sterile diluent (0.9% Saline / 0.1% peptone water / Buffer sodium chloride peptone /Buffer peptone water) for Raw water
ii. / process water/ Potable water and pretreated water such that the number of colonies on a plate shall be obtained between 30 and 300
iii. Immediately pour 15 to 20 ml of molten R2A agar or Plate count agar having temperature MT 45° C measure the temperature with calibrated IR gun and record in the TDS. Cover the petri plates and mix molten medium by swirling in'8' position or 'arc' position. Take precautions not to splash the medium over the edge of the plate. Allow the contents to solidify at room temperature.
iv. After the plates are solidified incubate the plates at 30- 35°C for 5 days or 20-25°C for 5-7 days at inverted position and document it.
v. For negative control aseptically pour the media in a single sterile Petri plate and Follow

step from 2 to 3 to complete the analysis.

vi. For the test negative control in case of using the diluent for the dilution of the sample pipette out the 1 ml of diluent in to the Petri plate and pour the media and incubate with the analysis plates generally using for the potable/raw water sample as due to higher bioburden.

3.14.3.3 Membrane Filtration Method:

i. Filter required volume of sample through a sterile 0.45 pm, membrane filter, under partial vacuum.

ii. Note: For the 1.0 mL sample quantity reconstruct the sample as 10 mL sample added in to the 90 mL 0.1% w/v peptone solution / Buffered Sodium Chloride Peotone /Buffer peptone water/Normal saline and further filter the 10 mL reconstructed sample quantity equivalent to 1 mL water sample quantity.

iii. Rinse the filtration assembly with 100 mL of sterile dilution water (0.1% w/ peptone solution / Buffered Sodium Chloride Peotone / Buffer peptone water /

iv. Normal saline) by rinsing the filtration portion of the filtration cup. Aseptically place the membrane filter on surface of the pre prepared / pre-incubated R2A agar or Plate count agar, which is validated to have an adequate recovery and growth promotion.

v. Incubate the plates in upright position at 30-350 C for 5 days or 20-25 °C for 5-7 days and document it.

vi. For negative control, Filter 100 mL sterile water through a sterile 0.45 m, membrane filter, under partial vacuum Follow steps 3 to 4 to complete the analysis.

vii. Any changes in the defined testing procedure due to the outcome of method suitability shall be specify in the site procedure because it depends on the source water quality and efficiency of the water purification system

viii. For e.g. change related to the water sample quantity, media, incubation condition etc.

3.14.3.5. Specified organism testing

- Filter 100 mL sample collected for microbiological analysis through a sterile 0.45pm membrane filter, under partial vacuum.
- Rinse the membrane filter with 100 mL of sterile diluent (0. 1% peptone solution/ sterile purified water or BSCP).
- After rinsing, aseptically transfer the membrane fitter into 100 mL of sterile Soyabean Casein Digest Medium and incubate at 30°C to 35°C for 18- 24 hours (solution A) and document it.
- Proceed for further testing for specified microorganisms as per applicable specifications

i.e. E. coli, Salmonella sp, P. aeruginosa, s. aureus, *Shigella* spp. etc.

- Filter 100 mL sterile diluent (0.1% peptone solution / sterile purified water or BSCP) through a sterile 0.45 um, membrane filter, under partial vacuum. Rinse the membrane filter with 100 ml sterile diluent. After rinsing, aseptically transfer the membrane filter in to 100 mL. of sterile Soyabean Casein Digest Medium. Incubate the tube at 30°C to 35°C for 18 - 24 hours this shall be treat as Negative control.
- Note: In case direct inoculation method to be followed for specified microorganisms testing, Double strength enrichment medium or as per specification (Soya bean Casein Digest Medium) shall be prepared and inoculate 100 ml Quantity of sample as per specification in to Soyabean Casein Digest Medium. Incubate at 30°C to 35°C for 18 - 24 hours (solution A) and document it.
- For Negative control take sterile diluent quantity as per specification in to Test tube / bottle of sterile double strength Soyabean Casein Digest Medium specification.
- Incubate the tube at 30°C to 35°C for 18 - 24 hours this shall be treat as Negative control.

3.14.3.5.1 Test for *Escherichia coli*:

- Selection - Mix & transfer 1 ml of solution from the incubated SCDM tube (sol. A) to 100 ml MacConkey broth, and incubate at 42-44°C for 24 to 48 hours. After incubation of 24 to 48 hours by means of an inoculating loop, streak a portion from the MacConkey broth on the surface of MacConkey agar medium, Cover, Reverse the dishes and incubate at 30°- 35° C for 18 to 72 hours.
- Interpretation- Growth of colonies specifies the possible presence of E. coli this is confirmed by identification tests. The sample complies with the test if no colonies are present or if the identification tests are negative.
- Negative control shall be performed the chosen diluent in place of the test preparation as per procedure applicable for test sample. There must be no growth. If growth observed the same shall be investigated as per applicable procedure

3.14.3.5.2 Test for Salmonella sp:

- Selection - Mix & transfer 0.1 mL of solution from the incubated SCDM tube (Solutionto 10 mL of Rappaport Vassiliadis *Salmonella* Enrichment broth and incubate at 30°35° C for 18 to 24 hours.
- After incubation of 18 to 24 hours by means of an inoculating loop, streak a portion from the Rappaport Vassiliadis *Salmonella* Enrichment broth on the surface of XLDA, Cover, invert the dishes and incubate at 30° - 35° C for 18 to 48 hours.
- Interpretation- The possible presence of *Salmonella* is indicated by the growth of well developed, red colonies with or without black center. That will be established by identification test.

- Negative control shall be performed the chosen diluent in place of the test preparation as per procedure appropriate for test sample. There must be no growth. If growth observed the same shall be investigated as per appropriate procedure.

3.14.3.5.3 Test for *Pseudomonas aeruginosa*:

- Selection and subculture- After incubation mix the SCDM tube (Solution A) and streak out a loop-full of solution on the surface of Cetrimide agar plate. Incubate at 30° C - 35° C for 18 to 72 hours.
- Interpretation- Growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests. The sample complies with the test if colonies are not present or if the confirmatory identification tests are negative.
- Negative control shall be performed the chosen diluent in place of the test preparation as per procedure applicable for test sample. There must be no growth. If growth observed the same shall be investigated as per applicable procedure.

3.14.3.5.4 Test for *Staphylococcus aureus*

- Selection and subculture- After incubation mix the SCDM tube (Solution A) and streak out a loop-full of solution on the surface of Mannitol Salt Agar. Incubate at 30° - 35° C for 18-72 hours.
- Interpretation- The possible presence of S. aureus is indicated by the growth of yellow or white colonies surrounded by a yellow zone. That will be confirmed by identification test.
- Negative control shall be performed the chosen diluent in place of the test preparation as per procedure applicable for test sample. There must be no growth. If growth observed the same shall be investigated as per applicable procedure.

3.14.3.5.5 Test for *Shigella* Spp.

- Selection and subculture- Transfer 1ml of Soyabean casein digest medium (Solution A) to 100 mL of GNB & incubate at 30-35°C for 24-48 hours after incubation subculture on a plate of Xylose Lysine Deoxycholate agar medium and incubate plate at 30-35°C for 24-48 hours.
- Interpretation- The possible presence of *Shigella* is indicated by the growth of well developed, red colored translucent colonies, with or without black centers. This is confirmed by identification test.
- Negative control shall be performed the chosen diluent in place of the test preparation as per procedure applicable for test sample. There must be no growth.

3.14.4 Procedure for analysis of Purified Water

3.14.4.1 Safety Precautions

- Minimize exposure to chemicals by using precautions such as wearing a lab coat, appropriate gloves and safety goggles.

3.14.4.2 Equipments:

- Hot air Oven
- Analytical balance
- Water Bath
- UV Spectrophotometer
- Conductivity Meter
- H.P.H.VG. Steam Sterilizer (Double door autoclave)
- Bacteriological Incubator
- Digital Colony Counter
- Desiccator
- Hot Plate
- Magnetic Stir
- TOC Analyze

3.14.4.3 Apparatus and Consumables:

- Volumetric flask class A: 100 mL, 1000Ml
- Pipette, class A: 2 mL ,5 mL, 10 Mi
- Beaker, Class A:20mL,50mL Conical Flask Test tubes
- Measuring Cylinder: 20 mL, 100 mL, 500Ml
- Nessler Cylinder
- Stopper
- Sintered Glass crucible
- Sterile Gloves
- Sterile pipette
- Sterile tube
- Sterile disposable petriplates 90 mm diameter, Make and Model: Himedia

- Hi-flexi loops, Make and model: Himedia or equivalent.
- Parafilm

3.14.4.4 Chemical and Reagents

- Purified water
- Milli-Q water
- Methyl red
- Nitric Acid
- Sodium hydroxide
- Hydrochloric acid
- Glacial acetic acid
- Ammonia solution
- Ammonium acetate
- Potassium chloride
- Diphenylamine
- Sulphuric acid
- Potassium nitrate
- Potassium permanganate
- Barium hydroxide
- Ethanol
- Sodium hydroxide
- 1,4-benzoquinone RS
- Sucrose RS
- Bromothymol Blue
- Ammonium Acetate
- Hydroquinoline
- Citric acid
- Nitrate standard solution
- Aluminium potassium sulphate
- Aluminium standard solution

- Hydroquinoline solution
- Lead nitrat
- Lead standard solution
- Thioacetamide
- Glycerol

3.14.4.5 Total Organic carbon or Oxidisable substances:

3.14.4.5.1 Total Organic Carbon:

Preparation of Reference standard (RS)

- Accurately weigh and transfer 11.9 mg of US Sucrose RS into 200 mL volumetric flask, dissolved and diluted with purified water.Further dilute with 10 ml of stock solution in to 500 mL volumetric to obtained concentration about 1.19 mg/L (0.50 mg/L carbon).

Preparation of system suitability solution:

- Accurately weigh and transfer 15 mg of US 1,4-benzoquinone RS in to 200 mL volumetric flask, dissolved and diluted with purified water, further diluted with 1 mI into 100 mL with purified water to obtained concentration about 0.75 mg/L. (0.50 mg/L carbon).

Reagent water control:

- Use suitable quantity of reagent water obtained at the same time as that used in the preparation of the standard solution and the system suitability solution.

Water Sample:

- Obtain an online or off-line sample that suitably reflects the quality of water used.

Other Control solutions:

- Prepare appropriate reagent blank solutions, and run the appropriate blanks to zero the instrument, if necessary.

System Suitability:

- Test the Reagent water control in the apparatus, and record the response, rw,
- Repeat the test using the Standard solution, and record the response, rs.
- Calculate the corrected Standard Solution response, which is also the limit response, by subtracting the Reagent Water Control response, from the response of the Standard Solution.

- The theoretical limit of 0.50 mg/L of carbon is equal to the corrected Standard Solution response, rs - rw, Test the System Suitability Solution in the apparatus, and record the response rss.
- Calculate the corrected System Suitability Solution response by subtracting the Reagent Water Control response, from the response of the System Suitability Solution rss – rw
- Calculate the percent response efficiency for the System Suitability Solution:

  % response efficiency = 100[(rss - rw)/(rs - rw)]

  Where, rss, is the instrument response to the System Suitability Solution. rw is the instrument response to the Reagent Water Control. rs is the instrument response to the Standard Solution.
- The system is suitable if the percent response efficiency is not less than 85% and not more than 115%.

3.14.4.5.2 By Oxidisable Substances:

Preparation of 0.02M Potassium permanganate

- Accurately weigh and transfer about 320 mg of potassium permanganate into 100 mL volumetric flask.
- Add about 70 mL of milli-Q water, sonicate to dissolve the content and dilute to volume with milli-O water.
- Heat the solution for 1 hour on water bath, allow it to cool and filter through a sintered glass filter.

Preparation of Dilute sulphuric acid:

- Accurately measure 5.5 mL of sulphuric acid into 100 mL of volumetric flask. already containing about 60 mL of milli-Q water. Swirl to mix thoroughly and allow it to cool. Dilute to volume with milli-Q water.

Procedure:

- Accurately measure and transfer about 100 mL of sample into a suitable volumetric flask. Add 10.0 ml of dilute sulphuric acid and 0.1mL of 0.02 M potassium permanganate and boil for 5 minutes. The solution remains faintly pink.

3.14.4.6 Conductivity:

Stage-I:

Measure the conductivity without temperature compensation, recording simultaneously the temperature. Temperature compensated measurement may be performed after suitable validation. The water to be examined meets the requirements if the measured conductivity at the recorded temperature is not greater than the value in the table mentioned below:

Table:4 Requirements of temperature and conductivity**.**

S.No.	Temperature (°C)	Conductivity (µS/cm)
1	0	2.4
2	10	3.6
3	20	4.3
4	25	5.1
5	30	5.4
6	40	6.5
7	50	7.1
8	60	8.1
9	70	9.1
10	75	9.7
11	80	9.7
12	90	9.7
13	100	10.2

1. Determine the temperature of the water and the conductivity of the water with a non-temperature compensated conductivity reading.

2. Using Table 4, find the temperature value that is not more than the measured temperature, i.e. the next lower temperature. The corresponding conductivity value on this table is the limit.

Note: Do not interpolate.

3. If the measured conductivity is not more than the table value determined in step 2, the water meets the requirements of the test for conductivity. If the conductivity is higher than the table value, proceed with Stage-2.

Table 5: Stage 1-Temperature and Conductivity Requirements (For non-temperature compensated conductivity measurements only)

Temperature (°C)	**Conductivity (µS/cm)**
0	0.6
5	0.8
10	0.9
15	1.0
20	1.1
25	1.3
30	1.4
35	1.5
40	1.7
45	1.8
50	1.9
55	2.1
60	2.2
65	2.4
70	2.5
75	2.7
80	2.7
85	2.7
90	2.7
95	2.9
100	3.1

Stage-2

Transfer an adequate volume of water to an appropriate container and agitate the test sample by stirring. Adjust the temperature if needed and maintain it at 25‡ 1°C while vigorously stirring the sample. Observe the conductivity periodically as you agitate the sample. When the change in conductivity (attributed to the absorption of atmospheric carbon dioxide) remains below a net value of 0.1 uS/cm over a 5-minute interval, record the conductivity measurement.

Please note that the conductivity measurements during this stage can either be compensated for temperature at 25°C or uncompensated for temperature. If the conductivity does not exceed 2.1 S/cm, the water meets the conductivity test criteria. In the event that the conductivity exceeds 2.1 uS/cm, proceed to Stage-3.

Stage-3

Conduct this test around 5 minutes after the conductivity determination in step 2 of Stage-2, while keeping the sample temperature at 25±1°C. Introduce a saturated potassium chloride solution into the same water sample (0.3 mL per 100 mL of the test specimen) and assess the pH to the nearest 0.1 pH unit, following the guidance provided in the pH section.

Consult Table-6 to establish the conductivity limit corresponding to the measured pH value. If the conductivity determined in step 1 of Stage-2 is equal to or lower than the value derived from the table in step 1 of Stage-3, the water satisfies the criteria of the conductivity test. However, if either the measured conductivity surpasses this value or the pH falls outside the range of 5.0 to 7.0, the water does not meet the conditions stipulated in the conductivity test.

Table-6 Stage 3 pH and Conductivity Requirements

pH	**Conductivity (µS/cm)**
5.0	4.7
5.1	4.1
5.2	3.6
5.3	3.3
5.4	3
5.5	2.8
5.6	2.6
5.7	2.5
5.8	2.4
5.9	2.4
6.0	2.4
6.1	2.4
6.2	2.5
6.3	2.4
6.4	2.3
6.5	2.2
6.6	2.1
6.7	2.6
6.8	3.1
6.9	3.8
7.0	4.6

3.14.4.7 Nitrates:

Preparation of Diphenylamine solution:

- Accurately weigh and transfer about 100 mg of diphenylamine into a 100 mL of volumetric flask. Add about 70 mL of sulfuric acid, sonicate to liquefy the content and dilute to volume with sulfuric acid.

Note: Store protected from light.

Preparation of Potassium chloride solution (10.0% w/v):

- Accurately weigh and transfer about 10 g of potassium chloride into a 100 ml of volumetric flask. Add about 70 mL of milli-Q water, sonicate to dissolve the content and dilute to volume with milli-O water, Preparation of Nitrate free water:
- Take 100 mL of purified water, add few milligrams of potassium permanganate and barium hydroxide. Distil using distillation apparatus. Reject the first 10 mL and collect the following 50 mL.

Preparation of Nitrate standard solution (100 ppm NO3):

- Accurately weigh and transfer about 815 mg of potassium nitrate into a 500 mL of volumetric flask. Add about 300 mL of milli-Q water, sonicate to dissolve the content and dilute to volume with milli-Q water.
- Accurately pipette and transfer 1.0 mL of above solution into a 10 mL of volumetric flask. Dilute to volume with milli-Q water and mix thoroughly.
- Note: Prepare the solution immediately before use.

Preparation of Nitrate standard solution (10 ppm NO:):

- Accurately pipette and transfer 1.0 mL of nitrate standard solution (100 ppm NO;) into a 10 mL of volumetric flask. Dilute to volume with milli-Q water and mix thoroughly.
- Note: Prepare the solution immediately before use.

Preparation of Nitrate standard solution (2 ppm NOs):

- Accurately pipette and transfer 1.0 mL of nitrate standard solution (10 pm NO3) into a 5 mL of volumetric flask. Dilute to volume with milli-Q water and mix thoroughly.
- Note: Prepare the solution immediately before use.

Procedure:

- Accurately pipette and transfer 5.0 mL of sample in a test tube immersed in iced water. Add 0.4 mL of potassium chloride solution (10% w/v), 0.1 mL of diphenylamine solution and dropwise with shaking, 5 mL of nitrogen free sulfuric acid. Transfer the tube to water bath at 50°C and allow to stand for 15 minutes. Any blue color in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 mL of nitrate-free water and 0.5 mL of nitrate standard solution (2 pm NO;).

3.14.4.8 Aluminum:

Preparation of Acetate buffer solution (pH 6.0):

- Accurately weigh and transfer about 100 g of ammonium acetate into a 500 mLvolumetric flask. Add about 300 mL of milli-Q water and sonicate to dissolve the content. Add 4.1 mL of glacial acetic acid, mix thoroughly and adjust the pH if necessary using ammonia or acetic acid and dilute to volume with milli-Q water.

Preparation of Hydroquinone solution (5 g/L):

- Accurately weigh and transfer about 5 g of Hydroquinone into a 1000 mL of volumetric flask. Add about 800 mL of chloroform, sonicate to dissolve the content and dilute to volume with chloroform.

Preparation of Dilute sulphuric acid:

- Accurately measure 5.5 mL of sulphuric acid into 100 mL of volumetric flask. already containing about 60 mL of milli-Q water. Swirl to mix thoroughly and allow it to cool. Dilute to volume with milli-Q water.

Preparation of Aluminum standard solution (2 pm AI):

- Accurately weigh and transfer about 352 mg of aluminum potassium sulphate into a 100 mL of volumetric flask. Add about 10 mL of dilute sulphuric acid and sonicate to dissolve the content. Dilute to volume with milli-Q water.
- Accurately pipette and transfer 1.0 mL of above solution into 100 mL of volumetric flask. Dilute to volume with milli-Q water and mix thoroughly.

- Note: Prepare the solution immediately before use.

Preparation of sample solution:

- Accurately measured about 400 mL of sample to be examined into a suitable volumetric flask. Add about 10 mL of acetate buffer solution pH 6.0 and 100 mL of milli-Q water. Mix thoroughly.

Preparation of Reference solution:

- Accurately pipette and transfer 2.0 mL of aluminum standard solution (2 ppm Al) into a suitable volumetric flask. Add about 10 mL of acetate buffer solution pH 6.0 and 98 mL of milli-O water. Mix thoroughly.

Preparation of Blank:

- Accurately pipette and transfer 10.0 mL of acetate buffer solution pH 6.0 and 100 mL of milli- Q water into a suitable volumetric flask. Mix thoroughly.

Procedure:

- Place the prescribed solution in a separating funnel and shake with 2 quantities, each of 20 mL and then with one 10 mL quantity of a Hydroquinone solution (5g/L).
- Dilute the combined chloroform solutions to 50 mL with chloroform (sample solution). Measure the intensity of the fluorescence of the sample solution, of the standard solution and of the blank using an excitant beam at 392 m and a secondary filter with a transmission band centered on 518 mm or a monochromatic set to transit at this wavelength.
- Note: Test conducted, if intended for use in the manufacture of dialysis solutions.

3.14.4.9 Acidity or Alkanity:

Preparation of 0.1 M Sodium hydroxide:

- Accurately weigh and transfer about 2 g of sodium hydroxide into a 500 mL of volumetric flask. Add about 300 mL of milli-Q water, sonicate to dissolve the content and dilute to volume with milli-Q water. Mix thoroughly.

Preparation of 0.02 M Sodium hydroxide:

- Accurately weigh and transfer about 400 mg of sodium hydroxide into a 500 mL of volumetric flask. Add about 300 mL of milli-Q water, sonicate to dissolve the content and dilute to volume with milli-Q water. Mix thoroughly.

Preparation of methyl red solution:

- Accurately weigh and transfer about 50 mg of methyl red into a 100 mL of volumetric flask. Add about 1.86 mL of 0.1 M sodium hydroxide and 50 mL of ethanol (95% /v), sonicate to dissolve the content. After the solution is effected, dilute to volume with milli-Q water. Mix thoroughly.

Sensitivity:

- A mixture of 0.1 mL of the above solution, 100 mL of milli-Q water and 0.05 mL. of 0.02 M hydrochloric acid is red. Not more than 0.1 mL of 0.02 M sodium hydroxide is required to change the color to yellow.

Preparation of bromothymol blue solution:

- Accurately weigh and transfer about 50 mg of bromothymol blue into a 100 mL of volumetric flask. Add about 4.0 mL of 0.02 M sodium hydroxide and 20 mL of ethanol (95% v/v), sonicate to dissolve the content. After the solution is effected, dilute to volume with milli-O water. Mix thoroughly.

Sensitivity:

- A mixture of 0.3 mL of the above solution and 100 mL of milli-Q water is yellow, not more than 0.1 mL of 0.02 M sodium hydroxide is required to change the color to blue.

Procedure:

- Accurately pipette and transfer 10.0 mL of sample, freshly boiled and cooled, into a borosilicate glass flask and add 0.05 mL of methyl red solution. The resulting
- solution is not red colored.
- Accurately pipette and transfer 10 mL of sample into a borosilicate glass flask and add

0.1 mL of bromothymol blue solution. The resulting solution is not blue colored.

3.14.4.10 Microbial contamination:

For Total Viable count - Take 10 mL of purified water sample and analyzed as per membrane filtration method. Results shall be reported as observed counts / 10 mL counts/mL.

4. RESULT AND DISCUSSION:

After sampling, analysis of water shall be carried out and results are recorded in respective test sheets.

Note-1: Test will be carried out at sampling points: After EDI system and before Purified water storage tank (OSD-PW-01), After UV of purified water storage tank (OSD-PW-04), Return loop (OSD-PW-05).

Note-2: In-case of failure of test result of Oxidisable substances for any sampling point, test for TOC shall be conducted for respective sampling point for evaluation.

Note-3: Test parameter: Aluminum: If intended for use in manufacture of dialysis solution.

Note-4: Test parameter: Heavy metals: If purified water in bulk comply with the requirements for conductivity prescribed for water for injection in bulk, it is not necessary to carry out the test for heavy metals.

Note-5: Protocol based study for monitoring purpose only. Test will be carried out at sampling points: After UV of purified water storage tank (OSD-PW-04) and Return loop (OSD-PW-05).

Table:7 - Phase III Test results of sampling point OSD-PW-05 in the month of April-2023

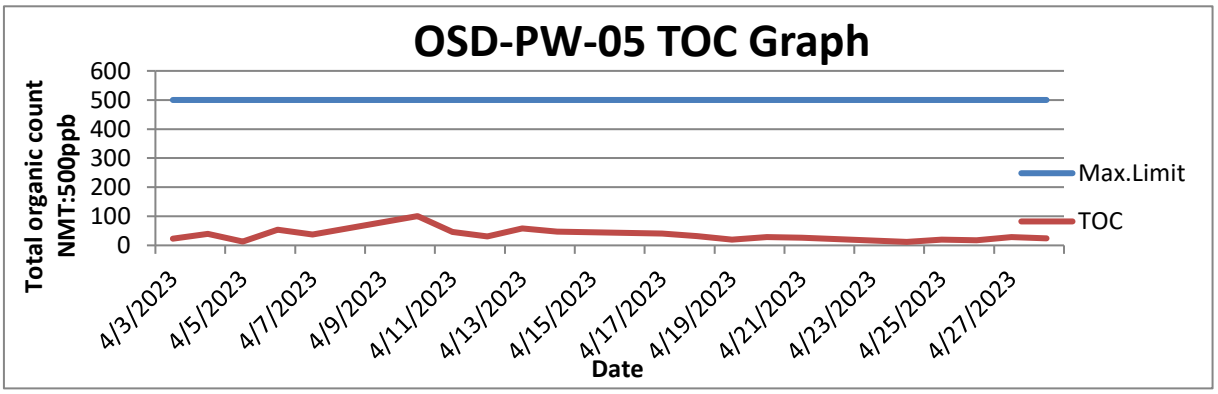
Sampling Point	OSD-PW-05								
No. of sample	Total number of samples is 22 , Number of purified water sample 13								
No.of days	21								
Type of Sample	Purified water								
Test►	Description	Total Organic Carbon	Oxidisable substance	Conductivity	Nitrates	Acidity	Alkalinity	Total viable count	Pathogens : Ecoli, S.aureus, Pseudomonas aeruginisa, Salmonella, *Shigella*
Specification	A Clear, Colorless, Odourless liquid	Not more than 0.5(NMT 500ppb)	Solution remains faintly pink	<1.3µs/cm	NMT 0.2ppm	Solution doesn't turn blue	Solution doesn't turn blue	NMT 100CFU/ml	Should be Absent/100mL
Date▼									
03-04-2023	A Clear, Colorless, Odourless liquid	23.07	Complies	0.887	Complies	Complies	Complies	Absent/mL	Absent/100mL
04-04-2023		39.08	Complies	0.0921	Complies	Complies	Complies	Absent/mL	Absent/100mL
05-04-2023		13.64	Complies	0.833	Complies	Complies	Complies	Absent/mL	Absent/100mL
06-04-2023		53.6	Complies	1.001	Complies	Complies	Complies	Absent/mL	Absent/100mL
07-04-2023		37.31	Complies	0.841	Complies	Complies	Complies	Absent/mL	Absent/100mL
10-04-2023		100.7	Complies	0.94	Complies	Complies	Complies	Absent/mL	Absent/100mL
11-04-2023		46.29	Complies	0.797	Complies	Complies	Complies	Absent/mL	Absent/100mL
12-04-2023		31.17	Complies	0.876	Complies	Complies	Complies	Absent/mL	Absent/100mL
13-04-2023		58.46	Complies	0.887	Complies	Complies	Complies	Absent/mL	Absent/100mL
14-04-2023		47.42	Complies	1.038	Complies	Complies	Complies	Absent/mL	Absent/100mL
17-04-2023		40.88	Complies	0.762	Complies	Complies	Complies	Absent/mL	Absent/100mL
18-04-2023		31.61	Complies	0.908	Complies	Complies	Complies	Absent/mL	Absent/100mL
19-04-2023		19.71	Complies	1.028	Complies	Complies	Complies	Absent/mL	Absent/100mL
20-04-2023		28.32	Complies	1.005	Complies	Complies	Complies	Absent/mL	Absent/100mL
21-04-2023		26.84	Complies	0.868	Complies	Complies	Complies	Absent/mL	Absent/100mL
24-04-2023		11.97	Complies	0.893	Complies	Complies	Complies	Absent/mL	Absent/100mL
25-04-2023		19.98	Complies	882	Complies	Complies	Complies	Absent/mL	Absent/100mL
26-04-2023		17.17	Complies	1.162	Complies	Complies	Complies	Absent/mL	Absent/100mL
27-04-2023		28.25	Complies	1.067	Complies	Complies	Complies	Absent/mL	Absent/100mL
28-04-2023		23.92	Complies	0.903	Complies	Complies	Complies	Absent/mL	Absent/100mL

Remarks:	Purified water sample from sampling point No.: OSD-PW-05 in the month of April from 03-04-2023 to 28-04-2023 is well within specification limit .

Table:8 Phase III Test results of TOC and Conductivity in the month of April 2023

Date	Max.Limit	TOC
03-04-2023	500	23.07
04-04-2023	500	39.08
05-04-2023	500	13.64
06-04-2023	500	53.6
07-04-2023	500	37.31
10-04-2023	500	100.7
11-04-2023	500	46.29
12-04-2023	500	31.17
13-04-2023	500	58.46
14-04-2023	500	47.42
17-04-2023	500	40.88
18-04-2023	500	31.61
19-04-2023	500	19.71
20-04-2023	500	28.32
21-04-2023	500	26.84
24-04-2023	500	11.97
25-04-2023	500	19.98
26-04-2023	500	17.17
27-04-2023	500	28.25
28-04-2023	500	23.92

Date	Max.Limit	Conductivity
03-04-2023	1.3	0.887
04-04-2023	1.3	0.0921
05-04-2023	1.3	0.833
06-04-2023	1.3	1.001
07-04-2023	1.3	0.841
10-04-2023	1.3	0.94
11-04-2023	1.3	0.797
12-04-2023	1.3	0.876
13-04-2023	1.3	0.887
14-04-2023	1.3	1.038
17-04-2023	1.3	0.762
18-04-2023	1.3	0.908
19-04-2023	1.3	1.028
20-04-2023	1.3	1.005
21-04-2023	1.3	0.868
24-04-2023	1.3	0.893
25-04-2023	1.3	0.882
26-04-2023	1.3	1.162
27-04-2023	1.3	1.067
28-04-2023	1.3	0.903



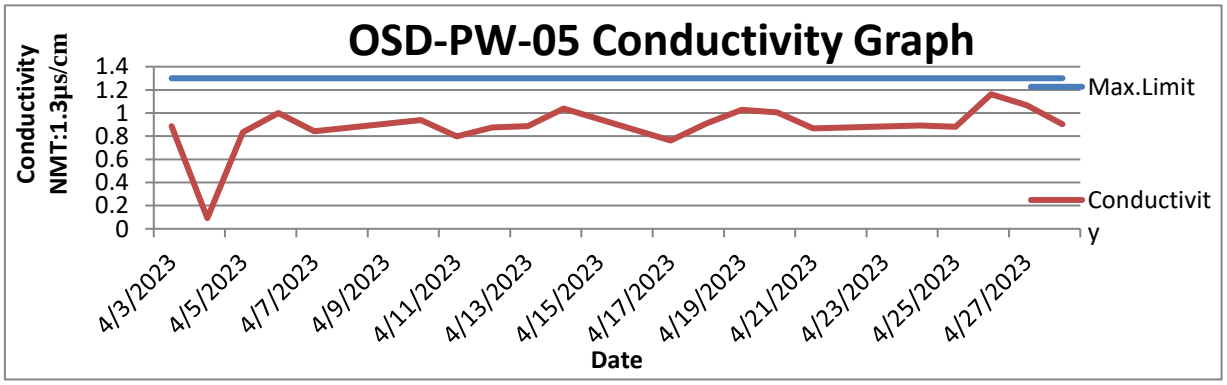


Fig:12 & 13: Total Organic Carbon and Conductivity test of Purified water sample from sampling point No.: OSD-PW-05 in the month of April from 03-04-2023 to 28-04-2023 is well within specification.

4.2 Interpretation of result

- For pour plate method analysis count the number of colonies in each plate by using colony counter after the incubation period.
- Calculate the mean (N) to determine the total viable count per mi. If the Average appears to be in decimal, round up the decimal to next whole Integer value.
- Report as a value in CFU / ml and CFU/100ml (as per requirement).
- For membrane filtration method count the number of colonies on the membrane filter paper after the completion of incubation period.
- For counting of spreader colonies, follow procedure described in procedure applicable for Microbiological test result interpretation.
- If number of colonies in a plate are more than 250 cfu / mL i.e. cannot be counted report the results as TNTC (too numerous to count).
- All the colonies observed in WFI and pure steam samples will be identified to species level.
- No growth should be observed in the negative controls and Test negative control after the completion of incubation period. If growth observed the same shall be investigated as per applicable procedure.

4.3 Test Results of Microbial Contamination

Fig.14 Sample A of MSA *Staphylococcus aureus* without growth

Fig.15 Sample A of MSA *Staphylococcus aureus*

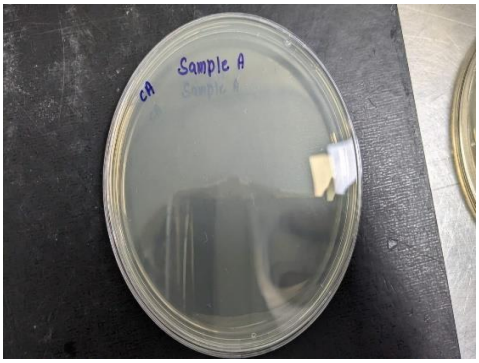


Fig.16 Sample A of MCA *Escherichia coli* without growth

Fig.17 Sample A of MCA *Escherichia coli* with growth

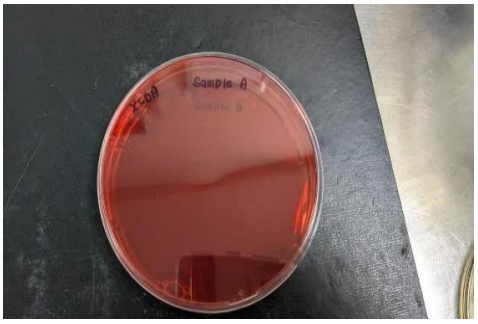


Fig 18: Sample A of XLDA *Salmonella sp.*without growth

Fig 19: Sample A of XLDA *Salmonella sp.*with growth

5.CONCLUSION:

On completion of PQ activity, data generated are collected, compiled, critically reviewed, evaluated Phase-1 & II: Based on water sample result trend review following action was recommended:

- HSRO membrane replacement frequency is defined to achieve consistent quality of purified water as product.
- Cleaning frequency of soft water storage tank.
- To control pH variation in feed water, pH dosing system was installed at RO2 plant of water treatment system.
- The total viable count of specified pathogen was found under specification as no growth was found exceeding the limit.
- PhaseIII: There is no impact on purified water quality during the six-month data review and trending. With additional recommended action implementation, the purified water quality was found complying with specification criteria. The purified water system is capable of producing purified water and performance was found consistent.

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