World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Review Article

Prospective validation for pharmaceutical industry water system

Anirban Rajkhuntia[1], Manoj Kumar Katual[2*], S.L. Harikumar[3]

[1]Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India.
[2]Department of Pharmacy, Rayat-Bahra Institute of Pharmacy, Education City, Hoshiarpur, Punjab.
[3]University School of Pharmaceutical Sciences, Rayat-Bahra University, Mohali, Punjab.

Received: 22-12-2015 / Revised: 25-01-2016 / Accepted: 27-01-2016 / Published: 30-01-2016

ABSTRACT

Purified water (PW) is a key component in the manufacturing of virtually all pharmaceutical products. PW is used in the pharmaceutical industry as a raw material in production or to clean equipment. PW tasteless, colorless, and odorless is often called the universal solvent. It is, therefore, important that the water meets the set standards and constantly provides the specified quality and quantity to ensure there is no contamination of the product or equipment. Depending on quality, raw water can be difficult to purify, and can require various processing stages to obtain PW quality.Pharmaceuticals are depended on the water purification systems due to holding there quality, safety and accuracy. The present context explains various validation techniques to determine that the produced water which is propose to use are suitable for all purpose as per specified in various monograph

Key words: *Bio-film, Industrial Water Validation, Pharmaceutical Water.*

INTRODUCTORY NOTES

Water is essential for industrial, pharmaceutical and hospital purposes, in the preparation and processing of pharmaceuticals and other health products. In the majority of cases, water is an input, which should be incorporated into the product during processing. At other times, even if it is not present in the preparation, it is especially used for cleaning and hygiene purposes. It is recognized that the greatest demand on water is destined for human consumption, its quality being relatively guarantied up to the point where the pipe transportation network terminates. For this reason, every industrial or pharmaceutical plant related to health products must rely on appropriate water purification systems, allowing it to meet its particular requirements, especially as to the problems related to storage and internal distribution. This procedure must guarantee supply according to the volume required and pursuant to the demanded quality consumption points. The purified water system that produces, stores, and circulates water under background conditions is susceptible to the establishment of adhesive biofilms or microorganisms, which can be the source of undesirable levels of viable microorganisms or endotoxins in the effluent water. Recent studies have shown that nearly all large water purification systems can cause the formation of biofilm in the piping. This biofilm can spread microorganisms within the system and contribute to an increase in particles, bacteria, and the level of total organic carbon (TOC). Contamination can affect the whole process in the pharmaceutical industry or hospital environment. These systems require frequent sanitation and microbiological monitoring to ensure water of the appropriate microbiological quality (microbial limit at the points of use) *[United States Pharmacopoeia National Formulary, edition 30, 2007]*.

Overview of pharmaceutical water system:

Types of water used for pharmaceuticals: The different types of water used in the manufacture of drug products are, non-potable, potable (drinkable) water, purified water, water for injection (WFI), sterile water for injection, sterile water for inhalation, bacterio-static water for injection, sterile water for irrigation. The all types of water are followed of an official monograph in the current U.S Pharmacopeia with various specifications for each type. There is no pure water in nature, as it can contain up to 90 possible unacceptable contaminants.The groups of contaminants are; Inorganic and Organic compounds, solids, gases and microorganism.

***Corresponding Author Address:** Manoj Kumar Katual, Department of Pharmacy, Rayat-Bahra Institute of Pharmacy, Education City, Hoshiarpur, Punjab; E-mail: manojkumar.katual@gmail.com*

Table- 1: Comparison of various water uses in Pharmaceuticals:

Types of tests	Purified water	Water for Injection	Sterile Purified water	Drinking water
Description	Clear, colourless liquid; odourless and tasteless	Clear, colourless liquid; odourless and tasteless	Clear, colourless liquid; odourless and tasteless	Colour 5 (Hazen units) maximum, 3 TON (threshold odor number), agreeable taste and Turbidity 5 NTU maximum.
pH	5.0 -7.0	5.0 - 7.0	5.0 - 7.0	6.5-8.5
Alkalinity	Should meet the requirement	Should meet the requirement	Should meet the requirement	Max 200 mg/lit.
Acidity	Should meet the requirement	Should meet the requirement	Should meet the requirement	NA
Ammonium	Maximum 20ppb.	Maximum 10 ppb.	Maximum10 ppb	50 to 200ppb
Calcium and magnesium	Should meet the requirement	Should meet the requirement	Should meet the requirement	300mg/lit(Total hardness)
Heavy metals	Not more than 10 ppb	Not more than 10 ppb	Not more than 10 ppb	50ppb
Chloride	Should meet the requirement	Should meet the requirement	Should meet the requirement	250 mg/lit
Nitrate	0.2 ppm	0.2 ppm	0.2 ppm	10ppm
Sulphate	Should meet the requirement	Should meet the requirements	Should meet the requirement	250 mg/l
Oxidisable substances	Should be meets the requirements	Should be meets the requirements	Should meet the requirement	< 15 mg/l
Total organic carbon	<500ppb	<500ppb	<500ppb	≥1ppm
Conductivity	at 25°c of 1.3 µS/cm	at 25°c of 1.3 µS/cm	at 25°c of 1.3 µS/cm	500 to 800 µS/cm at 25ºC
Residue on evaporation	NMT 0.001%	NMT 0.001%	NMT 0.001%	500 mg/L
Total viable aerobiccount	<100CFU/1ml	<10CFU/100ml	<100CFU/1ml	<500CFU/ml
specified microbs	Absents /100ml	Absents /100ml	Absents /100ml	Absents /100ml
Endotoxin Testing	< 1.0 Endotoxin Unit/ml	< 0.25 Endotoxin Unit per ml.	< 0.25 Endotoxin Unit per ml.	NA

Analytical Test Parameters: Specific analysis includes the following;

a) Chemical Tests:

Qualitative Chemical Tests: Acidity, Alkalinity , Ammonium, Calcium and magnesium character , Carbon dioxide, Heavy metals such as: (Cadmium, Chromium, Copper, Iron, Lead, Nickel), Zinc, Chloride, Nitrate, Sulphate and Oxidisable substances

Quantitative Chemical Tests: Total Organic Carbon, Conductivity, pH, Total Solids, Non-Volatile Solids and Residue on Evaporation

b) Microbial Limit Tests: Total viable count, Test for Pathogens, Bacterial Endotoxin Tests. *[http://www.pharmaceutical-testing.co.uk /Purified% 20Water% 20Analysis.asp]*.

OBJECTIVE OF THE STUDY:

The major problem in maintaining the water purification is biofilms growth; biofilms are a collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface. More than 99 percent of all bacteria live in biofilm communities. Some are beneficial. But biofilms can also cause problems by corroding pipes, clogging water filters, causing rejection of medical implants, and harboring bacteria that contaminate drinking water. So it is very objectionable for pharmaceuticals. *[http://www. edstrom .com/ Resources.cfm? docid=23]***.** We can realize from this condition by supplying chlorinated reverse osmosis water and by maintaining water quality through flushing and sanitization. *[Dreeszen, 1996]***.**Bacteria associated with biofilms are much more difficult to kill and remove from surfaces than planktonic organisms.

Incomplete removal of the biofilm will allow it to quickly return to its equilibrium state, causing a rebound in total plate counts following sanitization. The following figure shows typical regrowth following sanitization. Initially, the bulk water bacteria count dropped to zero after sanitization, but this was followed by a gradual increase in numbers to levels at or below the pretreatment levels. In this example, regrowth started after 2 days and was back up to equilibrium levels after 20 days. This is similar to results seen in in-house sanitization testing at Edstrom Industries.

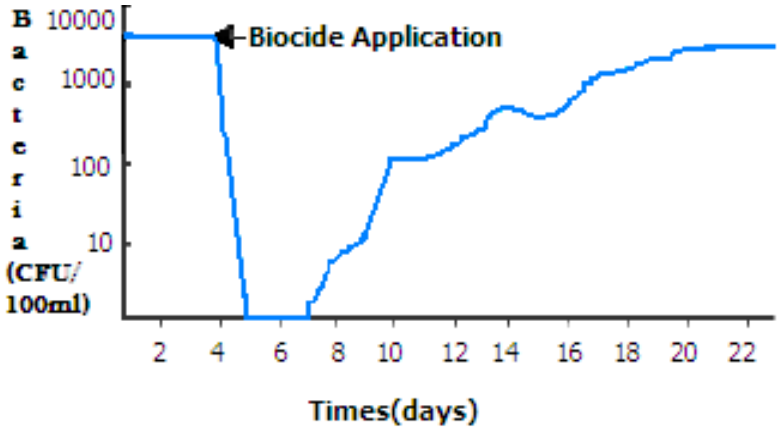

Figure 1: Example of sanitization followed by biofilm recovery (Bacteria count samples were taken on a daily basis). *[Mittelman, 1986]*

Biofilm recovery may be due to one or all of the following.

1. The remaining biofilm contains enough viable organisms that there is no lag phase in regrowth. Thus, biofilm recovery after shock chlorination is faster than initial accumulation on a clean pipe.
2. The residual biofilm on the surface makes it rougher than clean pipe. The roughness of the deposit may provide a stickier surface which adsorbs more microbial cells and other compounds from the water.
3. The chlorine preferentially removes extracellular polymers and not biofilm cells, thus leaving biofilm cells more exposed to the nutrients when chlorination ceases.
4. Surviving organisms rapidly create more slime (extracellular polymers) as a protective response to irritation by chlorine.
5. There is selection for organisms less susceptible to the sanitizing chemical. This is usually the organisms that produce excessive amounts of slime like Pseudomonas. *[Characklis and Marshall, 1990]*

Sanitization Methods: Biofilm can be removed and/or destroyed by physical and chemical treatments. Chemical biocides can be divided into two major groups: oxidizing and nonoxidizing. Physical treatments include mechanical scrubbing and hot water.

Physical Treatments:

Heat: Pharmaceutical Water-for-Injection systems use recirculating hot water loops (greater than 80°C) to kill bacteria. When these systems are used on a continuous basis, planktonic bacteria are killed and biofilm development is reduced *[Mittelman (1986)]*. Biofilms are even found in hot water (80°C). Periodic hot water sanitization can also be used to destroy bacteria in biofilm, but this requires a temperature of 95°C for a period in excess of 100 minutes *[Collentro, 1995]*.

Mechanical removal: Heavy biofilms cannot be removed from storage tank walls by the use of chemicals alone; mechanical scrubbing or scraping, high-pressure spraying, or a combination is also required. Mechanical removal of biofilm from distribution systems is impractical *[Mittelman, 1986]*. For RO system maintenance, we don't routinely scrub storage tanks, but there is usually a continuous low chlorine level in the stored water, so heavy biofilms aren't allowed to develop.

Chemical treatments:

Table- 2: Typical biocide dosage levels.

Biocide	Dosage Level (mg/l)	Contact Time (hours)
Chlorine	50-100	1-2
Ozone	10-50*	<1
Chlorine dioxide	50-100	1-2
Hydrogen peroxide	10%(v/v)	2-3
Iodine	100-200	1-2
Quaternary ammonium compounds	300-1000	2-3
Formaldehyde	1-2%	2-3
Anionic & nonionic surfactants	300-500	3-4

* Ozone dosage is 10-50 mg/l, but the residual levels in water were 1-2 mg/l. *[Mittelman, 1986]*

RATIONALE OF THE STUDY:

Quality and Project Plan: A quality and project plan (QPP) is devised at the beginning of a project. The purpose of this is to establish parameters for quality control (QC), inspections, approval, project execution, organization, responsibility and authorizations. This guarantees that activities are performed according to the requirements set within the agreed framework. It is also useful to write down practical details of project execution that are not dealt with in the URS. This would define:

- How communication is performed.
- How the information will be delivered to the project manager.
- Exceptions to the agreed protocols.
- The document approval process.
- The people responsible for reviewing and approving the documents.
- The number of days assigned to the review process.
- In which form comments must be returned.
- The amount of time allocated for amendments and updates, and how the conclusions and approvals are obtained.

Comments should be specified in writing and compiled in one document clarifying who has commented on what. For fast-track projects, these approval routines are particularly important and must be established at the beginning of the project. It is also recommended that the number of approving parties is kept to a minimum. The user should specify which routine applies to change requests in the project and from when it is applicable. A well-devised QPP, which has been agreed on and signed by both parties, saves time and makes it easier to complete activities such as design, installations and tests. An interface agreement should also be issued early in the project and will clarify details regarding tie-in points, control system interfaces and media. *[Hultqvist, 01 Dec 2007.]*

Validation for water systems consists of three phases;

Phase-1: Duration of this phase is 2-4 weeks (Investigational Phase), development of DQ, IQ and OQ are occurs in this phase. During this phase we develop the operational parameters and cleaning and sanitization procedures and frequencies. Sampling is daily at each point of use, End of Phase I; develop SOPs for the water system

Phase-2: Duration of this phase is 4-5 weeks (verifying control); during this phase demonstrate the system is in control. Same sampling as in phase 1

Phase-3: Duration of this phase is 1 year (verifying long-term control), making of PQ, demonstrate the system in control over a long period of time are done during this phase. Weekly sampling, Microbiological testing will be carried for all the sampling point by covering all the point's ones in a week and Physico-chemical testing will be carried for Point 13(Return loop) in once a week. *[Buckley, http://www.who.int/prequal /training resources / pq_pres/ workshop_RSA/ Water3_0506. ppt%20]*

Prospective Validation for New Pharmaceutical Water Systems:

Prospective validation started with the development of procedures, protocols and related documentation, to straightforward implementation against existing procedures established by the pharmaceuticals. Typically, the service will involve on and off-site work, regular performance reviews and on-going support for Continuous Compliance Assurance (CCA) which ensures the water system is in a continuous state of inspection readiness. *[http://www. honeyman .co.uk/ honeyman_water_system_validation.html]*. The 'life-cycle' of system validation includes;

❖ **Development of Validation Master Plan (VMP) and User Requirement Specification (URS) : User requirement specification:**

The prospective owner of the system creates a user requirement specification (URS). From a practical perspective, and to obtain good traceability, it is important that requirements are clear, well-structured, numbered and testable. It is preferable to assess the installation carefully at the start in the requirements specification. A risk analysis regarding the end product (e.g., water quality) should be performed before compiling the URS. The requirements relating to the safety of plant operators must be part of the risk analysis that occurs for CE marking of the installation, according to the machinery directive *[Hultqvist, 01 Dec 2007.]*. It is an advantage to divide the requirement specification into 'C' and 'Q' requirements with the help of the risk analysis performed. This is described in the ISPE *[ISPE, 2001.]*. C stands for commissioning and the requirement will then be tested under a factory acceptance test (FAT) or a site acceptance test (SAT). Q stands for qualification and the requirement is tested under an installation qualification (IQ) or an operation qualification (OQ). To find and select the typical Q requirements for the system, the ISPE's *[ISPE, 2001.]* and FDA's *[US FDA, 1993.* *http://www.fda.gov/ora/inspect_ref/igs/high.html]* can provide assistance.

❖ **Design Qualification (DQ) :**

During the design phase of the installation, the focus is on existing requirements and catering for them in the design. It is crucial to have an analysis of the incoming water to design the system correctly with the right pretreatment for the application. *[Collentro,1998.]*

Design documents: The following design documents are consulted for a water treatment system:

- piping and instrumentation diagram (P&ID)
- functional specification (FS)
- software design specification
- hardware design specification (HDS)
- electrical schematics
- layout drawing
- component list
- Instrument list
- Valve list.

Design verification: The design is verified in relation to the user's requirements, ensuring they will be complied with. This is easily done by establishing a traceability matrix in table form from the URS (Table 03).

Table -3: A traceability matrix showing in which protocols and tests the requirements

URS	Request number	QC Description	Protocol	Test
URS.2301-01	3.2.1	All instrumental monitoring sites (measurements sites) must be marked with the Tag number.	FAT-50303-01 SAT-50303-04	FAT-03 SAT-02

[Hultqvist, 01 Dec 2007]

Design approval: The design approval is an important milestone in a project as it makes it possible to progress with manufacturing and programming. To reach an approval it is necessary to review all design documents and drawings according to the requirements. *[Hultqvist, 01 Dec 2007]*

Installation Qualification (IQ): A protocol when completed ensures that the installation will meet the requirements of the design; this is performed by a number of different verifications, such as mechanical inspections, instrument calibrations and documentation verifications. It is recommended to include a review of the FAT/SAT reports at the start of the IQ to ensure that all deviations have been closed. The sequence of test performances also needs to be considered. The slope of the pipes must, for example, be measured before the distribution pipe is insulated in the case of a hot distribution system which often occurs before the IQ is started because the installation is ready. Documentation verification is a test where the status must be checked according to the project schedule on the IQ precisely, otherwise the IQ test could be open until both IQ and OQ are ready and the final documentation has been copied. A good way of performing document inspections is to have a document schedule clearly indicating which documents must be completed by when in the project. When the IQ is finished and reviewed, the result is presented in the IQ report and, if no critical deviations were identified, the OQ can begin.

Performance Qualification (PQ): To define the method and conduct chemical and microbiological testing for the PQ1, PQ2 and introductory PQ3 phases. The chemical and microbial quality of Purified waters, which are most widely used excipients, diluents, or solvents used in pharmaceutical manufacturing, is gauged only by a small number of generalized parameters

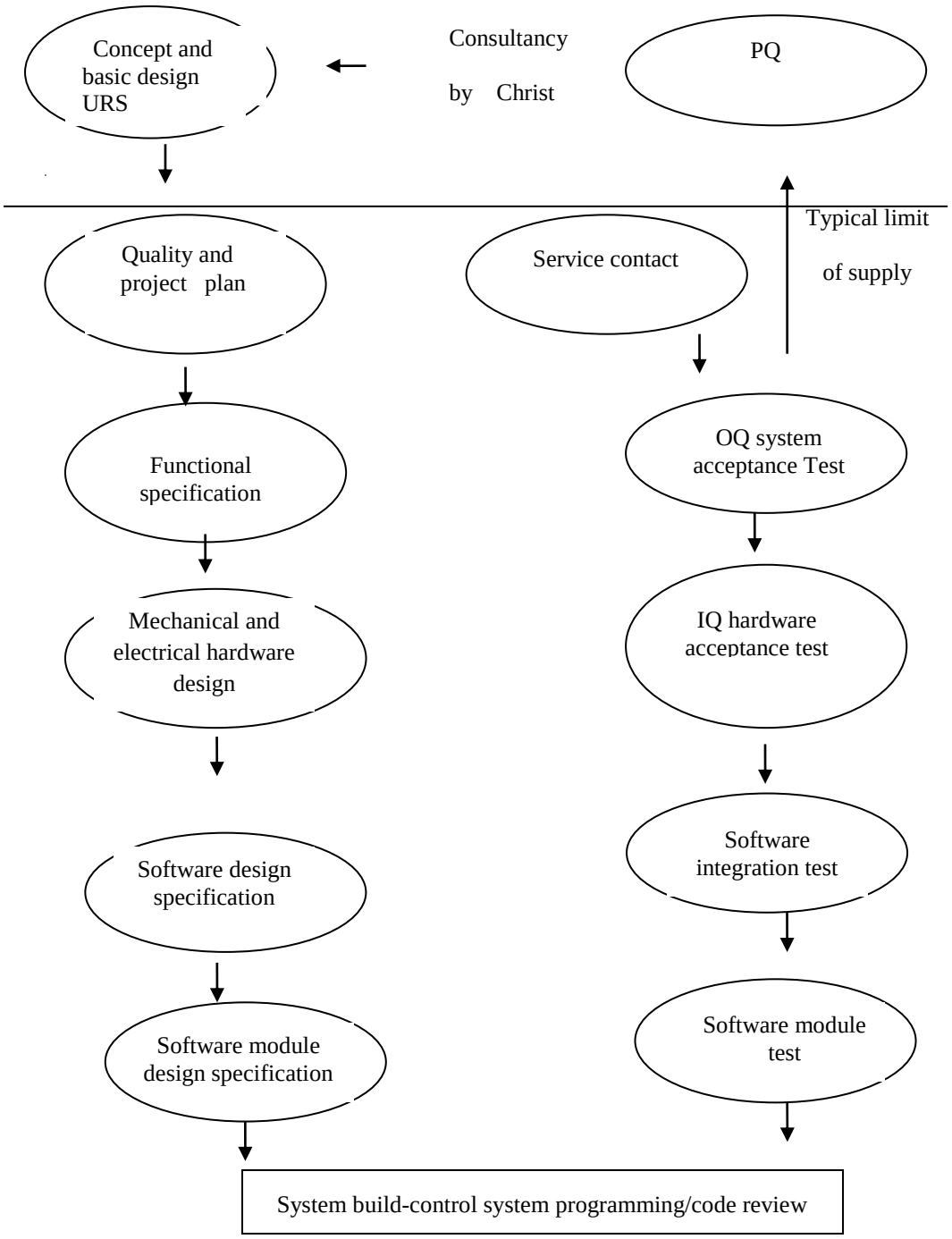

Figure 02: The verification of the system according to the design. *[Hultqvist, 01 Dec 2007.]*

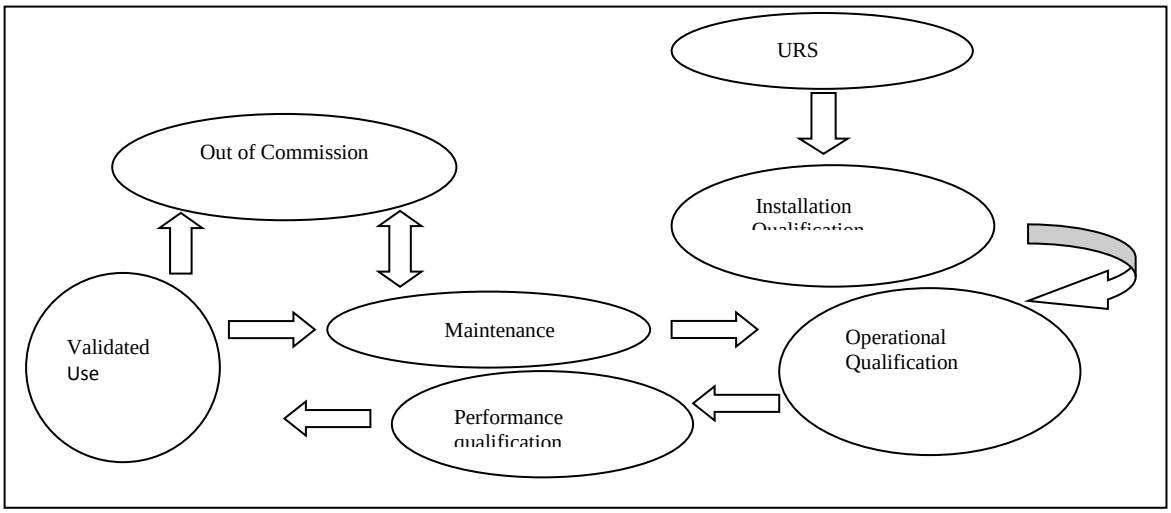

Figure -3: The relationship between the stage of qualification testing and validated use. *[http://www.cmscientific.co.uk/calibration_and_validation/validation]*

The above simplistic schematic of the qualification program shows the relationship between the stage of qualification testing and validated use.

Operational Qualification (OQ): A protocol when completed defines that the built system fulfils the URS and the system design intent, the OQ will verify the operation of the system according to the descriptions in the FS highlighted as critical for the product. The acceptance criteria, particularly for the OQ, must be carefully evaluated, which conductivity and temperature must be complied with the followings attributes:

i) Which flow
ii) What are the actual limits
iii) What is acceptable for the process and the product

The product requirements depend on the water quality that the system has been designed to achieve. The process engineer should also have evaluated suitable alert and action levels for the process, which form the basis for the alarms generated by the system. When all tests are performed and reviewed, the result of the OQ is presented in the OQ report. If no critical deviations were identified, the PQ can start. *[Hultqvist, 01 Dec 2007.]*

Retrospective Validation for Existing Pharmaceutical Water Systems: A Retro-validation exercise to determine the condition of the system. The industry is required to maintain water systems in a validated state, operating under proper procedures with full water system documentation, maintenance and management control that is supported by an appropriate monitoring regime. There are occasions; especially if there have been system modifications or alterations, when it is necessary to carry out a retrospective validation. Unlike generic validation houses, which lack industry specific knowledge and assess the water system against today's standards and criteria (often resulting in unnecessary system re-build and expense), the approach is to evaluate the system according to its age and design principles and retrospectively validate only to critical parameters. *[http://www.honeyman. co.uk/honeyman_water_system_ validation.html]*

On-Going Monitoring Regimes: This is also called Continuous Compliance Assurance or CCA, thus ensuring that the Purified Water / Water for Injection system are capable of audit at any time.

Re-Validation: The production of protocols and the re-validation of the system ensure that any changes made to the system are captured and that the system is inspection ready.

Documentation for the water treatment system: Test procedures should be written in a way that is complete, understandable and possible to repeat. With all qualifications, it is important to collect all relevant data, make clear references to documents used, mark attachments and review performed tests regarding completeness, traceability and signatures. It is fundamental that the structure of the documentation must be: Logical, trackable, simple and clear.

Change Control: Inauguration of a change control procedure to ensure compliance of any system change.

REGULATORY REQUIREMENTS FOR PHARMACEUTICAL WATER SYSTEMS: All pharmaceutical water systems delivering Purified Water, Water Highly Purified or Water for Injection must be validated to demonstrate that they meet, and will continue to meet, their quality specification as laid down in the monographs of the relevant pharmacopoeias (USP*[US Pharmacopoeia, Edition 30, 2007]* .There are also other requirements that do not stem from the product quality, but concern operator safety, including European directives 98/37/EC (Machinery*) [Directive98/37/EC of the European Parliament and of the Council of 22 June 1998 on the Approximation of the Laws of the Member States Relating to Machinery.*

Table-4: Testing-setting specifications for purified water or WFI in various Pharmacopeias:

Type of Test	Ph.Eur.	JP	USP	Int.Ph.
pH	5.0-7.0	5.0-7.0	5.0-7.0	Pass test
Cl	< 0.5mg/L	Pass test	-	Pass test
SO4	Pass test	Pass test	-	Pass test
NH4	< 0.2mg/L	< 0.05mg/L	-	Pass test
Ca/Mg	Pass test	-	-	Pass test
Nitrates	< 0.2ppm	Pass test	-	Pass test
Nitrites	-	Pass test	-	-
Conductivity (µS/cm)	-	-	< 1.3	-
Oxidizable subs.	Pass test	Pass test	-	Pass test
Solids (ppm)	< 10	< 10	-	NMT 10
TOC (ppm)	-	< 0.5	< 0.5	-
Heavy metals	-	-	-	Pass test
CO_2	-	-	-	Pass test
Total Viable Count	NMT 100CFU/ml for PW & 10 CFU/100ml for WFI	NMT 100CFU/ml for PW & 10 CFU/ 100ml for WFI	NMT 100CFU/ml for PW & 10 CFU /100ml for WFI	NMT 100CFU/ml for PW & 10 CFU/100ml for WFI
Pathogens	Absent/100ml	Absent/100ml	Absent/100ml	Absent/100ml
Endotoxin level	0.25 EU/ml for WFI	0.25 EU/ml for WFI	0.25 EU/ml for WFI	0.25 EU/ml for WFI

[Buckley, ftp://ftp.who.int/medicines/GMP/gmptrainsuplmt/Water02.ppt]

Pigmentation Test:

1. Streak representative suspect colonies from agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluorescein and pseudomonas agar medium for detection of pyocyanin contained in Petri dishes.
2. Cover and invert the inoculated media and incubate at 33°C to 37°C for not less than 3 days.
3. Examine the streaked surfaces under ultra-violet light.
4. Examine the plates to determine whether colonies conforming to the description in Table: 05 are present.

Table-5: Tests for *Pseudomonas aeruginosa*

Medium	Characteristic colonial morphology	Fluorescence in UV light	Oxidase test	Gram stain
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for detection of fluorescein	Generally colorless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for detection of Pyocyanin	Generally greenish	Blue	Positive	Negative rods

[Indian Pharmacopoeia, fourth edition, 1996.]

Test for Staphylococcus aureus:

1. Subculture a loopful of the enrichment culture on a plate of Baird – Parker Agar medium.
2. Inoculated with a loop full Staphylococcus aureus into Baird Parker Agar, used as positive control.
3. Randomly selects a Baird Parker Agar broth medium used as negative control.
4. Incubate all the plates at 35°C -37 °C for 18-72 h.
5. If any black colonies of gram-positive cocci surrounded by a clear zone indicate the presence of S. aureus.
6. Confirmation may be effected by suitable biochemical tests such as the coagulase test.
7. The product passes the test if colonies of the type described do not appear on Baird-Parker agar medium or if the confirmatory biochemical tests are negative. *[European Pharmacopoeia, Edition 6, 2008]*.

Table-6: Study by Qualitative Detection of Microorganisms.

S.No.	Name of the media	Test Organism	Incubation Condition
1	Baird Parker Agar	*Staphylococcus aureus*	35°C -37°C for 18-24 hours
2	Buffered sodium chloride Peptone Water	*Escherichia coli*	35°C -37°C for 18-24 hours
3	Cetrimied Agar	*Pseudomonas aeruginosa*	36°C -38°C for 18-24 hours
4	Deoxycholate Citrate agar	*Salmonella abony*	36°C -38°C for 18-24 hours
5	Mac Conkey Broth	*Escherichia coli*	43°C -45°C for 18-24 hours
6	Peptone water	*Escherichia coli*	37°C for 18-24 hours
7	Soyabean Casein Digest Broth	*Staphylococcus aureus* *Escherichia coli* *Bacillus subtlis* *Pseudomonas aeruginosa*	36°C -37°C for 24-48 hours
		Aspregillus niger *Candida albicans*	20°C -25°C for 48 hours-5 days
8	Tetrathionate Brillant Green Bile Broth	*Salmonella abony*	36°C -38°C for 48 hours
9	Triple Sugar Iron Agar	*Salmonella abony*	36°C -38°C for 18-24 hours
10	Pseudomonas Agar for Fluoroscein	*Pseudomonas aeruginosa*	36°C -38°C for 18-24 hours
11	Pseudomonas Agar for Pyocyanin	*Pseudomonas aeruginosa*	36°C -38°C for 72 hours
12	Xylose Lysine Deoxycholate Agar	*Salmonella abony*	36°C -38°C for 18-24 hours

Table -7: Study by Quantitative Detection of Microorganisms.

S.N.	Name of the media	Test Organism	Incubation Condition
1	R2A Agar	*Staphylococcus aureus* *Escherichia coli* *Bacillus subtlis* *Pseudomonas aeruginosa*	36°C -37°C for 24-48 hours
		Aspregillus niger *Candida albicans*	20°C -25°C for 48 hours-5 days

Sterility Check of Prepared Media:

A negative control is performed without inoculating the specific microorganism and incubated in specific condition. There must be no growth of micro-organisms. *[European Pharmacopoeia, Edition 6, 2008]*.

RESULT AND DISCUSSION:

By analyzing thirteen points of water system (in phase-1, phase-2and ongoing phase-3), we found that the all result are complies with in the following limits (the actual result are not disclosed due to Confidential matter of the industry), in table-8 and table -9.

Table-8: Acceptance criteria of various tests.

Types of tests	Acceptance criteria
Description	Clear, colourless liquid; odourless and tasteless.
pH	5.0- 7.0.
Alkalinity	The resulting solution is not blue.
Acidity	The resulting solution is not red.
Ammonium	The test solution is not more intensely coloured than a standard solution.
Calcium and Magnesium	Resultant solution should produce a pure blue colour.
Heavy Metals	Not more than 0.1 ppm
Chloride	The appearance of the test solution does not change for at least 15 min.
Nitrate	0.2 ppm.
Sulphate	The appearance of test the solution does not change for at least 1 hour.
Oxidisable substances.	The solution remains faintly pink.
Total Organic Carbon	Maximum 0.5 mg/l or <500ppb.
Conductivity	Conductivity at 25°c of 1.3 µS/cm.
Residue on evaporation	The residue weighs not more than 0.001%.
Total Viable Aerobic Count	<10,000CFU/100mL.
Test for Pathogens	Absents /100mL.

Table-9: Suggested bacterial limits (CFU /mL) in various points in water purified systems.

Sampling location	Target CFU /mL	Alert CFU /Ml	Action CFU /mL
Raw water	200	300	500
Post multimedia filter	100	300	500
Post softener	100	300	500
Post activated carbon filter	50	300	500
Feed to RO	20	200	500
RO permeate	10	50	100
Points of Use	1	10	100

[Buckley, ftp://ftp.who.int/medicines/GMP/gmptrainsuplmt/Water 02.ppt]

The water quality can be controlled by the industry as following;

In point 1, two multimedia filters parallel to each other offers a highly efficient removal of suspended fragmented matter from the water. The three layers of media (sand, anthracite and quartz) are selected in accordance with their particular size, specific gravity, and ability to trap particles of specific size ranges (≥ 10 micro). As the water flows downwards through the bed, it finds a layer of media with decreasing porous ness/permeability so that successively smaller particles are trapped in each layer, providing depth filtration. So by this process foreign particles are removed from the water. In point 2, two water softeners of an alternated sodium resin which remove hard minerals from the water. Ion exchange water softening exchanges the calcium and magnesium cations from water with an equivalent number of sodium cations. By this process hardness of the water can be reduced. In point 3, one filter of activated carbon is used to remove chlorine, chloramines, and dissolved organic substances from the water. Carbon filters are frequently used as the pretreatment to osmosis membranes and ion exchange resins, avoiding damage by oxidant substances, such as chlorine. By this process dissolved organic and inorganic matter can be reduced. In point 4, One 5.0 micro polyethylene microporous depth screen filter is often used ahead of other water purification operations, such as deionization, and reverse osmosis, as a polishing filter for removing resin, carbon fine colloids, and microorganisms. It also helps to removed objectionable material. In point 5, Reverse osmosis (RO) is the finest filtration available. The natural process of osmosis occurs when a solution with different concentrations of salts is separated by a semi-permeable membrane. As osmotic pressure drives the water through the membrane, the water dilutes more concentrated solutions, until equilibrium is achieved. The permeate pure water is collected on the downstream side of the membrane. Reverse osmosis removes 90%-99% of particles, colloids, bacteria, pyrogens, dissolved organic and inorganic substances greater than 200–300 molecular weight (MW) range or larger than the membrane's pore size of 150 to 200 angstroms. The conductivity of the water at the inlet is 150 µS and at the outlet is 5 µS. This process can reject bacteria, metals, protozoa, salts and viruses, and

can remove particles as small as ions. The membrane's shelf life is between 2 and 3 years and sanitation is carried out through the association of peracetic acid with a hydrogen peroxide solution. At this point conductivity measurement is not applicable because of conductivity is completely controlled by Electrical deionization system in Point 7. In Point 6, one continuous deionization column that removes dissolved minerals and salts, as well as some dissolved organic matter, from the water stream crossing ion exchange resins. The ion exchange operation removes positively charged cations, such as calcium, magnesium, and sodium from the water by cation exchange resins, which are replaced by hydrogen ions. Negatively charged anions such as chloride, nitrate, and silica are removed from the water by strong based anion exchange resins, and hydroxide ions then form water molecules. The water stream passes through a mixed bed of cation and anion exchange resins, which produces a very high quality of water with a resistance of up to 1.3 μS/cm (18.3 mega ohm-cm) at 25°C. Point 7, is for Ozone treatment to kills bacteria and viruses on contact and kills algae, mold and yeast spores. Due to this it is act is a strong disinfectant. This system removes excess iron, manganese and sulfur; removes color and odor. In point 8, Ultraviolet Light ($\lambda = 254$ nm) is used as a final step in the treatment for the purpose of preventing the growth of microorganisms, and reducing total organic carbon (TOC). This system also help to reduce the excess ozone added in previous system. In point 9, three 0.05 μm filters are set parallel to each other. Microporous filters are used to remove particles, and bacteria, ranging from 0.05 to 0.5 μm contaminants, which would not ordinarily be removed by depth filtration. Points of use 10, 11, and 12, every point of use is provided with 3 filters of 0.05 am set parallel to each other. From those points, the purified water for consumption is provided by a loop of distribution and is used for the cleaning of critical devices (membrane oxygenates and PVC tubes), the washing of semi critical areas, the preparation of chemical solutions, and the culture bacteriological media.

SUMMARY:

The main focus when validating water treatment systems should be on the requirements the water must comply with. This relates to parameters that control the current water quality, such as: conductivity, total oxidizable carbon (TOC), microbiological values and the presence of contaminants, including endotoxins, nitrates and heavy metals. A risk assessment for the system should be created based on these parameters, and the process steps and components required producing the desired quality need to be evaluated. The design of the water purification system should then be assessed and the appropriate inspections and tests developed. A thorough knowledge of the process is required to perform optimum qualification. Good communication and a comprehensive understanding of the requirements at the planning phase will guarantee a successful project and a water treatment system that performs well.

CONCLUDATORY COMMENTS:

Validation of water system is a part of quality control of water for pharmaceutical use. The water used in pharmaceutical industries should be periodically analyzed as a preventive measure against the spreading of microorganisms and controlling of other contaminations, allowing measures of improvement to be taken rapidly, as required. From the result of validation of water purification system we can come in this dissension that the system is suitable for purification of water, use in pharmaceuticals for any purpose, this due to continues circulation of water through the entire systems. If any result comes in outside that indicates the problems in water system, the necessary action should be taken immediately to keep the system in hand, otherwise it will hardly fall it effect in entire industry.

ACKNOWLEDGEMENT

The authors are extremely thankful to all the Faculty members/staffs of Dept. of Pharmacy, Annamalai University, Tamilnadu, Rayat-Bahra Institute of Pharmacy, Hoshiarpur, Punjab & USPS, Rayat-Bahra University, Mohali, and Punjab for their much needed help and for developing this work. The guidance and support received from all the members who contributed to this work, was vital for the success of the review. The authors are grateful for their constant support and help.

REFERENCES

1. ASME Codes and Standards. ASME Boiler and Pressure Vessel Code, (ASME, 2004) Section 8, Division 1, ASME Codes and Standards. ASME Bioprocessing Equipment (ASME, 2002) p 84.
2. Baron, EJ .; Janda, JM.;Schreckenberger, PC.; Wong, JD.“ Algorithms for identification of aerobic gram-negative bacteria”. In: Murray PR., editor. Manual of Clinical Micro biology. 7. Washington, American Society for Microbiology; 1999. pp. 438–452.
3. Baseline Guide, Volume 4, Water and Steam Systems (ISPE, 2001), p 228.
4. Baseline Guide, Volume 5, Commissioning and Qualification (ISPE, 2001), p 142.
5. Buckley.D.Richard,“Inspecting water treatment”, http://www.who.int/prequal/training resource/pq_pres/workshop_RSA/Water3_0506.ppt%20.
6. Buckley.D.Richard, “Inspection of water treatment system in pharmaceutical industry”,ftp://ftp. who.int/ medicines/GMP/gmptrainsuplmt /Water02.ppt.
7. Characklis, W.G.; Marshall, K.C. eds. “Biofilms”, John Wiley & Sons, Inc., New York, 1990.
8. Collentro. W.C., “Microbial Control in Purified Water Systems - Case Histories”, Ultrapure Water 12(3), April 1995. pp. 30-38.
9. Collentro. W.V., “Pharmaceutical Water System Design, Operation, and Validation” (Informa Healthcare USA, 1998) p 694.
10. Demadis.K. D.; Mavredaki .E.; Stathoulopoulou .A.; Neofotistou .E.; Mantzaridis .C., “Industrial Water System : problems, challenges and solutions for the process industries.”,23 january2006.pp38-46, www.desline.com/articoli/8606.pdf.
11. Directive 2006/95/EC of the European Parliament and of the Council of 12 December 2006 on the Harmonization of the Laws of Member States Relating to Electrical Equipment Designed for Use Within Certain Voltage Limits. http://ec.europa.eu /enterprise/electr_equipment/lv/direct/text.htm.
12. Directive 89/336/EC on the Approximation of the Laws of the Member States Relating to Electromagnetic compatibility, 2004. http://ec.europa. eu/enterprise/electrequipment/emc / directiv/text. html
13. Directive 97/23/EC of the European Parliament and the Council of 29 May 1997 on the Approximation of the Laws of the Member States Concerning Pressure Equipment. http://ec.europa.eu/enterprise/new approach /legislation/directives/table1.htm
14. Directive 98/37/EC of the European Parliament and of the Council of 22 June 1998 on the Approximation of the Laws of the Member States Relating to Machinery. http://europa. Eu/scadplus/leg/en/lvb/l21006a.htm
15. Dreeszen, P.H., “Microbiological Survey of Automated Watering Systems”, Edstrom Industries Inc. Document #D209, December 1996.
16. European Pharmacopoeia Edition 6 (EDQM.226.avenue de Colmar BP 907, F-67029 Strasbourg, France, 2008) p.167.
17. European Pharmacopoeia, Edition 6 (EDQM.226. avenue de Colmar BP 907, F-67029 Strasbourg, France, 2008) pp.173-177.
18. European Pharmacopoeia, Edition 6, EDQM.226. avenue de Colmar BP 907, F-67029 Strasbourg, France, 2008.pp.3209-3215.
19. FDA Current Good Manufacturing Practice regulations, Federal Register, Vol.43, No. 190-Sept. 29, 1978, I. General Comments and Subpart C, Para. 211.48.
20. Guide to Inspections of High Purity Water Systems (US FDA, 1993), http:// www.fda.gov/ora/inspect_ ref/igs/high.html.
21. Hultqvist. A.,“Practical guidelines for qualifying purified water systems”, Pharmaceutical Technology Europe, 01 Dec 2007. http://www. sabesp.com.br
22. http://http//:www.us.filter.com (Visited on January 25, 2008).
23. http://www.pharmaceutical-testing.co.uk/Purified%20Water%20Analysis.asp
24. http;//www.app1.fda.moph.go.th/drug/zone_gmp/files/GMP2548_2/Sep2105/P2/5.M&W.pdf.
25. http://en.wikipedia.org/wiki/Total_Organic_Carbon.
26. http;//www.app1.fda.moph.go.th/drug/zone_gmp/files/GMP2548_2/Sep2105/P2/5.M&W.pdf.
27. http://www.sgs.com/water_testing_system.pdf.
28. http://www.water-research.net/ph.htm.
29. http://www.freedrinkingwater.com/water-education/quality-water-ph.htm.
30. http://www.water-research.net/hardness.htm#test .
31. http://www.lenntech.com/sulfates.htm#Other%20problems%20caused%20by%20sulfate.
32. http://www.lenntech.com/Periodic-chart-elements/Pb-en.htm.