Shewhart Control Chart in Microbiological Quality Control of Purified Water and its Use in Quantitative Risk Evaluation

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Abstract

Water in pharmaceutical industry is a key ingredient in many industrial processes, including machine washing and preparation of most medicinal products. The process of regular monitoring for the microbiological quality of water at the points of use is a critical aspect in ensuring the compliance to the limit. However, the full value of the microbiological control could be accomplished by the use of control charts and statistical quality control to show the trend and behavior of bioburden. In addition, Shewhart charts provide insight for prediction of the future of the water quality. In the current study, purified water samples were analyzed microbiologically during a two-year period. The generated data were continuously interpreted using statistical software packages. The trend indicated that two regions in Shewhart chart were in the out-of-control state at these periods generating either out-of-trend (OOT) and/or out-of-specification (OOS). Investigations showed that these deteriorations in the performance were chronologically coincided with unusual rise in the microbial count of city water, although there was no direct correlation between the input and output water microbiologically. The rate of failure of product purified water was determined to be $7.69 \times 10^{-4}$ and $P_{pk}$ was 1.06 which were unsatisfactory performance. However, by removing the excursion periods due to the expected extraneous causes from the I-MR chart, a 337 times increase in the process performance was estimated with $P_{pk}$ index of 1.53 and quantitative performance failure risk (QPR) equals $2.28 \times 10^{-6}$. This finding highlighted the criticality of the quality of the feed city water and its impact on the final purified water. Thus, the processing stages in the water purification plant are not absolute barriers against microbial creeping into the distribution system, and the purification system of water has a certain tolerance limit after which an outbreak of microbial population occurs and hence compromising the system efficacy and performance. From the present study, it is recommended to design protective pretreatment system that buffers severe fluctuations in raw water quality so that water station possesses the ability to work optimally in an attempt of delivering safe water for medicinal and pharmaceutical manufacturing purposes.

1 Introduction

Purified water that is used for pharmaceutical production is a crucial component in several industrial steps, including bulk manufacturing of medicinal drugs. According to European Agency for the Evaluation of Medicinal Products\footnote{1}, the application of purified water in non-sterile manufacturing covering many preparations that are administered through several routes of administration in addition to isolation and purification of active pharmaceutical ingredients (APIs) during their processing. Machine cleaning, initial and terminal rinses, including Clean-In-Place (CIP) and cleaning and sanitization of clean rooms are conducted using water. In addition, water is the most commonly applied material, raw substance or starting ingredient in the production, processing and formulation of medicinal products such as granulation, tablet coating and as an ingredient in the formulation prior to non-sterile lyophilization. Regular monitoring of source water...
and treated water must be performed for chemical and microbiological contaminant. Hence, the efficacy of water purification, storage and distribution systems should be monitored regularly. Quality control results, trended data and any events such as corrective and preventive actions should be analyzed and recorded. Indeed, purified water (PW) is frequently used as an ingredient in the manufacturing of official products and in many medicinal applications; therefore, it must achieve the compendial chemical purity quality and safeguards must be ensured against microbial proliferation. PW is prepared using potable water as income raw water and is purified using processing stages that include deionization, distillation, ion exchange, reverse osmosis, filtration or other known techniques or technologies, which necessitates the qualification of PW systems. Production, storage and circulation of water under ambient conditions in PW systems are susceptible to the development and establishment of coherent biofilms of microorganisms, which can be the origin of excursions in counts of viable microorganisms in the output water. Therefore, these systems require frequent disinfection and microbiological quality control monitoring in order to ensure delivering water of appropriate microbiological quality at the application point.

Implementation of statistical and analytical tools to monitor the pharmaceutical water processing stations is very critical to control them and makes any improvements. Six sigma is an important tool and well known and proven method for improving processes. Application of such important tool allowed for achieving quality that meets customer requirements that is simultaneously integrated with the “benefit” for the stakeholders. Interestingly, six sigma is applicable in all industries and services branches in addition to its broad acceptance on capital and labor markets. The use of Six Sigma tools allows for achieving balance between quality and cost.

The aim of the current study was the investigation of the final purified water quality during long term inspection through approximately 14 months of monitoring. In addition, the state of control and its level was determined, and the points of defects investigated. Moreover, if the excursions could be related to definite special cause variations, then reassessment would be conducted to find the impact of omitting such an assignable cause of the water station performance.

2 Materials and Methods

Before application of statistical processing on gathered results, analysis was made to verify normality of data distribution. If data did not pass a normality test, they were subjected to transformation procedure to approach Gaussian distribution. New water treatment plant was installed in pharmaceutical facility for production of purified water. This water is used mainly in medicinal oral solid dosage forms manufacturing clean class D area (granulation, blending, washing, coating solutions preparation and coating machines rooms). Since samples were collected on a weekly (excluding shutdown times) basis, I-MR chart type was selected. I-MR chart is a good graphical method of displaying the process performance based on continuous data measurement with subgroup of size one. Average transformed results of nine points of use of purified water samples was interpreted as it was more convenient for analysis and provided a better view for the overall performance under the same conditions of sampling. According to Minitab Stat Guide: Individual-Moving Range (I-MR) chart is composed of 2 charts; Individuals (I) chart plus Moving Range (MR) chart. The I chart allows investigators to track the process level (observe common and special cause variation through drifts in the trends) and the MR chart allows to track process variation (assessing process stability). First examination of the MR chart was performed, which tells whether the process variation was in control. If the MR figure showed out of control state, the control limits on the I chart will be imprecise. On other words, this means any absence of control in the I graph may be because of unsteady but not actual changes in the process mean. If the MR chart is in the state of control, it can be concluded that an out-of-control in I chart is due to changes in the process center. However, if the data were normally distributed, then any out of control situation would be noticed on both I chart and MR chart simultaneously. So either chart can be used to check if the process is in control or not.

Results that do not pass Minitab's tests are checked with a red marks on the diagram. A measure of potential process capability ($C_{pk}$) and an indicator of total process capability ($P_{pk}$) benchmark value of 1.33 was used as a reference value in many industries. Upper Specification Limit (USL) was taken at 10,000 CFU/100 ml (four in log10 scale) as stated by USP but there was no Lower Specification Limit (LSL). As the microbial count decreases, the water quality is getting better even at zero CFU/100 ml. All procedures, statistical analysis, limits and criteria were established as those described by Eissa et al., 2015.

A normal probability plot is an important mean to determine the degree of normality of data distribution. A p-value lower than 0.05 indicates the data is not normally distributed. Output results that is not normally distributed can’t be processed further in the capability assessment. Transformation of non-normal data, or determination and elimination of the cause of the non- normality are methods to obtain normally distributed results. The better option is to improve the process so that the data is normally distributed. In order to measure the efficacy of purified water quality microbiologically, throughput yield was used (TPY) from which a quantitative risk of failure in the quality of water station performance could be calculated.

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and denoted by quantitative performance failure risk (QPR). Throughput yield is based on the defects. It uses DPU (Defects per unit) for its calculation. Thus, DPU = Total number of defective items/Total items and TPY = e^{DPU}. Hence, the QPR = 1-e^{DPU} and the increase in its value indicates deterioration in microbiological water quality with the consequent increase of the adverse impact on the product bioburden. Statistical analysis was conducted using GraphPad Prism® v6.01 for Windows and control charts and six sigma analysis were performed using Minitab® v17.1.0 while other calculations were carried out using Microsoft office excel 2007.

3 Results and Discussions

Although rapid microbiological methods (RMM) proved to be critical tools in microbiological quality control - especially in situations where microorganisms are stressed and/or viable - but-not-culturable (VBNCl) due to hostile environment, e.g. long exposure to residual chlorine in city water - yet conventional methods are still widely used and accepted. Hence, it is not surprising that the bioburden of waters is still evaluated on the basis of the number of CFUs in a fixed sample volume tested. Microbiological testing of drinking water (potable water), which is used as the main source water (feed water) in pharmaceutical manufacturing industries, is subject to the National Primary Drinking Water Regulations (NPDWR) issued by the EPA. For pharmaceutical-grade waters, there is no specific official standard recovery method; however, the USP recommends, in the informational Chapter <1231>, the recovery techniques that are detailed in other references. Interestingly, performing a transformation process of the original raw output results to a new different data that is normally distributed is a pivotal initial step in assessing the quality of water. These transformations are useful for (a) achieving normality of measured results, (b) meeting the assumption of equal sample variances required in certain tests, and (c) satisfying the hypothesis of additivity of effects in certain tests as stated by Juran et al., 1974. Water is the most commonly consumed component in pharmaceutical manufacturing, and the basic ingredient required for machines and instruments washing and cleaning. However, it is hard to provide proper control on the microbiological quality of water obtained from either municipal or non-municipal sources which could by reflected on the final grade pharmaceutical water. There are numerous types of microorganisms in pharmaceutical water with notably that Gram-negative bacteria are the most abundant. Interestingly, several different types of microbes can overcome water treatment barriers and are found in pharmaceutical waters. It is subsequently, not astonishing that legitimate control and observation of feed water as crude materials are significant to guarantee acceptable quality of output water. Statistical analysis in table 1 showed that the process of transformation converted results from non-normally distributed to Gaussian-distributed data, and these are illustrated as Normal Quantile (Q-Q) Plot in Fig. 1. Box plot diagram showed the difference in profile between both transformed and non-transformed data with the former one showing two outliers values (asterisks) related to the two excursions in the control chart. Histogram in Fig. 2 showed that 75% of samples counts were between 41 CFU/100 ml to 646 CFU/100 ml, 21% of samples ≤16 CFU/100 ml and 4% of samples ≥1622 CFU/100 ml. Capability plot diagram showed that both overall and within the process, capability met the specification yet Pp value was below the acceptance criteria and this is evident in Fig. 3.

Although there was no correlation between microbial count in city (raw) water and purified loop water yet Fig. 4 demonstrated that out-of-control conditions no.1 in I-MR chart were almost chronologically synchronized with two abnormal out-of-trend water count of the city water.

Table 1: Statistical analysis showing the effect of logarithm to the base ten transformation of the raw data from non-normally distributed results to exhibit bell curve one (Generated using GraphPad Prism® v6.01 for Windows)

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Raw Data</th>
<th>Transformed Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>25% Percentile</td>
<td>26</td>
<td>1.4</td>
</tr>
<tr>
<td>Median</td>
<td>53</td>
<td>1.7</td>
</tr>
<tr>
<td>75% Percentile</td>
<td>176</td>
<td>2.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>10360</td>
<td>4.0</td>
</tr>
<tr>
<td>10% Percentile</td>
<td>6.0</td>
<td>0.85</td>
</tr>
<tr>
<td>90% Percentile</td>
<td>437</td>
<td>2.6</td>
</tr>
<tr>
<td>Mean</td>
<td>273</td>
<td>1.8</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1119</td>
<td>0.70</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>107</td>
<td>0.067</td>
</tr>
<tr>
<td>Lower 95% CI of mean</td>
<td>60</td>
<td>1.6</td>
</tr>
<tr>
<td>Upper 95% CI of mean</td>
<td>485</td>
<td>1.9</td>
</tr>
<tr>
<td>Lower 95% CI of median</td>
<td>41</td>
<td>1.6</td>
</tr>
<tr>
<td>Upper 95% CI of median</td>
<td>85</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Shapiro-Wilk normality test

| W         | 0.20 | 0.99 |
| P value   | < 0.0001 | 0.2942 |
| Passed normality test (alpha=0.05)? | No | Yes |
| P value summary | **** | ns |
| Skewness  | 7.9  | 0.071 |
| Kurtosis  | 67   | 0.62  |

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Fig. 5 illustrates the relation between seasonal temperature variation with microbial count in the distribution loop. There was some degree of agreement between both temperature fluctuation and microbial count change with time if extraneous excursion regions were ignored, although there was no direct statistical correlation was found between them. By omitting out-of-control regions from the control chart, Fig. 6 was obtained, which showed better performance by about 337.1 from the original shewchart with QPR of 0.000228% and 0.076858% respectively. Significant improvement of the performance was evident by $P_{pk} = 1.53$. Interestingly, the control chart showed another pattern of out-of-control condition, although less in magnitude yet it requires further investigation to elucidate its origin. However, at this level the major defects of output water apparently came from raw water of uncontrolled quality. Moreover, control charts are necessary in determining the quality control specifications that should be updated regularly.

Because alert and action levels should be based on historical data, it is a not uncommon practice to reestablish and recalculate these figures on a periodical basis. Actually, industrious assessment and presentation of, and instant response to, results gathered are pivotal aspects of a functional management platform for water systems. There is no true agreement on the best approach of setting up alert and action levels according to the trended results of analysis. On the other hand, PDA Technical Report No. 13, Fundamentals of an Environmental Monitoring Program gave some ways to deal with decision of alarm and action limits. Finally, the method applied for microbial count transformation proved to be useful and effective in statistical analysis and quality control interpretation of data as discussed by other researchers.

Fig 1: Boxplot diagram of raw and log$_{10}$ transformed microbiological results of purified loop water showing the distribution pattern of upper and lower whisker, interquartile range box and the distribution of outliers.

Fig 2: Histogram showing the distribution of transformed microbial counts among purified water samples.
Fig 3: Net performance of purified water treatment station measured through the average microbiological quality of points-of-use samples.

Fig 4: Synchronized transformed microbial distribution of raw city water (blue) and purified loop water (red). General trend of each is shown in black line.
Fig 5: Chronological relation diagram between seasonal temperature variation and transformed averaged microbial count of the distribution loop water.

Fig 6: Significant improvement in the key performance measurement parameters after removing microbiological excursions in purified water that was chronologically coincided with that of the raw city water.
4 Conclusions

The processing stages in the water purification plant are not absolute barriers against microbial creeping into the distribution system, and the purification system of water has a certain tolerance limit after which an outbreak of microbial population occurs and hence compromising the system efficacy and performance. The findings of study recommended to design protective pretreatment system that buffers sever fluctuations in raw water quality so that water station possesses the ability to work optimally in an attempt of delivering safe water for medicinal and Pharmaceutical manufacturing purposes.

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6 Competing interests

MEE declares no competing interests.

7 Author’s contributions

MEE conceived idea, planned, performed research and wrote the manuscript.

8 References

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