

Development, Verification and Evaluation of Container Closure Integrity Assessment of Prefilled Syringes Using Fluorescence Spectroflurometer

Deepak D. Kayande^{1*} Shripad Deshpande¹, Mazahar Farooqui², Parag Deshmukh³

¹ S.B.E.S. College of science, Aurangabad, Maharashtra, India-431001.

² Maulana Azad College, Aurangabad, Maharashtra India-431004.

³ ICICI knowledge park Shameerpet, Hyderbad, 500078 India.

Abstract

The preference of Prefilled syringes is increased over all the vials as container closure systems for liquid injections, when facilitated or self-administration is required. However, prefilled syringes are more complex compared to container closure system (CCS) consisting of vial, rubber stopper and flip off. Container closure integrity assurance and verification has been a specific challenge for prefilled syringes as they feature several sealing areas. A comprehensive understanding of the container closure system is necessary for an appropriate container closure integrity assessment as well as for packaging development and qualification.

Method for the measurement of container closure integrity (CCI) of prefilled syringes using fluorescence spectrophotometry was developed and validated with a spectroflurometer. Methylene blue solution was initially evaluated as the fluorophore in a syringe with excitation at 605 nm and emission at 678 nm, which generated a limit of detection of 0.06 µg/mL. Further studies were conducted using Rhodamine 123, a dye with stronger fluorescence. Using 482 nm excitation and 527 nm emission, the dye in the syringe could be easily detected at levels as low as 0.001 µg/mL. The relative standard deviation for six measurements of three different samples with different concentration was less than 2.8%. A number of operational parameters were optimized, including the photomultiplier tube voltage, excitation, and emission slit widths. The specificity of container closure integrity was checked by using marketed drug products sample, which showed no interference to the rhodamine detection. Results obtained from this study demonstrated that using rhodamine 123 for container closure integrity testing with in syringe fluorescence measurements significantly enhanced the sensitivity and robustness of the testing and effectively overcame limitations of the traditional methylene blue method with visual or UV-visible absorption detection.

Keyword: Prefilled Syringes, Container Closure System, Methylene Blue, Fluorescence-UV Detection.

Introduction

Prefilled glass syringes have been increasingly used for delivery of parenteral drugs and biological products (1, 2). Prefilled syringes function as a primary packaging component that provides protection and maintains efficacy and product sterility prior to use. Development of drug product using such syringes, and testing to demonstrate the sterile product packaging integrity, must follow regulatory agency requirements (3, 4). The U.S. Food and Drug Administration (FDA) requests the use of the USP sterility test as a part of the stability protocol for sterile products, with testing at initial release and at the stability end point (5). The FDA further provided guidance for industry to use container closure integrity testing (CCIT) as an alternative to sterility testing, performed throughout the product shelf life. In the USP guidance, it is recommended to perform integrity testing at three phases throughout the life cycle of the sterile product: initial development of the product packaging, routine manufacturing, and shelf life stability assessment (6). Many physical or chemical methodologies have been proposed and described for CCIT (7–9). More detailed

research and development work on CCIT has been published, including pressure/vacuum decay (10 –13), trace gas permeation/leak tests, dye ingress tests, electrical conductivity and capacitance tests, and microbial challenge or immersion tests. These methods exhibit many advantages compared to conventional USP sterility testing in demonstrating the potential for product contamination over the product shelf life.

Among the many physical or chemical testing methodologies, dye ingress testing is the most commonly used method for CCIT. Dye ingress testing historically uses methylene blue dye. Besides a vacuum vessel, it does not require special instruments or technology. Detection is typically based on visual observation. A failure is determined when the dye is observed in the container, which proves ingress. This method is simple, inexpensive, widely accepted by industry and health authorities, and recommended by most compendia. However, the dye ingress method is a limit test and not a quantitative approach. Traditional dye methods are also generally not as sensitive as some of the methods mentioned earlier using modern

Technologies:

UV-visible spectrophotometry has been applied to the detection of dye ingress for CCIT in order to overcome the limitations of visual detection. UV-vis spectrophotometric detection is more robust and typically offers lower detection limits in comparison with visual analysis. UV-vis spectrophotometric detection is more robust and typically offers lower detection limits in comparison with visual analysis. However, significant challenges were encountered in our laboratories when applying methylene blue dye immersion with UV-Vis spectrophotometric detection to drug product prefilled glass syringes. Due to the small diameter of the syringe (6.4 mm internal diameter) and possible low concentration of the dye intruded, direct spectrophotometric scanning of the intact syringe could not detect a signal comparable to that seen in glass vials and a similar detection limit could not be reached. An alternative method was evaluated in which the sample solution in the syringe was transferred to a cuvette to increase the effective pathlength. While the measurement in the cuvette improved sensitivity, the transfer procedure was labour-intensive and required multiple extra steps to reduce the potential for contamination, which increased the complexity, variability, and false-positive risk of the CCIT measurement. Consequently, this approach was not desirable for routine use.

In this study, fluorescence spectrophotometry was evaluated for developing a sensitive and robust method for the dye ingress CCIT of prefilled glass syringes. The evaluations include optimization of operational parameters, comparison with visual and UV-Vis detection, specificity, linearity, limit of detection and precision with actual drug products. The objectives were to enable the fluorescence measurement of the unopened prefilled syringes after dye immersion with liquid transfer, develop a sensitive method with a better limit of detection compared to visual and UV-Vis methods, and to simplify the testing procedure to fit the needs for quality control (QC) and stability studies.

Materials and Methods

The study used prefilled glass syringes that have a staked needle with a rubber needle shield was taken from the vendor of IKP. Methylene blue (3,7-bis(dimethylamino)phenazathioniumchloride), was purchased from Sigma Aldrich (St. Louis, MO). Rhodamine 123, (2-(6-amino-3-imino-3H-xanthen-9-yl) benzoic acid methyl ester), Bio-Reagent, was also purchased from Sigma Aldrich. For assessment of the specificity of the fluorescence method, several injectable drug products were purchased in assistance with IKP. The reagents, chemicals and marketed samples were used from IKP.

Detection of Dye Ingress by UV-Vis Spectrometric Method:

UV-vis has been adopted for the detection of dye ingress in CCIT of glass vials. However, the conventional spectrometric dye method for CCIT of vials was shown not to be robust for a QC environment when applied to syringes. Two approaches were evaluated



using UV-vis spectrometry for the measurement of the dye ingress in prefilled glass syringe: (1) direct scan through the syringe barrel, and (2) analysis after transferring the liquid in the syringe into a cuvette. Results from the scan through the barrel showed significant noise that was 10 times higher than that from the scan through the cuvette. The use of the cuvette for the UV-vis measurement of dye ingress improves the sensitivity, but it does have a significant drawback in that the sample solution needs to be transferred to the cuvette from the syringe after the dye immersion treatment. Thorough washing of the syringe with the needle shield attached is challenging. Some post-rinse samples had evidence of dye in the shield housing, and the dye occasionally transferred to the syringe tip as the needle shield was removed, resulting in dye carry-over and contamination during the solution transfer to the cuvette, making the procedure problematic for use in a QC environment.

UV-Vis testing:

An aliquot of the testing medium was used as a negative control. A series of solutions of methylene blue in WFI at different concentrations was prepared for determination of the method sensitivity. UV-vis spectrophotometer (Shimadzu UV-2600) was used for the UV-vis measurement at 635 nm. The samples were measured either through a 5 mm quartz cuvette. The testing results were statistically evaluated. The limit of detection (LOD) for the UV-vis measurement was determined based on the baseline variation. The LOD for the UV-vis method was set as being equivalent to the signal from the concentration of dye in the syringe that resulted from the 5 μ m capillary breach. The syringe sample treated by dye immersion was analysed after transferring the liquid in the syringe into the cuvette. For the dye immersion-treated syringe samples, the syringe was subjected to six rinse cycles using fresh deionized water for each cycle. To transfer the liquid in the syringe into the cuvette, the syringe needle shield was carefully removed, and the first several drops of the liquid in the needle were discarded to avoid dye carryover. A CCIT failure was identified when the detected absorption value was greater than that of the LOD.

Development of a Fluorescence based method:

Fluorescence spectrometry was considered for the development of a sensitive and robust method for the dye ingress CCIT of syringes because it generally has higher sensitivity and selectivity in comparison to UV-vis. It could enable the in situ measurement of prefilled syringes after dye immersion without liquid transfer, simplify the testing procedure to fit QC

needs, as well as provide a better LOD and robustness compared to the visual method and the UV-vis method.

A Fluorescence spectrophotometer from Agilent Technologies was used for the detection of the dye ingress in the prefilled glass syringes. It has the following wavelength ranges: excitation 200~900 nm and emission 200~900 nm. The wavelength accuracy and resolution were ± 1.5 nm. The data collection and processing were performed by using Scanwin lab software. Both methylene blue and rhodamine 123 were evaluated as fluorescence indicators for dye ingress CCIT of prefilled syringes. Key operational parameters were optimized including the voltage of the photomultiplier tube (PMT), standard voltage settings of high (800 V), medium (600 V), and low (400 V) and the width and shape of the excitation and emission slits (1.5, 2.5, 5, 10, and 20 mm rectangular; 10 mm round).

Rhodamine for Dye Ingress Testing:

Further studies were performed using rhodamine 123, a dye widely used in biological applications with stronger fluorescence. Rhodamine 123 has a molar extinction coefficient of UV-vis absorption that is similar to methylene blue. However, the fluorescence quantum yield of rhodamine is 30 times that of methylene blue. Greater sensitivity is thus expected from using rhodamine 123 for dye ingress detection. The intensity of the fluorescence band at 525 nm is significantly higher than the methylene blue band at 678 nm for equivalent concentration levels. This result shows that rhodamine 123 in the syringe can be easily detected by fluorescence as low as 0.001 ug/mL without optimization. The detected rhodamine 123 fluorescence signal

was more intense than methylene blue under the same measurement condition (PMT voltage: 600 V; excitation slit: 5; emission slit: 5).

Optimization of Instrument Parameters:

The Fluorescence spectrophotometer allows selection of a number of operational parameters. The sensitivity of fluorescence detection for rhodamine 123 in prefilled syringes was further enhanced by optimization of the key instrument parameters, including the voltage of the PMT, and the width and shape of the excitation and emission slits. Results showed the fluorescence signal increases with the increased PMT voltage. However, the noise also increases, and the S/N becomes worse than at 800 V. Furthermore, the dynamic range of the recorded signal becomes narrower when a higher photomultiplier voltage is used, and the detector is easily saturated. Development trial shows that use of 800 V voltage setting is the optimal for these measurements on this specific type of fluorometer. When the PMT voltage was fixed, and the bigger the excitation and emission slits used, the higher the S/N obtained. In addition, the measurements using a round shape excitation slit (10 R) generated a better S/N than those using the rectangular excitation slit. However, the measurements using a round shape emission slit (10 R) generated a much poorer S/N than that using rectangular shape emission slit. Results showed that a suitable measurement was obtained using PMT voltage: high; excitation slit: 10 R; and emission slit: 10. Under such conditions, the

S/N for the syringe with 0.001 µg/mL rhodamine 123 was determined as >24, and rhodamine 123 in the syringe can be detected at concentrations as low as 0.0001 µg/mL.

Method verification:

The specificity was done by measuring the spectra of the medium and samples in the syringe using the fluorescence methodology: (1) WFI, (2) methylene blue in WFI solutions,

and (3) rhodamine 123 in WFI solutions. The excitation and emission wavelengths (605 nm and 678 nm for methylene blue, 482 nm and 527 nm for rhodamine 123) were determined based on the wavelength of maximum fluorescence of the dyes at wavelengths with negligible background interference. The further study using the available market drug products further confirmed the method specificity. With the excitation at 480 nm, no fluorescence peak was detected at or close to 525 nm for any of the tested commercial drug products sample. In addition, the fluorescence peak was detected at 525 nm in all these rhodamine dye spiked commercial drug products. The serial dilution of rhodamine dye was prepared for the lowest detection. With a low LOD, this method can provide a sensitive and robust approach that can detect the dye in the syringe. The accuracy and precision were assessed by forcefully adding the dye at different three different concentration ranging from LOD (0.0001 µg/mL) to 0.05 µg/mL, the percentage recovery was calculated. Similarly, the precision of drug product is performed by measuring the percentage of relative standard deviation of six treated prefilled syringes containing the different filling of drug product in it. The linearity of the method is plotted from 0.001 µg/mL to 0.05 µg/mL. The range of method is decided based on the results obtained from precision linearity and accuracy.

Results and discussion

Table 1 shows the difference of sensitivity between methylene blue and rhodamine dye. Which clearly informs the very high sensitivity of rhodamine. The detection limit is at the level of 0.01 µg/mL. As an alternative, rhodamine dye has stronger fluorescence and can meet the application need. A detection limit of less than 0.0001 µg/mL or 0.1 ppb is feasible to detect dye in the syringe. The comparison of results between the methylene blue dye and rhodamine dye shows the greater sensitivity, the method is linear over the range of 0.001 µg/mL to 0.05 µg/mL, the regression coefficient value more than 0.999 proves the linearity. The added and found amount of rhodamine dye at different concentration proves the accuracy. The precision for each six preparations of market sample containing different drug concentration was below 2.8%.

Conclusion

We successfully demonstrated in this study that it is feasible to develop a sensitive and robust CCIT method for prefilled syringes by using dye ingress with in syringe fluorescence detection. The conventionally used dye for CCIT, methylene blue, could not provide the sensitivity needed for in syringe measurements. The method developed in this study using fluorescence detection is not only suitable for the WFI-filled syringes for component qualification and filling process validation, but is also applicable to testing injectable drug products for stability, especially for biologics and drug products.

Acknowledgement

The authors would like to thank IKP laboratory for providing dyes and samples, and Vishal Khadke and Venkatesh Apte for their assistance in preparing syringes and testing samples.

References

1. Adler, M. Challenges in the development of prefilled syringes for biologics from a formulation scientist's point of view. *Am. Pharm. Rev.* 2012, 15 (1), 96 –101.
2. Kiang, P. Recent developments in prefilled syringes. *Am. Pharm. Rev.* 2012, 15 (1), 104 –107.
3. U. S. Food and Drug Administration (FDA). Guidance for Industry Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products; FDA: Silver Spring, MD, 1994.
4. European Commission. EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use. Annex 1, Manufacture of Sterile Medicinal Products; European Commission: Brussels, Belgium, 2008.
5. FDA. Guidance for Industry Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products; FDA: Silver Spring, MD, 2008.
6. USP. General Chapter <1207> Sterile Product Packaging—Integrity Evaluation; United States Pharmacopeia; United States Pharmacopeial Convention: Rockville, MD, 2014; Vol. 37.
7. Carroll, M. C.; Danny, V. F.; Guazzo, D. M.; Kaiser, M. W.; Kirsch, L. E.; Ludwig, J. D.; Masover, G. K.; May, J. L.; Moldenhauer, J. E.; Olsen, J. I.; Polson, T. M.; Wright, G. E. Pharmaceutical Package Integrity; PDA Technical Report #27; Parenteral
8. Li, L. Container closure integrity testing method development and validation for prefilled syringes. *Am. Pharm. Rev.* 2013, 16 (1), 48-52.
9. Simonetti, A.; Amari, F. Non-destructive vacuum decay method for pre-filled syringe closure integrity testing compared with dye ingress testing and high-voltage leak detection. *PDA J. Pharm. Sci. and Technol.* 2015, 69 (1), 108 –122.
10. Morton, D. K.; Lordi, N. G.; Ambrosio, T. J. Quantitative and mechanistic measurements of parenteral vial container/closure integrity, leakage quantitation. *J. Parenter. Sci. Technol.* 1989, 43(2), 88 –97.
11. Wolf, H.; Stauffer, T.; Chen, S.-C. Y.; Lee, Y.; Foster, R.; Ludzinski, M.; Kamat, M.; Godorov, P.; Guazzo, D. M. Vacuum decay container/closure integrity testing technology. Part 1. ASTM F2338 – 09 precision and bias studies. *PDA J. Pharm. Sci. Technol.* 2009, 63 (5), 472 – 488.

12. Wolf, H.; Stauffer, T.; Chen, S.-C. Y.; Lee, Y.; Foster, R.; Ludzinski, M.; Kamat, M.; Mulhall, B.; Guazzo, D. M. Vacuum decay container/closureintegrity testing technology. Part 2. Comparisonto dye ingress tests. PDA J. Pharm. Sci. Technol. 2009, 63 (5), 489 – 498.
13. Patel, J.; Mulhall, B.; Wolf, H.; Klohr, S.; Guazzo, D. M. Vacuum decay container closureintegrity leak test method development and validationfor a lyophilized product-package system.PDA J. Pharm. Sci. Technol. 2011, 65 (5), 486 –505.

Table 1: Comparison for sensitivity

Name of Dye	Concentration of Dye	Readings (RFU)
Methylene blue	0.05 mg/mL	47.9
	0.1 mg/mL	56.8
	1 mg/mL	689.1
Rhodamine 123	0.005 mg/mL	109.2
	0.01 mg/mL	221.6
	0.05 mg/mL	984.3

Table 2: Precision on different filling with different concentration of rhodamine 123.

Sr. No.	0.25 mL/PFS+0.05µg/mL rhodamine 123	0.75 mL/PFS + 0.01µg/mL rhodamine	1.5mL/PFS + 0.005µg/mL rhodamine
1	994.2*	223.5*	108.2*
2	996.9*	217.9*	110.7*
3	989.2*	216.8*	109.8*
4	978.9*	219.8*	112.1*
5	928.8*	220.1*	107.9*
6	948.8*	216.8*	109.8*
Average	972.8*	219.15*	109.8*
SDV	27.77	2.56	1.57
% RSD	2.86	1.17	1.43

(*)- Values are in Reference fluorescence unit (RFU)

Table 3: Accuracy at three different levels

Sr.No.	Amount added(µg/mL)	Amount found (µg/mL)	% Recovery
Sample-1 (Level-1)	0.0010	0.001011	101.1
Sample-2 (Level-1)	0.0010	0.001095	109.5
Sample-3 (Level-1)	0.0010	0.001076	107.6
Sample-4 (Level-1)	0.0010	0.001052	105.2
Sample-5 (Level-1)	0.0010	0.001098	109.8
Sample-6 (Level-1)	0.0010	0.001037	103.7
Level-2			
Sample-1 (Level-2)	0.0020	0.002078	103.9
Sample-2 (Level-2)	0.0020	0.002012	100.6
Sample-3 (Level-2)	0.0020	0.002096	104.8
Sample-4 (Level-2)	0.0020	0.00199	99.5
Sample-5 (Level-2)	0.0020	0.00208	104.0
Sample-6 (Level-2)	0.0020	0.00204	102.0
Level-3			
Sample-1 (Level-3)	0.050	0.05143	102.9
Sample-2 (Level-3)	0.050	0.04981	100.0
Sample-3 (Level-3)	0.050	0.05112	102.3
Sample-4 (Level-3)	0.050	0.05208	104.2
Sample-5 (Level-3)	0.050	0.05209	104.2
Sample-6 (Level-3)	0.050	0.05087	101.7

