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

Deepak D. Kayande[1]* Shripad Deshpande[1], Mazahar Farooqui[2], Parag Deshmukh[3]

[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.

Drkayande@gmail.com

(Received: 2020-04-21, Accepted: 2020-05-16)

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 packagingdevelopment and qualification.

Method for the measurement ofcontainer 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 ina syringe with excitation at 605 nm and emission at 678 nm, which generated a limit of detection of 0.06µg/mL. Furtherstudies were conducted using Rhodamine 123, a dye with stronger fluorescence. Using 482 nm excitation and 527 nmemission, the dye in the syringe could be easily detected at levels as low as 0.001 µg/mL. The relative standard deviationfor sixmeasurements of three different sample with different concentration was less than 2.8%. A number of operational parameters were optimized, including the photomultiplier tube voltage, excitation, andemission 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 usingrhodamine 123 for container closure integrity testing with in syringe fluorescence measurements significantlyenhanced the sensitivity and robustness of the testing and effectively overcame limitations of the traditional methylene bluemethod 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 usedfor delivery of parenteral drugs and biological products(1, 2). Prefilled syringes function as a primarypackaging component that provides protection andmaintains 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 DrugAdministration (FDA)requests the use of the USP sterility test as a part of thestability protocol for sterile products, with testing atinitial release and at the stability end point (5). The FDA further provided guidance for industry to use container closure integrity testing (CCIT) as an altar-native to sterility testing, performed throughout the product shelf life. In the USP guidance, it is recommendedto perform integrity testing at three phases throughout the life cycle of the sterile product: initialdevelopment 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 commonlyused method for CCIT. Dye ingress testing historically uses methylene blue dye. Besides a vacuum vessel, itdoes 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. Traditionaldye methods are also generally not as sensitive as some of the methods mentioned earlier using modern

Technologies:

UV-visible spectrophotometry has been appliedto the detection of dye ingress for CCIT in orderto overcome the limitations of visual detection. UV-vis spectrophotometric detection is more robustand typically offers lower detection limits in comparisonwith 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-Visspectrophotometric detection to drug product prefilledglass syringes. Due to the small diameter of the syringe (6.4 mm internal diameter) and possible lowconcentration of the dye intruded, direct spectrophotometric scanning of the intact syringe could not detecta signal comparable to that seen in glass vials and a similar detection limit could not be reached. An alternativemethod 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 forcontamination, 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 wasevaluated for developing a sensitiveand robust method for the dye ingress CCIT of prefilledglass syringes. The evaluations include optimization of operational parameters, comparisonwith visual and UV-Vis detection, specificity, linearity, limit of detection and precision with actual drug products. The objectives were to enablethe fluorescence measurement of the unopened prefilledsyringes 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 simplifythe testing procedure to fit the needs for quality control(QC) and stability studies.

Materials and Methods

The study used prefilled glass syringes that have astaked 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 fluorescencemethod, 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 hasbeen adopted for the detection of dye ingress in CCITof glass vials. However, the conventional spectrometric dye method for CCIT of vials wasshown not to be robust for a QC environment whenapplied to syringes. Two approaches were evaluated

using UV-vis spectrometry for the measurementof the dye ingress in prefilled glass syringe:(1) direct scan through the syringe barrel, and (2)analysis after transferring the liquid in the syringe intoa cuvette. Results from the scan through the barrelshowed significant noise that was 10 times higherthan that from the scan through the cuvette. The use of the cuvette for the UV-vis measurementof dye ingress improves the sensitivity, it doeshave a significant drawback in that the sample solutionneeds to be transferred to the cuvette from the syringeafter the dye immersion treatment. Thorough washingof the syringe with the needle shield attached is challenging.Some post-rinse samples had evidence of dyein the shield housing, and the dye occasionally transferredto the syringe tip as the needle shield wasremoved, resulting in dye carry-over and contaminationduring the solution transfer to the cuvette, making the procedureproblematic for use in a QC environment.

UV-Vis testing:

An aliquot of the testing medium was usedas a negative control. A series of solutions of methyleneblue in WFI at different concentrations wasprepared for determination of the method sensitivity.UV-vis spectrophotometer (Shimadzu UV-2600) was used for the UV-vismeasurement at 635 nm. The samples were measuredeither through a 5 mm quartz cuvette. The testingresults were statistically evaluated. Thelimit of detection (LOD) for the UV-vis measurement was determined based on the baseline variation. The LOD for the UV-vismethod was set as being equivalent to the signal fromthe concentration of dye in the syringe that resultedfrom the 5µm capillary breach. The syringe samplestreated by dye immersionanalysed after transferring the liquid inthe syringe into the cuvette. For the dye immersion–treated syringe samples, the syringe was subjected tosix rinse cycles using fresh deionized water for eachcycle. To transfer the liquid in the syringe into thecuvette, the syringe needle shield was carefully removed,and the first several drops of the liquid in theneedle were discarded to avoid dye carryover. A CCITfailure was identified when the detected absorptionvalue was greater than that of the LOD.

Development of a Fluorescence based method:

Fluorescence spectrometry was considered for the developmentof a sensitive and robust method for the dyeingress CCIT of syringes because it generally hashigher sensitivity and selectivity in comparison toUV-vis. It could enable the in situ measurement ofprefilled syringes after dye immersion without liquidtransfer, simplify the testing procedure to fit QC

needs, as well as provide a better LOD and robustnesscompared to the visual method and the UV-vismethod.

A Fluorescence spectrophotometer from Agilent Technologies was used for the detectionof the dye ingress in the prefilled glasssyringes. It has the following wavelength ranges: excitation200~900 nm and emission 200~900 nm. Thewavelength accuracy and resolution were ±1.5 nm.The data collection and processing were performed byusing Scanwin lab software. Both methylene blue andrhodamine 123 were evaluatedas fluorescence indicators for dye ingress CCIT ofprefilled syringes. Keyoperational parameters were optimized including thevoltage of the photomultiplier tube (PMT),standard voltage settings of high (800 V), medium(600 V), and low (400 V) and the width and shape ofthe excitation and emission slits (1.5, 2.5, 5, 10, and20 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 rhodamineis 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. Thesensitivity 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 ofthe 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 doneby measuring the spectra of the medium andsamples 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 emissionwavelengths (605 nm and 678 nm for methyleneblue, 482 nm and 527 nm for rhodamine 123)were determined based on the wavelength of maximum fluorescence of the dyes at wavelengths withnegligible background interference. The further study using the available market drug productsfurther confirmed themethod specificity. With the excitation at 480 nm, nofluorescence peak was detected at or close to 525 nmfor any of the tested commercial drug products sample. In addition, the fluorescence peak wasdetected at 525 nm in all these rhodaminedye spikedcommercial drug products. The serial dilution of rhodamine dye was prepared for the lowest detection. With a low LOD, this method can provide asensitive and robust approach that can detect the dyein 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 0.05µg/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 0.001µg/mL to 0.05µg/mL. The range of method is decided based on the results obtained from precision linearity and accuracy.

Results and discussion

Table 1 show the difference of sensitivity between methylene blue and rhodamine dye. Which clearly informs the very high sensitivity of rhodamine. The detection limit is at thelevel of 0.01µg/mL. As an alternative, rhodaminedye has stronger fluorescence and can meet theapplication need. A detection limit of less than 0.0001 µg/mL or 0.1ppb 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 0.001µg/mL to 0.05µg/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 isfeasible to develop a sensitive and robust CCIT method for prefilled syringes by using dye ingress with in syringe fluorescence detection. The conventionallyused dye for CCIT, methylene blue,could not provide thesensitivity needed for in syringe measurements.The method developed in this study using fluorescencedetection is not only suitable for the WFI-filled syringesfor component qualification and filling processvalidation, but is also applicable to testing injectabledrug 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 Documentationfor Sterilization Process Validation in Applicationsfor Human and Veterinary Drug Products;FDA: Silver Spring, MD, 1994.

4. European Commission. EU Guidelines to GoodManufacturing Practice Medicinal Products forHuman and Veterinary Use. Annex 1, Manufactureof Sterile Medicinal Products; EuropeanCommission: Brussels, Belgium, 2008.

5. FDA. Guidance for Industry Container and ClosureSystem Integrity Testing in Lieu of SterilityTesting as a Component of the Stability Protocolfor Sterile Products; FDA: Silver Spring, MD,2008.

6. USP. General Chapter <1207> Sterile ProductPackaging—Integrity Evaluation; United StatesPharmacopeia; 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.; Polsen,T. M.; Wright, G.E. Pharmaceutical Package Integrity;PDA Technical Report #27; Parenteral

8. Li, L. Container closure integrity testing methoddevelopment and validation for prefilled syringes.Am. Pharm. Rev. 2013, 16 (1), 48-52.

9. Simonetti, A.; Amari, F. Non-destructive vacuumdecay method for pre-filled syringe closure integritytesting compared with dye ingress testing andhigh-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 ofparenteral vial container/closure integrity, leakagequantitation. 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/closureintegrity testing technology. Part 1. ASTMF2338 – 09 precision and bias studies. PDAJ. 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