USP Class VI Testing for Dursan Coated Samples

Technical Insight

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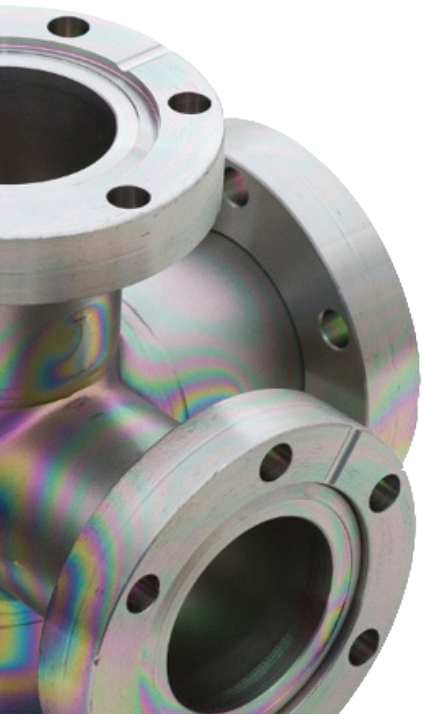

Synopsis

The Dursan coating process has been used in diagnostic equipment for its anti-sticking properties, particularly for the analysis and transfer of protein-containing biofluids. Bio-compatibility has been questioned by numerous customers looking to use Dursan in their process or instruments. This TI will discuss USP biological reactivity tests that Dursan coated parts went through, and what it means to be USP Class VI compliant.

Background

The U.S. Pharmacopeia (USP) group is a non-government, non-profit organization that sets the standards for the production of drugs (both human and animal), food ingredients, and dietary supplements. While enforcement of such standards is typically handled by the U.S. Food and Drug Administration, USP regularly sets new standards for strength, quality, purity and consistency of ingredients and components necessary for such production.

General chapter <88> of USP: “Biological reactivity tests, in vivo”, is of importance to a wide variety of industries including packaging, medical devices, implants, and drug manufacturers. It tests whether there is a biological response in animals to materials that are either directly or indirectly in contact with the material of choice. There are three main tests that are done: a systemic injection test, an intracutaneous test, and an implant test. These testing parameters are specifically for plastics; however, non-plastic items, such as a Dursan coated stainless steel part, can be put through the same testing procedures to determine compliance with the standard.

This TI will discuss the details of this test, and the results of an investigation into samples that were coated with Dursan and subjected to Class VI testing.

Data and Discussion:

USP General chapter <88> outlines six different classes of plastics based on the testing protocols that they undergo. The six classes and required tests are listed in Table 1. Each extraction can be performed at different temperatures depending on the nature of the plastic material. The three standard temperatures used are 50°, 70°, and 121° C. The 121° C extraction temperature is the harshest condition possible and is the temperature used for Dursan evaluations. Our testing facility (NAMSA) separated the reports into three categories: injections into mice, injections into rabbits, and implantation tests. Here we will discuss the details of those three tests.

Testing to be done				Plastic classes					
Test material	Animal	Dose	Procedure	I	II	III	IV	V	VI
Extract of 0.9% sodium chloride solution	Mouse	50 mL/kg	Intravenous injection	X	X	X	X	X	X
	Rabbit	0.2 mL in 10 sites	Intracutaneous injection	X	X	X	X	X	X
Extract of 1:20 Ethanol: Saline	Mouse	50mL/kg	Intravenous injection		X	X	X	X	X
	Rabbit	0.2 mL in 10 sites	Intracutaneous injection		X	X	X	X	X
Extract of polyethylene glycol	Mouse	10g/kg	Intraperitoneal injection			X		X	X
	Rabbit	0.2 mL in 10 sites	Intracutaneous injection					X	X
Extract of vegetable[A] oil	Mouse	50mL/kg	Intraperitoneal injection			X	X	X	X
	Rabbit	0.2 mL in 10 sites	Intracutaneous injection				X	X	X
Implant of test strips	Rabbit	4 implants per animal	Intramuscular implantation				X		X

A: The vegetable oil used here was sesame oil

Test 1: Acute Systemic Toxicity Study in Mice

The Dursan coated samples were evaluated for systemic toxicity in mice. The four separate studies are necessary to be Class III, V, and VI compliant. In this test, the extracts were created from an alcohol saline solution, polyethylene glycol solution, 0.9% sodium chloride solution, and finally sesame oil. These extracts were then injected into a group of five separate animals. Additionally, a group of five animals were injected with the extraction fluid without exposure to the Dursan coupons to act as a control. These mice were then monitored for signs of toxicity immediately after injection and at 4, 24, 48, and 72 hours after injection. The body weights of the mice were also recorded prior to dosing and then at the end of the 72-hour period.

For the results to be considered harmful, the treated animals would have had to die, show abnormal behavior such as convulsions or prostration, or lose greater than 2 grams of weight after the 3 days of observation. The results showed that there was no mortality of any of the mice in the study. All mice were lethargic immediately after the injection (both for extracted samples and control samples) but recovered to normal activity after 4 hours. And finally, all mice saw normal weight over the 3-day period.

Through the entirety of this test there was only one difference between the control group and the Dursan coated group. After the extraction in sesame oil, the oil had an opaque clarity to it where the control group remained clear. Despite this change, there seemed to be no adverse impact to the injection of the mice. The coated sample extracts passed the entirety of this test.

Test 2: Intracutaneous Study in Rabbits

This study utilizes the same 4 extracts as the acute systemic toxicity study in mice and all four are required for USP Class V and VI compliance. In this test each rabbit was clipped free of fur from the top and both sides of the spinal column. Each rabbit was then injected five separate times on the right side of the spine with the extracts made in contact with the Dursan coating, and five separate times on the left side of the spine with the control substances. The animals were then evaluated at 24, 48, and 72 hours after the injections. Evaluations looked for signs of erythema (reddening of the skin) and edema (swelling). Scores were given according to the following subjective rating scale:

Score	Erythema	Edema
0	No sign of erythema	No sign of edema
1	Very slight reddening (barely perceptible)	Very slight swelling (barely perceptible)
2	Well-defined reddening	Slight edema (edges of area well defined by definite raising)
3	Moderate to severe reddening	Moderate edema (raised approximately 1 mm)
4	Severe redness (beet red) or slight eschar formation	Severe edema (raised more than 1 mm and extending beyond the area of exposure)

To pass this study, each injection site of the extract must be on average 1.0 or less difference from the control group. The entirety of this test (both control and extracts) did not score anything other than zero for all evaluations except in the case of the sesame oil extraction. Once again, the extracted version of sesame oil turned opaque, and scores for both the control group and extracted group of the oil scored between 0 and 1. The overall average score for erythema and edema for the sesame oil injections were 0.3 for both the control and extract groups. This yielded a difference of zero and the Dursan coated article passed the test.

Test 3: Muscle Implantation Study in Rabbits

The samples for this study were 1 mm x 10 mm cylindrical pins made of stainless steel that were coated with Dursan. The negative control samples were similarly shaped high density polyethylene reference samples that were purchased from the US Pharmacopeial Convention. These pins were cleaned with steam and then surgically implanted into a rabbit's paravertebral muscle tissue and the rabbits were then monitored for 7 days. At the end of the week of the rabbit were euthanized and the implantation sites were investigated. With the assistance of an auxiliary light source and low magnification, the examiner looks for signs of tissue damage such as hemorrhages, necrosis, discoloration, and infection. If there are no signs of damage, the bio-capsule that forms around the sample is measured if it is present. In this study, neither the controls nor the coated sample showed any measurable capsule or signs of tissue damage.

Conclusion:

The Dursan coating process makes stainless steel surfaces inert to numerous chemicals as well as creates a non-stick surface for the release of proteins, plastics, and many other materials. Many customers have inquired about its cytotoxicity (which SilcoTek has previously showed it to be non-toxic) and its reactivity with biological samples. Here, Dursan was subjected to the most rigorous biological reactivity tests and passed all of them. While the USP Class VI testing is specifically for plastic testing, Dursan was shown to be compliant with the Class VI testing performed after a 121°C extraction for 1 hour in various solvent systems.

