

<381> Elastomeric Closure for Injections

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In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the General Chapters—Packaging and Distribution Expert Committee has revised General Chapter <381> *Elastomeric Closure for Injections*.

In *USP 41–NF 36* General Chapter <381> *Elastomeric Closure for Injections* references <231> *Heavy Metals*. With the impending omission of <231> on January 1, 2018, <381> was revised to include the necessary information to allow execution of the outlined test.

The <381> *Elastomeric Closure for Injections* Revision Bulletin will supersede the currently official General Chapter. The Revision Bulletin will be incorporated in the *Second Supplement to USP 41–NF 36*.

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〈381〉 ELASTOMERIC CLOSURES FOR INJECTIONS

INTRODUCTION

Elastomeric closures for containers used in the types of preparations defined in the general test chapter *Injections and Implanted Drug Products* (1) are made of materials obtained by vulcanization (cross-linking) polymerization, polyaddition, or polycondensation of macromolecular organic substances (elastomers). Closure formulations contain natural or synthetic elastomers and inorganic and organic additives to aid or control vulcanization, impart physical and chemical properties or color, or stabilize the closure formulation.

This chapter applies to closures used for long-term storage of preparations defined in the general test chapter *Packaging and Storage Requirements* (659), *Injection Packaging*. Such closures are typically used as part of a vial, bottle, or pre-fill syringe package system.

This chapter applies to closures formulated with natural or synthetic elastomeric substances. This chapter does not apply to closures made from silicone elastomer; however, it does apply to closures treated with silicone (e.g., Dimethicone, *NF*). When performing the tests in this chapter, it is not required that closures be treated with silicone, although there is no restriction prohibiting the use of siliconized closures.

This chapter also applies to closures coated with other lubricious materials (e.g., materials chemically or mechanically bonded to the closure) that are not intended to, and in fact do not provide, a barrier to the base elastomer. When performing the tests, closures with lubricious nonbarrier coatings are to be tested in their coated state.

The following comments relate solely to closures laminated or coated with materials intended to provide, or in fact function as, a barrier to the base elastomer (e.g., PTFE or lacquer coatings). It is not permissible to use a barrier material in an attempt to change a closure that does not meet compendial requirements to one that does conform. Therefore, all *Physicochemical Tests* apply to the base formula of such closures, as well as to the coated or laminated closure. To obtain *Physicochemical Tests* results, the tests are to be performed on uncoated or nonlaminated closures of the same elastomeric compound, as well as to the laminated or coated closure. The *Functionality Tests* apply to and are to be performed using the laminated or coated elastomeric closure. *Biological Tests* apply to the lamination or coating material, as well as to the base formula. *Biological Tests* may be performed on the laminated or coated closure, or they may be performed on the laminate/coating material and the uncoated or nonlaminated closures of the same elastomeric compound. In the latter case, the results are to be reported separately. The base formula used for physicochemical or biological tests intended to support the compendial compliance of a barrier-coated closure should be similar to the corresponding coated closure in configuration and size.

For all *Nephelometry, Turbidimetry, and Visual Comparison* (855) tests performed on any closure type, it is important to document the closure being tested, including a full description of the elastomer, and any lubrication, coating, laminations, or treatments applied.

This chapter states test limits for Type I and Type II elastomeric closures. Type I closures are typically used for aqueous preparations. Type II closures are typically intended for nonaqueous preparations and are those which, having properties optimized for special uses, may not meet all requirements listed for Type I closures because of physical configuration, material of construction, or both. If a closure fails to meet one or more of the Type I test requirements, but still meets the Type II requirements for the test(s), the closure is assigned a final classification of Type II. All elastomeric closures suitable for use with injectable preparations must comply with either Type I or Type II test limits. However, this specification is not intended to serve as the sole evaluation criteria for the selection of such closures.

It is appropriate to use this chapter when identifying elastomeric closures that might be acceptable for use with injectable preparations on the basis of their biological reactivity, their aqueous extract physicochemical properties, and their functionality. The following closure evaluation requirements are beyond the scope of this chapter:

- The establishment of closure identification tests and specifications
- The verification of closure–product physicochemical compatibility
- The identification and safety determination of closure leachables found in the packaged product
- The verification of packaged product closure functionality under actual storage and use conditions

The manufacturer of the injectable product (the end user) must obtain from the closure supplier an assurance that the composition of the closure does not vary and that it is the same as that of the closure used during compatibility testing. When the supplier informs the end user of changes in the composition, compatibility testing must be repeated, totally or partly, depending on the nature of the changes. Closures must be properly stored, cleaned for removal of environmental contaminants and endotoxins, and, for aseptic processes, sterilized prior to use in packaging injectable products.

CHARACTERISTICS

Elastomeric closures are translucent or opaque and have no characteristic color, the latter depending on the additives used. They are homogeneous and practically free from flash and adventitious materials (e.g., fibers, foreign particles, and waste rubber.)

IDENTIFICATION

Closures are made of a wide variety of elastomeric materials and optional polymeric coatings. For this reason, it is beyond the scope of this chapter to specify identification tests that encompass all possible closure presentations. However, it is the responsibility of the closure supplier and the injectable product manufacturer (the end user) to verify the closure elastomeric formulation and any coating or laminate materials used according to suitable identification tests. Examples of some of the analytical test methodologies that may be used include specific gravity, percentage of ash analysis, sulfur content determination, FTIR-ATR test, thin-layer chromatography of an extract, UV absorption spectrophotometry of an extract, or IR absorption spectrophotometry of a pyrolysate.

TEST PROCEDURES

Elastomeric closures shall conform to biological, physicochemical, and functionality requirements both as they are shipped by the closure supplier to the injectable product manufacturer (the end user), and in their final ready-to-use state by the end user.

For those elastomeric closures processed by the supplier prior to distribution to the end user, the supplier shall demonstrate compendial conformance of closures exposed to such processing and/or sterilization steps. Similarly, if elastomeric closures received by the end user are subsequently processed or sterilized, the end user is responsible for demonstrating the continued conformance of closures to compendial requirements subsequent to such processing and/or sterilization conditions (i.e., in their ready-to-use state). This is especially important if closures shall be exposed to processes or conditions that may significantly impact the biological, physicochemical, or functionality characteristics of the closure (e.g., gamma irradiation).

For closures that are normally lubricated with silicone prior to use, it is permissible to perform physicochemical testing on nonlubricated closures, in order to avoid potential method interference and/or difficulties in interpreting test results. For closures supplied with other lubricious nonbarrier coatings, all tests are to be performed using the coated closure.

For closures coated or laminated with coatings intended to provide a barrier function (e.g., PTFE or lacquer coatings), physicochemical compendial tests apply to the uncoated base elastomer, as well as to the coated closure. In this case, suppliers are responsible for demonstrating physicochemical compendial compliance of the coated closure, as well as of the uncoated closure, processed or treated in a manner simulating conditions typically followed by the supplier for such coated closures prior to shipment to the end user. The uncoated closure subject to physicochemical tests should be similar to the corresponding coated closure in size and configuration. End users of coated closures are also responsible for demonstrating the continued physicochemical compendial conformance of the coated closure, processed or treated in a manner simulating conditions typically employed by the end user prior to use.

In all cases, it is appropriate to document all conditions of closure processing, pretreatment, sterilization, or lubrication when reporting test results.

Table 1 summarizes the testing requirements of closures, and the responsibilities of the supplier and the end user.

Table 1

Closure Types (As Supplied or Used)	Test Requirements		
	Physicochemical Tests	Functionality Tests	Biological Tests
Closure with or without Silicone Coating	• Tests are to be performed.	• Tests are to be performed.	• Tests are to be performed.
	• Silicone use is optional.	• Silicone use is optional.	• Silicone use is optional.
	• Responsibility: supplier and end user	• Responsibility: supplier and end user	• Responsibility: supplier and end user
Closures with Lubricious Coating (Nonbarrier Material; Not Silicone)	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.
	• Responsibility: supplier and end user	• Responsibility: supplier and end user	• Responsibility: supplier and end user
Closures with Barrier Coating	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.	• Tests are to be performed on coated closures.
	• Responsibility: supplier and end user		OR:
	AND:		• Tests are to be performed on uncoated closures (base formula) and the laminate/coating material (report results separately).
	• Tests are to be performed on uncoated closures (base formula).	• Responsibility: supplier and end user	
• Responsibility: supplier		• Responsibility: supplier and end user	

BIOLOGICAL TESTS

Two stages of testing are indicated. The first stage is the performance of an in vitro test procedure as described in general test chapter *Biological Reactivity Tests, In Vitro* (87). Materials that do not meet the requirements of the in vitro test are subjected to the second stage of testing, which is the performance of the in vivo tests, according to the procedures set forth in the general test chapter *Biological Reactivity Tests, In Vivo* (88), *Systemic Injection Test* and *Intracutaneous Test*. Materials that meet the requirements of the in vitro test are not required to undergo in vivo testing.

Type I and Type II closures must both conform to the requirements of either the in vitro or the in vivo biological reactivity tests. [NOTE—Also see the general information chapter *The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants* (1031).]

Change to read:

PHYSICOCHEMICAL TESTS

Preparation of Solution S

Place whole, uncut closures corresponding to a surface area of $100 \pm 10 \text{ cm}^2$ into a suitable glass container. Cover the closures with 200 mL of *Purified Water* or *Water for Injection*. If it is not possible to achieve the prescribed closure surface area ($100 \pm 10 \text{ cm}^2$) using uncut closures, select the number of closures that will most closely approximate 100 cm^2 , and adjust the volume of water used to the equivalent of 2 mL per each 1 cm^2 of actual closure surface area used. Boil for 5 minutes, and rinse five times with cold *Purified Water* or *Water for Injection*.

Place the washed closures into a Type I glass wide-necked flask (see *Containers—Glass* (660)), add the same quantity of *Purified Water* or *Water for Injection* initially added to the closures, and weigh. Cover the mouth of the flask with a Type I glass beaker. Heat in an autoclave so that a temperature of $121 \pm 2^\circ$ is reached within 20 to 30 minutes, and maintain this temperature for 30 minutes. Cool to room temperature over a period of about 30 minutes. Add *Purified Water* or *Water for Injection* to bring it up to the original mass. Shake, and immediately decant and collect the solution. [NOTE—This solution must be shaken before being used in each of the tests.]

Preparation of Blank

Prepare a blank solution similarly, using 200 mL of *Purified Water* or *Water for Injection* omitting the closures.

APPEARANCE OF SOLUTION (TURBIDITY/OPALESCEANCE AND COLOR)

Determination of turbidity (opalescence)

[NOTE—The determination of turbidity may be performed by visual comparison (*Procedure A*), or instrumentally using a suitable ratio turbidimeter (*Procedure B*). For a discussion of turbidimetry, see *Nephelometry, Turbidimetry, and Visual Comparison* (855). Instrumental assessment of clarity provides a more discriminatory test that does not depend on the visual acuity of the analyst.]

Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water and dilute with water to 100.0 mL. Allow to stand for 4 to 6 hours.

Hexamethylenetetramine solution: Dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water in a 100-mL glass-stoppered flask.

Opalescence stock suspension: Add 25.0 mL of *Hydrazine sulfate solution* to the *Hexamethylenetetramine solution* in the flask. Mix, and allow to stand for 24 hours. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Opalescence standard suspension: Prepare a suspension by diluting 15.0 mL of the *Opalescence stock suspension* with water to 1000.0 mL. *Opalescence standard suspension* is stable for about 24 hours after preparation.

Reference suspensions: Prepare according to *Table 2*. Mix and shake before use. [NOTE—Stabilized formazin suspensions that can be used to prepare stable, diluted turbidity standards are available commercially and may be used after comparison with the standards prepared as described.]

Table 2

	Reference Suspension A	Reference Suspension B	Reference Suspension C	Reference Suspension D
Standard of Opalescence	5.0 mL	10.0 mL	30.0 mL	50.0 mL
Water	95.0 mL	90.0 mL	70.0 mL	50.0 mL
Nephelometric Turbidity Units	3 NTU	6 NTU	18 NTU	30 NTU

Procedure A: visual comparison: Use identical test tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm. Fill one tube to a depth of 40 mm with *Solution S*, one tube to the same depth with water, and four others to the same depth with *Reference suspensions A, B, C, and D*. Compare the solutions in diffuse daylight 5 minutes after preparation of the *Reference suspensions*, viewing vertically against a black background. The light conditions shall be such that *Reference suspension A* can be readily distinguished from water and that *Reference suspension B* can be readily distinguished from *Reference suspension A*.

REQUIREMENT: *Solution S* is not more opalescent than *Reference suspension B* for Type I closures, and not more opalescent than *Reference suspension C* for Type II closures. *Solution S* is considered clear if its clarity is the same as that of water when examined as described above, or if its opalescence is not more pronounced than that of *Reference suspension A* (refer to Table 3).

Procedure B: instrumental comparison: Measure the turbidity of the *Reference suspensions* in a suitable calibrated turbidimeter (see (855)). The blank should be run and the results corrected for the blank. *Reference suspensions A, B, C, and D* represent 3, 6, 18, and 30 Nephelometric Turbidity Units (NTU), respectively. Measure the turbidity of *Solution S* using the calibrated turbidimeter.

REQUIREMENT: The turbidity of *Solution S* is not greater than that for *Reference suspension B* (6 NTU FTU) for Type I closures, and is not greater than that for *Reference suspension C* (18 NTU FTU) for Type II closures (refer to Table 3).

Table 3

Opalescence Requirements	Comparison Method	
	Procedure A (Visual)	Procedure B (Instrumental)
Type I closures	No more opalescent than Suspension B	No more than 6 NTU
Type II closures	No more opalescent than Suspension C	No more than 18 NTU

Determination of color

Color standard: Prepare a solution by diluting 3.0 mL of *Color and Achromicity (631)*, *Color Determination and Standards, Matching Fluids, Matching Fluid O* with 97.0 mL of diluted hydrochloric acid.

Procedure: Use identical tubes made of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm. Fill one tube to a depth of 40 mm with *Solution S*, and the second with the *Color standard*. Compare the liquids in diffuse daylight, viewing vertically against a white background.

Requirement: *Solution S* is not more intensely colored than the *Color standard*.

Acidity or Alkalinity

Bromothymol blue solution: Dissolve 50 mg of bromothymol blue in a mixture of 4 mL of 0.02 M sodium hydroxide and 20 mL of alcohol. Dilute with water to 100 mL.

Procedure: To 20 mL of *Solution S* add 0.1 mL of *Bromothymol blue solution*. If the solution is yellow, titrate with 0.01 N sodium hydroxide until a blue endpoint is reached. If the solution is blue, titrate with 0.01 N hydrochloric acid until a yellow endpoint is reached. If the solution is green, it is neutral and no titration is required.

Blank correction: Test 20 mL of *Blank* similarly. Correct the results obtained for *Solution S* by subtracting or adding the volume of titrant required for the *Blank*, as appropriate. (*Titrimetry (541)*.)

Requirement: Not more than 0.3 mL of 0.01 N sodium hydroxide produces a blue color, or not more than 0.8 mL of 0.01 N hydrochloric acid produces a yellow color, or no titration is required.

Absorbance

Procedure: [NOTE—Perform this test within 5 hours of preparing *Solution S*.] Pass *Solution S* through a 0.45- μ m pore size filter, discarding the first few mL of filtrate. Measure the absorbance of the filtrate at wavelengths between 220 and 360 nm in a 1-cm cell using the blank in a matched cell in the reference beam. If dilution of the filtrate is required before measurement of the absorbance, correct the test results for the dilution.

Requirement: The absorbances at these wavelengths do not exceed 0.2 for Type I closures or 4.0 for Type II closures.

Reducing Substances

Procedure: [NOTE—Perform this test within 4 hours of preparing *Solution S*.] To 20.0 mL of *Solution S* add 1 mL of diluted sulfuric acid and 20.0 mL of 0.002 M potassium permanganate. Boil for 3 minutes. Cool, add 1 g of potassium iodide, and titrate immediately with 0.01 M sodium thiosulfate, using 0.25 mL of starch solution TS as the indicator. Perform a titration using 20.0 mL of blank and note the difference in volume of 0.01 M sodium thiosulfate required.

Requirement: The difference between the titration volumes is not greater than 3.0 mL for Type I closures and not greater than 7.0 mL for Type II closures.

Heavy Metals

• **Lead nitrate stock solution:** Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard lead solution: On the day of use, dilute 10.0 mL of *Lead nitrate stock solution* with water to 100.0 mL.

pH 3.5 acetate buffer: Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard preparation: Into a 50-mL color-comparison tube pipet 2 mL of *Standard lead solution* (20 µg of Pb) and dilute with water to 25 mL. Using a pH meter or short-range pH indicator paper as external indicator, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH of between 3.0 and 4.0, dilute with water to 40 mL, and mix.

Test preparation: Into a 50-mL color-comparison tube place 10.0 mL of *Solution S*.

Procedure: To each of the two tubes containing the *Standard preparation* and the *Test preparation*, add 2 mL of *pH 3.5 acetate buffer*, then add 1.2 mL of thioacetamide–glycerin base TS. [NOTE—In countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared hydrogen sulfide TS to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface.] Dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation*. • (RB 1-Jan-2018)

Requirement: *Solution S* contains not more than 2 ppm of heavy metals as lead.

Extractable Zinc

Test solution: Prepare a *Test solution* by diluting 10.0 mL of *Solution S* to 100 mL with 0.1 N hydrochloric acid. Prepare a test blank similarly, using the *Blank* for *Solution S*.

Zinc standard solution: Prepare a solution (10 ppm Zn) by dissolving zinc sulfate in 0.1 N hydrochloric acid.

Reference solutions: Prepare not fewer than three *Reference solutions* by diluting the *Zinc standard solution* with 0.1 N hydrochloric acid. The concentrations of zinc in these *Reference solutions* are to span the expected limit of the *Test solution*.

Procedure: Use a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a zinc hollow-cathode lamp and an air–acetylene flame. An alternative procedure such as an appropriately validated inductively coupled plasma analysis (ICP) may be used.

Test each of the *Reference solutions* at the zinc emission line of 213.9 nm at least three times. Record the steady readings. Rinse the apparatus with the test blank solution each time, to ensure that the reading returns to initial blank value. Prepare a calibration curve from the mean of the readings obtained for each *Reference solution*. Record the absorbance of the *Test solution*. Determine the ppm zinc concentration of the *Test solution* using the calibration curve.

Requirement: *Solution S* contains not more than 5 ppm of extractable zinc.

Ammonium

Alkaline potassium tetraiodomercurate solution: Prepare a 100-mL solution containing 11 g of potassium iodide and 15 g of mercuric iodide in water. Immediately before use, mix 1 volume of this solution with an equal volume of a 250 g per L solution of sodium hydroxide.

Test solution: Dilute 5 mL of *Solution S* to 14 mL with water. Make alkaline if necessary by adding 1 N sodium hydroxide, and dilute with water to 15 mL. Add 0.3 mL of *Alkaline potassium tetraiodomercurate solution*, and close the container.

Ammonium standard solution: Prepare a solution of ammonium chloride in water (1 ppm NH₄). Mix 10 mL of the 1 ppm ammonium chloride solution with 5 mL water and 0.3 mL of *Alkaline potassium tetraiodomercurate solution*. Close the container.

Requirement: After 5 minutes, any yellow color in the *Test solution* is no darker than the *Ammonium standard solution* (no more than 2 ppm of NH₄ in *Solution S*).

Volatile Sulfides

Procedure: Place closures, cut if necessary, with a total surface area of 20 ± 2 cm² in a 100-mL flask, and add 50 mL of a 20 g per L citric acid solution. In the same manner and at the same time, prepare a control solution in a separate 100-mL flask by dissolving 0.154 mg of sodium sulfide in 50 mL of a 20 g per L citric acid solution. Place a piece of lead acetate paper over the mouth of each flask, and hold the paper in position by placing over it an inverted weighing bottle. Heat the flasks in an autoclave at 121 ± 2° for 30 minutes.

Requirement: Any black stain on the paper produced by the test solution is not more intense than that produced by the control substance.

FUNCTIONALITY TESTS

[NOTE—Samples treated as described for preparation of *Solution S* and air-dried should be used for *Functionality Tests of Penetrability, Fragmentation, and Self-Sealing Capacity*. *Functionality Tests* are performed on closures intended to be pierced by a hypodermic needle. The *Self-Sealing Capacity* test is required only for closures intended for multiple-dose containers. The needle specified for each test is a lubricated long bevel (bevel angle $12 \pm 2^\circ$) hypodermic needle¹.]

Penetrability

Procedure: Fill 10 suitable vials to the nominal volume with water, fit the closures to be examined, and secure with a cap. Using a new hypodermic needle as described above for each closure, pierce the closure with the needle perpendicular to the surface.

Requirement: The force for piercing is no greater than 10 N (1 kgf) for each closure, determined with an accuracy of ± 0.25 N (25 gf).

Fragmentation

Closures for liquid preparations: Fill 12 clean vials with water to 4 mL less than the nominal capacity. Fit the closures to be examined, secure with a cap, and allow to stand for 16 hours.

Closures for dry preparations: Fit closures to be examined into 12 clean vials, and secure each with a cap.

Procedure: Using a hypodermic needle as described above fitted to a clean syringe, inject into each vial 1 mL of water while removing 1 mL of air. Repeat this procedure four times for each closure, piercing each time at a different site. Use a new needle for each closure, checking that it is not blunted during the test. Filter the total volume of liquid in all the vials through a single filter with a nominal pore size no greater than 0.5 μm . Count the rubber fragments on the surface of the filter visible to the naked eye.

Requirement: There are no more than five fragments visible. This limit is based on the assumption that fragments with a diameter $>50 \mu\text{m}$ are visible to the naked eye. In case of doubt or dispute, the particles are examined microscopically to verify their nature and size.

Self-Sealing Capacity

Procedure: Fill 10 suitable vials with water to the nominal volume. Fit the closures that are to be examined, and cap. Using a new hypodermic needle as described above for each closure, pierce each closure 10 times, piercing each time at a different site. Immerse the 10 vials in a solution of 0.1% (1 g per L) methylene blue, and reduce the external pressure by 27 kPa for 10 minutes. Restore to atmospheric pressure, and leave the vials immersed for 30 minutes. Rinse the outside of the vials.

Requirement: None of the vials contain any trace of blue solution.

¹ Refer to ISO 7864, Sterile hypodermic needles for single use with an external diameter of 0.8 mm (21 gauge).