Lined and Unlined Rubber Stoppers for Multiple-Dose Vial Solutions I

Sorption of Preservatives and Leaching of Extractives

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The protective action of epoxy lining on rubber stoppers of natural, neoprene, and butyl rubber was evaluated against unlined stoppers of the same composition for sorption of preservative from vial solutions and extractives leached from closures into the solution. For the preservatives tested—namely, *p*-chloro-β-phenylethyl alcohol and its nonchlorinated analog, phenylethyl alcohol—no protective action against sorption was exhibited by the lined closures. The solutions used in the extractives study were water, 10 per cent ethanol, 50 per cent polyethylene glycol 300, 50 per cent *N,N*-dimethylacetamide, and 2 per cent benzyl alcohol. The lining on the closures was found to afford protection against leaching. For both the sorption and extractives studies, the butyl rubber closures exhibited optimal stability.

RECENT PUBLICATIONS from this laboratory and others have shown that rubber closures used for multiple-dose vial solutions can be detrimental to the stability of the vial contents through sorption of materials from solution (1-7) or through the action of closure extractives on the solution (7-10). To alleviate these inadequacies, one approach used by certain rubber stopper manufacturers is to line the closure surface that will come in contact with the vial solution with a lacquer containing polymeric materials as its major component.

Lacquered stoppers have been in use as closures for multiple-dose vial solutions for about 5 years. However, reports relative to the effectiveness of the linings against sorption of materials from solution into the rubber and leaching of extractives from the rubber by the solution appear to be lacking.

Accordingly, this study was initiated to determine the degree of protection against sorption and leaching contributed to the rubber stopper by an epoxy lining. Lined and unlined natural, neoprene, and butyl rubber stoppers were used in the evaluation. The sorption characteristics were tested with aqueous solutions of the preservatives, phenylethyl alcohol and *p*-chloro-β-phenylethyl alcohol, stored in multiple-dose vials. The leaching tendencies of 10% ethanol, 50% polyethylene glycol 300, 50% *N,N*-dimethylacetamide, 2% benzyl alcohol, and water on the stoppers were evaluated. Identification of the extractives leached from the stoppers was performed.

EXPERIMENTAL

Materials.—*p*-Chloro-β-phenylethyl alcohol, Ciba, b.p. 80-83°; phenylethyl alcohol, Eastman Organic Chemicals; thiazole type accelerator; substituted butylphenol, diphenylamine, substituted carbamic acid, thiuram, thiazole dicarbamate reaction product, imidazoline type accelerator, from rubber manufacturer were employed.

Polyethylene glycol 300, Union Carbide; *N,N*-dimethylacetamide, E. I. du Pont; benzyl alcohol, reagent grade, Fisher Scientific Co., double distilled at Ciba; 0.275*M* citric acid-sodium phosphate buffer of pH 4.0; natural rubber, neoprene rubber, and butyl rubber stoppers, epoxy lined and unlined, 13 mm., West Co.; U.S.P. type 1, 10-ml. clear ampuls and vials, Kimble Glass Co.; three-piece aluminum caps for vials, West Co., No. 13-30 were also utilized.

Equipment.—Beckman model DU spectrophotometer, Cary model No. 11 recording spectrophotometer, Beckman model G pH meter, and microdistillation apparatus (as described in an earlier report) (7) were employed.

Preparation of Ampuls, Vials, and Stoppers.—These units were washed and dried in accordance with the methods described in a previous publication from our laboratories (6).

Sorption of Preservative from Solution.—To evaluate the effectiveness of the epoxy lining against sorption of antibacterial preservatives from vial solutions, lined and unlined closures of the same basic rubber formulations were used. The preservative solutions used in the evaluation were 0.3% *p*-chloro-β-phenylethyl alcohol and 0.5% phenylethyl alcohol prepared on a weight-to-volume basis with water for injection buffered to a pH of 4.0. Each preservative solution was filtered through a medium porosity sintered-glass filter before filling into 10-ml. clear glass ampuls and vials. The ampuls were closed by customary pull sealing technique with an oxygen-gas flame. The vials of each preservative solution were divided into six equal parts and stoppered with epoxy lined and unlined natural, neoprene, and butyl rubber stoppers. The stoppered vials were then sealed with three-piece aluminum caps at a constant head pressure of 50 p.s.i. with a hydraulic capper. The preservative solutions in the ampuls and vials were placed into constant temperature cabinets regulated at 25, 40, 50, and 60 ± 1.5°.

Half of the vials were stored upright and half

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TABLE I.—CLOSURE COMPOSITION

Natural Rubber	Neoprene Rubber	Butyl Rubber
Natural Rubber[a]	Neoprene rubber[b]	Butyl rubber[c]
Zinc oxide	Natural rubber	Aluminum silicate
Stearic acid	Aluminum silicate	Zinc oxide
Microcrystalline wax	Zinc sulfide	Iron oxide
Sulfur	Barium sulfide	Stearic acid
Thiazole-type accelerator	Iron oxide	Sulfur
Thiuram-type accelerator	Zinc oxide	Thiazole-type accelerator
Substituted butylphenol	Magnesium oxide	Substituted carbamic acid
...	Stearic acid	Butoxy phosphate
...	Light mineral oil	...
...	Diphenylamine	...
...	Imidazoline-type accelerator	...
...	Thiazole-type accelerator	...

[a] Rubber content: 95.8% of total composition. [b] Rubber content: 43% of total composition—38.3% neoprene and 4.6% natural. [c] Rubber content: 48.1% of total composition.

inverted. At designated time intervals, samples were withdrawn and evaluated for loss in preservative content.

Rubber Closure Extractives.—To determine the effectiveness of the epoxy lining on the three composition closures against leaching of unreacted materials and reaction products from the rubber stoppers into the vial solution, several solvents used in practice for the formulation of injectable preparations were employed. These include water, 10% ethanol, 50% polyethylene glycol 300, 50% *N,N*-dimethylacetamide, and 2% benzyl alcohol. These solvents were filled into 10-ml. clear glass vials; each set of vials for a particular solvent was divided into six parts. These were then stoppered with the unlined and epoxy-lined natural, neoprene, and butyl closures. The stoppered vials were then sealed with three-piece aluminum caps at a constant sealing head pressure of 50 p.s.i. with a hydraulic operated capper. Half of each of these vials were autoclaved in an inverted position at 115° for 6 hours at 10 p.s.i., the other half were autoclaved in an upright position at 115° for 30 minutes at 10 p.s.i. After treatment at these two conditions, the solutions in the vials were scanned spectrophotometrically to obtain the ultraviolet absorption curve of the rubber extractives.

Identification of Extractives.—In order to obtain a qualitative identification of the rubber closure extractives, the accelerators, activators, antioxidants, and possible reaction products for the three rubber compositions used in this study were obtained. These materials were dissolved in the solvents used for the extractives study and their ultraviolet absorption spectra determined.

Analytical Method.—*Phenylethyl Alcohol.*—The concentration of preservative was determined by pipeting 3 ml. of solution into a microsteam distillation apparatus and the sample steam distilled to 25 ml. The absorbance was measured at 257 mμ where A (1% 1 cm.) = 18.

p-Chloro-β-phenylethyl Alcohol.—Residual preservative was determined by pipeting 2 ml. of solution into the microsteam distillation apparatus and the sample steam distilled to 25 ml. Absorbance was measured at 267 mμ where A (1% 1 cm.) = 22.

TABLE II.—EFFECTIVENESS OF EPOXY LINING ON NEOPRENE RUBBER STOPPERS AGAINST SORPTION OF *p*-CHLORO-β-PHENYLETHYL ALCOHOL FROM VIAL SOLUTIONS REPRESENTED AS PER CENT RESIDUAL PRESERVATIVE

Time, Days	Temp., 25° C. Upright L[a]	Upright P[b]	Inverted L	Inverted P	A[c]	Temp., 40° C. Upright L	Upright P	Inverted L	Inverted P	A
0	..	..	..	..	100	..	..	..	..	...
2	96	96	91	91	100	96	96	87	87	98
7	..	..	..	..	...	..	..	..	..	...
9	95	93	81	81	...	91	89	74	75	97
11	95	95	81	79	97	91	92	74	75	97
14	93	91	79	79	99	89	89	74	73	99
28	91	92	76	73	101	85	85	68	68	99
43	92	91	73	74	101	79	80	65	63	99
56	88	89	83	72	99	79	79	61	62	99
84	86	87	69	70	101	79	79	61	62	99

Time, Days	Temp., 50° C. Upright L	Upright P	Inverted L	Inverted P	A	Temp., 60° C. Upright L	Upright P	Inverted L	Inverted P	A
2	96	96	85	86	99	96	96	85	85	98
7	..	..	..	..	...	..	..	..	..	...
9	86	88	72	75	...	83	83	74	72	...
11	86	88	70	72	99	83	83	70	70	98
14	82	84	72	71	99	81	81	71	70	99
28	80	78	63	62	99	72	72	61	61	99
43	71	72	58	59	98	65	66	56	57	101
56	70	69	56	57	100	61	62	53	53	101
84	..	61	54	54	102	56	56	50	51	104

[a] L = Epoxy lined. [b] P = Plain. [c] A = Ampul.

TABLE III.—EFFECTIVENESS OF EPOXY LINING ON NATURAL RUBBER STOPPERS AGAINST SORPTION OF *p*-CHLORO-β-PHENYLETHYL ALCOHOL FROM VIAL SOLUTIONS REPRESENTED AS PER CENT RESIDUAL PRESERVATIVE

Time, Days	Temp., 25° C. Upright L[a]	P[b]	Inverted L	P	A[c]	Temp., 40° C. Upright L	P	Inverted L	P	A
0	..	..	..	..	100	..	..	..	..	...
3	97	97	91	93	99	96	96	88	89	99
5	97	97	89	92	101	94	95	82	89	99
10	97	97	88	91	103	94	94	81	84	99
12	96	97	88	91	101	93	94	81	84	100
14	97	96	87	91	101	93	93	80	82	100
28	94	96	82	87	101	87	88	75	78	100
34	93	94	83	86	100	84	86	74	76	100
56	93	94	83	79	102	87	90	74	75	101
84	91	91	78	80	99	77	78	70	75	100
Time, Days	**Temp., 50° C. Upright L**	**P**	**Inverted L**	**P**	**A**	**Temp., 60° C. Upright L**	**P**	**Inverted L**	**P**	**A**
3	94	97	86	88	98	92	94	84	86	98
5	94	94	81	86	100	90	93	80	82	100
10	91	91	78	80	99	86	88	76	80	100
12	91	93	79	81	97	84	87	75	78	100
14	89	89	76	80	100	82	84	73	76	100
28	82	84	74	75	100	74	79	68	74	100
34	79	79	69	74	100	74	73	68	68	100
56	75	78	69	72	101	69	71	64	65	101
84	70	73	68	68	101	63	62	59	63	101

[a] L = Epoxy lined. [b] P = Plain. [c] A = Ampul.

RESULTS AND DISCUSSION

The effectiveness of the epoxy lining on natural, neoprene, and butyl rubber stoppers against sorption of antibacterial preservatives from solution and leaching of extractives from the stoppers by several solvents used in injectable formulations was investigated. The formulations for the three different rubber stoppers used in this investigation are not the same as used in our previous studies (6, 7) because of required modifications to permit the application of the epoxy lacquer. The composition and per cent rubber content of these stoppers is presented in Table I.

Sorption Studies.—The sorption tendencies of the epoxy lined and unlined rubber stoppers were evaluated with vial solutions of phenylethyl alcohol and *p*-chloro-β-phenylethyl alcohol buffered to a pH of 4.0. These two preservatives were chosen for this study since their partition tendencies between rubber and solution are substantially different, with the chlorinated analog favoring rubber to a much larger extent than phenylethyl alcohol (7).

TABLE IV.—EFFECTIVENESS OF EPOXY LINING ON BUTYL RUBBER STOPPERS AGAINST SORPTION OF *p*-CHLORO-β-PHENYLETHYL ALCOHOL FROM VIAL SOLUTIONS REPRESENTED AS PER CENT RESIDUAL PRESERVATIVE

Time, Days	Temp., 25° C. Upright L[a]	P[b]	Inverted L	P	A[d]	Temp., 40° C. Upright L	P	Inverted L	P	A
0	..	...	..	...	100	..	..	..	..	...
3	97	100	97	100	100	96	99	97	99	100
7	99	100	96	98	100	98	98	96	98	100
8	97	100	96	99	100	97	99	94	98	100
10	98	99	97	99	100	97	98	95	97	100
13	97	99	95	98	101	96	99	95	97	100
16	96	97	95	98	100	93	96	93	95	99
28	97	98	94	99	99	94	94	93	97	99
42	97	98	95	99	100	93	95	93	96	100
50	95	96	93	97	100	92	94	91	96	99
84	95	97	95	97	100	91	94	93	99	99

Time, Days	Temp., 50° C. Upright L	P	Inverted L	P	PC[c]	A	Temp., 60° C. Upright L	P	Inverted L	P	PC	A
3	96	99	94	99	...	101	96	97	96	97	..	100
7	97	97	95	97	...	100	95	96	95	96	..	100
8	95	97	94	97	...	99	95	96	93	96	..	98
10	96	97	95	97	...	100	95	96	95	96	..	100
13	95	96	93	97	...	100	95	96	93	96	..	99
16	94	95	93	97	...	99	93	96	92	95	..	97
28	94	94	93	98	...	99	92	94	92	97	..	100
42	93	96	93	100	98	99	93	95	92	100	97	100
50	94	91	91	103	97	100	90	93	91	107	95	99
84	93	94	91	103	96	100	90	93	91	104	96	101

[a] L = Epoxy lined. [b] P = Plain. [c] PC = Plain corrected. [d] A = Ampul.

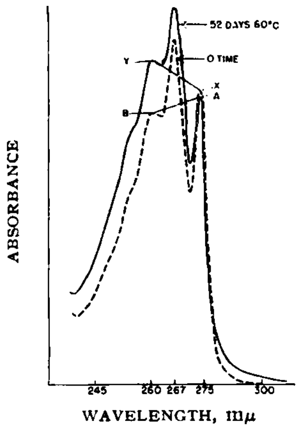

Fig. 1.—Plots showing the influence of butyl rubber extractives on the absorption characteristics of *p*-chloro-β-phenylethyl alcohol.

The data in Tables II–IV summarize the sorption tendencies of the lined and unlined closures for *p*-chloro-β-phenylethyl alcohol from vial solutions stored at several temperature conditions. It is evident from the results in these tables that the epoxy lining imparts *no* protective action against preservative sorption by the three different composition closures for vials stored upright or inverted. Only for the neoprene closures do the vial solutions stored in an inverted position show greater loss of preservative than for the vials stored upright. The vial solutions stoppered with the neoprene closures showed the greatest loss in preservative concentration, while the vials stoppered with the butyl closures showed the least. The vials stoppered with the natural rubber closures showed losses in preservative content between that found with the other two closures. Since there was no diminution of preservative concentration in the ampul solutions, the loss in the vial solutions must be attributed to closure effect.

The data for the butyl closures given in Table IV appear to show that the vials stoppered with unlined closures are increasing in preservative concentration with time, instead of decreasing or remaining at initial concentration. The same stoppers having the epoxy lining show the expected decrease in preservative concentration with storage. This would indicate that an extractive from the unlined closure is entering the vial solutions and influencing the analysis for residual *p*-chloro-β-phenylethyl alcohol. This is not surprising, in light of our recent report (7), where we had experienced this situation with benzyl alcohol.

The effect of closure extractive on the absorption characteristics of *p*-chloro-β-phenylethyl alcohol in a buffered solution stored in vials stoppered with the butyl rubber closures is shown in Fig. 1. By comparing the curve for zero time and storage at 60° for 52 days, it is obvious that the absorbance difference between 260 and 275 mμ changes greatly. This effect at 260 and 275 mμ causes a concurrent effect at 267 mμ, the absorption maximum used for the preservative analysis.

The equations developed to correct for the interference of rubber closure extractive on the analysis for residual *p*-chloro-β-phenylethyl alcohol were

$$C = D - [(X - A) + (Y - X) + K]$$

where X is lower than Y

$$C = D - [(X - A) - (X - Y) + K]$$

where X is higher than Y and C = corrected absorbance at 267 mμ; D = uncorrected absorbance at 267 mμ; A = absorbance at 275 mμ for standard solution; B = absorbance at 260 mμ for standard solution; X = absorbance at 275 mμ for test sample; Y = absorbance at 260 mμ for test sample; and $K = A - B = 0.09$.

Using the above equation, the data for the butyl rubber at the elevated temperatures were recalculated and are presented as corrected values in Table IV. These data now represent the true loss in concentration of preservative due to the closure effect.

Table V.—Effectiveness of Epoxy Lining on Neoprene Rubber Stoppers Against Sorption of Phenylethyl Alcohol from Vial Solutions Represented as Per Cent Residual Preservative

Time, Days	Temp., 25° C. Upright L[a]	P[b]	Inverted L	P	A[c]	Temp., 40° C. Upright L	P	Inverted L	P	A
0	..	..	..	..	100	..	..	..	..	...
3	97	97	97	97	99	97	96	95	95	99
5	99	98	97	99	101	97	97	96	97	100
7	97	97	96	98	100	97	95	97	95	100
10	96	96	95	95	100	94	94	92	93	99
14	95	96	94	95	100	93	93	93	93	99
28	95	95	94	94	99	91	93	90	92	100
42	90	94	93	94	100	92	89	89	89	99
56	94	93	92	93	100	88	89	88	88	99
84	93	93	91	92	97	88	87	86	86	97

Time, Days	Temp., 50° C. Upright L	P	Inverted L	P	A	Temp., 60° C. Upright L	P	Inverted L	P	A
3	97	96	95	95	99	95	95	94	95	99
5	97	95	95	95	100	95	95	93	94	100
7	95	96	94	95	98	94	94	93	94	100
10	93	93	93	92	99	93	93	89	89	100
14	92	90	90	90	99	90	90	88	88	99
28	89	89	89	88	100	87	87	86	86	100
42	86	87	86	86	100	85	84	84	84	100
56	85	88	85	86	99	83	83	81	82	101
84	84	82	82	80	100	78	80	79	80	103

[a] L = Epoxy lined. [b] P = Plain. [c] A = Ampul.

Table VI.— Effectiveness of Epoxy Lining on Natural Rubber Stoppers Against Sorption of Phenylethyl Alcohol from Vial Solutions Represented as Per Cent Residual Preservative

Time, Days	Temp., 25° C. Upright L[a]	P[b]	Inverted L	P	A[c]	Temp., 40° C. Upright L	P	Inverted L	P	A
0	..	..	..	..	100	..	..	..	..	...
3	98	98	98	98	100	97	97	97	97	99
7	98	98	98	98	100	97	97	98	96	99
10	98	98	97	97	100	96	97	95	96	100
14	98	98	98	98	100	96	96	96	96	100
28	97	96	96	97	100	94	94	94	95	99
42	96	96	95	96	99	94	92	90	93	98
56	96	96	95	96	100	93	91	92	91	99
Time, Days	**Temp., 50° C. Upright L**	**P**	**Inverted L**	**P**	**A**	**Temp., 60° C. Upright L**	**P**	**Inverted L**	**P**	**A**
3	98	96	96	96	99	96	98	95	96	98
7	96	96	95	96	99	94	94	94	95	98
10	95	95	94	96	100	94	94	93	94	100
14	96	96	94	95	100	94	94	94	94	101
28	93	96	92	92	100	89	89	88	89	100
42	89	90	87	88	98	84	85	84	83	99
56	88	88	86	87	102	81	82	81	83	101

[a] L = Epoxy lined. [b] P = Plain. [c] A = Ampul.

A comparison of phenylethyl alcohol loss from vial solutions stoppered with the epoxy lined and unlined closures is presented in Tables V–VII. It is evident from the results shown in the tables that, as in the case for the chlorinated analog, the epoxy lining on the closures affords no protection against sorption of phenylethyl alcohol from the vial solutions. For this preservative, both the natural and neoprene stoppers cause about the same loss of phenylethyl alcohol from solution and, again, the butyl rubber closure shows the least tendency to absorb the preservative. Compared to the chlorinated analog, phenylethyl alcohol is absorbed by the closures to a lesser degree. This can be explained by the relative tendencies of these two preservatives to distribute between rubber and water, the chlorinated analog favoring rubber to a greater extent than phenylethyl alcohol (7).

A situation was observed for this preservative similar to that which existed for *p*-chloro-*β*-phenylethyl alcohol with vials stoppered with butyl rubber closures. Table VII shows that the vial solutions (stored at the elevated temperature conditions and stoppered with the unlined butyl rubber closures) exhibited an increase in assay for residual preservative with storage, instead of the expected decrease or maintenance of initial concentration. This is indicated by absorption curves presented in Fig. 2. Here again the extractives being leached from the rubber stoppers by the preservative solution in the vials were interfering with the analysis for preservative content. Using the equations derived for *p*-chloro-*β*-phenylethyl alcohol, corrections were made for the influence of closure extractives on residual preservative assays.

For this preservative, C and D of the equation

Table VII.—Effectiveness of Epoxy Lining on Butyl Rubber Stoppers Against Sorption of Phenylethyl Alcohol from Vial Solutions Represented as Per Cent Residual Preservative

Time, Days	Temp., 25° C. Upright L[a]	P[b]	Inverted L	P		A[d]	Temp., 40° C. Upright L	P	Inverted L	P	PC[c]	A
0	..	..	...	..		100	..	..	..	...	..	..
2	98	98	98	99		98	98	98	98	98	..	99
5	99	99	99	99		99	98	99	98	98	..	99
8	97	99	97	98		99	98	99	97	98	..	97
13	99	99	99	99		99	99	99	98	99	..	99
15	99	98	99	99		99	99	98	98	99	..	99
28	99	99	99	99		100	99	99	98	99	..	99
42	98	99	99	99		100	98	99	98	101	98	99
56	98	99	101	99		99	99	99	99	99	..	99
84	..	..	...	..		...	..	..	..	...	..	..
Time, Days	**Temp., 50° C. Upright L**	**P**	**Inverted L**	**P**	**PC**	**A**	**Temp., 60° C. Upright L**	**P**	**Inverted L**	**P**	**PC**	**A**
2	98	98	97	98	..	99	98	99	98	99	..	98
5	99	100	98	99	..	99	97	99	98	97	..	99
8	97	97	97	98	..	100	97	99	97	98	..	99
13	99	99	98	99	..	99	99	99	97	102	98	99
15	99	99	99	99	..	99	97	99	98	101	99	99
28	97	98	98	101	98	99	97	98	97	112	95	99
42	98	98	97	110	96	99	98	99	97	110	94	99
56	98	98	99	99	..	99	99	99	99	115	95	100
84	..	...	..	...	..	...	..	..	..	...	..	...

[a] L = Epoxy lined. [b] P = Plain. [c] PC = Plain corrected. [d] A = Ampul.

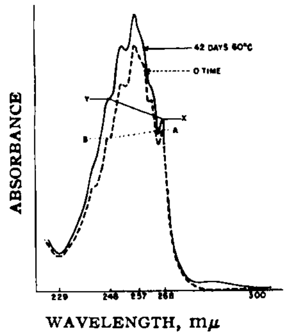

Fig. 2.—Plots showing the influence of butyl rubber extractives on the absorption characteristics of phenylethyl alcohol.

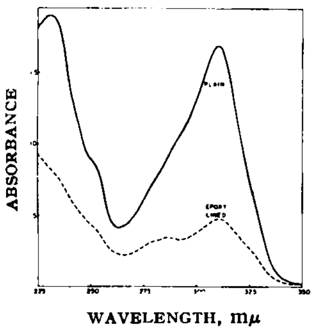

Fig. 3.—Ultraviolet absorption curve of leached extractives. Rubber, natural; solvent, water; storage condition, 6 hours at 115° C., vial inverted.

were measured at 257 mμ, *A* at 268 mμ, and *B* at 248 mμ, and *K* had a value of 0.06. The recalculated data showing the true loss of phenylethyl alcohol are presented in Table VII as the corrected values. These data are now representative of the loss in preservative concentration due to closure effect.

These findings regarding the ineffectiveness of the epoxy lining on rubber stoppers toward preservative sorption from solution were recently substantiated in a report by Garnet (11), who studied the effect of closures on antiseptics in biological products. He found that lacquered rubber stoppers did not solve the problem of loss of thiomersol from vaccine solutions. He reasoned that this lacquer was ineffective because the lacquer membrane was semipermeable and too fragile to withstand the slight distortion brought about by manipulation.

Leaching of Extractives.—The influence of several solvents commonly employed in the preparation of injectable solutions on the leaching of extractives from epoxy lined and unlined stoppers when used on multiple-dose vials was also investigated.

A representative ultraviolet absorption spectrum for the extractives found in the vial solutions containing water, and stoppered with natural rubber closures, is given in Fig. 3. The curves in this figure indicate that considerable absorbance is present and more than one peak in the curve exists. For each solvent the lined closures gave considerable protection against leaching of extractives as evidenced by a two to threefold decrease in absorbance given by the solvents from the vials stoppered with the lined closures compared with the solvents from vials stoppered with unlined closures. This is clearly shown in Table VIII, which summarizes the absorbance values at the peaks in the curves for the extractives found in the solvents from the three rubber stopper compositions tested. It is evident from the data in the table that the protection afforded by the epoxy lining on the neoprene stoppers is considerably less than that found for the natural rubber.

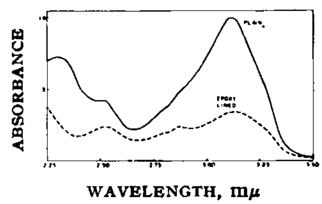

Fig. 4.—Plot showing ultraviolet absorption spectra of extractives leached from the closures after retreatment. Rubber, natural; solvent, water; storage condition, 3 hours at 115° C., vial inverted, second treatment.

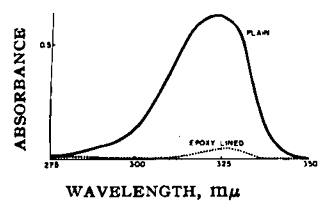

Fig. 5.—Ultraviolet absorption spectra of extractives leached from the rubber stopper after autoclaving for 30 minutes at 115° C., 10 p.s.i. Rubber, natural; solvent, *N,N*-dimethyl acetamide (50%); storage condition, 30 minutes at 115° C. upright.

The butyl rubber closures exhibit the least tendency for extractives to be leached into the solvents.

Table VIII.—Absorbance Values of Extractives Leached by Several Solvents in 10-ml. Vials from Epoxy Lined and Unlined Rubber Stoppers after Autoclaving the Vials in an Inverted Position at 115° C. for 6 Hours

	Neoprene			Natural			Butyl		
Solvent	Wavelength, mμ	Absorbance P	Absorbance L	Wavelength, mμ	Absorbance P	Absorbance L	Wavelength, mμ	Absorbance P	Absorbance L
Water	232	2.01	1.59	310	1.59	0.52	...	...	...
				232	1.38	0.60			
Ethanol, 10%	232	2.30	1.85	312	2.49	0.73	...	...	...
Benzyl alcohol, 2%	...	...	...	312	2.36	0.71	...	...	...
PEG 300, 50%	232	1.81	1.40	325	1.26	0.44	250	0.23	0.26
	256	0.67	0.62	...	...	...	...	...	...
	275	0.55	0.49	...	...	...	...	...	...
N,N-Dimethylacetamide, 50%	275	1.39	1.14	325	3.80	1.52	280	...	0.08
				282	0.99	0.43	...	...	...

TABLE IX.—ABSORBANCE VALUES OF EXTRACTIVES LEACHED BY SEVERAL SOLVENTS IN 10-ml. VIALS FROM EPOXY LINED AND UNLINED RUBBER STOPPERS AFTER AUTOCLAVING THE VIALS IN AN UPRIGHT POSITION AT 115° C. FOR 30 MINUTES

	Neoprene			Natural			Butyl		
Solvent	Wave-length, mμ	Absorbance P	Absorbance L	Wave-length, mμ	Absorbance P	Absorbance L	Wave-length, mμ	Absorbance P	Absorbance L
Water	232	0.60	0.30	...	...	...	...	...	...
Ethanol, 10%	232	0.76	0.51	230	0.09	0.11	...	...	...
Benzyl alcohol, 2%	...	...	...	312	0.07	0.03	...	...	...
PEG 300, 50%	275	0.03	0.03	325	0.16	0.04	...	...	...
N,N-Dimethylacetamide, 50%	...	...	...	325	0.31	0.02	...	...	...

In fact, with four out of the five solvents investigated, essentially no extractives were leached from the rubber. Only with the 50% polyethylene glycol 300 solvent is there significant extractive leached from the closure. With this solvent the same amount of extractive is in the vial solutions stoppered with the lined and unlined closures.

Treatment of the natural rubber closures used in the 6-hour autoclave experiment with water for injection for an additional 3 hours under the same temperature condition resulted in additional extractives coming into the water as shown by the absorption curves in Fig. 4. This illustrates the presence of considerable extractives in the rubber available for leaching by the solutions in the vials.

To determine whether normal autoclaving conditions cause extractives to be leached from the closures, lined and unlined stoppers were used with the vial solutions containing the solvents and autoclaved in an upright position at 115°, 10 p.s.i. for 30 minutes. A representative ultraviolet absorption curve for the extractive found in the vial solutions containing 50% *N,N*-dimethylacetamide and stoppered with natural rubber closures is shown in Fig. 5. The data in Table IX summarize the results obtained with the three closures and several solvents.

The natural rubber stoppers exhibit extractives in four out of five solvents, the neoprene stoppers in three out of five solvents, and the butyl rubber stoppers exhibit no extractives in any of the five solvents. Here, as under the more drastic testing conditions, the epoxy lining affords greater protection against leaching for natural rubber than neoprene rubber.

Although the epoxy lining employed for the three closures was the same, it is interesting that the lining afforded greater protection to the natural than the neoprene rubber stoppers against the leaching of extractives by the solvents used in this study. This may be explained on the basis that after the lacquer is applied to the rubber stoppers they require a curing period at elevated temperatures. During this period, it is possible for ingredients in the rubber formulation to enter the lining and subsequently influence the properties of the lining on the closures. Consequently, although the lacquer applied on the three different rubber stoppers is the same, the lining that results after curing of the lacquer on the rubber may possess different properties, depending on what materials migrated into the lining from the stopper composition.

Identification of Extractives.—In an attempt to identify qualitatively the extractives leached from the three composition closures by the several solvents, the formulation of these closures was studied to determine which ingredients would tend

TABLE X.—ULTRAVIOLET ABSORPTION MAXIMA OF RUBBER CLOSURE INGREDIENTS

	Wavelength, mμ				
Rubber Stopper	Water	10% Ethanol	2% Benzyl Alcohol	50% *N,N*-Dimethyl Acetamide	50% Polyethylene Glycol 300
Neoprene					
1 Imidazoline-type accelerator	232	232	...	...	232
2 Thiazole-type accelerator	...	...	312	320	255
				275	320
					275
3 Diphenylamine antioxidant	...	...	...	285	285
Natural					
1 Thiazole accelerator	312	312	312	325	257
	232	232	...	...	325
	...	...	...	...	230
2 Thiuram accelerator	275	275	275	275	275
3 Substituted butyl phenol antioxidant	...	...	282	282	282
Butyl					
1 Thiazole-type accelerator	312	312	312	325	257
	232	232	...	...	325
	...	...	...	...	230
2 Substituted carbamic acid accelerator	282	282	282	282	277
	258	258	...	...	...
3 Thiazole dicarbamate reaction product	...	...	...	294	...
	...	...	...	282	...
	...	...	...	275	...

to give absorption in the ultraviolet region of the spectrum. Samples of these ingredients were obtained from the rubber stopper manufacturer, and ultraviolet absorption spectra were obtained on these ingedients in the solvents studied. The absorption peaks obtained are summarized in Table X. With this information available, it was possible to identify the ingredient or ingredients being extracted by the solvents in contact with the rubber closures used on the vials.

For the neoprene closures, the material responsible for the absorption at 232 mμ was the primary accelerator (imidazoline type) in the rubber formula. The agent responsible for absorption at 256 and 275 mμ is the secondary accelerator (thiazole type).

In the case of the natural rubber stoppers, the absorption at 230–232, 312, and 325 mμ is due to the primary accelerator (thiazole type). The absorption at 282 mμ is caused by the antioxidant used in the rubber formulation, a substituted butyl phenol.

For the butyl rubber stoppers, the absorbance at 250 mμ is caused by the secondary accelerator (thiazole type). The absorbance at 280 mμ is caused by the primary accelerator, a substituted carbamic acid.

It is important to realize that in addition to the extractives that have been identified, there may be others present in the solvents. These would be materials that do not give an absorption curve in the ultraviolet region of the spectrum.

From the results obtained in this investigation, it is evident that lacquered closures are ineffective in preventing the sorption of materials from solution, are partially effective in preventing extractives from being leached from the rubber by solvents used in the formulation of parenteral solutions. In addition, it appears that the effectiveness of the epoxy lining against leaching of extractives may be dependent upon the rubber composition on which the lining is applied.

Appreciable extractives can be leached from the vial stoppers under normal autoclaving conditions. Subsequent storage of these vials at room temperature for the shelf-life of pharmaceuticals, which nowadays is considered to be approximately 3 years, would no doubt cause further leaching of extractives into solution. Since, for the most part, the materials extracted from the closures by the vial solutions are reactive chemicals, they can cause serious stability, toxicity, and assay problems with the vial contents. Accordingly, before selecting a rubber stopper composition for a vial solution, the closure must be evaluated as to its sorption and extractive characteristics with the solution with which it will be used. This is now of particular importance in light of the proposed regulations on drugs issued by the Food and Drug Administration on February 14, 1963 (12), which state under Section 133.13b that pharmaceutical manufacturers "make adequate provision to determine the stability of products in containers in which they are marketed to insure among other things, that the container is non-reactive and non-absorptive to the drugs." Pharmaceutical and rubber closure manufacturers can no longer disregard the importance of the closure on the stability, toxicity, and assay of the parenteral solution for which it is to be used.

SUMMARY

Epoxy-lined and unlined closures of the same composition were evaluated regarding their relative sorption and leaching of extractives characteristics. Aqueous buffered solutions of the preservatives phenylethyl alcohol and *p*-chloro-β-phenylethyl alcohol filled into multiple-dose vials and stoppered with the lined and unlined closures were used for the sorption studies. In the leaching investigation, several solvents commonly used in parenteral formulations were evaluated with the lined and unlined stoppers. The solvents used were water, 10% ethanol, 2% benzyl alcohol, 50% polyethylene glycol 300, and 50% *N,N*-dimethylacetamide. The results obtained were:

1. The epoxy lining is ineffective as a barrier against closure sorption of the preservatives from solution.
2. The lining for the most part is effective in reducing the amount of extractives being leached from the closures. The degree of effectiveness is dependent upon the closure composition.
3. Of the three rubber compositions evaluated, the butyl rubber exhibited the least tendencies toward sorption of preservative from solution and the leaching of extractives into solution.
4. The extractives leached from the natural, neoprene, and butyl rubber closures by the several solvents were identified and found for the most part to be the accelerators used in the rubber formulations.
5. The extractive leached from the unlined butyl rubber closure interfered with the analysis for the preservatives. The lined closures of the same composition did not exhibit this interference.
6. Extractives were found to be leached from the closures under normal autoclaving conditions used for multiple-dose vial solutions—namely, 115°, 10 p.s.i. for 30 minutes.

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