tains its prestige among similar publications. The unselfish and scientific character of pharmacopœial work must be maintained if Pharmacy is to merit this rich heritage, and our generation must look well to the spirit with which we approach the forthcoming Pharmacopœial Convention. The eyes of the coöperating scientific world are upon us and we who love our profession must zealously guard the essential standards.

With the limited time allotted for this presentation it is possible to touch only a few outstanding features of the revision. Detailed changes, in abstract, in many departments are being published in the A. PH. A. JOURNAL during the next two months and will provide an opportunity for many to obtain information concerning the changes.

THE TESTS FOR REDISTILLED WATER IN THE NATIONAL FORMULARY VI MONOGRAPH.*

BY R. S. ADAMSON, R. K. SNYDER, E. N. GATHERCOAL.

The tests in the N. F. VI monograph for redistilled water are as follows:

Tests for Purity.—Evaporate 100 cc. of Redistilled Water to dryness on a water-bath, and subsequently dry the residue in an oven to constant weight at 100° C.: not more than 0.0005 Gm. of *residue* remains.

Separate portions of 10 cc. each of Redistilled Water are not affected by the addition of barium chloride T.S. (*sulfate*); silver nitrate T.S. (*chloride*); ammonium oxalate T.S. (*calcium*); hydrogen sulfide T.S. (*metals*).

Redistilled Water shows not more than a faint yellow color when 0.1 cc. of alkaline mercuric potassium iodide T.S. is added to a 100-cc. potion (*ammonia*).

Add 10 cc. of calcium hydroxide T.S. to 5 cc. of Redistilled Water: the mixture remains clear and transparent (*carbon dioxide*).

Heat 100 cc. of Redistilled Water to boiling, acidulate with 10 cc. of diluted sulfuric acid, and subsequently add 0.1 cc. of twentieth-normal potassium permanganate: the color of the liquid is not completely destroyed by boiling for 10 minutes (*oxidizable substances*).

Test for Sterility.—Follow the general methods given on pages 24 to 26 for the Testing of Ampul Solutions for Sterility. If the sample to be examined is in a bulk package, follow Section D; plant 10 fermentation tubes with 1 cc. of the sample in each: if growth appears in any of the fermentation tubes, the test may be repeated. If growth appears in any of the second lot of fermentation tubes, the water in the bulk package shall not be used in any product intended for parenteral use. If the sample to be examined is taken from ampuls, follow Section E; if growth appears in any of the fermentation tubes planted, the test may be repeated. If growth appears in any of the second lot of fermentation tubes planted, the whole lot of ampuls shall be discarded.

QUANTITY OF WATER REQUIRED FOR THE TESTS.

Considerable objection has been raised to the use of a large quantity of Redistilled Water (when in ampuls) for making the tests for purity. In U. S. P. X, about 725 cc. of distilled water is required to make the purity tests. Therefore, in the redistilled water monograph, the 100 cc. quantities have been reduced to 10 cc. for the tests for *sulfate*, *chloride*, *calcium* and *metals*, and a 5-cc. quantity for *carbon dioxide*. The 100-cc. quantities are retained for determining the *residue*, *oxidizable impurities* and *ammonia*. This requires a total of 345 cc. of redistilled water.

THE TESTS FOR SALTS.

A solution was prepared as follows:

Sodium Chloride	1.0 Gm.
Sodium Sulfate, anhydrous	1.0 Gm.
Calcium Oxide	5.0 Gm.

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* Scientific Section, A. PH. A., Portland meeting, 1935.

Iron Nitrate	2.0 Gm.
Copper Acetate	1.0 Gm.
Stronger Ammonia Water	3.5 cc.
Acetic Acid,	
Doubled Distilled water, each a sufficient quantity	
To make	1000.0 cc.

Dissolve the salts and the oxide in 750 cc. of the doubled-distilled water with the aid of a little acetic acid, add the stronger ammonia water and then sufficient acetic acid to make slightly acid. Filter, and add enough of the double-distilled water through the filter to make 1000 cc.

Dilute 100 cc. of the solution with double-distilled water to make 1000 cc. and mix well.

Dilute 50 cc. of the dilution with double distilled water to make 500 cc. and mix well.

The final dilution contains 10 parts per million of non-volatile solids.

Two 100-cc. samples of the final dilution were evaporated to dryness. The residues weighed 0.0010 Gm. and 0.0011 Gm.

The qualitative tests were made by adding 1 cc. of the test solution to 10 cc. of the double-distilled water containing the 10 parts per million of added salts and to a "blank" of 10 cc. of the double-distilled water. Also, a few drops of nitric acid were added in the chloride test, and a few drops of hydrochloric acid in the sulfate test. Comparison was made between the "blank" and the "test" after the two mixtures had stood for five minutes or longer in cylindrical graduated tubes. The results were as follows:

Test for:	Redistilled Water.	Redistilled Water with 10 Parts per Million of Added Salt.
Chloride	Unaffected	Very slight cloudiness
Sulfate	Unaffected	Unaffected
Calcium	Unaffected	Cloudiness
Iron	Unaffected	Slight darkening
Copper	Unaffected	
Ammonia	Very light yellow	Light yellowish orange

These results were due to:

	Parts per Million.							
Test for:	Present before T.S. Was Added.		Present after T.S. Was Added.		Solubility in Water.*		Insoluble Residue.	
Chloride	NaCl	1.00	AgCl	2.45	AgCl	1.52	AgCl	0.93
Sulfate	Na_2SO_4	1.00	$BaSO_4$	1.64	$BaSO_4$	1.74	$BaSO_4$	0.00
Calcium	CaO	5.00	CaC_2O_4	11.55	CaC_2O_4	5.54	CaC_2O_4	6.01
Iron	$Fe(NO_3)_3$	2.00	FeS	0.64	FeS	8.9	FeS	0.00
Coppper	$Cu(C_2H_3O_2).H_2O$	1.00	CuS	0.48	CuS	0.33	CuS	0.15
Ammonium Acetate	$NH_4C_2H_3O_2$	4.50						

* "Handbook of Chemistry and Physics," 18th Edition, Charles D. Hodgman, *Editor*.

These results indicate that if but 5 parts per million of chemical salts be present in the water, no one of them will appear in the qualitative tests unless it be predominant in the mixture of salts.

A similar set of tests made on 100-cc. quantities of the same dilution of the solution of salts in water indicated almost the same results. In 50-cc. Nessler tubes the cloudiness might be slightly more evident and in 100-cc. Nessler tubes perhaps slightly more evident than in the 50-cc. tubes.

THE TEST FOR OXIDIZABLE MATTER.

The U. S. P. X uses 0.1 cc. of *tenth-normal* potassium permanganate to 100 cc. of distilled water, and requires that the pink color shall not be entirely discharged.

Early in the work on ampuls, Dr. E. B. Carter published an extensive investigation on pyrogens and claimed that one-tenth cc. of *twentieth*-normal potassium permanganate was ample for this test (see last paragraph of the monograph on redistilled water).

The Joint Contact Committee of the Manufacturers' Associations recommended a modification of this test as follows:

"To 100 cc. of distilled water, add 10 cc. of diluted sulfuric acid, and bring to a boil. Add twentieth-normal potassium permanganate until a faint pink color is obtained, then add an additional 0.2 cc. of twentieth-normal potassium permanganate. Add a measured amount of the redistilled water being tested until the pink color is discharged, maintaining the solution at the boiling point throughout the test. The quantity of this water required to completely discharge the pink color shall not be less than 100 cc. (*oxidizable impurities*)."

After a rather extensive investigation by Raymond S. Adamson, as reported below, it would seem as though there were several objections to the test for *oxidizable impurities* as presented by the Joint Contact Committee.

1. The addition of $N/20$ $KMnO_4$, in appreciable quantity to the preliminary 100 cc. of distilled water, introduces an excess of the reagent unless the distilled water be highly contaminated with oxidizable matter.

2. The use of 0.2 cc. of $N/20$ $KMnO_4$ introduces so much of the reagent that the test becomes very coarse. A much decreased amount should be used to insure a reasonable sensitiveness.

3. The quantity of redistilled water, added to the original 100 cc. of distilled water and the reagents, is so great that the color of the permanganate is lost by dilution rather than by a chemical reduction.

4. It is a question whether a chemical test can be devised that is sufficiently delicate to detect dangerous quantities of bacteria and of their decomposition or metabolic products. Mr. Adamson's report indicates a possibility of distinguishing between the presence of 3 bacteria per cc. as compared with 5 bacteria per cc. To do this, he uses 0.5 cc. of $N/1000$ $KMnO_4$ in 100 cc. of redistilled water and determines the excess $KMnO_4$ by the use of KI T.S. and starch T.S. However, the reading of the test is such a delicate matter that it probably will not be acceptable to some workers. It is estimated that one trillion small bacteria yield about 13 mg. of protein, and that possibly 500 mg. of $KMnO_4$ will be chemically reduced by 1 mg. of bacterial protein. Therefore, the infinitesimal amount of $KMnO_4$ (about 0.1 cc. of $N/1{,}000{,}000$) reduced by 500 bacteria is almost too small to measure (0.1 cc. of $N/10$ $KMnO_4$ requires about 50,000,000 small bacteria to reduce it).

The report by Mr. Adamson is as follows:

In the descriptions of these experiments, certain abbreviated forms are used as follows:

H_2O = recently prepared double-distilled water or such water sterilized within 2 hours after being prepared.

H_2SO_4 = Diluted sulfuric acid, U. S. P.

$KMnO_4$ = Potassium permanganate in the designated volumetric solution.

Boil = Heated over a flame in a flask to boiling and kept gently boiling for 10 minutes.

Experiment 1.—To 100 cc. of ordinary distilled water, add 10 cc. of H_2SO_4 and bring to a boil; then add 0.05 cc. of $N/20$ $KMnO_4$, and boil. A pink color remains. It is evident, therefore, that 0.05 cc. of $N/20$ $KMnO_4$ added to a preliminary 100 cc. of distilled water is an excess of the reagent, unless the distilled water be badly contaminated with oxidizable matter.

Experiment 2.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.2 cc. of $N/20$ $KMnO_4$, and boil. Maintain the solution at the boiling point, add a measured amount of tap water until the pink color is completely discharged. Twenty cc. of Chicago chlorinated tap water completely discharges the color after boiling for 2 minutes.

Experiment 3.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.2 cc. of $N/20$ $KMnO_4$, and boil: a pronounced pink color remains.

Experiment 4.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.1 cc. of $N/20$ $KMnO_4$, and boil: a distinct pink color remains.

Experiment 5.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.1 cc. of $N/20$ $KMnO_4$, and boil. Add to the boiling solution a measured quantity of H_2O, maintaining the solution at the boiling point. At least 350 cc. of the water is added before the pink color is no longer visible.

Experiment 6.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.1 cc. of $N/20$ $KMnO_4$,

and boil. Add to the boiling solution a measured quantity of H_2O containing 25,000 bacteria per cc. Seventy cc. of this bacterial suspension, or 1,750,000 bacteria are required to destroy the pink color.

Experiment 7.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.1 cc. of $N/100$ $KMnO_4$ and boil: no pink color is visible. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S., and mix: a distinct blue color is produced.

Experiment 8.—To 300 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.2 cc. of $N/100$ $KMnO_4$, and boil: no color is visible. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S., and mix: a light pink color is produced which gradually darkens to a light purple.

Experiment 9.—To 300 cc. of boiling H_2O add 10 cc. of H_2SO_4 but no $KMnO_4$, and boil: the solution is colorless. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S., and mix : no color is produced. After standing one hour, some darkening occurs.

Experiment 10.—To 300 cc. of boiling H_2O containing 750,000 bacteria (2500 per cc.), add 10 cc. of H_2SO_4 and 0.2 cc. of $N/100$ $KMnO_4$, and boil: no color is visible. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S., and mix: no color is produced immediately. After standing one hour, the solution becomes darker.

Experiment 11.—To 100 cc. of boiling H_2O add 10 cc. of H_2SO_4 and 0.5 cc. of $N/1000$ $KMnO_4$, and boil. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S., and mix: a slight purple-pink color is produced.

Experiment 12.—To 100 cc. of boiling H_2O containing 250 bacteria (2.5 per cc.) add 10 cc. of H_2SO_4 and 0.5 cc. of $N/1000$ $KMnO_4$, and boil. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S., and mix: a faint pink color is produced immediately.

Experiment 13.—To 100 cc. of boiling H_2O containing 500 bacteria (5 per cc.) add 10 cc. of H_2SO_4 and 0.5 cc. of $N/1000$ $KMnO_4$, and boil. Cool the solution, add 1 cc. of potassium iodide T.S. and 2 cc. of starch T.S. and mix: no color is produced immediately.

The colors in these experiments are seen to best advantage by using Nessler tubes on a white background and looking down through the depth of the solution in the tube. The bacteria used were *Bacillus Coli.*

Instead of using starch T.S. to detect the presence of the iodine liberated by the excess of $KMnO_4$, chloroform may be used. To the cool solution add 1 cc. of potassium iodide T.S. and 5 cc. of chloroform, and mix. The chloroform layer receives the iodine color, varying from the faintest trace of pink to quite a pronounced iodine color.

The presence of the excess $KMnO_4$ may also be detected by adding a few drops of diphenylamine T.S. to the solution after cooling. The solution acquires a blue color, varying in depth according to the amount of $KMnO_4$ present.

THE TESTS FOR BACTERIA PRESENT IN REDISTILLED WATER.

A test for the sterility of the Water should be included among the tests for purity, and also a test for dead bacteria.

As Redistilled Water is practically always intended for parenteral use, the presence of any bacteria, either dead or alive, is objectionable, for their protein or their metabolic products may be toxic or anaphylactic when the Water is so used. When it is considered that there are many natural spring waters that will show no living bacteria in at least 8 tests out of 10, we certainly should require Redistilled Water, prepared especially to show complete purity, to be free from microbic contamination. The test for sterility indicated at the beginning of this paper is satisfactory.

A test for dead bacterial bodies can quite easily be carried out as follows:

Centrifuge a measured quantity of the water, but not less than 10 cc. in a suitable centrifuge tube, at not less than 2500 revolutions per minute for 30 minutes. Pour off the supernatant water and remove the last 0.1 to 0.2 cc. from the tip of the tube with a capillary pipette, and place it as a drop upon a glass microscopic slide. Rinse the tip of the tube and the pipette with a drop of Redistilled Water and add to the drop on the slide. Evaporate the water from the drop on the slide, fix the residue to the slide by heat, and stain it with a suitable bacterial stain. Not more than five bacterial bodies per cc. of water are present in the mount.