

STANDARDIZED COLORED FLUIDS AND SOME OFFICIAL
COLORIMETRIC TESTS.*

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INTRODUCTION.

This is the ninth of a series of papers reporting the work of one of us and his co-workers¹ and our recent work reported in this paper confirms our opinion as to the practicability of the standardized colored fluids proposed by us.

STANDARDIZED COLORED FLUIDS.

As previously reported, a large percentage of the colors produced in various color tests can be matched by two sets of fluids, each set consisting of three liquids representing the three primary colors.

Co-Fe-Cu Fluids.—These represent half-normal acidulated solutions of cobalt chloride, of ferric chloride, and of copper sulphate. The cobalt solution contains 59.4965 grams $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ to the liter dissolved in approximately 1 per cent. hydrochloric acid (25 cc 32 per cent. HCl and 975 cc of water). The iron solution contains 45.054 grams of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to the liter dissolved in approximately 1 per cent. hydrochloric acid (25 cc 32 per cent HCl and 975 cc of water). The copper solution contains 62.43 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to the liter dissolved in approximately 1 per cent. hydrochloric acid (25 cc 32 per cent. HCl and 975 cc of water).

In this connection attention must be called to the blunder made in our paper on the standardization of the color of cottonseed oil² in which it was stated that the cobalt, iron and copper salts were dissolved in 15 per cent. hydrochloric acid. A fluid prepared with 15 per cent. HCl is absolutely unfit for use as a color standard; hence the blunder in statement is deeply regretted.

Co-Cro-Cu Colors.—These represent fiftieth-normal solutions of roseo-cobaltic chloride, of ammonium chromate and of copper sulphate in 2.8 per cent. ammonia water. As these do not figure in the experimental work reported in this paper, the reader is referred for details to a previous article on the subject.³

STANDARDIZING THE COLORED FLUIDS.

A careful study of various colorimetric tests now in vogue shows that they are as unscientific and as inaccurate as it would be to use in an acidimetric assay a normal KOH made by merely dissolving 56 grams of stick potassium hydroxide in water enough to make a liter. Colorimetric tests, like volumetric assays, must be carried out under exact conditions, otherwise they are inaccurate.

This being so, the standard colors—the red cobalt solution, the yellow ferric solution and the blue copper solution—should be prepared with the same care that characterizes the preparation of a volumetric solution. The standardization of the ferric and the copper solutions can be accomplished without real difficulty by volumetric assay. The standardization of the cobalt solution was a less simple matter and constituted a weak point in the proposed use of the "Co-Fe-Cu" fluids. This question of a simple cobalt assay occupied our attention during the past winter and after a study of all of the methods found in the literature, one of us (Taub) found

* Scientific Section, A. Ph. A., Asheville meeting, 1923.

a slight modification of the Engle and Gustavson assay⁴ furnished a very satisfactory volumetric cobalt assay. The modified assay follows:

To 5 cc of the cobalt solution in a 250-cc Erlenmeyer flask are added 15 cc 20 per cent. NaOH, and 5 cc 3 per cent. H_2O_2 . After boiling for ten minutes, the solution is cooled rapidly and 2 grams KI are added followed by 20 cc H_2SO_4 (1:4). When the precipitate has dissolved, the solution is titrated with $N/10 Na_2S_2O_3$.

Each cc of $N/10 Na_2S_2O_3$ is equivalent to 0.005897 gram of Co, or 0.023799 gram $CoCl_2 \cdot 6H_2O$.

This *cobalt assay* gave the following figures with our standard half-normal cobalt chloride:

(a) *Volumetric Assay*.—5 cc of solution consumed 12.4 cc of $N/10 Na_2S_2O_3$, corresponding to 0.0730 gram Co in 5 cc, or 58.9 grams $CoCl_2 \cdot 6H_2O$ in 1000 cc.

(b) *Volumetric Assay*.—2.5 cc consumed 6.2 cc of $N/10 Na_2S_2O_3$, corresponding to 0.0365 gram Co in 2.5 cc, or 58.9 grams $CoCl_2 \cdot 6H_2O$ in 1000 cc.

(c) *Electrolytic Assay*.—7 cc yielded 0.1025 gram Co, corresponding to 14.65 grams Co, or 59.1 grams $CoCl_2 \cdot 6H_2O$ in 1000 cc.

(d) *Electrolytic Assay*.—7 cc yielded 0.1020 gram Co, corresponding to 14.6 grams Co, or 58.9 grams $CoCl_2 \cdot 6H_2O$ in 1000 cc.

Our method of *copper volumetric assay* is substantially that directed by U. S. P. IX for *Cupri Sulphas*. We treated 10 cc of our acidulated copper solution with acetic acid and potassium iodide and then titrated the liberated iodine with $N/10$ sodium thiosulphate. This assay gave the following results with our standard half-normal copper sulphate:

(a) *Volumetric Assay*.—10 cc consumed 25 cc of $N/10$ thiosulphate, corresponding to 15.9 grams Cu, or 62.3 grams $CuSO_4 \cdot 5H_2O$ in 1000 cc.

(b) *Volumetric Assay*.—10 cc consumed 24.8 cc of $N/10$ thiosulphate, corresponding to 15.8 grams Cu, or 62.0 grams $CuSO_4 \cdot 5H_2O$ in 1000 cc.

(c) *Electrolytic Assay*.—5 cc yielded 0.0789 gram Cu, corresponding to 15.8 grams Cu, or 62.1 grams $CuSO_4 \cdot 5H_2O$ in 1000 cc.

(d) *Electrolytic Assay*.—5 cc yielded 0.0788 gram Cu, corresponding to 15.76 grams Cu, or 61.8 grams $CuSO_4 \cdot 5H_2O$ in 1000 cc.

As to the *iron volumetric assay* we have already reported⁵ that the regular iodometric assay with potassium iodide, hydrochloric acid and sodium thiosulphate V. S. gave entirely satisfactory results.

COLORIMETRIC STANDARDIZATION OF THE Co-Fe-Cu FLUIDS.

Elsewhere⁶ we have given our opinion of the Lovibond Tintometer, so here we need only say that it is an exceedingly convenient instrument, that it gives very accurate results when the same set of glasses is used for all color comparison, and that its only drawback is the fact reported by Priest⁷ that different glasses labelled as of the same arbitrary Lovibond numbers, differ distinctly in color value. During the ten years we have been engaged in color work we have found our own Lovibond instrument and its glasses of great value in keeping check on our own standard colored fluids, and it might be of interest to state at this place that the Lovibond readings of our standard half-normal cobalt, iron and copper solutions made in 1913, 1915, 1917 and 1923 by different operators, each using solutions prepared and standardized by himself, are in close agreement. It might also be stated that

the standard half-normal fluids prepared in 1914 were reëxamined in 1919, and were found to possess the same Lovibond values—a convincing indication of the permanence of our colored fluids.

At this time it might be in place to answer a question sometimes put as to why we chose as our standard half-normal cobalt, iron and copper solutions instead of those of greater color value. This may be answered by saying that our own experience has been that when the color of a fluid is greater than 10 Lovibond units (either a single color or combined) the color matching is more difficult for the average eye than when the color is below that Lovibond number.

As the half-normal solutions approach the maximum of color value just mentioned, it is found best to utilize fluids of this strength and to dilute color tests down to such values, rather than use darker colored fluids.

THE U. S. P. COLOR TEST FOR LIQUID PETROLATUM.

In U. S. P. IX under *Petrolatum Liquidum* there is inserted a test for *limit of carbonizable impurities* which reads as follows:

Introduce into a glass-stoppered cylinder, which has been previously rinsed with sulphuric acid, 5 mils of liquid petrolatum and 5 mils of colorless sulphuric acid; heat in a water-bath during ten minutes, shaking well at intervals of thirty seconds; the oil remains unchanged in color and the acid does not become darker than pale amber (*carbonizable impurities*).

This test is fairly simple and is quite effective, but in reading it one is confronted with the question, "What is meant by 'pale amber?'"

In attempting to answer this question we secured fourteen samples of liquid petrolatum, many obtained from producers of this official substance, and some purchased in the open market.

We conducted the U. S. P. test on each of these samples, using 1-ounce glass-stoppered French square bottles instead of the glass-stoppered cylinders directed by the U. S. P. This we did for the following reasons: First, the test involves the use of an all-glass container; second, because the French square bottle fitted into the Lovibond apparatus and could replace therein the cell used in Lovibond readings (the latter cell not standing the action of concentrated acid); and third, because the determination of the layer of fluid within the French square bottle was a matter of no great difficulty.

Examination of several dozen French square bottles brought to light two that upon calibration were found to hold in each bottle a layer of liquid exactly 16 mm. in thickness. These bottles were carefully set aside as "all-glass cells" and in these all of the liquid petrolatum tests were run and, after running, the color of the resulting sulphuric acid layer was read without transferring the fluid to another container.

As mentioned above, the test was run upon fourteen samples of liquid petrolatum and immediately upon the expiration of the ten minutes specified in the official test the bottle and its contents were cooled to room temperature and read in our Lovibond apparatus. In the case of Samples Nos. 4, 13 and 14 reported in the following table the Lovibond reading of a second test of the same batch of liquid petrolatum is also given in order to show that the test is uniform if carried out under uniform conditions.

TABLE I.

Lovibond Readings of "Carbonizable Impurities" Test of Liquid Petrolatum.

NOTE.—In this and all other Lovibond readings in this paper, the red Lovibond glasses were No. 200, the yellow were No. 510, and the blue were No. 1180. In Tables I and II the fluid was read in the 16-mm. "all-glass cell" described above; in all other tables the regular 1/2-inch Lovibond cell was used.

Sample No.	Red.	Lovibond reading.		Blue.
			Yellow.	
1	0.7 + 0.1		1.3	
2	0.7 + 0.2		1.5	
3	1 + 0.2		2 + 0.2	
4	1 + 0.1		2 + 0.4	
4a	1 + 0.1		2 + 0.3	
5	1 + 0.4 + 0.1		3	
6	2		5 + 0.4	
7	2 + 0.1		5 + 0.7	
8	2 + 0.7 + 0.2		5 + 1.6 + 1	
9	2 + 0.7 + 0.2 + 0.1		5 + 1.8 + 1	
10	3 + 0.2		4 + 3 + 2 + 1	
11	4 + 0.2		5 + 4 + 3 + 2	
12	4 + 0.4		5 + 4 + 3 + 2 + 0.4 + 0.1	
13	4 + 0.4		5 + 4 + 3 + 2 + 1.3	0.1
13a	4 + 0.5		5 + 4 + 3 + 2 + 1.5	0.1
14	4 + 0.4		5 + 4 + 3 + 2 + 1.9 + 0.7	
14a	4 + 0.4 + 0.1		5 + 4 + 3 + 2 + 1.8 + 1	

A study of the foregoing table clearly shows the variation in the quality of liquid petrolatum furnished to the American market; that is, if the carbonizable impurities test is a criterion of quality; for it will be seen that under this sulphuric acid color test some samples show more than 10 times as much color as others. Some samples gave so little color that matching the hue of the sulphuric acid layer with our Lovibond glasses was a difficult matter; on the other hand, the samples at the lower end of the table gave a sulphuric acid layer of brown rather than amber color. Making an arbitrary decision we found that of the fourteen samples, seven would fail to pass a standard set at the color of Sample No. 7, while three would fail to pass were the standard color set to match Sample No. 11. To set a more lenient standard than the one last mentioned would in our opinion transcend the pharmacopœial requirement of "pale amber color."

TABLE II.

Lovibond Readings of Co-Fe-Cu Matching Fluids for "Carbonizable Impurities" Test.

NOTE.—Fluid read 16-mm. "all-glass cells."

Standard Fluid.	Composition.	Lovibond reading.		
		Red.	Yellow.	
F ₂	$\left\{ \begin{array}{l} N/2 \text{ ferric soln. } 2.1 \text{ cc; } N/2 \\ \text{cobalt soln. } 0.75 \text{ cc; } N/2 \text{ cop-} \\ \text{per soln. } 0.15 \text{ cc; water } 2 \text{ cc.} \end{array} \right\}$	1.9	5 + 0.4	
		(Compare with Sample No. 6 above)	2.0	5 + 0.4
		(Compare with Sample No. 7 above)	2.0 + 0.1	5 + 0.7
G	$\left\{ \begin{array}{l} N/2 \text{ ferric soln. } 3.0 \text{ cc; } N/2 \\ \text{cobalt soln. } 1.5 \text{ cc; } N/2 \text{ copper} \\ \text{soln. } 0.5 \text{ cc; water none} \end{array} \right\}$	4.0	5 + 4 + 3 + 2 + 0.2 + 0.1	
		(Compare with Sample No. 11 above)	4.2	5 + 4 + 3 + 2
		(Compare with Sample No. 12 above)	4.4	5 + 4 + 3 + 2 + 0.4 + 0.1

Choosing these two samples as norms, we proceeded to match up these respective hues, matching the mixed Co-Fe-Cu fluids overlaid with liquid petrolatum in the glass-stoppered French square bottles previously set aside as our standard "all-glass cells." Incidentally it might be stated that the difference in specific gravity of the concentrated acid and of the aqueous Co-Fe-Cu fluids had to be taken into consideration, but that we succeeded in striking satisfactory matches as Table II shows.

Summarizing our work on this sulphuric acid color test for liquid petrolatum we find no difficulty in fixing a definite standard for the maximum degree of color that should be produced by the test. Whether this color should be the greater or the less tint suggested above is a matter for the proper sub-committee of the U. S. P. Revision Committee to decide.

We therefore suggest that the paragraph describing the test be made to read as follows:

Introduce into a glass-stoppered cylinder, which has been previously rinsed with sulphuric acid, 5 cc of liquid petrolatum and 5 cc of colorless sulphuric acid; heat in a water-bath during ten minutes, shaking well at intervals of thirty seconds; the oil remains unchanged in color and the acid does not become darker than the standard color produced by mixing in a similar glass-stoppered cylinder 2.1 cc of colorimetric ferric T. S., 0.75 cc of colorimetric cobalt T. S., 0.15 cc of colorimetric copper T. S., and 2 cc of distilled water; this mixed fluid being overlaid with 5 cc of liquid petrolatum.

If the more lenient standard set by Fluid G of Table II be adopted, the last lines of the test would read, "mixing in a similar glass-stoppered cylinder, 3 cc of colorimetric ferric T. S., 1.5 cc of colorimetric cobalt T. S., and 0.5 cc of colorimetric copper T. S., this mixed fluid being overlaid, etc."

This test being accepted it would necessitate the inclusion among the reagents in Part II of the Pharmacopœia of the following new test solutions:

Colorimetric Cobalt T. S.—Dissolve 59.4965 grams (or 59.5 grams) of cobalt chloride, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, in enough of a fluid made by mixing 25 cc of hydrochloric acid U. S. P. with 975 cc of distilled water to make 1000 cc of test solution. This test solution should be standardized to the above half-normal strength by the following assay: Place 5 cc in a 250-cc flask; add 15 cc of 20 per cent. sodium hydroxide and 5 cc of solution of hydrogen dioxide (3 per cent.), boil for ten minutes, cool, add 2 grams potassium iodide, followed by 20 cc sulphuric acid (1:4). When the precipitate has dissolved titrate with tenth-normal sodium thiosulphate. Each cubic centimeter of *N*/10 thiosulphate corresponds to 0.023799 gram $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$.

Colorimetric Ferric Test Solution.—Dissolve about 50 grams of ferric chloride U. S. P. in enough of a fluid made by mixing 25 cc of hydrochloric acid U. S. P. with 975 cc of distilled water, to make 1000 cc of test solution. This test solution must be standardized to half-normal strength (45.054 grams $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to the liter) by the hydrochloric acid, potassium iodide, sodium thiosulphate volumetric assay, found under *Ferri Chloridum* (p. —); 10 cc of the test solution being employed. Each cubic centimeter of *N*/10 thiosulphate corresponds to 0.027032 gram $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$.

Colorimetric Copper Test Solution.—Dissolve 62.43 grams of copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, in enough of a fluid made by mixing 25 cc of hydrochloric acid U. S. P. and 975 cc of distilled water, to make 1000 cc of test solution. This test solution should be standardized to the above half-normal strength by the acetic acid,

potassium iodide, sodium thiosulphate volumetric assay, found under *Cupri Sulphas* (p. —); 10 cc of the test solution being employed. Each cubic centimeter of *N/10* thiosulphate corresponds to 0.024972 gram $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

COLORIMETRIC SUPRARENAL ASSAY.

In U. S. P. IX appears the following assay for Dried Suprarenals:

Add 0.005 gram of finely powdered manganese dioxide and 10 cc of distilled water to 0.01 gram of Dried Suprarenals; thoroughly shake the mixture several times during one hour and filter. Compare the color of the filtrate in a test-tube or in any other convenient manner with the color of cobaltous chloride T. S. and diluted gold chloride T. S. combined as follows: Mix 1.85 cc of cobaltous chloride T. S. with 0.95 cc of diluted gold chloride T. S. and 7.2 cc of distilled water; the color corresponds to 0.2 per cent. of epinephrine in the filtrate obtained above; 2.95 cc of cobaltous chloride T. S. with 1.25 cc of diluted gold chloride T. S. and 5.8 cc of distilled water corresponds in color to 0.4 per cent. of epinephrine; 4.05 cc of cobaltous chloride T. S. with 1.35 cc of diluted gold chloride T. S. and 4.6 cc of distilled water corresponds in color to 0.6 per cent. of epinephrine; 5.15 cc of cobaltous chloride T. S. with 1.55 cc of diluted gold chloride T. S. and 3.3 cc of distilled water corresponds in color to 0.8 per cent. of epinephrine.

The percentages of epinephrine indicated by the above color standards are based upon the maceration of 0.01 gram of the Dried Suprarenals in 10 cc of distilled water as directed above and filtering. In samples containing more than 0.8 per cent. of epinephrine 0.005 gram of the Dried Suprarenals may be taken, in which case the percentage stated above, as indicated by the color standards, should be doubled.

This assay is far from satisfactory. The actual manipulation of the suprarenals is good but the preparation of the matching fluid could be improved upon. In the first place no concordant color results can be obtained among different operators unless the exact strength of the standard color solutions are known. Merely weighing out 59.4965 grams of cobalt chloride and dissolving it in 1000 cc of acidulated water does not produce fluids of uniform hue although in our own work we have found in the case of cobalt and copper solutions fairly good approximate results may be obtained at the hands of an experienced worker. But why select as the yellow standard a solution of gold chloride when equally good results can be obtained by the use of inexpensive ferric chloride solution? Another objection to the standard set is that the resultant color is too light for accurate matching, our own experience being that colors not darker than 10 Lovibond color units and not lighter than 2 Lovibond units represent the limits of accurate color valuation. For purposes of comparison, however, we tried out twenty samples of dried suprarenals by the exact terms of U. S. P. IX and compared these with the cobalt-gold fluids directed in the official assay.

All of the samples were furnished by pharmacist friends and were taken directly from their stock bottles. While most of the samples were in powdered form, some were in tablets and in handling the latter we used the aliquot part representing the amount of pure dried gland directed in the assay of U. S. P. IX. In all cases the amount of suprarenal used was that directed by U. S. P. IX, *viz.*, 0.01 gram. For purposes of comparison the Lovibond readings of the U. S. P. matching fluids representing 0.4 per cent. and 0.6 per cent. of epinephrine are given. Incidentally we prepared a match from our Co-Fe-Cu fluids containing 1.7 cc of half-normal cobalt chloride, 1 cc of half-normal ferric chloride and 10 cc of water, but it does

not figure in this paper since we prefer to base our suggested assay against solutions of ten-fold greater epinephrine content than that provided by the assay of U. S. P. IX.

TABLE III.

Lovibond Readings of Suprarenal Assay of U. S. P. IX.

NOTE.—Fluids read in $\frac{1}{4}$ -inch Lovibond cell.

Sample No.	Lovibond readings.	
	Red.	Yellow.
10 Powder	1.8	1 + 0.2 + 0.1
11 Tablet	0.7	0.4 + 0.1
12 Powder	2 + 0.2 + 0.1	1 + 0.7
13 Powder	1 + 0.7 + 0.1	0.7 + 0.4
14 Powder	1.9	1 + 0.4
15 Powder	1 + 0.1	0.7
16 Powder	1 + 0.7	1 + 0.2
17 Powder	1 + 0.7 + 0.2	1 + 0.2 + 0.1
18 Powder	2 + 0.4	1.8
19 Powder	2 + 0.1	1 + 0.7
20 Powder	2 + 0.1	1.8
21 Powder	2 + 0.2	1.5 + 0.2 + 0.1
22 Powder	1.9	1 + 0.4
23 Tablet	0.4 + 0.1	0.4
24 Powder	2 + 0.2	1 + 0.4 + 0.1
25 Powder	1 + 0.2	0.7 + 0.1
26 Tablet	1 + 0.1	0.7
27 Powder	2 + 0.2	1 + 0.7
28 Powder	1 + 0.7	1 + 0.2 + 0.1
29 Powder	2 + 0.1	0.7 + 0.2
Cobalt-gold solution of U. S. P. IX representing		
0.6 per cent. epinephrine	1.6	1 + 0.1
0.4 per cent. epinephrine	1 + 0.1	0.4 + 0.2 + 0.1

Finding the assay of U. S. P. IX far from satisfactory we tried checking the epinephrine color reaction against our Co-Fe-Cu colored fluids.

As we found the tints produced in the official assay (10 cc containing, respectively, 0.00004 gram and 0.00006 gram epinephrine) too light for exact comparisons, we used solutions, 10 cc of which contained, respectively, 0.0002 gram, 0.0004 gram and 0.0006 gram epinephrine. That is, we established our Co-Fe-Cu standards at the color of fluids containing 10 times as much epinephrine as the standards set by U. S. P. IX. This was apparently the strength of the adrenalin solutions employed by Scoville in his work commented upon below.⁸

As to the Scoville paper, since our work is based upon the suprarenals assay while his dealt with the assay of adrenalin solutions his results, while highly interesting and important, are of value to our work only by way of comparison.

Scoville studied the adrenalin-manganese dioxide reaction from the standpoint of temperature and acidity. He also studied the adrenalin-potassium iodate color reaction and found the latter preferable to the former test. He then formulated a matching liquid for the adrenalin-potassium iodate reaction, dissolving cobalt chloride in water and adding thereto ammonium carbonate solution, thereby changing the red color of the cobalt chloride solution to the purplish red color

required to match the hue of adrenalin-potassium iodate reaction. Of his matching solution, Scoville says, "The ammoniacal cobalt chloride solution therefore differs enough in color to limit its usefulness, but it can be used to verify the claim for a 1:1000 solution of adrenalin, provided the test be applied with proper precautions."

As our preliminary work we tried comparison tests using the same solutions of adrenalin with manganese dioxide and with potassium iodate. As our standard adrenalin we used a sample kindly furnished by Parke, Davis and Co., and later securing through the courtesy of the H. A. Metz Laboratories, Inc., a sample of synthetic epinephrine (laevo-methyl-amino-ethanol-catechol), we used this as a check against the Parke, Davis adrenalin. As the synthetic product yielded a solution that became slightly colored on standing we were not able to conduct as many tests with it as with the natural product.

However, as shown in Table IV the color obtained with the standard natural adrenalin closely agreed with the synthetic sample.

TABLE IV.

Lovibond Readings of Solutions of Pure Adrenalin and of Synthetic Epinephrine.

NOTE.—Fluids read in $\frac{1}{2}$ -inch Lovibond cell.

Source.	Milligram per 10 cc.	Red.	Lovibond readings.	
			Yellow.	
Natural	0.2	3 + 0.4 + 0.1	1 + 0.7 + 0.1	
Synthetic	0.2	3 + 0.4 + 0.2	1 + 0.7	
Natural	0.4	5 + 1 + 0.7 + 0.1	3 + 1 + 0.1	
Synthetic	0.4	5 + 1 + 0.7 + 0.1	3 + 1	
Natural	0.6	5 + 4 + 0.7 + 0.1	5 + 1 + 0.7	
Synthetic	0.6	5 + 4 + 0.7	5 + 1 + 0.7	

Taking up Scoville's results as to the influence of temperature he reports that different colors were obtained with the same solution when the reaction was conducted at 15°, 20°, 38° (optimum) and 80°. In the case of adrenalin solutions, variations in the temperature during reactions may be of importance but in our own work we feel that the conditions of average room temperature are about all with which we need to concern ourselves.

Below is given Lovibond readings of the same adrenalin solution, the reactions being carried out at room temperatures of 22° C. and 28° C., respectively.

TABLE V.

Lovibond Readings of the Same Adrenalin Solution at 22° C. and 28° C.

Temperature.	Red.	Lovibond reading.	
		Yellow.	
22° C.	5 + 4 + 0.7 + 0.1	5 + 1 + 0.7	
28° C.	5 + 4 + 0.7 + 0.1	5 + 1 + 0.4 + 0.2	

As to acidity, it will be noted that the suprarenal manganese dioxide test of U. S. P. IX is carried on in a non-acid medium, hence Scoville's statement as to acidity, while of the utmost importance in assaying adrenalin solutions, does not necessarily apply to the suprarenal test. However, for purposes of comparison we ran a few suprarenal tests: (a) exactly as per U. S. P. IX, and (b) as per U. S. P. IX after adding 0.1 cc of *N* HCl. In each case we noted the fading of color pointed out by Scoville.

The work just reported did not convince us of the superiority of the potassium iodate reaction over the manganese dioxide method and as the latter assay is the simpler of the two and as it yields a color more easily matched, we feel that this manganese dioxide method is the best to follow.

Incidentally, while U. S. P. IX directs in its suprarenal assay the use of "manganese dioxide" the absence of capitals would seem to indicate that Precipitated Manganese Dioxide, U. S. P. was not intended. However, among the reagents in Part II of the Pharmacopœia no mention of manganese dioxide can be found. In our work we experimented with natural black oxide of manganese and also with a sample of the official brownish dioxide. The Lovibond readings given in Table VI show that both types of manganese dioxide give about the same color value.

TABLE VI.

Lovibond Readings of the Same Adrenalin Solution with Different Dioxides.

Reagent.	Lovibond reading.	
	Red.	Yellow.
Ppt. MnO ₂	5 + 4 + 0.7 + 0.1	5 + 1 + 0.4 + 0.2
Natural MnO ₂	5 + 4 + 0.7 + 0.1	5 + 1 + 0.7

Having established the color values of the epinephrine-manganese dioxide test, we next duplicated the tints with our Co-Fe-Cu solutions. Below are given Lovibond readings of several blends we tried out.

TABLE VII.

Lovibond Readings of Co-Fe-Cu Matching Fluids.

No.	Co soln. Cc.	Blends. Fe soln. Cc.	H ₂ O. Cc.	Lovibond readings.	
				Red.	Yellow.
A	2.8	1.1	6.1	3 + 0.4 + 0.1	1 + 0.7 + 0.2
B	2.7	1.1	6.2	3 + 0.2 + 0.1	1 + 0.7 + 0.1
C	2.8	1.2	6.0	3 + 0.4	1 + 0.7 + 0.4
0.2 mg. epinephrine per 10 cc (Table IV)				3 + 0.4 + 0.1	1 + 0.7 + 0.1
G	5.4	0.8	3.8	5 + 1 + 0.4	3 + 0.7 + 0.2
H	6.4	0.9	2.7	5 + 1 + 0.7 + 0.1	3 + 1 + 0.1
0.4 mg. epinephrine per 10 cc (Table IV)				5 + 1 + 0.7 + 0.1	3 + 1 + 0.1
D	9.1	0.9		5 + 4 + 0.4	5 + 1 + 0.2
E	9	1		5 + 4 + 0.2	5 + 1 + 0.2 + 0.1
F	9.15	0.85		5 + 4 + 0.7	5 + 1 + 0.2 + 0.1
0.6 mg. epinephrine per 10 cc (Table IV)				5 + 4 + 0.7	5 + 1 + 0.4 + 0.2

Table VII shows that the best Co-Fe-Cu match of a epinephrine solution (0.2 mg. to 10 cc and corresponding, in an assay where 0.1 gram of suprarenals is employed, to a 0.2 per cent. epinephrine content) is a blend of 2.8 cc of half-normal cobalt solution, 1.1 cc of half-normal iron solution and 6.1 cc of distilled water; that the best match for 0.4 per cent. epinephrine content is 6.4 cc of half-normal cobalt solution, 0.9 cc of half-normal iron solution and 2.7 cc of distilled water; and that the best match for 0.6 per cent. epinephrine content is 9.15 cc of half-normal cobalt solution, 0.85 cc of half-normal iron solution and no water. We therefore suggest the following wording for the U. S. P. assay of Dried Suprarenals:

Add 0.005 gram of finely powdered manganese dioxide and 10 cc of distilled water to 0.1 gram of Dried Suprarenals. Thoroughly shake the mixture several times during one hour and filter. Compare the color of the filtrate in a test-tube or in any other convenient manner with the color of the following blends of colorimetric cobalt T. S. (p. —) and colorimetric ferric T. S. (p. —). Mix 6.4 cc of

colorimetric cobalt T. S., 0.9 cc of colorimetric ferric T. S. and 2.7 cc of distilled water; the color corresponds to 0.4 per cent. epinephrine in the filtrate obtained above. Mix 9.15 cc of colorimetric cobalt T. S. with 0.85 cc of colorimetric ferric T. S.; the color corresponds to 0.6 per cent. of epinephrine in the filtrate obtained above.

The percentages of epinephrine indicated by the above color standards are based upon the maceration of 0.1 gram of Dried Suprarenals in 10 cc of distilled water as described above. In samples containing more than 0.6 per cent. of epinephrine, 0.05 gram of Dried Suprarenals may be taken, in which case the percentage stated above, as indicated by the color standards, should be doubled.

Having established the foregoing quantitative test, we applied it to eight samples of commercial solutions of suprarenal solution (labelled 1 in 1000) diluting each for purposes of comparison, the basis of 0.0006 gram Adrenalin in 10 cc.

It is obvious that the test applied to commercial solutions is an uncertain one, for, as Scoville says, "commercial solutions always contain an excess of acid, and many of them a reducing agent also;" the latter usually being sodium bisulphite.

Our results are tabulated below.

TABLE VIII.

Lovibond Readings of Commercial Epinephrine Solutions.

NOTE.—Fluid read in $\frac{1}{2}$ -inch Lovibond cell. All solutions labelled 1 in 1000. In tests, we used 0.6 cc of the solution in each 10 cc of filtrate.

Solution No.	Red.	Yellow.
1	5 + 4 + 1 + 0.1	5 + 2 + 0.1
2	5 + 4 + 1 + 0.1	5 + 2 + 0.1
8	5 + 4 + 0.1	5 + 1
7	5 + 1 + 0.7 + 0.1	4 + 1 + 0.2
4	5 + 1	5 + 0.7
6	4 + 0.4	3 + 0.2
5	4 + 0.2 + 0.1	3 + 0.1
3	3 + 0.4 + 0.2	3 + 0.4

Lastly we applied our proposed assay to the twenty samples of Dried Suprarenals already described above and reported on in Table III. Our results are tabulated below.

TABLE IX.

Lovibond Readings of Proposed Suprarenal Assay.

NOTE.—Fluids read in $\frac{1}{2}$ -inch Lovibond cell.

No.	Red.	Yellow.
10	5 + 4 + 3 + 2 + 1.8	5 + 4 + 2 + 0.1
11	5 + 0.7 + 0.2	3 + 1 + 0.1
12	5 + 4 + 3 + 2 + 1.3 + 1 + 0.1	5 + 4 + 2 + 0.7
13	5 + 4 + 3 + 1	5 + 4 + 1.6
14	5 + 4 + 3 + 2 + 0.1	5 + 4 + 2 + 0.4
15	5 + 4 + 0.2 + 0.1	5 + 2 + 0.4 + 0.1
16	5 + 4 + 3 + 1.5	5 + 4 + 2 + 0.1
17	5 + 4 + 3 + 0.2 + 0.1	5 + 4 + 2 + 1 + 0.4
18	5 + 4 + 3 + 2 + 1.6 + 0.1	5 + 4 + 3 + 0.7
19	5 + 4 + 3 + 2 + 1.5 + 0.1	5 + 4 + 3 + 1 + 0.4
20	5 + 4 + 3 + 1.8 + 0.1	5 + 4 + 3 + 1

21	5 + 4 + 3 + 2 + 0.2 + 0.1	5 + 4 + 3 + 2
22	5 + 4 + 3 + 0.7	5 + 4 + 3 + 1
23	4 + 0.7	3 + 0.7
24	5 + 4 + 3 + 2 + 1.8 + 0.1	5 + 4 + 2
25	5 + 4 + 1.8 + 0.1	5 + 4 + 3
26	5 + 3 + 0.7	5 + 2 + 0.7
27	5 + 4 + 3 + 2 + 1.3 + 0.1	5 + 4 + 3 + 1 + 0.7
28	5 + 4 + 2 + 0.2	5 + 4 + 2 + 0.7 + 0.1
29	5 + 4 + 3 + 2 + 1 + 0.1	5 + 4 + 3 + 0.7

Studying Tables VIII and IX we find that of the twenty samples of Dried Suprenals examined two showed colors suggesting an adrenalin content between 0.4 per cent. and 0.6 per cent. Sixteen were above this standard and two were sub-standard. Of the eight commercial solutions examined three were apparently up to the labelled 1-1000 strength, none were above standard and five were sub-standard. In fairness, however, it must be stated again that degree of acidity and presence of reducing agents cause the colorimetric assay of solutions to be far from reliable.

SUMMARY.

1. This paper discusses the Co-Fe-Cu standard colorimetric solutions pointing out their simplicity, accuracy and practicability.
2. It describes a simple volumetric assay of cobalt solutions.
3. It establishes Lovibond color values for the test for "carbonizable impurities" in liquid petrolatum prescribed in U. S. P. IX and offers an improved method of carrying out this test by use of a standard color prepared from the Co-Fe-Cu fluids.
4. It likewise establishes Lovibond color values for the colorimetric assay of Suprarenal Glands as prescribed in U. S. P. IX and offers an improved assay based upon the Co-Fe-Cu fluids.

BIBLIOGRAPHY.

1. (a) *Am. Drug.*, 60, 35, 1912; (b) *Proc. 8th Int. Cong. App. Chem.*, 26, 319, 1912; (c) *D-A. Apoth. Ztg.*, 33, 165, 1913; (d) *Drug. Circ.*, 57, 131, 1914; (e) *J. Frank. Inst.*, 180, 199, 1915; (f) *JOUR. A. PH. A.*, 4, 1294, 1915; (g) *J. Ind. Eng. Chem.*, 8, 309, 1916; (h) *Ibid.*, 11, 950, 1919.
2. See item (h) in Reference 1.
3. See item (e) in Reference 1.
4. Engel and Gustavson, *J. Ind. & Eng. Chem.*, 8, 901, 1916.
5. See item (b) in Reference 1.
6. See item (d) in Reference 1.
7. Priest, *Proc. Soc. Cotton Products Analysts*, 6, 1913.
8. Scoville, *J. Ind. & Eng. Chem.*, 12, 769, 1920.

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AUGUST 1923.