THE "CO-FE-CU" FLUIDS AND CERTAIN PHARMACOPŒIAL TESTS.*,†

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INTRODUCTION.

This is the thirteenth of a series of papers on the "Co-Fe-Cu" standardized colored fluids which have been published since 1912 by the senior author and his co-workers. These fluids have been described at length in the other papers so at this place, we need only present recipes for their manufacture couched in pharmacopœial phraseology.

THE CO-FE-CU FLUIDS.

Colorimetric Cobalt T.S.—Dissolve about 60 grams of cobalt chloride, $CoCl_2.6H_2O$, 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 $^1/_4$ molar strength (59.496 grams $CoCl_2.6H_2O$ to the liter) 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 $CoCl_2.6H_2O$.

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 $^1/_6$ molar strength (45.054 grams $FeCl_2.6H_2O$ to the liter) by the hydrochloric acid, potassium iodide, sodium thiosulphate volumetric assay, found under *Ferri Chloridum* (p. ...); 10 cc. of the test solution is employed. Each cubic centimeter of $N/10$ thiosulphate corresponds to 0.027032 gram $FeCl_3.6H_2O$.

Colorimetric Copper Test Solution.—Dissolve about 65 grams of copper sulphate, $CuSO_4.5H_2O$, 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 $^1/_4$ molar strength (62.43 grams $CuSO_4.5H_2O$ to the liter) by the acetic acid, potassium iodide, sodium thiosulphate volumetric assay found under *Cupri Sulphas* (p. ...); 10 cc. of the test solution is employed. Each cubic centimeter of $N/10$ thiosulphate corresponds to 0.024972 gram $CuSO_4.5H_2O$.

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[*] Scientific Section, A. Ph. A., Madison meeting, 1933.

[†] The work described in this paper was performed at the request of Dr. George D. Beal, chairman of the U. S. P. sub-committee on organic chemicals. Our thanks are due to the Abbott Laboratories, Mallinckrodt Chemical Works, Merck & Company, New York Quinine and Chemical Works, Charles Pfizer & Co. and E. R. Squibb and Sons, for samples of the chemicals studied and to Messrs. Armao, Brown, Cooper, D'Orazio, Ginsberg, Givens, Glockner, Héiko, Hoehler, Isacoff, Kleinsinger, Sher, Silver, Vigilante, Wight and Ziliotto, fourth-year students of Columbia University College of Pharmacy, for the preliminary work upon which our final standards were based. [A. and T.]

POSSIBLE PHARMACOPŒIAL APPLICATIONS OF THESE FLUIDS.

In 1919, Arny, Kish and Newmark used these fluids to set the color standard for cottonseed oil; in 1923, Arny and Taub used them for setting color standards for (*a*) the "carbonizable impurities" test for Liquid Petrolatum, (*b*) the colorimetric suprarenal assay of U. S. P. IX; in 1933, Taub reported "Co-Fe-Cu" matches for the color of cod liver oil, almond oil and castor oil.

During the past year, Dr. George D. Beal requested us to set standards for all of the "readily carbonizable substances" tests of U. S. P. X. Thanks to the sixteen senior students mentioned in the foot-note, a preliminary study of these tests was made. Three to five different samples of the chemical were treated with concentrated sulphuric acid as directed in U. S. P. X and the resulting color (if any) was determined on the Lovibond tintometer that has been used in our color work of the past twenty years.

A color match was then prepared from the "Co-Fe-Cu" standard fluids. The results obtained by these students were then checked up by the authors of the present paper with three basic principles in mind: (*a*) Was the darker color produced by a fair sample? (*b*) Could the test of U. S. P. X be simplified? (*c*) Should prescribed quantities be placed upon a uniform basis?

Before discussing these principles, a typical "carbonizable substances" test of U. S. P. X should be quoted:

> The solution of 0.5 Gm. of Acetanilid in 5 cc. of sulphuric acid is colorless or only faintly yellow.

If the color matches given below are accepted by the Committee on Revision, the color phraseology given above will be revised. At this time, however, attention should be called to the uncertainty of color description. Thus, among the 51 descriptions of these sulphuric acid color tests of U. S. P. X we find "faintly yellow," "light yellow," "slightly yellow," "light yellow or slightly brown," "transient pinkish tint," "colorless or nearly so," "pale amber" and "no appreciable darkening." These descriptions are bewildering, especially when our tests showed that a sample producing a supposedly "light yellow" color revealed to our eyes a liquid that was greenish rather than yellow.

Taking up the three principles enunciated above, the following comments are in order:

As to fairness of sample, we frequently found that the deepest color produced in the preliminary work was that resulting from the action of the acid on a sample that had been in our college stock for some time. In all such cases, we [A. and T.] set our standard upon the poorest sample of the three or more sent in for this particular test by our manufacturing friends. We must, moreover, hasten to say that the phrase "poorest sample" just used, is scarcely fair, since all of the samples sent us this year were of excellent quality. In fact, we were confronted by the embarrassing situation that many of our recently received samples were colorless after treatment with sulphuric acid.

As to simplification of testing, there is little to be said and that topic is carried over to general directions given farther along in this paper.

As to uniformity in quantities, we believe that attempts in this direction should be made. The foregoing transcript of the wording of the test for Acetanilid calls for 0.5 Gm. of the chemical and 5 cc. of the acid. The 5-cc. volume is the proper one for all tests, whenever it is practical. We believe that 5 cc. of acid should be used in the chloroform test even as now obtains in the test for carbon tetrachloride. The 2-cc. quantities of sulphuric acid prescribed in some thirty of the tests of U. S. P. X are almost impracticable because of the difficulty in determining a match for so small an amount of liquid. Because the fluid to be matched is essentially concentrated sulphuric acid, colorimeter cells can scarcely be used and 2 cc. of fluid in a test-tube or bottle is almost unmatchable. Of course, we realize that most of the chemicals tested with 2 cc. of acid are expensive. One (Dichloramine) explodes when mixed in the proportion 0.5 Gm. in 5 cc. of acid, but reacts without violence in the proportion 0.1 Gm. to 1 cc. of acid. However, a revision as to volume of acid is desirable, and in our findings given below, we have generally put the test upon a 5-cc. basis.

In testing the approximately fifty chemicals for which U. S. P. X prescribes the sulphuric acid color test, we discovered twenty blends which provided matches for all of the chemicals tested. These are tabulated below where columns headed "Co," "Fe" and "Cu," respectively, mean the volume (in cubic centimeters or fractions thereof) of the standard cobalt or iron or copper solutions required in 5 cc. of the finished standard matching fluid. The only exception is blend "B" which is adjusted to a 10-cc. basis. Of course H_2O means in this case, the amount of water used as the diluent.

Table of Matching Fluids.

	Co.	Fe.	Cu.	H_2O.
"Grayish"				
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	8.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
"Fawn Colored"				
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
"Yellow"				
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0
L	0.8	3.8	0.1	0.3
"Green"				
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0
"Pink"				
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

These are the twenty blends just as we found them as matches for the colors produced by action of concentrated sulphuric acid upon the approximately fifty chemicals studied by us. There is little doubt that a check-up of our work by the U. S. P. sub-committee on organic chemicals may reduce the matches to 2 "grayish," 1 "fawn," 2 or 3 "yellows," 2 "greens" and 2 "pinks." If this lessening of the number of matches is agreed upon, the wording of the test in the individual monograph may be materially simplified.

SUGGESTED OUTLINE OF THE PROPOSED SULPHURIC ACID TEST.

First, there will be introduced among the test solutions described in the rear pages of U. S. P. XI, specifications for colorimetric test solutions of cobalt, iron and copper, with wording substantially as given in the first part of this paper.

Secondly, there should appear among the "General Tests, Processes and Apparatus" of U. S. P. XI a concise description of "Colorimetric Test for Readily Carbonizable Substances in Organic Chemicals."

This description should include:

I. GENERAL DIRECTIONS AND WARNINGS.

(*a*) *Finely Powder the Chemical Prior to Weighing.*—Otherwise solution is delayed beyond reasonable limits of time.

(*b*) *Time Limit.*—Matching should be performed not less than 15 or more than 30 minutes after complete solution has been obtained.

(*c*) *Gas Evolution.*—Some chemicals upon addition of the acid produce vigorous effervescence. Do not stopper container until effervescence is completed.

(*d*) *Matching Containers.*—As already mentioned, the strongly acid liquid should not be poured into the average colorimeter cell. In our experience with the 5-cc. batches of the acid liquid, we find matching in test-tubes is far from satisfactory. Above all, the operator should be warned not to attempt matching by looking down on the surface of the liquid in a Nessler tube placed on a white surface. Our own experience indicates that the best method of matching is by using a 15-cc. ($^1/_2$ ounce) glass stoppered "French square" bottle of clear flint glass. In the run of a dozen such bottles, a large percentage of them will be found to be uniform as to interior area, at least as far as the accuracy of this acid test demands.

(*e*) *The Act of Matching.*—If "French square" bottles are employed, the best match is secured by viewing the fluids transversely against a white background (porcelain or white glass plate).

II. TABLE OF MATCHING FLUIDS.

Similar to that given above. With this information in condensed form, the wording of the test in the monograph may be expressed something like this:

> The solution of 0.5 Gm. of Acetanilid in 5 cc. of sulphuric acid has no more color than matching fluid A, described under the "test for carbonizable substances" (p. . . .).

As to these "carbonizable substances" tests, all remaining to report are the colors produced with concentrated sulphuric acid.

COLOR MATCHES.

Blend A.—Acetanilidum, Aethylis Aminobenzoas, Atropina, Atropinæ Sulphas, Barbitalum, Barbitalum Solubile, Caffeinæ Sodio-Benzoas, Carbonei Tetrachloridum, Carbromalum, Chloramina, Chloroformum, Cocaina, Glusidum, Glusidum Solubile, Phenobarbitalum, Pilocarpinæ Nitras, Strychninæ Sulphas, Theobrominæ Sodio-Salicylas and Theophyllina.

Blend B.—Glycerinum.
Blend C.—Acidum Salicylicum.
Blend D.—Caffeina.
Blend E.—Pelletierinæ Tannas.
Blend F.—Cocainæ Hydrochloridum.
Blend G.—Procainæ Hydrochloridum.
Blend H.—Emetinæ Hydrochloridum.
Blend I.—Physostigminæ Salicylas.
Blend J.—Caffeina Citrata.
Blend K.—Acidum Citricum.
Blend L.—Strychninæ Nitras.
Blend M.—Quinidinæ Sulphas, Quinina, Quininæ Bisulphas, Quininæ Dihydrochloridum, Quininæ et Ureæ Hydrochloridum, Quininæ Sulphas.
Blend N.—Cinchophenum.
Blend O.—Santoninum.
Blend P.—Chloralis Hydras, Dichloramina.
Blend Q.—Acidum Acetylsalicylicum, Acidum Benzoicum.
Blend R.—Guaiacolis Carbonas.
Blend S.—Codeina, Codeinæ Sulphas.
Blend T.—Acetphenetidinum.

The foregoing tabulation suggests a number of special remarks explaining certain deviations from the fixed rules outlined above:

Blend A represents a grayish tint just beyond the clear color of distilled water. Among the chemicals listed in this group are found some that are directed in the U. S. P. X to be "colorless or nearly so." Among these are the two barbitals, chloral hydrate, eucaine hydrochloride, dichloramine and procaine hydrochloride. In our experiments, at least one of the samples of each chemical just mentioned showed a faint suggestion of color; hence we included all of these chemicals in the group matched by "Blend A."

Lactic Acid.—U. S. P. X gives a sulphuric acid test for this chemical. The specifications call for "no dark zone," hence it fell outside the class of exact color matching.

Carbon Tetrachloride and Chloroform Tests call for the shaking of 20 or 40 cc. of the chemical with 4 or 5 cc. of concentrated sulphuric acid in a glass-stoppered cylinder and viewing color of acid after shaking. We recommend that both tests be run with 40 cc. of the chemical and 5 cc. of the acid. The surplus of the chemical is to be decanted and the acid residue transferred to the 15-cc. glass-stoppered "French square" bottle for matching against the standard blend.

Codeine.—The pink color produced is not so transient as the Pharmacopœia suggests. That is, the acid matched the same pink fluid (Blend S) both 15 and 60 minutes after complete solution.

Dichloramine.—This test must be handled with care. When 0.5 Gm. is mixed with 5 cc. of sulphuric acid, an explosion occurs. For this reason, as mentioned above, we advise the present U. S. P. proportion 0.1 Gm. to 1 cc. of the acid.

Gluside.—We recommend that the heating of this chemical with the acid be continued at 48° to 50° C. but we suggest that the phrase "on a water-bath" be deleted.

HYOSCYAMINE HYDROBROMIDE AND SCOPOLAMINE HYDROBROMIDE.

While U. S. P. X gives the sulphuric acid test for these chemicals, we find it is not practical since the yellow color produced in both cases is largely due to bromine.

Pelletierine Tannate.—The process of U. S. P. X is to view the color change as a "spotting test" upon a white porcelain surface. We recommend that the residue be treated with 2 cc. of sulphuric acid and that this be matched in the 15-cc. "French square" bottle.

Liquid Petrolatum.—This color test was reported in a previous paper (2). At this time we need only state that the match then reported (Co, 1.5 cc.; Fe, 3.0 cc.; Cu, 0.5 cc.; H_2O 0.0 cc.) is more amber than the five yellows (H – L) of the tabulation given above).

MATCHING THE COLOR OF FIXED OILS.

In previous papers (3) we have published the composition of blends made from the Co-Fe-Cu fluids for matching the color of cottonseed oil, cod liver oil, almond oil and castor oil. For details, the reader is referred to the original articles listed in the bibliography with which this paper ends. Chairman Beal has requested us to attempt to devise a blend matching the color of halibut liver oil. Securing 8 commercial samples of this now popular oil, we were surprised to note that the color ranged from a light yellow to an amber that was almost brown. As all of the oil samples examined were marketed for medicinal purposes and, as the darker samples in our opinion are not attractive, we picked as our "average sample" one that was darker than 3 samples and lighter than 5 samples. It appears to us that the first step, if the color of halibut oil is to be standardized will be the setting, by mutual agreement, of a proper shade of yellow for medicinal halibut oil. Tentatively, we have ventured to select our "average sample" and this sample is matched by a blend of Co, 4.5 cc.; Fe, 10 cc.; Cu, 0.5 cc. and H_2O, none. This halibut oil problem was submitted to us only recently. Our conclusions are therefore tentative and we recommend that the marketers of this new product give attention to further refining and to establishing a more uniform color.

CONCLUSIONS.

1. Report of the study of the color changes produced when certain U. S. P. chemicals are treated with concentrated sulphuric acid, and a tabulation of 20 blends of the "Co-Fe-Cu" standardized colored fluids by which the colors produced by the "readily carbonizable substances" test of U. S. P. X upon fifty chemicals may be accurately matched.
2. Consideration of the standardizing of the color of official fixed oils already reported by the authors, and publication of a "Co-Fe-Cu" color blend which matches an "average sample" of halibut liver oil.

BIBLIOGRAPHY.

(1) (*a*) *Am. Drug.*, 60 (1912), 35; (*b*) *Proc. 8th Int. Cong. App. Chem.*, 26 (1912), 319; (*c*) *D.-A. Apoth. Ztg.*, 33 (1913), 165; (*d*) *Drug. Circ.*, 57 (1914), 131; (*e*) *J. Frank. Inst.*, 180 (1915), 199; (*f*) JOUR. A. PH. A., 4 (1915), 1294; (*g*) *Ind. & Eng. Chem.*, 8 (1916), 309; (*h*) *Ibid.*, 11 (1919), 950; (*i*) JOUR. A. PH. A., 12 (1923), 839; (*j*) *Ibid.*, 16 (1927), 116; (*k*) *Am. J. Pharm.*, 104 (1932), 272; (*l*) JOUR. A. PH. A., 22 (1933), 195.

(2) See item (*i*) in Reference (1).

(3) See items (*h* and *l*) in Reference (1).