COLOR STANDARDS AND CUDBEAR.*

H. V. ARNY AND C. H. RING.

In 1908 the Committee of Revision of the National Formulary appointed a sub-committee of which one of the writers was chairman, to devise a means of standardization of the color of tincture of cudbear and tincture of caramel made by the methods proposed for the next issue of the Formulary. The difficulties of establishing such standards were explained in detail in a paper that appeared in the *American Druggist* (60-1912-35); the finding of standard color fluids was announced at the Eighth International Congress of Applied Chemistry (Jour. A. Ph. A., 2-1913-76), while the elaboration of the work to practical completion is the subject of a paper appearing in the *Journal of the Franklin Institute* for August, 1915. At this time we will merely demonstrate the standard fluids and will announce that the most difficult problem of all, the matching of cudbear tinctures, has been accomplished.

The result of the work first outlined was the devising of three sets of standard colored fluids, which we have designated as the "Co-Fe-Cu" the "Co-Cro-Cu" and the "Cro-Manganate" blends.

The "Co-Fe-Cu" tints have as their basis: *Red Acidulated Half-Normal Cobalt Solution* containing 59.49 gm. of cobalt chloride, $CoCl_26H_2O$ to the liter, the solvent being one percent hydrochloric acid; *Yellow Acidulated Half-Normal Ferric Solution,* containing 45.05 gm. of ferric chloride, $FeCl_36H_2O$ to the liter, the solvent being one percent hydrochloric acid; *Blue Acidulated Half-Normal Copper Solution,* containing 62.43 gm. copper sulphate, $CuSO_45H_2O$ to the liter, the solvent being one percent hydrochloric acid.

The "Co-Cro-Cu" tints are prepared from ammoniacal solutions of the three elements mentioned, the solvent in each case being 2.8 percent ammonia water. These consist of *Red Ammoniacal Fiftieth-Normal Cobalt Solution,* containing 2.7 gm. of roseo-cobaltic chloride, $CoCl_35NH_3H_2O$ to the liter; *Yellow Ammoniacal Fiftieth-Normal Chromium Solution,* containing 0.420 gm. of ammonium dichromate, $(NH_4)_2Cr_2O_7$ to the liter; and *Blue Ammoniacal Fiftieth-Normal Copper Solution* containing the equivalent of 2.49 gm. copper sulphate to the liter.

Details concerning the preparation of these "Co-Cro-Cu" fluids and their blends will be found in the *Journal of the Franklin Institute* for August, 1915.

The blending of the acidulated fluids to make the "Co-Fe-Cu" tints, and of the ammoniacal fluids to make the "Co-Cro-Cu" hues can, of course, be performed in any proportion that fancy suggests. The 91 samples of each series exhibited include the possible blends produced in making 12 cc. of finished fluid when the ingredients are mixed in even (non-fractional) cubic centimeter quantities. The nomenclature devised is of the simplest kind. Thus the original red fluid is "R. Y. B. 12-0-0" the original yellow is "R. Y. B. 0-12-0" and the original blue is "R. Y. B. 0-0-12." The sample designated as "R. Y. B. 6-6-0" will of

* Presented in Scientific Section A. Ph. A., San Francisco meeting.

course be an orange hue, that called "R. Y. B. 0-6-6" will be green, while "R. Y. B. 6-0-6" has a purplish hue.

That the intensity of color of each of the three basic fluids is about the same as shown by the fact that "R. Y. B. 4-4-4" closely approached, both in the "Co-Fe-Cu" and in the "Co-Cro-Cu" series, the "neutral gray" which is the nearest that blended reds, yellows and blues can approach to pure white in solids or transparent colorlessness in fluids.

As to the permanency of these fluids: the original acidulated cobalt, iron and copper solutions and their blends neither fade nor precipitate to a perceptible degree until at least two years old: the ammoniacal cobalt and chromium solutions have now been under observation for over a year without any fading being detected. The ammoniacal copper on the other hand precipitates and consequently undergoes color change after a few weeks, but our experiments so far show that the blends keep satisfactorily when sealed in ampuls. Moreover the ammoniacal copper solution is in practice prepared extemporaneously by diluting the permanent half-normal acidulated copper solution to fiftieth-normal strength by addition of ammonia water and water, hence the preparation of the "Co-Cro-Cu" blends is merely a matter of mixing solutions that can be kept in stock for months without deterioration.

Having prepared a line of color fluids, the next move was their application as standards. As announced in the paper read before the Eighth International Congress of Applied Chemistry it was found that a standard *Caramel* solution prepared by heating 1 gramme of sugar to 180° C and then diluting with water to 500 cc. matched the "Co-Fe-Cu" blend, R. Y. B. 4-7-1. During the past year, we have found that in a properly conducted *Nessler Test,* an ammonia dilution representing a nitrogen content of 1 to 500,000 matched the "Co-Fe-Cu" blend 3-9-0, when this half-normal mixture was diluted to "50%" of its original strength by addition of an equal volume of water: that the color of the *Phenol-Sulphonic Acid Test for Nitrates* when the nitrogen content was 1 in 500,000 was matched by "Co-Fe-Cu" blend 0-12-0 when this was diluted to "66%" strength: that the *Molybdate Assay for Phosphoric Acid,* 1 in 20,000 gave a yellow color exactly matched by "Co-Cro-Cu." 0-12-0 diluted to "15%" strength: that *Folin's Vanillin Test* when the vanillin content was 1 in 100,000 gave a color matched by "Co-Cro-Cu" blend 3-3-10: that *Riegler's Uric Acid Test* of a uric acid content of 1 in 40,000 had the same tint as "Co-Cro-Cu" blend 2-2-8: and that a salicylic acid dilution of 1 in 50,000 when treated with the proper amount of ferric chloride solution produced a color exactly matching "Co-Cro-Cu" blend 7-1-5, that had been diluted to 65% strength.

In carrying out these colorimetric tests, exactitude of manipulation is essential and the procedure to be followed will be found in detail in a paper which will be published in the near future.

As mentioned above, the two sets of colored fluids "Co-Fe-Cu" and "Co-Cro-Cu" fail when it comes to certain shades of red. Thus the color produced in the *Naphthylamine-Sulphanilic Acid Test for Nitrites* had no match in the pink fluids of the two sets of standard blends. This led to the study of other possible standard fluids that would supply the hues not attained by the two sets of blends just mentioned and these were found in two solutions kept in every well equipped

analytical laboratory, the volumetric solutions of potassium dichromate and potassium permanganate.

An investigation of these fluids showed that a one-thousandth-normal permanganate volumetric solution containing 0.0313 grammes of $KMnO_4$ to the liter has about the same intensity of color as a one-hundredth-normal dichromate solution containing 0.487 grammes of $K_2Cr_2O_7$ to the liter and blends of these two fluids we have designated as the "Cro-Manganate" tints. As might be expected these blends are extremely unstable and must be used for matching within one or two hours after mixing.

Comparing blends of these with the nitrite test mentioned above, we found that the hue produced by a nitrite dilution representing 1 part of nitrogen in 10 million was matched by the standard "Cro-Manganate" blend, 15-1, diluted to 55%.

There still remained unsolved, a part of the original quest, which had started this line of color investigation: a search for standardized matches for caramel and for cudbear. The match for caramel is given above: but the curious reddish blue tint of cudbear had no match in the "Co-Fe-Cu" or the "Co-Cro-Cu" or even in the "Cro-Manganate" blends. The match has however been found and we take pleasure in reporting the news to the Association by whom the color standard work was entrusted to us.

The first step in the work of matching cudbear is to produce a standard cudbear solution of known strength representing a constant color value with any other cudbear solution of the same strength. The difficulty of this task lies in the fact that the official methods of preparing cudbear extracts and tinctures are extremely unsatisfactory, in that they do not produce anything like constant color results.

The only methods of preparation by which we were able to secure satisfactory results in constant color values, were the ones explained in detail by one of us in the *Journal of the American Pharmaceutical Association* for 1913, page 47. These are the *Alcohol-Chloroform-Acetone* and the *Chloroform-Acetone* extracts, both of which produce color values, when made into tinctures and diluted in exactly the same manner and quantity, which are fairly uniform in tinctorial power.

We had on hand some extract of cudbear, made by this method over two years ago. To 0.2 grammes of this was added 0.4 cubic centimeters of 10% ammonia water. Alcohol was then added to make a total volume of 200 cc. This tincture represented 1 part of the extract in 1000. Part of this was then diluted with water to a volume in which cudbear extract content was 1 part in 50,000. The result was a perfectly clear reddish violet liquid.

The Lovibond reading of this in a half-inch cell was as follows:

Red	*Yellow*	*Blue*
5.0+4.0+0.4	—2.2	+2.0

We also prepared a dilution of cudbear representing 1 part in 50,000 from an old tincture prepared over two years ago from the *alcohol-chloroform-acetone* extract mentioned above. This liquid had been in a cork stoppered bottle during all that time: and while the dilution of this was not the same shade as the dilu-

tion of the freshly prepared tincture, still the liquid was perfectly clear. We made a Lovibond reading of it as follows:

Red	*Yellow*	*Blue*
5.0+1.8	—0.7	1.0+0.2

We now added one drop of 10% ammonia to this dilution and the color immediately changed to the standard reddish violet tint which was produced in the dilution from the fresh tincture. Note the difference in the Lovibond reading which we now give:

Red	*Yellow*	*Blue*
5.0+4.0+1.5	—2.5	+1.6

It will be seen that the total net color value after adding the ammonia closely approximates that of the fresh dilution of the same strength. It is not surprising that the color of this old tincture faded slightly during its two years standing in a cork stoppered bottle: but it was very gratifying to see that the original color returned on the addition of a small quantity of ammonia.

A standard color match was found for several of these cudbear samples: but we considered it advisable at this time to match only the dilution of what we consider our best cudbear tincture, where the cudbear content was 1 part of extract in 50,000.

We found that no blend could be obtained from either the "Co-Fe-Cu" or the "Co-Cro-Cu" lines of colored fluids that would properly match the cudbear hue. Even the permanganate-dichromate combination was of no avail in this case: but we found that by blending the potassium permanganate solution with our copper solution, we could get the desired result.

We finally found a practically perfect match by blending 10 volumes of N/400 potassium permanganate with 4 volumes of the half-normal acidulated copper sulphate solution. We found the Lovibond reading of this blend was as follows:

N/400 Mn	N/2 Cu	*Red*	*Yellow*	*Blue*
10	4	5.0+4.0	—1.9	0.7+0.2

We will give Lovibond readings of a few of the other blends which were examined, from which we selected the best match for the cudbear tint.

Lovibond readings all in ½ inch cells.

N/400 Mn	N/2 Cu	*Red*	*Yellow*	*Blue*
10	3	5.0+4.0	—1.8	+0.7
10	4	5.0+4.0	—1.9	0.7+0.2
10	5	5.0+3.0+0.2	—2.2	1.0
10	6	5.0+3.0	—2.0	1.3
N/300 Mn	N/2 Cu			
10	2	5.0+4.0+3.0+2.0+0.2	—3.5	none
10	3	5.0+4.0+2.0+0.7	—3.1	+0.4
10	4	5.0+4.0+1.6	—2.5	0.7+0.1

It must be borne in mind that while the "Co-Fe-Cu" tints are practically permanent and that the "Co-Cro-Cu" tints are stable for several weeks, those blends containing permanganate must be used within a half-hour after mixing.

Before leaving the subject of cudbear, we wish to again emphasize that the matching of this or any other color with our standard colored fluids is now merely a matter of routine work. We hope that others will become sufficiently

interested to check up our work, in order that uniformity in the color of pharmaceuticals ultimately obtain. We further predict that it will be only the matter of a few years before color standardization by methods similar to those we have devised will be given pharmacopœial recognition.

COLUMBIA UNIVERSITY COLLEGE OF PHARMACY, July, 1915.

ABSTRACT OF DISCUSSIONS.

Dr. Schneider: I want to ask Dr. Arny several questions. Does the thickness of the container have to be considered? What would you suggest as determining a recognizable difference in tint?

Dr. Arny: I might say, Mr. Chairman, it would facilitate matters if our friends will ask questions and I will answer them to the best of my ability when they have finished. I am also sorry that I have not a few copies of the Institute paper with me. The details will be found in that.

Dr. Turner: In preparing these colors by means of a colorimeter, is it necessary to come to an absolute match of colors, or will a slight difference of tint affect it enough to change the effect of the color?

Mr. J. U. Lloyd: This question of standard colors confronts us in whatever line of experimental demonstration we may engage. It confronts the botanist and especially the bacteriologist; it confronts the entomologist; it confronts all who attempt to make a description of a substance and describe the color so a person understandingly can get that color. Now, we know that the vegetable colors are, some of them, very permanent. I know that when I went to the Orient to study, that one of the subjects committed to me by the Department of Agriculture was to study the color used in Persia and Turkey in the making of the dyes used in coloring those carpets in the olden times. And I found there carpet that was unquestionably hundreds of years of age, back of Smyrna, carpets that were taken from the floors where they had been for hundreds of years unquestionably, with the colors as bright almost as the day they were made, and vegetable unquestionably. These people were so exceedingly careful concerning the origin of the dyes that they make, which seem to be empirical. Unfortunately the aniline dyes have crept into that country so now it seems the natural dyes of the olden times will be swept out. These vegetable dyes give the mellow shades of the Oriental carpet. These vegetable dyes I consider to be as permanent as any of the dyes we can make on the inorganic side. My brother who is a mycologist, has studied and devoted much of his time in the attempt to arrive at a method of making standard descriptions of the different colors in his line. You know how they are described, how weak some of the descriptions are, and the impossibility of setting them down in black and white. The lithograph man has been appealed to; the painter has been appealed to. It is almost impossible to get the standard shade. Now, there is one red I would like to ask Dr. Arny to think of. It is a vegetable red in the line of a standard acid color. That is the shade produced when sanguinarine is dissolved in sulphuric acid. I believe you have a red there you will find to be pretty nearly perfect, and that you can make almost any shade you desire by using the exact amount of sanguinarine dissolved in the exact amount of acid water. It is a very clear cut red, and red is a color that you have trouble with in using cudbear. I would like to have you try sanguinarine.

A Member: The average pharmacist is interested in making mixtures, so that in filling prescriptions the patient will not notice any difference in the color. I have found that the darker the color, even if it does not match the former one, the patient will not notice the difference. If the color is lighter, the least difference makes quite an impression on the patient.

Dr. Arny: I will answer the questions. As far as Dr. Schneider is concerned, he speaks of the thickness of the container. Of course, in matching the colors there are several ways it can be done. It can be done with the colorimeter. In my own work I have found that I have had satisfactory results by the use of Blake bottles. I started in on the work not to reach scientific exactness, but from the standpoint of matching colors in everyday pharmacy. We use the Blake bottle, holding about an ounce. I might say in our most exact work we use two Lovibond cells, each of which is uniform in thickness.

On the subject of color, it is true that the darker the color is the less we can discern differences in shade in it. That is the reason why in delicate work we always use a half-normal solution, because we see distinctions more clearly in these than in stronger solutions.

Another point is the fact which may be in the mind of some of you, although you have not asked the question: "How do you know the uniformity of these colors?" All the work we have done in our laboratory during the past eight years has been handled as "unknowns." We take these Blake bottles and number them and put the solutions in, and I myself keep the record of them. Then I take a student and ask him to put them in the proper sequence, first the darkest and then the lighter. It is strange how close they can get to it. That opens the question which Dr. Schneider has asked about, the question of how far I can discern distinction on colors, and I am glad that question is asked.

I wish at this time to sound a warning (for it is needed) on the idea of doing quantitative analysis by colorimetric schemes. We recommend colorimetric tests only when the quantity of material to be tested is too small to assay gravimetrically or volumetrically. The sharpest discernable difference in color work is five per cent. We have one solution representing 1 part in 100; another 1 in 110 and another 1 in 90. Dozens of people coming in will put them in the proper sequence. If you have solutions 1 in 90, 1 in 95 and 1 in 100, it takes a fairly good eye to make the distinction, the eye of someone who has studied the subject. If you make then 1 in 92, 1 in 93, 1 in 94 and 1 in 95, nobody can tell. So the limitations of the colorimetric test is say 5 percent which is not, of course, exact scientific work.

The next was the question Dr. Turner asked. I don't know that I have that question exactly.

Dr. Turner: Whether it is necessary to match the color absolutely.

Dr. Arny: I will answer by saying that if we have two colored solutions, one consisting of 50 cc. of the cobalt solution, 4 cc. of the iron and 9 cc. of copper and the second made from 50 cc. of cobalt solution, 5 cc. of iron and 8 cc. of copper, the difference in the tints of these two fluids is easily discernable.

As far as Professor Lloyd's statements are concerned, I want to confirm all he says about the uselessness of lithograph work on color standardization. The first thing we did was to find out from the leading lithographers all we could about the subject and they told us that there is considerable difference between the fiftieth and the hundredth impression from the same plate. Therefore with the same ink they cannot guarantee the uniform coloring of successive impressions.

NOTES ON THE HISTOLOGY OF AN AMERICAN CANNABIS.*

C. W. BALLARD, A. M., PHAR. D.

There is a difference of opinion relative to the therapeutic value of foreign and native Cannabis; in this article their histological characters are compared.

A number of factors are responsible for the revival of interest in American Cannabis sativa. The foreign drug is apparently becoming less plentiful and is consequently increasing in price. Recent legislation has forced some manufacturers to use this drug in place of opium in their remedies. Last but most important is the accumulated data as to the therapeutic value of the native drug as compared with the foreign and although there is yet a lack of complete agreement among authorities, the weight of experimental evidence seems to indicate that preparations of American grown cannabis are almost, if not fully, as active therapeutically as those prepared from the foreign drug.

*Presented at Scientific Section, A. Ph. A., San Francisco meeting.