6-1963

The Development of a New Staining Technique for the Colorimetric Identification of Neutral Sulfite Semichemical Fibers

John F. Kratochvil
Western Michigan University

The Development of a New Staining Technique for the Colorimetric Identification of Neutral Sulfite Semichemical Fibers

A

Dissertation Submitted to the Faculty

of

Western Michigan University

Kalamazoo, Michigan

by

John F. Kratochvil

In Fulfillment of Course No. 571

Problem Analysis

June 1963

Abstract

A new staining technique has been developed for the colorimetric separation of unbleached neutral sulfite semichemical fibers from other types of fibers. It involves the use of two reagents, a 2% solution by weight of p-nitroaniline in 3N HCl and Herzbergs stain. The former solution is applied to the fibers, the excess is removed, and Herzberg stain is applied. This process will produce an orange color on groundwood, a brownish green color on unbleached neutral sulfite semichemical fibers, and a color on chemical fibers and bleached neutral sulfite semichemical hardwood fibers that will vary from blue to deep purple, depending upon the type of fiber and cook. The new technique produces much better color separations between these classes of fiber cooks than those obtained through the use of "C" stain or Herzberg stain, when used alone.

Table of Contents

A. Introduction

A great deal of experimental research work has been done on the color reactions of cellulose and lignin with various stains and dyes, during the last seventy years. Many notable stains, dyes, and techniques have been developed for the colorimetric differentiation of groundwood and chemical pulps and for the identification of the different types of chemical pulps and their degree of cooking and bleaching. However, the neutral sulfite semichemical pulping process is a relatively recent development and, to the knowledge of the author, not much work has been done in the field of colorimetric fiber identification of this type of pulp through the use of stains and dyes. Therefore, it is the purpose of this investigation to develope a staining technique that will produce a distinctly different color reaction with chemical, neutral sulfite semi-chemical, and groundwood pulps.

B. Literature Survey

All material that was written before 1952, on the staining of paper fibers, may be found in Graff's (1) literature survey.

Only the important articles with respect to this thesis, covering all of the journals commonly in use, will be given in this literature survey.

No solution to the problem was found in the literature. An attempt was made by Neumann and Herb (2) to differentiate between semichemical pulp and chemigroundwood by color, but they were unsuccessful. Their work was not directly in line with the problem but it was related.

The most important articles, with regard to the problem, were by Rowe (3), Crocker (4), and Berge (5).

Rowe states that the staining of fibers by iodine stains is a colloidal phenomenon, involving the uniform adsorption of small particles of iodine on unhydrated lignin to produce a yellow color or the formation of iodine, iodide, and water molecule complexes around the hydroxyl groups of cellulose to give a color that varies from orange to red to violet to blue with increasingly larger complexes.

Crocker conducted a study in the field of lignin color reactions. The colors produced by the reactions of many different phenols, aniline and its derivatives, and other compounds with lignin were given. Also, some of the mechanisms of the reactions were revealed.

According to Berge, a solution of p-nitroaniline in dilute sulfuric acid produces a stain that will not decompose with time. This solution will stain mechanical pulp orange to red but will not give a color reaction with unbleached chemical pulps.

C. Analysis of the Problem

In the development of a stain for the colorimetric separation of three classes of fibers, that is groundwood, semichemical, and chemical fibers, it is desirable that one class should have a color, due to the stain, at one end of the visible spectrum, the second should produce a color at the other end of the spectrum, and the color of the third should be in between those of the other two. As a result, there would be a maximum of color separation with a minimum of color overlap. In the original outline of the research program, it was decided to use an iodine-iodide type of stain to produce blue and violet colors on the chemical fibers, since two of these stains and their color reactions were already known, and to find or develope another stain that would produce a red or orange color on the groundwood. It was hoped that the use of the above two stains in either separate applications or in a common mixture would produce the desired colors on groundwood and chemical fibers and that semichemical fibers would be stained a color intermediate between those of the other two, since its degree of lignin exposure to cooking liquor is intermediate between those of the other fibers.

If the neutral sulfite semichemical fibers did not give

the color reaction that was wanted, which was green, a triple stain would have to be developed that would stain them green directly, while maintaining the red or orange color on the groundwood and the violet color on the chemical pulps.

D. Experimental Program

The first step that was taken was to stain 1/3 of a microscope slide, that was covered with equal amounts of a mixture of unbleached softwood kraft, unbleached neutral sulfite semichemical hardwood, and groundwood fibers with 5 drops of "C" stain. The kraft stained a faint purple gray. The color of the semichemical hardwood varied from a gray to a green and the groundwood stained yellow. None of the colors were very intense.

A similar slide was stained in the same manner with Herzbergs stain. The kraft stained a deep purple, semichemical fibers stained a dark gray while the vessels were brownish gray, and the groundwood stained a weak yellow gray. The purple color was very intense although the color differentiation was poor.

A study was made of Rowe's article and attempts were made to produce an iodine-iodide stain that would give even more intense violet and blue colors with chemical pulps than with Herzbergs stain but no real improvement could be developed.

Full attention was then given to the finding of a suitable dye that would color groundwood red or orange, stain unbleached neutral sulfite semichemical fibers to a much lesser degree,

and have no color reaction with chemical pulps. Some of the dye solutions that were tested are as follows.

A 1.2% solution by weight of phloroglucinol in 7 N HCl was made and tested on various fibers. It stained groundwood a deep violet, which made it undesirable. Some of its other drawbacks were that it colored unbleached neutral sulfite semichemical fibers light rose, stained unbleached kraft fibers a redish violet, and did not completely dissolve in the acid.

A 2% solution by weight of pyrogallol in 6 N HCl was prepared. It could not be used since it gave the same faint blue color with each type of fiber tested.

A 6% solution of pyrrole, by volume, in 3 N HCl did not give the desired color reactions and it also polymerized in the presence of the acid so that it could not be used upon standing.

A 4% solution by volume of aniline in 3 N HCl had improved staining characteristics over those of the previous solutions. It stained groundwood yellow but stained other fibers only faintly. If a double staining technique is used by first adding a few drops of the aniline solution to a fiber covered slide, allowing the aniline to react and then letting the excess flow off of the slide, followed by several drops of Herzbergs stain, the following results are obtained. Groundwood is yellow, unbleached softwood kraft is purple, and unbleached hardwood neutral sulfite semichemical is brownish

gray, with the vessels staining brown. There was not too great of an improvement of this stain over "C" stain, except that the color separation of the kraft was much better and it was more intense. The great disadvantage of this solution is that it slowly changes its staining properties and color with time and becomes unusable after a few days.

Several attempts were made to produce appropriate solutions of other dyes, such as indole, but they met with failure.

Finally, a solution of p-nitroaniline was made by dissolving 2.0 grams of the substance in 100 ml. of 3 N HCl. This solution was tested for its staining properties. It colored groundwood an intense orange, stained unbleached neutral sulfite semichemical fibers a very faint yellow, and did not stain unbleached kraft!

There was no difference in the staining properties of this solution after two months time, either alone or in combination with Herzberg stain, although a very small amount of a light brown precipitate slowly collected on the bottom of the container. It is probably best to avoid exposing this solution to light.

After much experimentation with this stain, in combination with Herzberg stain, the following technique was developed that gives very close color reactions, with various different types of fiber cooks, to the ones that were originally desired.

The microscope slide is prepared by applying an aqueous dispersion of the fibers to be studied on its surface. The water is then evaporated from the slide by heating it on a

warm plate. A grease pencil is used to divide the face of the slide into three equal squares by drawing two streaks through the fiber surface layer.

After the slide is ready, five drops of the 2% p-nitroaniline solution are applied to it so that the entire surface of one of the squares is completely covered. After a 20 to 30 second reaction time, the slide is tilted and touched to a blotter paper so that all of the excess dye solution is removed. Immediately after this, the slide is placed on a flat surface and five drops of Herzberg stain are added evenly to the fibers that had been stained with the p-nitroaniline solution. These drops are allowed to react for 20 to 30 seconds. Finally, a cover glass is placed over this section and the slide is again tilted on a blotter paper to remove the excess stain.

It is important to remove as much of the p-nitroaniline solution as possible before the addition of the Herzberg stain because its presence will destroy the staining power of the Herzberg stain. However, the p-nitroaniline is so firmly adsorbed onto the fibers with which it reacts that it is not removed by the addition of the Herzberg stain.

All of the color reactions given in the following data tables were obtained through the use of the p-nitroaniline - Herzberg double staining technique.

Herzbergs stain may be made in the following manner. Dissolve 0.25 grams of iodine and 5.25 grams of potassium

iodide in 12.5 ml. of water. Add to this, 25 ml. of a zinc chloride solution, with a specific gravity of 1.80, at 28°C. The specific gravity of a mixture of 50 grams of dry zinc chloride and 25 ml. of distilled water will be approximately equal to 1.80. Allow the iodine, iodide, zinc chloride mixture to stand until it is clear, which will require from 12 to 24 hours. Decant the supernatant liquid into an amber-colored glass bottle and add a crystal of iodine to the solution. Avoid exposure to light and air. This solution will stain somewhat more to the blue with the addition of more zinc chloride. Like the p-nitroaniline solution, it should retain its staining properties for several months, although this is not always true.

Although the p-nitroaniline - Herzberg double stain gives intense colors on groundwood and chemical pulps, the color produced with unbleached neutral sulfite semichemical hardwood fibers is not as intense. A triple separate staining program was devised, using the p-nitroaniline solution, Herzberg stain, and a special $K_3Fe(CN)_6$, $FeCl_3$ solution, that was given by Maddox (6), for the purpose of intensifying the green color on semichemical fibers. It was found that the third stain was not specific for just semichemical pulps but also gave green colors with groundwood and unbleached kraft. No improvement could be made upon using this stain in any combination with the other two, either. A final drawback

was that this stain rapidly reacted with itself and after a few hours became unusable. As a result, the $K_3Fe(CN)_6$, $FeCl_3$ stain was not used.

No further attempt was made to improve upon the staining characteristics of the previously given p-nitroaniline-Herzberg staining technique.

E. Presentation of Data

1. The Following are the color reactions given by the p-nitroaniline - Herzberg staining technique, with various different paper making fibers and types of cooks. All colors are given at a magnification of 100 times.

Type of Fiber and Cook	Color
Bl. Hardwood Kraft Vessels	Medium Blueish Purple
Bl. Hardwood Kraft Fibers	Very Dark Purple
Bl. Ponderosa Pine Kraft	Medium Purple
Semi Bl. Ponderosa Pine Kraft	Medium Purple
Un. Bl. Ponderosa Pine Kraft	Medium Purple
Un. Bl. Shortleaf Pine Kraft	
Spring Fibers	Medium Purple
Summer Fibers	Brownish Purple
Bl. Mead Soda Vessels	Lavender
Bl. Mead Soda Fibers	Dark Lavender to Blue
Bl. Softwood Sulfite	Light Rose Purple
Un. Bl. Softwood Sulfite	Light Rose Purple
Cotton	Red Violet (Magenta)
Bl. Aspen Watervliet NSSC Vessels	Light Blue
Bl. Aspen Watervliet NSSC Fibers	Blue
Bl. Red Oak NSSC Vessels	Light Blue
Bl. Red Oak NSSC Fibers	Blue
Un. Bl. Aspen Watervliet NSSC Vessels	Brownish Green

Un. Bl. Aspen Watervliet NSSC Fibers	Grayish Green
Un. Bl. Red Oak NSSC Vessels	Greenish Blue
Un. Bl. Red Oak NSSC Fibers	Brownish Green
Un. Bl. Balsam Poplar NSSC Vessels	Brownish Green
Un. Bl. Balsam Poplar NSSC Fibers	Grayish Green
Un. Bl. Basswood NSSC Vessels	Brownish Green
Un. Bl. Basswood NSSC Fibers	Grayish Green
Un. Bl. Hardwood Otsego NSSC	Brownish Green
Black Tupelo NSSC Vessels	Light Green
Black Tupelo NSSC Fibers	Brownish Green
Paper Birch NSSC Vessels	Light Green
Paper Birch NSSC Fibers	Greenish & Blueish Brown
Aspen NS Vessels	Light Green
Aspen NS Fibers	Brownish Green
Un. Bl. Jack Pine High Yield NSSC	Light Brownish Green
Un. Bl. Slash Pine High Yield NS	Light Brownish Green
Parama Pine NSSC	Light and Dark Brownish Green
Slash Pine Medium Kraft Semichemical	Redish Brown with an Orange Hue
Bl. Spruce Groundwood	Orange
Un. Bl. Spruce Groundwood	Orange
Aspen Groundwood	Orange
Sheet Harbour Groundwood	Orange
Ontario Paper Co. Groundwood	Orange

2. Below is a table of pairs of groups of separate paper making cooks, in which the fibers of each group are easily differentiated from the other by color, through the use of the new staining technique. Except where noted, 100 X magnification is a good magnification to use.

(a) Any bleached NSSC hardwood, bleached soda, or sulfite or kraft with any degree of bleaching, except for certain unbleached summer softwood kraft fibers and unbleached kraft hardwood fibers which were not available to test, give colors upon staining that vary from blue to purple.

All types of unbleached NSSC fibers give brownish green or grayish green colors. 60 X magnification is the best magnification to use when comparing these fibers with sulfite fibers.

(b) Any softwood kraft or sulfite gave various shades of purple.

Bleached NSSC hardwood fibers stain blue.

The above color comparison is best made at 60 X magnification. No bleached NSSC softwood fibers were available to stain.

(c) Any type of groundwood stains orange, although very highly fibrilated fragments may be colored greenish orange.

All types of unbleached NSSC fibers give brownish green or grayish green colors.

60 X magnification is best when comparing groundwood containing fibrilated fragments and unbleached NSSC softwoods.

(d) Groundwood stains orange.

Any fully cooked chemical fiber or bleached NSSC hardwood fiber stains from purple to blue.

(e) Cotton fibers stain a weird red violet color that is unlike the color reaction given by any other type of fiber.

(f) Kraft semichemical softwood fibers are stained a color similar to that of groundwood and may easily be identified when compared with chemical or NSSC fibers. The best magnification to use is 60 X when comparing these fibers with unbleached neutral sulfite semichemical hardwood or parama pine fibers.

3. The following table is made up of pairs of groups of fibers that produce similar colors when the new staining technique is applied to them.

(a) Certain unbleached summer softwood kraft fibers are stained dark brownish purple.

Some unbleached NSSC hardwood fibers are brown.

A quantitative colorimetric fiber analysis of a mixture of these fibers might take quite a while to work out but could be done at 60 X magnification. A qualitative colorimetric separation should always be possible due to the presence of the purple, kraft, springwood fibers.

(b) Bleached hardwood soda or kraft vessels are stained a lavender color while the fibers vary from blue to dark purple.

Bleached hardwood NSSC vessels are light blue and the fibers are blue.

A qualitative colorimetric analysis can be made between the vessels of the bleached soda and bleached NSSC cooks.

(c) Kraft semichemical fibers stain redish brown, with an orange hue.

Groundwood stains orange.

To the human eye this color difference is very slight. A magnification of 60 X is again best for a quantitative colorimetric separation but structural differences are probably as important as are the color differences between these types

of cooks.

(d) Although the colors given by unbleached NSSC hardwood and softwood fibers are often quite similar, a quantitative fiber analysis can always be made on a pulp mixture containing both of these types of fibers by using 60 X magnification and basing the separation upon structural differences.

F. Discussion of Results

Every effort was made to develope a stain that would differentiate between chemical, semichemical, and groundwood pulps, by color alone and not by structure.

As far as unbleached neutral sulfite semichemical pulps are concerned, they can easily be distinguished from groundwood and chemical pulps, with the exception of some unbleached summer softwood kraft fibers, by using the new staining technique. A very good color separation also exists between the chemical pulps and groundwood. Kraft semichemical fibers give very close color reactions to those of groundwood fibers and as a result they can easily be separated by color from NSSC and chemical pulps. This is both an asset and a drawback for this combination stain.

Bleached hardwood NSSC fibers are stained practically the same color as bleached soda or bleached kraft hardwood fibers, using the p-nitroaniline-Herzberg stain, which will not allow for the quantitative colorimetric separation of these pulps. However, bleached NSSC hardwood fibers may be easily differentiated by color from any softwood kraft or sulfite.

The results of using the p-nitroaniline-Herzberg stain seem to indicate that the color reactions of various fibers with this stain are dependent mainly upon the degree of oxidation of the lignin on the fibers and secondly upon the amount of lignin that is present on them. With increasing degrees of lignin oxidation and decreasing amounts of lignin, fiber colors

vary from bright orange to deep purple. Considerable variation in color is noted between unbleached NSSC fibers. This is due to the nature of the fiber being pulped and to differences in the amount and degree of oxidized lignin present on the fibers, which is the result of different degrees of pulp cooking and the position of the fiber in the chip.

Bleaching does not change the color reactions of chemical or groundwood fibers with this stain, although there is a great color difference between unbleached and bleached NSSC fibers.

The color of the groundwood is due to the action of the p-nitroaniline stain while the color reactions of the chemical pulps are almost entirely the result of the Herzberg stain. The colors of the unbleached NSSC fibers are due to the presence of both stains.

Microscope slides were again made containing equal amounts of unbleached softwood kraft, unbleached NSSC hardwood, and groundwood fibers. These slides were stained separately with "C" stain, Herzberg stain, and the p-nitroaniline-Herzberg double stain, then they were compared. The double stain produced much more intense colors on the unbleached kraft and groundwood fibers, along with an improvement in color differentiation, than those fibers stained with "C" stain. The new stain also produced much more intense colors on groundwood and NSSC fibers than those fibers stained by Herzberg's stain alone. Finally, there was a much better color separation between the fibers by using the double stain than by using only

Herzberg stain.

G. Conclusion

A staining technique has been developed which will separate unbleached neutral sulfite semichemical fibers, by color alone, from groundwood, chemical, and bleached hardwood neutral sulfite semichemical fibers. In this respect, this technique is a great improvement over "C" or Herzbergs stain, when used alone.

The new p-nitroaniline-Herzberg double stain has several other attributes. It will differentiate by color between: (1) chemical pulps and groundwood, (2) bleached neutral sulfite semichemical hardwood fibers and softwood kraft or sulfite fibers, (3) cotton and other fibers, and (4) kraft semichemical pulps and all other pulps, except groundwood.

Literature Cited

1 Graff, John H. Bibliographic Series Number 177, Microscopy of Pulp and Paper. The Institute of Paper Chemistry. Appleton, Wisconsin. 1952.

2 Herb, Klare and Neumann, Fritz. Microscopic Differentiation of Semichemical Pulp and Chemigroundwood. Chem. Abstract 52:722.

3 Rowe, Herbert W. The Nature of Fiber Staining by Iodine Stains. Paper Trade Journal, 116 no. 10:26 - 34 (March 11, 1943).

4 Crocker, Ernest C. An Experimental Study of the Significance of "Lignin" Color Reactions. Journal of Industrial and Engineering Chemistry 13, no. 7:625 - 627 (July 1921).

5 Berge, A. A Reagent for Detecting Mechanical Wood Pulp. Journal of the Society of Chemical Industries 25:912 (1906).

6 Maddox, Harry A. Characteristics of Paper Fibers. IV. Application of Coloring Stains and Solutions. Pulp and Paper Mag. Can. 15, no. 18:435 - 437 (May 3, 1917).