# HILGARDIA

# PREVENTION OF COPPER AND IRON TURBIDITIES IN WINE

M. A. JOSLYN and AARON LUKTON

A search was conducted for a satisfactory substitute for potassium ferrocyanide in removing copper from wine to prevent formation of copper casse, at present the most important problem in wine stabilization. The most promising among the relatively nontoxic organic compounds tested was rubeanic acid. Cysteine and related sulfhydryl compounds and ascorbic acid precipitate excess copper, but further tests under winery conditions are needed. Aside from potassium ferrocyanide itself, the only other material that gave satisfactory results was an adsorption complex of potassium ferrocyanide and Prussian blue. Properly prepared and used—possibly with the addition of denatured protein—this complex would avoid contamination of the wine with ferrocyanide. Neither it nor rubeanic acid was as effective as the soluble ferrocyanide in removing iron from wine; but iron casses if troublesome might be dealt with by other means.

The rest of the materials tested—a number of organic chelating agents, calcium phytate, phytic acid, chlorophyll, and a number of ion-exchange resins—were unsatisfactory either because they did not remove copper adequately or because they had an adverse effect on wine flavor. The results suggested, however, that it might be feasible to develop a special cation-exchange resin for this purpose.

The rate of formation and precipitation of copper rubeanate and of copper and iron ferrocyanides in wine was found to depend on the proportion of reagent used, the initial copper content, and the type of wine.

Sodium ethylenediaminetetraacetate was found to sequester copper and iron as soluble metal chelates in wine, but it did not bind them tight enough to prevent casse formation in some wines; and since it leaves a residue in the wine it does not appear to offer a practical solution to the casse problem.

Some tests were conducted on the corrosion of various metals with and without hard chrome-plating, when stored in wine under oxidizing and nonoxidizing conditions. The preliminary results indicated that such plating would reduce copper and iron pickup during wine processing and might be a practical means of salvaging corrodible winery equipment now in use.

## PREVENTION OF COPPER AND IRON TURBIDITIES IN WINE[1]

### M. A. JOSLYN and AARON LUKTON[2]

### INTRODUCTION

EXCESSIVE AMOUNTS of dissolved copper and iron in wine result in undesirable changes in the flavor and appearance of the wine. The change in flavor is due to the catalysis by these ions of oxidative and reductive reactions. The change in appearance is due to the development of casse: turbidity (haze) or sediment or both. Casse occurs by the formation of cupric sulfide (or some other compound or complex of copper—see next paragraph), ferric phosphate, or ferric tannate by reaction between copper or iron and certain other constituents of the wine. These relatively insoluble compounds appear as a haze when held in suspension, as a sediment when precipitated. Copper casse is favored by reducing conditions, iron casse by oxidative ones.

The actual nature of the turbidities produced by excess copper in wine has not yet been satisfactorily determined. Copper casse in wine was reported by Ribéreau-Gayon (1933a) to be due to the formation and flocculation of colloidal cupric sulfide. Subsequently turbidities in white Swiss wines were reported to be due to formation of cuprous sulfite (Rentschler and Tanner, 1951) and to copper proteins (Kielhöfer, 1942; and others). Whether three different types of copper casse occur according to conditions or whether all copper casse is of one kind is not known. If proteins or particularly their degradation products are involved—as suspected by Kean[3]—the nature of the bonding of copper by these compounds still remains to be elucidated. Thompson, Kocher, and Fritzche (1948) have discussed briefly the problem of bonding of copper by proteins and amino acids and have reported that copper-protein complexes can promote browning. The copper bonding in the compounds involved in haze formation may be by the amino acids or amino-acid nuclei of the peptide chains, by chemical forces existent in polypeptides and not in amino acids, or by particular reactive groups common to the amino acids and polypeptides. If the copper

bonding is through sulfhydryl groups of the sulfur-containing amino acids, their formation from disulfide groups during reduction, before combination with copper, might explain the fact that copper casse forms usually only under reducing conditions.

The rate of formation of turbidities depends on the concentration of copper or iron ions present, on the concentration of other consitutents which are involved (polypeptides and other nitrogenous compounds, sulfurous acid, phosphate, tannic acid, organic acids, pH, residual oxygen content, and others) and on conditions of storage (temperature, exposure to light). In the presence of 5 p.p.m. of copper at 40° to 50° C, haze formation in the absence of oxygen may occur in 24 hours. With low copper and iron content the reactions involved are extremely slow, and haze may appear only after several months of storage.

Copper and iron casse are old problems, common to all wine-producing regions of the world. But changes in the methods of making, bottling, and handling wine in this country since Repeal have recently aggravated the problems, particularly that of copper casse. Wine is now bottled at the winery and shipped in the bottle, instead of being shipped in bulk to consuming centers—a change necessitated by laws prohibiting bulk sale of wine at retail in most states. Better closures for wine bottles have been developed; these prevent losses in quality by leakage and oxidation, but produce reducing conditions favorable to the formation of copper casse. Wine is now often stored in the bottle at the winery or the bottling plant, and there is a longer period between bottling and consumption during which the wine is expected to remain bottle-bright. Equipment and fittings of brass, bronze, and other copper alloys are now widely used by the industry in an attempt to prevent iron casse. Finally, sulfurous acid and sulfites have been increasingly used to control the growth and activity of undesirable microörganisms and prevent overoxidation under the prevailing winery conditions; but these compounds render the wine more susceptible to copper casse.

Solution of the casse problem has previously been sought along two general lines: removing excess copper and iron ions from the wine and reducing the contamination of wine with these ions.

**Removing Excess Copper and Iron.** Until very recently the most effective method known of reducing the iron and copper content was by "blue-fining"—precipitation by the addition of potassium ferrocyanide under carefully controlled conditions. This method has been used in Germany extensively under strict government supervision but is proscribed in France, Spain, Italy, and other European wine regions. When properly used, under conditions that eliminate any possibility of the presence of even traces of excess ferrocyanide, blue-fining will effectively remove both copper and iron without adversely affecting the palatability of wine or changing its chemical composition. Proper blue-fining will stabilize the wine and even improve it organoleptically (Couerbe, 1949). The reluctance to use it in many countries, including the United States,[4] is based upon the possible

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[4] The Commissioner of Internal Revenue, U. S. Treasury Department, has pointed out that the fact that the use of potassium ferrocyanide is not mentioned in Regulations No. 7

health hazards involved in the use of a cyanogenetic compound. The limited literature on the toxicology of ferrocyanides is reviewed in a later section.

Potassium ferrocyanide has been used for many years in the removal of heavy metals from wine (Fessler, 1949; Garino-Canina, 1950a; von der Heide, 1933; Miconi, 1948; Ribereau-Gayon, 1935a; Saywell, 1934b; de Waal, 1932), vinegar (Saywell, 1934a), and organic acids and other products (Brown, 1932). The conditions under which it was used, however, were developed empirically for the most part, without due consideration of the physical-chemical properties of the ferrocyanides. The literature dealing with these properties was surveyed as the basis of our investigations. Much of it is not readily available, and it has sometimes been misinterpreted. Furthermore, the evidence on the properties and structure of these compounds has in the past been contradictory and inconclusive; and while in recent years our knowledge of them has been greatly expanded (Davidson, 1937a, 1937b; Weiser, 1949), the new information has not been applied as yet. For these reasons it seemed worth while to include a fairly comprehensive review of this material. In addition, published reports on the tolerance of wine for heavy metals and on the corrodibility of various metals by wine and similar liquids are briefly reviewed.

## Previous Experiments on Other Methods of Preventing Turbidity

**Removing Copper and Iron from Wine.** There have been many attempts to develop suitable substitutes for soluble ferrocyanides. These have included storage of the wine under conditions favoring the formation of copper and iron casse and the subsequent separation of the insoluble compounds by fining and filtration; precipitation with inorganic reagents; and more recently the addition of organic acids which would form soluble stable complexes with copper or iron ions and so prevent their reacting with phosphates, tannates, or other constituents.

Aeration with or without refrigeration, followed by fining and filtration to remove excess of iron from wine, has not been successful because it adversely affects the quality of the wine and does not accomplish the removal of copper.

Storage of wine in completely filled tanks with or without heating and additional sulfiting to promote formation of copper casse and the subsequent removal of copper casse by filtration has been of limited value because of inconsistent results in removal of copper and adverse effect on quality of wine so treated. Kite[5] has successfully removed excess copper by heating wine containing 25 p.p.m. of free sulfur dioxide to 135°F, and storing the hot wine in narrow-topped completely filled tanks maintained under reducing condi-

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should not be construed as meaning that the Bureau sanctions its use in the treatment of wines. The Food and Drug Administration considers potassium ferrocyanide toxic and "while it has not expressly prohibited the use of potassium ferrocyanide in the manufacture of wine it does not approve the use thereof and will hold the winemaker responsible in the event the use of the material has a deleterious effect on the consumer or consumers of wine." (Personal communication from C. E. Mealey, Deputy Commissioner of Internal Revenue, 1948.)

[5] Kite, W. E., in a report at a meeting of the Technical Advisory Committee of the Wine Institute May 15, 1953.

tions by introducing sulfur dioxide into the top layers where necessary. When the wine had cooled to about 110°, bentonite was added and the wine after 3 days was filtered in a closed air-free system. This treatment reduced the copper content to 0.0 to 0.2 p.p.m. and stabilized the wine. Its applicability to all wines and under all conditions remains to be proved and the possibility of damaging the flavor of the more heat-sensitive wines is also present. Precipitation with sodium sulfide (Ribéreau-Gayon, 1935b), while it reduces the copper content, also impairs the quality of wine.

**Reducing Contamination of Wine with Copper and Iron.** Another approach to the casse problem—reducing contamination of the wine with copper and iron—has received little attention until comparatively recent years.

One possibility for reducing metal pickup is to reduce the number of winery operations. At the present time wines are subjected to repeated pumping, filtration, chilling, pasteurizing, and other treatments to obtain the desired degree of bottle-stability. Reduction in the number of operations, particularly in sterilization filtration, has been an important development in the industry. Improvements in this direction cannot be expected to solve the entire problem, however. Some operations that cannot be materially reduced present conditions especially favorable to metal pickup.

Another possibility for reducing copper and iron pickup is to replace these metals with other materials in winery equipment. Though there are limited opportunities for the use of glass linings and other coatings for some types of equipment, for the most part this means the use of corrosion-resistant metals. The first studies in this country on resistance of metals to corrosion with wine were reported by Searle, LaQue, and Dohrow in 1934. In 1937 Mrak, Caudron, and Cash reported experiments with stainless steels, Inconel, and aluminum alloy 76. Results of both these investigations are reviewed later. Hard chrome-plating was suggested for reducing corrodibility in food processing by Scull (1949) and in wines by Joslyn (1950). Recently Rossi (1951) published some data on its effectiveness with wines.

## The Present Investigations

**Possible Substitutes for Soluble Ferrocyanides.** This paper presents the results of a search for a practical solution to the casse problem under present practices of handling and shipping wine. The development of a possible substitute for soluble ferrocyanide was our major concern. Search was undertaken for a precipitant for heavy metals that would remove them as effectively and as economically as ferrocyanide, that could be added directly to the wine without introducing equipment or operations other than those normally used in fining and filtration, and that would be perfectly safe to use.

The investigations included tests of three naturally occurring substances—calcium phytate, phytic acid, and chlorophyll—and several types of ion-exchange resins. The addition of naturally occurring substances, such as alpha cellulose (Ramage, 1938b), lignic acid (Nelson, Levine, and Lynch, 1939) and calcium phytate (Cohee and Steffen, 1949) as iron-binding and -precipitating agents has been suggested for other products. Natural or synthetic ion-exchange resins have been proposed (Austerweil and Pêcheur, 1951; Felton, 1949; Garino-Canina, 1950b, 1951; Kunin and Myers, 1950;

Ramage, 1938*a*; Trevor, 1949*a*). At the time our investigations were undertaken in 1948, however, there were no data in the literature on the removal of copper and iron from wine by synthetic or natural ion-exchange resins or adsorption or precipitating compounds.

Another group of materials tested was synthetic organic precipitating agents. One of these was an adsorption complex of Prussian blue and ferrocyanide. The others were organic chelating agents.[6] In recent years, as a result of a better understanding of the chemistry of organometallic compounds, particularly the metal chelate compounds (Johnson, 1938; Martell and Calvin, 1952), attention has been paid to the possible uses of chelating compounds for binding or separation of metals. Such compounds have already been introduced for deactivating copper in the catalytic deterioration of gasoline during storage (Pederson, 1949; Watson and Tom, 1949) and in water softening and other uses (Anonymous, 1952*a*, *b*). At the outset of the investigations we had proposed to use chelating agents for metal removal either as such or in the form of specific tailor-made cation resins prepared by adsorbing them upon the surface of an inert carrier. Such ion-exchange resins had been proposed for atomic-energy projects and for possible use to facilitate recovery of rare metals from low-grade ore. Because these were not available to us, we decided to test the chelating agents by direct addition to the wine. An exploratory survey of sixteen chelating agents was made. Tests on formation and precipitation were run on the most promising of these, rubeanic acid. Similar tests of potassium ferrocyanide were run for comparison.

Preliminary results of our investigations in metal removal from wine were presented in part in 1949[7] and 1952[8] (Joslyn, Lukton, and Cane, 1953). We then reported that of the reagents and treatments tested, rubeanic acid and an adsorption complex of Prussian blue and ferrocyanide were most promising. Subsequently the use of a compound of ferrocyanide developed by Fessler was reported by Fessler (1952) and Marsh (1952).

**Sequestering Copper and Iron in Soluble Complexes.** The investigations also included some exploratory experiments along a somewhat different line: testing the possibility of sequestering excess copper and iron in soluble complexes to bind them so that they could not form casse. At the time our investi-

[6] A complex, or coördination compound, is formed when a metal ion combines with an electron donor. If the substance which combines with the metal ion contains two or more donor groups, so that one or more rings are formed, the resulting structure is a metal chelate compound and the donor is a chelating agent. "Chelate" is derived from the root *chela*, a pincerlike claw. Metal chelation is also referred to in the literature as "sequestering" or "metal deactivation." Metal chelation may occur with the formation of either soluble or insoluble chelates. In a recent paper (Anonymous, 1952*a*), a complexing agent is defined as any compound that will inactivate a metallic ion; a chelating agent as any compound that will inactivate a metallic ion with the formation of an inner ring structure in the molecule, the metallic ion becoming part of the ring; and a sequestering agent as any complexing or chelating compound that forms a water-soluble metal-complex compound.

[7] Presented at the Wine Technology Conference held at Davis, California, August 10–12, 1949, and at the annual meeting of the Northern California Section of the Institute of Food Technologists on December 8, 1949.

[8] Presented at the meeting of Fresno Wine Chemists on May 2, 1952, and at the annual meeting of the Institute of Food Technologists at Grand Rapids, Michigan, June 8–12, 1952.

gations were begun, ethylenediaminetetraacetic acid and its sodium salts[9] (Bersworth, 1949) were already in use as a powerful chelating or sequestering agent for metals in water softening, in pharmaceutical preparations, and other uses. We therefore determined on a test of the sodium salt for sequestering copper and iron in wine—that is, binding these ions in soluble complexes that would remain in the wine but that would prevent casse formation. Unfortunately, even if successful, this method is open to the objection that it leaves a residue in the wine. Nevertheless, an investigation of the possibilities in this direction was thought to be worth while. After these investigations were under way, Krum and Fellers (1952) reported the clarification of cloudy wine upon addition of ethylenediaminetetraacetate.

**Hard Chrome-plating.** Finally, our investigations included some tests of hard chrome-plating as a means of preventing copper and iron contamination. Corrosion tests were made with hard-chrome-plated copper, brass, bronze, steel, and cast iron, under oxidizing and reducing conditions; and with thick and thin plating.

## REVIEW OF THE LITERATURE

### Tolerance of Wine for Metals

In order to determine the amounts of copper and iron that must be removed to attain stability, it is necessary to know the tolerance of wine for metals. That is, we must know the maximum concentration of these metals at which undesirable changes in appearance and flavor will not occur in a particular wine under particular storage conditions.

The tolerance of wine for metals and the conditions under which the white ferric phosphate casse, the blue ferric tannate casse, and the white to reddish-brown copper casse will form have been described in the publications of Amerine and Joslyn (1940, 1951), Casale (1934), de Castella (1925), Cruess (1947), Joslyn and Amerine (1941), Marsh (1940), de Waal (1932), and others. The most extensive investigations of the factors influencing the distribution of iron and copper ions in wine in various states of oxidation and combination and of the conditions affecting formation of their colloidal suspensions and deposits were those of Ribéreau-Gayon (1933a, b, 1935a, b, 1947).

Some data on the distribution of iron in California wines, using the methods described by Ribéreau-Gayon (1933a, b), have been reported by Marsh and Nobusada (1938). Large changes in total iron content are known to occur in wines exposed to air during fermentation, storage, and refrigeration.

**The State of Iron in Wines.** Ribéreau-Gayon (1933a, b) and others have shown that under reducing conditions, the iron in wine exists in the ferrous state largely as free $Fe^{++}$ and to a smaller extent in soluble organometallic complexes. On contact with air, the ferrous ions are rapidly oxidized to the ferric state in which they can form colloidal suspensions or precipitates

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[9] The remarkable ability of ethylenediaminetetraacetic acid and its salts to form stable complexes with nearly every metal has been known for several years. A survey of a number of common-metal complexes was made by Pecsok (1952) and also by Martell and Calvin (1953). Pecsok (1953) also investigated the stability of copper versenate and reported the stability constant to be $10^{18.8}$.

of ferric tannates (blue or ferric casse), or of the very slightly soluble ferric phosphate (white casse) (see Casale, 1934). Phosphate casse, however, occurs only in the region of pH 2.9 to 3.6. The ferric ions, however, readily form complexes with citric acid and other organic acids.

Michaelis and Smythe (1931), Smythe (1931*a*, *b*), and Smythe and Schmidt (1930), have studied the types of complexes which can be formed with ferric and ferrous ions and the effect of complexing on rate of oxidation of ferrous ions. Smythe and Schmidt found that ferric ions will form stable complexes with the anions of many organic acids and polyhydroxy alcohols. These complexes are of four types: (1) positively charged cation; (2) negatively charged anion; (3) neutral un-ionized complex; and (4) a complex in which the iron atom is partly electropositive and partly electronegative. The rate of oxidation of ferrous compounds by molecular oxygen was greatly dependent upon the electrical condition of the ferrous ion. The ferrous ion, for example, was oxidized more slowly than the electrically neutral ferrous iron atom in certain compounds. Citric and pyrophosphoric acid were found always to form electronegative complexes, tartaric acid complexes were but slightly charged. The substances with the strongest tendency to complex iron in acid solution at pH 2.5, in order of decreasing affinity, were saccharic acid, citric acid, orthophosphoric acid, tartaric acid, oxalic acid, pyrophosphoric acid, malic acid, gluconic acid, lactic acid, and succinic acid. Smythe (1931*b*) presented some evidence based on titration curves that both ferric and cupric ions formed complexes with hydroxy organic acids (lactic, glycollic, malic, tartaric, and citric acids) in which the metal replaced the hydrogen of the hydroxyl group and increased its acid properties.

**Copper and Its Reduction in Wines.** The catalysis of oxidative reactions in wine by copper or iron ions is markedly increased over that of the individual ions by their presence together. This synergistic effect is well established in biological oxidations. The catalytic properties of copper in oxidations—for example, in oxidation of ascorbic acid—are changed greatly by the presence of proteins. Stotz, Harrer, and King (1937) reported that more copper was required to produce a given rate of oxidation of ascorbic acid in presence of protein than with copper alone. Ovalbumin reduced the catalysis of oxidation of ascorbic acid by copper more than did gelatin or edestin. Heating the copper-protein mixtures caused marked decrease in catalytic power due to coagulation of the protein and precipitation of copper bound to the protein. Heat-coagulation of protein was shown to involve binding of copper in a noncatalytic form since the precipitated copper was practically without effect on oxidation of ascorbic acid. Copper-proteins are also known to be involved in nonenzymatic browning of foods (Thompson, Kocher, and Fritzche, 1948). The proteins and derived products in wine as well as the copper and iron ions are involved in the undesirable oxidative and nonoxidative changes that occur during aging and storage of wine.

The occurrence and possible role of proteins in the clouding of wine have been reported by several investigators, particularly Ribéreau-Gayon (1932), Hennig (1944), and Kielhöfer (1942, 1948, 1949, 1951). Saywell (1934*a*) showed that the removal of excess iron from and the clarification of wine

and cider vinegars by potassium ferrocyanide was greatly improved by the addition of small amounts of egg albumen. The addition of 36 mg of egg albumen per 100 ml of vinegar after treatment with ferrocyanide, storage for 48 hours, and filtration produced additional precipitate. After filtration the treated samples were clearer and more stable during storage and did not contain residual cyanide even when blue-fined in moderate excess. A decrease in protein content of blue-fined wines was reported by Kielhöfer (1942) and Hennig (1944). Kielhöfer recommended blue-fining as a means of reducing protein content and, in some cases, also for the elimination of protein-clouding in white wines.

Under reducing conditions, according to Ribéreau-Gayon (1933a), the copper in wine forms colloidal cupric sulfide. Ribéreau-Gayon (1933a, 1935a) postulated a preliminary reduction of $Cu^{++}$ by some reducing agent to $Cu^+$, then reduction of sulfite to $H_2S$ by $Cu^+$ and formation and subsequent flocculation of colloidal cupric sulfide. Copper is apparently necessary for the reduction of sulfite in wine. Whether the white cloudiness which forms in wine in the presence of excess $Cu^{++}$ under reducing conditions is actually due to formation of CuS is not known nor is it known that the deposit, which readily dissolves on aeration or oxidation, is only CuS.

The evidence available at present indicates that the copper content of the sediment formed in wines stored under reducing conditions is about 30 per cent, as compared with 66.7 per cent in cupric sulfide. It has been reported to be cuprous sulfite and not cupric sulfide (Rentschler and Tanner, 1951). Kean[10] has not found sulfur in preparations of the copper deposit he has been investigating.

Turbidity in wines susceptible to copper casse stored in absence of air is prevented by heating or by adding a stabilizing colloid like gum arabic. Prolonged storage of clarified wine also increases its tolerance for added copper. This may be due to removal of the polypeptides that are involved in copper casse formation. Flocculation of the colloidally dispersed cupric sulfide occurs as a result of the action of electrolytes present, the wine becomes turbid, and finally a deposit of reddish-brown cupric sulfide containing occluded organic matter (largely proteinaceous) forms.

The ability of hydroxy acids to complex copper has been investigated by Cannan and Kilbrick (1938), Peacock and James (1951), Pederson (1952), and Williams (1953). The stability constants for the copper complexes of malonic, succinic, oxaloacetic, and pyruvic acids, as log $K$, are respectively 4.80, 3.33, 4.00, and 2.50. Malonic acid has been used in sequestering traces of copper in yeast fermentation liquors (Pinkalla[11]).

**Characteristics of Casses and Mechanisms of Their Formation.** The characteristics of iron and copper casse are summarized in table 1 and the proposed mechanism of iron and copper casse formation in table 2. It is now known that the state of oxidation or reduction of the wine, its pH value, its content of sulfur dioxide or of organic acids capable of complexing ferric ions (citric, malic, tartaric), of tannins, anthocyanin and related flavonols,

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[10] Kean, C., unpublished data.

[11] Pinkalla, M. J. Personal communication from American Bio-synthetic Corporation, Milwaukee, Wisconsin, 1953.
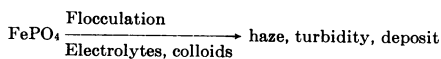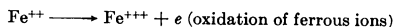
TABLE 1

CHARACTERISTICS OF IRON AND COPPER CASSE*

| Characteristic | White iron casse | Blue iron casse | Copper casse |
|---|---|---|---|
| Cation involved............... | $Fe^{+++}$ | $Fe^{+++}$ | $Cu^{++}$ |
| Anion involved................ | $(PO_4)^{---}$ | Tannate | $S^{--}$ |
| Compound formed............ | $Fe(PO_4)$ | Ferric tannate | CuS |
| Appearance: | | | |
|   Initial....................... | Bluish-white opalescent haze | Blue haze | White haze |
|   Final........................ | White deposit | Blue-black deposit | Reddish-brown deposit |
| Behavior: | | | |
|   With access to air............ | Appears | Appears | Disappears |
|   On exclusion of air.......... | Disappears | Disappears | Appears |
|   In light..................... | Disappears (hinders, decreases) | ............ | Appears (hastens, increases) |
|   In darkness................. | Appears | ............ | Disappears |
|   With temperature........... | Most at low | Most at low | Most at high |
|   On addition of $H_2O_2$......... | Appears | Appears | Disappears |
|   pH range.................... | 2.9–3.6 | ............ | ............† |
|   Effect of citric acid.......... | Inhibits | Inhibits | No effect |

\* After Marsh (1940).

† Some evidence has been obtained recently by A. Lukton which indicates that copper casse formation also is affected by pH. Maximum turbidity in a sauterne wine occurred at pH 4 and little or no turbidity at pH 5.

TABLE 2

MECHANISM OF IRON AND COPPER CASSE*

1. Ferric phosphate casse

$$Fe^{++} \longrightarrow Fe^{+++} + e \text{ (oxidation of ferrous ions)}$$

$$Fe^{+++} + PO_4^{---} \longrightarrow FePO_4$$

$$FePO_4 \xrightarrow[\text{Electrolytes, colloids}]{\text{Flocculation}} \text{haze, turbidity, deposit}$$

2. Copper casse

$$Cu^{++} + RH \longrightarrow Cu^+ + R + H^+ \text{ (reduction of cupric ions)}$$

$$6\,Cu^+ + H_2SO_3 + 6H^+ \longrightarrow 6Cu^{++} + H_2S + 3H_2O \text{ (reduction of sulfite ions)}$$

$$Cu^{++} + H_2S \longrightarrow CuS + 2H^+$$

$$CuS \xrightarrow[\text{Electrolytes, colloids}]{\text{Flocculation}} \text{haze, turbidity, deposit}$$
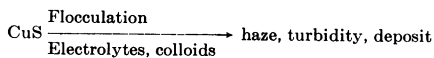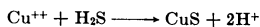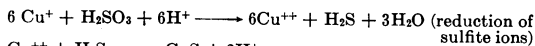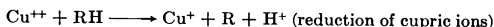
\* As proposed by Ribéreau-Gayon (1933a).

of phosphate and sugars and sugar derivatives, and other constituents, all influence the tolerance of the wine for metals.

The effect of oxidation-reduction level on formation of iron and copper casse is shown in figure 1. These data on changes in redox potential of a dry sauterne wine during storage under oxygen-free conditions are plotted against changes in turbidity as measured in a photoelectric turbidity meter. The high turbidity of the wine initially is due to ferric phosphate casse. This decreases as the redox potential drops, until at an observed redox
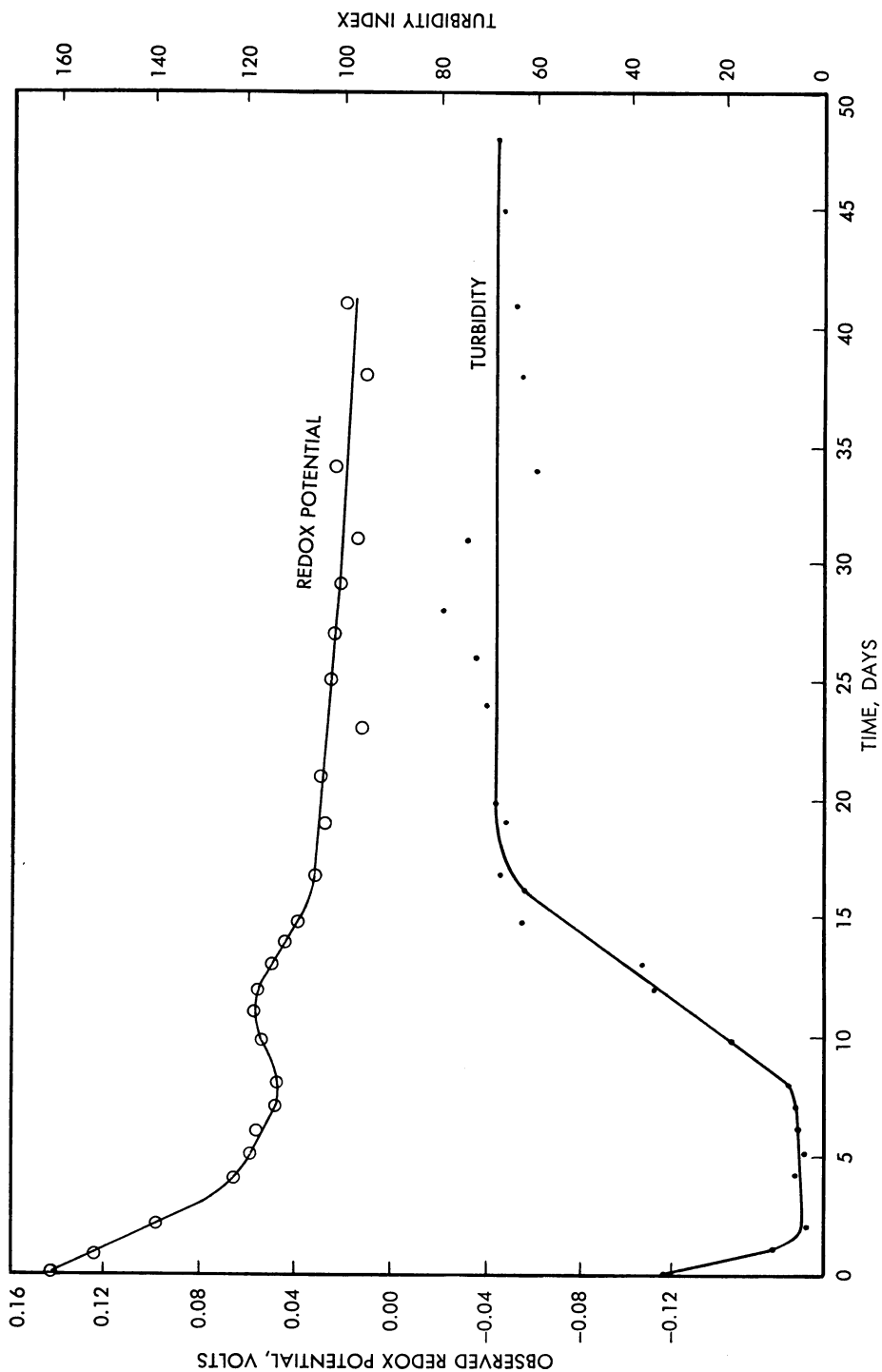
Fig. 1. Changes in turbidity and oxidation-reduction potential of a sauterne wine adjusted to 3 p.p.m. of copper, 10 p.p.m. of iron, and 333 p.p.m. of sulfur dioxide.

potential of 0.10 volts (against a saturated calomel reference electrode) it disappears. Then when the redox potential drops to 0.048 volts, copper casse begins to form and increases until the potential reaches 0.02 volts, and then remains constant.

**Tolerance Data.** Failure to control these variables led Searle, LaQue, and Dohrow (1934) to report that the tolerance of wines for metals is 1 p.p.m. for iron and tin, 15 to 30 p.p.m. for chromium, 60 to 100 p.p.m. for copper, 100 to 200 p.p.m. for aluminum, and over 200 p.p.m. for nickel and zinc. Mrak, Cash, and Caudron (1937) reported the tolerance of California chablis and sauterne white table wines to be 2 p.p.m. for aluminum, chromium, copper, and iron; 5 p.p.m. for tin and zinc; and 50 p.p.m. for nickel. Jaulmes (1951) recently reported that French wines will not be susceptible to iron casse below 12 to 15 mg of iron per liter for white wines and 20 to 25 mg per liter for red wines. Copper casse in French wines will occur at 0.5 mg of copper per liter.

Amerine and Joslyn (1940) reported that the tolerance of sulfited white table wines for copper was between 0.5 and 0.8 p.p.m.; Joslyn and Amerine (1941) reported about 0.5 p.p.m. for dessert wines. At the present time the general concensus in the industry supports the conclusions of Nasledov (1949) that for a California wine to be metal-stable it must contain less than 0.2 to 0.3 p.p.m. of copper and 3 to 4 p.p.m. of iron. These tolerances, however, depend on the composition of wine and the conditions of bottling and storage.

**Copper Pickup.** The widespread use of equipment and fittings of brass, bronze, or other copper alloys in the industry, originally done to minimize contamination of wine with iron, and the reduced tolerance of wines to copper has focused attention on copper pickup. Iron casse has been controlled by lessened exposure to iron-bearing metals and by the use of citric acid as stabilizing agent. Hence at the present the chief cause of metal clouding is excess of copper. Data on copper content of California grapes and on the extent of copper pickup during crushing, fermenting, pumping, filtering, and bottling were not available until recently. But the development of a rapid sensitive method of determining copper by Marsh (1941) finally led to its application to determination of changes in copper content of musts and wines by Nasledov (1949). The latter reported that new wines less than 5 months old are practically copper-free but that nearly finished wines may contain from 1.4 to 2.0 p.p.m. of copper. He also showed that this excess of copper was picked up by contact of the wine with pasteurizers, pumps, chilling equipment, filters, or filling machines constructed in part or entirely of brass or other copper alloys. The increase in copper content of wines was reported to be 0.5 p.p.m. for pumping, 1.7 p.p.m. for filtering, and 0.6 p.p.m. for bottling.

Copper pickup does not occur during fermentation; it occurs largely during aging and processing. Pumps, pipelines, and valves contribute more to copper pickup than other equipment used in the wine cellars. Repeated pumping, filtration, chilling, pasteurization and other treatments to obtain the desired degree of bottle-stability increase copper pickup. Reduction in the number of operations used can markedly reduce metal pickup.

## Corrodibility of Metals by Wines

Metals are widely used in the wine industry, not only in the construction of crushers, pumps, filters, pasteurizers, refrigerators, and bottling machinery, but also in the construction of storage tanks, tank cars, trucks, pipes, and pipe and hose fittings. Their superior physical properties, and the ease with which they can be cleaned and sterilized have influenced their use. Where economically possible corrosion-resistant metals have been selected, but metal-clad, metal-lined or glass-lined or otherwise coated baser metals and alloys are used. The selection of the metal to be used for construction depends on its physical and chemical properties such as its resistance to corrosion by wine, the tolerance of the wine for the metal picked up by the wine during its passage over or contact with the metal surface, the ease with which the metal can be fabricated into the equipment desired, and the structural properties of the metal.

Shortly after the resumption of commercial production of wine in California following Repeal, copper and copper-bearing metals were widely used in the construction of winery equipment and replaced much of the steel and iron equipment still available in the older wineries. At that time there was little information available on the corrosion resistance of metals in wine making and on the tolerance of wines, particularly California wines, for metals.

Anticipating the revival of wine making in California, Searle, LaQue, and Dohrow in 1934 reported the results of their investigations on the resistance of seven metals to wines made from Labrusca grapes (grown in Canada) while they were being processed by commercial equipment. They also reported observations on the changes produced in brilliance, color, flavor, and bouquet of a dry white wine (sauterne), a sweet red wine (port), and a dry red wine (claret) 2 days and 51 days after the addition of the citrates of aluminum, chromium, copper, iron, nickel, tin, and zinc. The wines were bottled, capped, or corked, and the dry wines subjected to bottle-pasteurization. These workers concluded that Inconel is adequate for all winery uses and that nickel, Monel metal, 18–8 alloy, aluminum, and copper were adequate for most winery purposes except in equipment in which the relation between liquid volume and exposed metal area was unfavorable.

Mrak, Cash, and Caudron, using similar methods, investigated the corrosion of metals by musts and wines in two wineries at Lodi and one at Livermore, and reported their data in 1937. They also reported the effects of a number of metals and alloys on California claret and sauterne. They reported that the stainless steels, Inconel, and aluminum alloy 76 were most resistant to corrosion and found corrosion to be most severe at the crushers. In their tests white grape juice and wine were usually more corrosive than red, but, in general, the conditions existing at the point of test determined the behavior of the metal or alloy.

Although both of these investigations were satisfactory in determining the general corrodibility of the metals and alloys under the conditions tested, they were not sufficient to measure the effects of all the variables that occur

in winery operations. It was recognized that the velocity of flow of wine over the metal surface, temperature, extent of aeration, composition of wine (particularly its acidity, sulfur dioxide content, and pigment content) were important factors governing corrodibility. But mechanical strains and actual physical and chemical changes induced by fabrication (for example, welding), electrolysis caused by the use of different metals in the same piece of equipment, and abrasion or erosion produced by pumping or existing in crushers, must lines, filters, and filter-aid mixers were not assessed.

The corrodibility of copper and copper-containing alloys is determined by the oxygen content of the wine or its content of hydrogen acceptors such as the anthocyanin pigments of red grapes. Copper will not react with non-oxidizing inorganic acids or with the commonly occurring organic acids in the absence of oxygen or other hydrogen acceptors. Copper-bearing surfaces that are bright and free of oxide films are relatively inert. Copper pickup usually is greater when wine is passed over surfaces that have been exposed to air. Valves and hose and pipe fittings that have been in contact with air, particularly in the presence of water or wine, are an important source of contamination. The ready formation of verdigris on wet bronze tank valves is a common experience. However, the complete removal of all preformed oxide films and the complete exclusion of oxygen and other hydrogen acceptors is difficult and may be impractical.

Sulfites will also increase the rate of copper solution, and the pasteurization of sulfited wines in pasteurizers built of copper-bearing metals is a serious source of contamination.

The replacement of processing equipment, tank valves, and hose fittings now in use with stainless steel is not only uneconomical but may be impractical. Although suitably selected and properly fabricated stainless steels are ideal, the construction of machinery entirely of stainless steels presents mechanical difficulty. It is well known that bearing surfaces must be constructed of dissimilar metals to prevent seizing or binding, and for this reason the housing and shaft and rotors of pumps, for example, cannot be constructed of stainless steel. Furthermore while stainless steels are chemically inert, the steel surfaces may not be sufficiently hard to withstand abrasion or erosion such as occurs in pumps, crushers, and filter-aid premixers and coaters.

As mentioned earlier, hard chrome-plating was suggested for reducing corrodibility of equipment and machinery used in the food industry by Scull (1949). Joslyn (1950) suggested the use of hard chrome-plating for winery equipment and machinery on the basis of preliminary investigations. Rossi (1951) reported on laboratory and winery work on hard chrome-plating. He found that where there was metal-to-metal contact, where the parts were difficult to plate, where mechanical wear occurred, or where wine and air were in contact with equipment, hard chrome-plating did not prevent corrosion and was not as satisfactory as stainless steel. Chemical plating, by which a more uniform coating of a metal like nickel can be obtained than possible by electroplating, has been introduced recently, but so far not applied to winery equipment.

## Structure and Composition of Heavy Metal Ferrocyanides[12]

In the nearly two hundred and fifty years since the discovery of Prussian blue in 1704 by Diesbach, extensive investigations have been made on the conditions of formation and the properties and structure of the ferrocyanides. The structure of even the simple ferrocyanides was early recognized to be complex. Werner in 1907 on the basis of his theory of coördination complexes suggested the most reasonable structure for ferrocyanide ions with single covalent bonds from the iron atom to each of the six carbon atoms of the cyanide groups. Pauling (1939) pointed out that this structural formulation would place a charge of $-4$ on the iron atoms, whereas actually iron tends to assume a positive charge. Pauling modified the structure proposed by Werner by assuming that the cyanide group in this complex can function as an acceptor of electrons and that the iron–carbon bonds are resonance hybrids of three types: an electrostatic bond between the iron atom and the cyanide ion, a single covalent bond from iron to carbon, and a double covalent bond that utilizes another third orbital of the iron atom. Although absolute proof of the structure of ferrocyanide ion is still unavailable, most investigators accept the postulate of Pauling.

The metallic ferrocyanides in order of increasing solubility in dilute acid (Anonymous, 1953a) are: silver, ferric iron, cupric, zinc, ferrous iron, alkaline earths, and alkali metals. Relative concentrations as well as solubilities will determine the order of precipitation from solutions containing mixtures of metal ions.

The investigations leading to our present knowledge of the chemistry of heavy metal ferrocyanides has been reviewed by Holtzman (1945) and more recently by Anonymous (1953a). These pigments have been investigated by chemical analysis of the precipitates themselves and by analysis of the supernatant liquids obtained from precipitation reactions. Physical chemical methods utilizing X rays and electron diffraction, magnetic moment and susceptibility, and absorption spectroscopy have been used also. These investigations, however, have been hampered by such phenomena as colloid and gel formation, adsorption and occlusion of electrolytes by the gels formed, and changes in the structures during analysis or preparation due to oxidation, reduction, or variation in pH. Because of these effects much of the experimental data reported are conflicting and considerable errors have been introduced in the analytical data and in their interpretation. Strict chemical formulas cannot be written for many of the "iron blues" because their composition is indefinite. The more important concepts, interpretations, and formulations are reviewed in this section.

The early identification of Prussian blue as ferric ferrocyanide and that of Turnbull's blue as ferrous ferricyanide was discarded years ago in favor of a concept that both are ferrocyanides. This was followed by a formulation of both as ferricyanides, and even today complete agreement on their

[12] This section is based largely on the review prepared for the Wine Advisory Board by A. Cane. (Cane, A. Structure and composition of ferrocyanides of heavy metals: review of literature. Unpublished report, May, 1948). A review of the chemistry of the ferrocyanides has recently been published by the American Cyanamid Company (Anonymous, 1953a).

structures is lacking. Although the ferrocyanide designation is gaining more and more support, as late as 1942 evidence for the ferricyanide structure was offered (Weiser, Milligan, and Bates, 1942).

It is interesting to note that many elementary chemistry texts still differentiate Prussian and Turnbull's blues as products of the interactions of ferric with ferrocyanide ion and ferrous with ferricyanide ion respectively. This classical formulation is given in table 3, and that proposed by Davidson (1937*b*) in table 4.

TABLE 3

CLASSICAL FORMULATION OF IRON FERROCYANIDE

|  | $Fe^{++}$ | $Fe^{+++}$ |
|---|---|---|
| Ferrocyanide: $Fe(CN)_6^{-4}$ .............................. | $K_2Fe[Fe(CN)_6]$ white precipitate | $Fe_4[Fe(CN)_6]_3$ blue precipitate Prussian blue |
| Ferricyanide: $Fe(CN)_6^{---}$ ............................ | $Fe_4[Fe(CN)_6]_3$ Turnbull's blue precipitate Prussian blue | $Fe[Fe(CN)_6]$ deep-brown solution |

The addition of ferrocyanide to ferric ions results in the production of blue substances generally called "Prussian blues." When an excess of ferric ions is present an insoluble Prussian blue forms, but with equimolecular quantities of ferric ion and ferrocyanide a colloidal solution forms. The formation of Prussian blue, however, is not simply a mutual precipitation of ferric and ferrocyanide ions and is not an instantaneous reaction, but actually has been found to be a relatively slow reaction. Davidson (1937*a*), for example, points out that when equal volumes of 0.0002 formal $K_4Fe(CN)_6 \cdot 3H_2O$ and ferric alum, $NH_4Fe(SO_4)_2 \cdot 12H_2O$ are mixed, the original colorless liquids form a yellow solution which quickly changes to green and finally becomes blue in one minute. If the solutions taken are three times as strong the succession of colors is complete in one second, while if the solutions are only one third as strong as the original the reaction requires the better part of an hour. The formation of Prussian blue also is known to be hindered by excess of ferric ions and hastened by excess of ferrocyanide. Davidson has explained this paradox by pointing out that, preceding the formation of Prussian blue, the ferric ion is almost completely reduced to the ferrous ion by the ferrocyanide ion, which is simultaneously oxidized to ferricyanide ion. If ferric and ferrocyanide ions are actually involved in the formation of Prussian blue, then it is slow because the ions required for it practically disappear when mixed, owing to the ionic redox reaction which they undergo instantaneously.

For this reaction,

$$Fe^{+++} + Fe(CN)_6^{-4} \rightleftarrows Fe^{++} + Fe(CN)_6^{---},$$
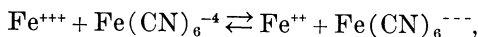
the equilibrium constant, $K$, calculated from standard electrode potential data, is 100,000. The equilibrium thus lies far to the right, on the side of ferrous and ferricyanide ions. The concentrations of ferric and ferrocyanide

TABLE 4

## TABLE OF THE PRUSSIAN BLUES*

| Common name | Prepared by | Old formula | Old name | New formula | New name |
|---|---|---|---|---|---|
| Soluble Prussian blue | Ferric ion on ferrocyanic acid<br>Oxidation of ferroferrocyanic acid<br>Acid on soluble Prussian blue | $HFe^{+++}[Fe(CN)_6]^{-4}$, | Hydrogen ferric ferrocyanide | $H^+[[Fe(CN)_6]Fe]^-$ | Berlinic† acid |
| | Ferric ion on ferrocyanide<br>Ferrous ion + ferricyanide<br>Oxidation of ferrous ferrocyanide<br>Reduction of ferric ferricyanide | $KFe^{+++}[Fe(CN)_6]^{-4}$,<br>or<br>$KFe^{++}[Fe(CN)_6]^{---}$ | Potassium ferric ferrocyanide, or potassium ferrous ferricyanide | $K^+[[Fe(CN)_6]Fe]^-$ | Potassium berlinate† |
| Insoluble Prussian blue | Excess ferric ion on ferrocyanide<br>Ferric ion on soluble Prussian blue | $Fe_4^{+++}[Fe(CN)_6]_3^{-4}$ | Ferric ferrocyanide | $Fe^{+++}[[Fe(CN)_6]Fe]_3^-$ | Ferric berlinate† |
| Turnbull's blue | Excess ferrous ion on ferricyanide<br>Ferrous ion on soluble Prussian blue | $Fe_3^{++}[Fe(CN)_6]_2^{-4}$ | Ferrous ferricyanide | $KFe^{++}[[Fe(CN)_6]Fe]_3$<br>$or$<br>$KFe_2^{++}[[Fe(CN)_6]Fe]_6$ | Potassium ferrous berlinate† |

* After Davidson (1937b.)

† Berlinate is a complex of ferric ion and ferrocyanide ion.

ions available for Prussian blue formation thus are relatively small and lead to slow rate.

In confirmation of this, Davidson (1937b) showed that mixtures of ferrous ions and ferricyanides behave similarly to those of ferric ions and ferrocyanides. Here an excess of ferrous ions favors formation of a blue product from ferricyanide ion.

As the precipitate forms, however, the redox equilibrium shifts to maintain a constant but small concentration of ferric and ferrocyanide ions, allowing the reaction to proceed. Davidson hypothesized the existence of two competing parallel reactions, the fast redox reaction and Prussian blue precipitation, made slow by the depletion of its component ions. Davidson demonstrated that the rate of Prussian blue precipitation varies with the concentrations of the reactants.

The acceleration provided by excess of ferrocyanide ions can be explained by this redox reaction. From what has been said, one would expect, by similar reasoning, that the excess ferrocyanide would also drive the redox equilibrium to the right. However, assuming that it does, Davidson shows that a precipitation of ferrous ferrocyanide would follow, because a 10:1 ratio of ferrocyanide to ferric ion would give a far greater value of the ion product $(Fe^{+++})$ $(Fe(CN)_6^{-4})$ than a 10:1 ratio of ferric to ferrocyanide would give.

Assuming the validity of the above reasoning, the substitution of ferrous and ferricyanide ions in equivalent amounts should have no effect on the final product. This was found to be the case by several investigators in addition to Davidson.

Vörlander in 1913 (cited in Davidson, 1937a), however, reported that unlike the formation of a blue from ferric and ferrocyanide ions, which is slow, the production of a blue from ferrous and ferricyanide ions is instantaneous.

Davidson (1937b) accounted for the color and composition of soluble (colloidal) Prussian blue by assuming polynuclear complex formation between ferric ion and the nitrogen end of the cyanide groups present in ferrocyanide, and proposed "berlinate" as a name for the complex of ferric and ferrocyanide ions.

Using reasoning similar to the above, excess ferrous ion should accelerate and excess ferricyanide retard the formation of Turnbull's blue. This can be readily predicted from a calculation of the ion products at equilibrium.

McBain (1950), in discussing the preparation of colloidal sols, explains their formation in accordance with von Weimarn's precipitation laws. Von Weimarn (1925) assumes that usually the reaction producing insoluble substances is instantaneous and that to form a particle from such molecules it is necessary that the concentration of the reaction product exceed its true solubility. Each particle grows from an initial nucleus so that in the formation of colloidal sols one must consider, besides the stabilizing agent, the rate of formation and number of nuclei present and the rate of growth of nuclei. Von Weimarn showed that particles will be at their finest when formed from very dilute or from very concentrated solutions. In very dilute

solutions diffusion is very slow because the concentration gradient is so small, and sufficient nuclei will usually appear to relieve the slight super-saturation locally. In extremely concentrated solutions nuclei appear so quickly and in such numbers that supersaturation is immediately relieved before appreciable diffusion can occur. McBain illustrates these effects by pointing out that if very dilute solutions of ferric chloride and potassium ferrocyanide are mixed there is formed a transparent sol of Prussian blue that passes unaltered through filter paper. At a more concentrated range a precipitate of Prussian blue is formed a few moments after mixing which is completely retained on the filter paper. When one part of a saturated solution of ferric chloride is poured into the chemically equivalent amount (two parts) of saturated potassium ferrocyanide solution, a thick cheesy paste results.

Both the colloidal aspects and the redox equilibrium must be considered in developing the optimum conditions for heavy metal removal by ferrocyanide. The conditions must be such that a readily filterable insoluble fer-rocyanide precipitate rather than a colloidal sol is formed. Empirically it has been found in the industry that the quantity of potassium ferrocyanide required must be added in a fairly dilute but not too dilute solution, and introduced into the wine so that it is thoroughly and completely dispersed throughout to avoid local supersaturation, that aeration during fining should be avoided, and that sufficient time should be allowed for precipitation of the heavy metal ferrocyanides before filtration. Additional fining agents, gelatin–tannin, casein, or bentonite, if used, should be added after the intro-duction of potassium ferrocyanide and initial formation of particles of metal ferrocyanides.

**Evidence from Analytical Chemical Methods.** Much of the conflicting evidence in the literature on the chemical composition of the ferrocyanides is due to the disregard or poor control of such factors as: the redox reaction, which could result in unsuspected changes in chemical composition before analysis could be performed (Bhattacharya, 1934; Bhattacharya and Gaur, 1948); the need to rigidly control the precipitation reaction to obtain repro-ducible results because of strong adsorption, tending to give impure gels (Weiser, 1938; Weiser, Milligan, and Bates, 1938); pH, which if too high would introduce coprecipitation of iron hydroxides (Holtzman, 1945); and hydrolysis during the washing of precipitates (Bhattacharya, 1941$b$). Direct analysis and indirect methods based on analysis of supernatant liquors obtained from precipitation reactions have been used in preparing mate-rial balances and calculating formulas for the precipitates. Müller, as a result of extensive investigations during 1908–1922 (Holtzman, 1945), concluded that both Prussian and Turnbull's blue were ferrocyanides, but designated the so-called "soluble" blue as $KFe^{+++}(Fe(CN)_6)^{-4}$. Prussian blue was considered to be $Fe_4^{+++}(Fe(CN)_6)_3^{-4}$ and Turnbull's $KFe^{++} Fe_3^{+++}(Fe^{++}(CN)_6)_3^{-4}$. Müller's contemporaries. Woringer and, independently, Eibner and Gerstacker, disagreed and countered with the ferricyanide for-mulation as the basic nucleus of the "iron blues" (See Holtzman, 1945).

Reihlen and Zimmerman (1927), on the basis of general properties, con-cluded that the complex metal cyanides were best represented as complex

polynuclear structures, rather than salts of hydroferrocyanic acid. Davidson and Welo (1928) were in disagreement with this concept at that time.

Justin-Mueller (1931), concluded that Prussian and Turnbull's blues were ferricyanides, namely, ferric triferro ferricyanide and ferrous diferro ferricyanide, respectively. The experimental work consisted of a series of reactions with oxalic acid and KOH.

Britton and Dodd (1933) followed the reactions of copper, zinc, cadmium, cobalt, nickel, lead, and silver with potassium ferrocyanide conductimetrically. The metal salts used were all sulfates except with lead and silver, where the nitrates were employed. They found that only the lead salt gave a normal endpoint, that is, the break occurred when a stoichiometric amount of potassium ferrocyanide had been added. In all other cases excess ferrocyanide was required before the conductivity rose sharply.

Britton and Dodd used a direct conductimetric titration and also determined the conductivity of a series of mixtures of the reactants in well-stoppered bottles, placed in a thermostat until equilibrium had been obtained. Each bottle was vigorously shaken daily. The results obtained by the two methods did not check too closely, the second giving higher values for adsorbed potassium ferrocyanide. With copper the value of $X$ in the formula $Cu_2 Fe (CN)_6 - XK_4 Fe (CN)_6$ varied from 0.52 to 0.71, according to precipitate analyses, but the titrations revealed two breaks in the curve: at 0.16 and 0.40 with direct titration, and at 0.12 and 0.52 with the equilibrium reaction.

The strength of hydroferrocyanic acid was also determined by Britton and Dodd by following changes in pH with a glass electrode during titration with sodium hydroxide. They found this acid to be just slightly weaker than sulfuric acid in all four stages of ionization. Hence, basic salts do not form when potassium ferrocyanide reacts with heavy metal salt solutions. These workers conclude, "On the present evidence it does not seem justifiable to discriminate between the double salts as to which are definite compounds. Such information can only be satisfactorily determined by phase-rule study."

About this same time Bhattacharya and Dhar (1933) suggested that both Prussian and Turnbull's blue approach the intermediate formula $Fe_{12}(CN)_{30}$, or, empirically, $Fe_2(CN)_5$. The "soluble blues" were considered to be colloidal precipitates stabilized by adsorption of potassium ferro- or ferricyanide.

Bhattacharya (1934) investigated whether mutual oxidation and reduction actually take place in the precipitation of the blues. The filtrates of the blues were prepared by mixing equivalent proportions of the reactant solutions, and the supernatant liquid was tested for the products of the redox reaction as well as for residual reactants. Mixing ferric chloride and potassium ferrocyanide in equivalent proportion gave no residual reactants in the filtrate, but ferricyanide ion did appear as indicated by the liberation of iodine from potassium iodide. Tests for the ferrous ion were negative, regardless of the proportion of the reactant.

Ferrous sulfate and potassium ferricyanide in equivalent proportion yielded a filtrate containing appreciable ferrous sulfate and a trace of ferric ion. Adding excess potassium ferricyanide produced some ferrocyanide as indicated by the decolorization of potassium permanganate solution by a

filtrate sample. Even after removal of the ferrous ion using ammonium hydroxide, the filtrate decolorized permanganate solution. Tests for cyanide ion were negative. Filtrates from Prussian blue tested negative for ferric and ferrous ions and oxalic acid whether kept in the light or dark.

Bhattacharya explained the appearance of ferricyanide in the preparation of Prussian blue using an excess of ferrocyanide and the absence thereof when equivalent proportions of reactants are used on the basis of the probable adsorption of the free ferricyanide in the latter case.

Davidson (1937b) suggested that the assumption of a polynuclear complex between the ferric ion and the nitrogen end of the CN group of the ferrocyanide was plausible and helped to account for the color and other properties of "soluble" Prussian blue. He favored a ferrocyanide structure on the basis of the evidence given in an earlier paper (1937a), and along with other evidence from analytical methods, he designates "soluble" Prussian blue as $KFe^{+++}(Fe(CN)_6^{-4})$. Assuming that CN groups have the nitrogen available for coördination, ferric ion with a coördination number of six could be coördinated with a ferrocyanide thus: $(Fe^{+++}(CN)_6^{-6}Fe^{++})$, but spatial limitations would prevent a ferric ion from being coördinated with one ferrocyanide by six bonds. The possible alternatives are a ferrocyanide coördinated with a ferric ion by one, two, or three bonds, the six bonds being obtained by involving six, three, or two ferrocyanide ions, respectively.

Three configurations are possible in this light, and no one can be eliminated. Hence Davidson merely designates the ion as "berlinate," with a formula $(Fe^{+++}(NC)_6Fe^{++})^-$. The name is derived from the German name for Prussian blue, *Berlinerblau*. Thus, although the supercomplex theorized above is highly polymerized, it may be represented in terms of its structural unit, the berlinate ion.

Likewise the compositions of the other heavy metal ferrocyanides can be accounted for by assuming polynuclear complexes based on structural units which consider the coördination numbers of the metals involved.

In reference to the berlinate ion, Emeléus and Anderson (1938) suggest that the question as to whether the "iron blues" are ferro- or ferricyanides becomes meaningless if we assume the existence of resonance stabilization. Thus the hybrid may be symbolized by

$$(Fe^{+++}(CN)_6Fe^{++})^- \leftrightarrow (Fe^{++}(CN)_6Fe^{+++})^-.$$

A statistical distribution of the resonance forms which depends on electron densities would probably indicate unequal contributions of the hybrids, since we would expect differentiation between the Fe–C and Fe–N bonds involved. The bronzy luster and deep color of Prussian blue are understandable from a consideration of the above.

The role of adsorption in reactions of the "iron blues" has been extensively studied by several workers. Kanning and Campbell (1939) suggest that adsorption of ions such as potassium, ferric, and ferrocyanide account for all the deviation from the old Prussian blue formula, $Fe_4(Fe(CN)_6)_3$.

Bhattacharya (1941a) reported on a series of adsorption experiments in which various electrolytes were added to Prussian and Turnbull's blue suspensions shaken and left overnight to settle. The equilibrium concentration

of ferric ion was estimated gravimetrically. Those of ferrous and ferro-
cyanide ions were determined volumetrically with 0.005 $N$ permanganate
solution. Ferricyanide was estimated iodometrically with KCl and titrating
with 0.005 $N$ sodium thiosulfate.

The results show that both Prussian and Turnbull's blues have high
adsorptive tendencies which are later modified by the effects of chemical
reaction between the adsorbent and the adsorbate. Prussian blue prepared
with a 3:2 ratio of ferric ion was found to adsorb more potassium ferro-
cyanide than that made from equivalent amounts of reactants. Conversely,
the blue prepared using a 3:2 ratio of potassium ferrocyanide adsorbed less
of the ferrocyanide than the precipitate resulting from equivalent reactant
concentrations.

The high adsorptive properties of Prussian blue indicate particles of
very small size. Various methods have been used to estimate their average
diameter. These are as follows:

| Method | Average diameter, microns | Observer | Date | Reference |
|---|---|---|---|---|
| Various colloidal | 1.14 | Nistler | 1930 | Weiser (1938) |
| Graded ultrafilter | 0.15 | Bargues | 1942 | Bargues (1942) |
| Electron microscope | 0.05 | Green and Fullam | 1943 | Green and Fullam (1943) |

Although the conditions of preparation and the methods of evaluation
would be expected to affect the results obtained, it is obvious that Prussian
blue particles are very small indeed. The last value, probably the most
accurate, indicates the proximity to molecular dimensions approached by the
particles. In addition, the electron microphotograph revealed, probably for
the first time, the definitely cubic crystals of Prussian blue (Green and
Fullam, 1943).

Weiser (1938) characterizes the Prussian blue sol as stable at room tem-
perature but coagulated by boiling and by visible and ultraviolet light but
not by alpha, gamma, or X rays. The optimum wave length for most rapid
coagulation is given as approximately 420 millimicrons. A pressure of 2,000
atmospheres is reported to, cause immediate coagulation. Hazel and Sorum
(1930) reported that continued boiling of a dilute Prussian blue sol yields
a negatively charged sol of hydrous ferric oxide.

The coagulating action of electrolytes on Prussian blue sols has been
demonstrated by many investigators. Multivalent cations exhibit the
greatest effect, the relative order of precipitating power being given as
Fe, Al, $Cr > Ba$, $Cd > Sr$, $Ca > H > Cs > Rb > K > Na > Li$ (Weiser, 1938).
The precipitating action of electrolytes decreases with dilution, irrespective
of valence. Also, the addition of electrolytes to a mutual coagulation will
widen the range of sensitivity.

The effect of nonelectrolytes on the coagulation of colloids was studied by
Chaudbury and Chatterjee (1929). The sol used was cupric ferrocyanide,
which was prepared by mixing equivalent amounts of cupric sulfate and
potassium ferrocyanide, washing thoroughly to effect peptization, and dialyz-
ing to remove any sulfate or ferrocyanide present. Then equicoagulating con-

centrations of different electrolytes in the presence of nonelectrolytes were determined by noting the time when the light of a filament lamp just disappears owing to increased turbidity of the sol. The limiting concentrations are said to be sensitive within a drop, that is, 0.05 cc of electrolyte. The nonelectrolytes tested were methanol, ethanol, urea, cane sugar, gelatin, and acetic acid. These investigators found that both methanol and ethanol, in combination with the electrolyte, sensitized the sol. Urea also sensitized the sol. Urea also sensitized for most of the electrolytes used, but it stabilized the sol against KCl. Sugar stabilized the sol against KCl and $H_2SO_4$ but had no effect on the coagulating concentrations of the other electrolytes.

The theoretical considerations useful in explaining these phenomena are the contributions of the nonelectrolyte in changing the dielectric constant of the medium and the interfacial tension (surface tension). Thus, an increase in interfacial tension tends to coagulate a sol (reduce the total surface) while, on the other hand, an increase in dielectric constant tends to retard coagulation by decreasing the available coulombic forces between charged particles. However, the experimental results did not completely correlate with the above reasoning. Hence these investigators concluded that other factors, such as electrical forces opposing collision, changes in energy associated with agglomeration, and the changes in the critical potential of the sol, must be considered. In addition, the data on the dielectric constants and surface tensions involved were possible sources of error.

The effect of hydrolysis on dilution of the blue suspensions was demonstrated in experiments by Bhattacharya (1941b). It was found that the ratio of Fe to CN varied with dilution. A plot of these ratios indicated that at a concentration of about 0.125 $M$, the blues have identical compositions.

The identical composition of the blues, at least under specific conditions, is likewise demonstrated in electrometric titrations using (a) $FeCl_3$ with $K_4Fe(CN)_6$ and reverse; (b) $FeCl_2$ with $K_3Fe(CN)_6$ and reverse. The titration curves all have a common point of intersection corresponding to the same composition (Weiser, 1938). Weiser cautions, however, that electrometric titrations cannot give conclusive results where gels with high adsorptive capacity are involved. Nevertheless, on this particular question, both X-ray and electron-diffraction studies, discussed in more detail later, indicate that Prussian and Turnbull's blue are identical in structure (Weiser and Milligan, 1942).

Weiser and Milligan (1942), and Weiser, Milligan, and Bates (1942), reopened the controversy concerning the identification of the blues of ferro- or ferricyanides by claiming the latter as the more representative formula. On the basis of the ionic redox equilibrium (discussed previously) which lies far to the right on the side of ferrous and ferricyanide ions and the fact that ferricyanide ion is detected in the Prussian blue filtrate, they felt that the blues could be ferricyanides. Although quantitative agreement between the amount of ferricyanide detected and the theoretical quantity has not been obtained, they argue that adsorption phenomena could account for this seeming discrepancy. If one assumes the validity of the resonance hybrid configuration as suggested by Emeléus and Anderson (1938) the entire question is meaningless.

The chemical methods of investigating the properties and structure of cupric ferrocyanide are likewise limited in scope, but nevertheless are useful. The role of adsorption in producing what some investigators have called "double salts" is highly significant. Using equivalent amounts of a cupric salt and potassium ferrocyanide, precipitation of the copper was found to be incomplete due to adsorption of some of the ferrocyanide (Weiser and Milligan, 1942). With hydroferrocyanic acid, however, pure $Cu_2Fe(CN)_6$ is said to be obtained by mixing equivalent solutions of the reactants.

Eméleus and Anderson (1938) assign 7 to 10 $H_2O$ of hydration to $Cu_2Fe(CN)_6$, which is known as Hatchett's brown. They consider this compound also as a salt of a polynuclear complex anion, $CuFe(CN)_6^{--}$, comparable to that of Prussian blue or ferrous ferrocyanide.

Rigamonti (1937, 1938) studied the equilibrium of cupric ferrocyanide in solutions containing ions of the alkali, alkaline earth, and heavy metals. After allowing the precipitates to age 24 hours, he analyzed the filtered products. The results show relative tendencies of the cations to displace copper from cupric ferrocyanide according to the following reactions:

$$Cu_2Fe(CN)_6 + 2M^+ \leftrightarrows CuM_2^+Fe(CN)_6 + Cu^{++}$$
$$Cu_2Fe(CN)_6 + M^{++} \leftrightarrows CuM^{++}Fe(CN)_6 + Cu^{++}$$
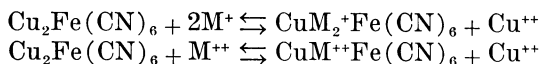
**Evidence from Physical Methods.** On the basis of purely chemical studies, no final solution to this problem could be found, but the polynuclear structure proposed by Reihlen and Zimmerman (1927) and later by Davidson (1937a) gained prominence in the preceding decade. This was supported by the application of such methods as X ray, absorption spectra, magnetic moment and susceptibility, and electron diffraction.

In 1928, Davidson and Welo reported their studies of Prussian blue utilizing magnetic-susceptibility measurements. The ferrocyanides, long known to be diamagnetic, should indicate paramagnetism only because of contributions from any ferric ion present in the structure. In the insoluble blue, $Fe_4(Fe(CN)_6)_3$ only 4 out of 7 iron atoms per molecule would be expected to contribute to the paramagnetic effect. Their experimental data checked these predictions within rather wide limits, but on the basis of a polynuclear formulation, improbable values were obtained. This led these workers to conclude that the polynuclear configuration was not compatible with experimental fact.

Light-absorption studies of Prussian and Turnbull's blues by Bhattacharya (1935) indicated that the two compounds are quite similar and are even more so as the aging of the precipitates continues. The various gels were prepared and aged 24 hours before filtering with most samples, although some were aged 5 weeks in stoppered bottles. He took absorption photographs in the visible, ultraviolet, and infrared regions. He found the shapes of the absorption curves for the two blues to be quite similar except for a slight shift in range. The shifts of absorption bands were explained as the result of formation of different proportions of Prussian and Turnbull's blues during the reaction between the iron and ferrocyanogen salts or due to differences in particle size. The greater initial absorption capacity of Turnbull's blue could possibly be contributed by an excess of ferrous molecules, since it is well accepted

that unsaturated (with respect to valence) organic compounds show greater absorption than saturated ones.

The X-ray powder analysis, applied to various ferro- and ferricyanides by Keggin and Miles (1936), revealed a cubic structure with unit edges of about 5.1 Å or 10.2 Å. The colorless ferrous alkali ferrocyanides were found to contain a lattice of ferrous atoms linked by CN groups with alkali atoms at the center of each cube. The unit edges of this cube were 10.2 Å. On oxidation Keggin and Miles believed that alternate alkali atoms were removed and, alternate iron atoms were oxidized to ferric state yielding Prussian blue. In Prussian blue the iron atoms were postulated to be arranged, ferrous and ferric alternately, at the corners of a cubic lattice of 5.1 Å edge length, with the CN group lying along these edges. The alkali atoms were assigned the centers of alternate small cubes.

The oxidation product of Prussian blue, known as Berlin green, was designated as $Fe^{+++}Fe^{+++}(CN)_6$, or ferric ferricyanide. In this case the unit cell would be 5.1 Å since all the iron atoms are the same, and of course no alkali atoms are involved.

The cupric ferricyanides were designated as $RCu^{++}Fe^{+++}(CN)_6$, which has the same structure as Prussian blue except for the cupric copper replacing all the ferrous iron. The edge of this unit cube was found to be slightly less than 10.2 Å.

It was noted that the water content of the gels used above was very high. Weiser (1938) stated that ten waters of hydration are contained in cupric ferrocyanide molecules in equilibrium with 5 per cent sulfuric acid and only six or three when dried over concentrated sulfuric acid. Bhattacharya (1935) reported the moisture content of air-dried Prussian and Turnbull's blues as 16 and 18 per cent, respectively.

Weiser, Milligan, and Bates (1941) carried out a study of the water involved in gels of $Cu_2Fe(CN)_6$, $Cu_3(Fe(CN)_6)_2$, and Prussian and Turnbull's blues. By means of high-vacuum desiccation, all water was removed from some samples, and these were compared with moist gels by means of electron-diffraction analyses. It was found that the patterns did not change after being exposed to the high vacuum for periods as long as 24 to 48 hours. This indicates the absence of any definitely hydrated compounds. These results were later borne out by Sutra (1946), who compared Stokes' radii for ions with the limiting ionic radii in solution. For these ferrocyanides, the values turned out to be the same. This again indicates lack of hydration. Hence the water in the samples may be adsorbed on the surface of the finely divided crystals or may be held in the lattices themselves in the manner of zeolite crystals.

Electron-diffraction methods were applied by Fordham and Tyson (1937) to a study of semipermeable membranes of inorganic salts, among which were copper, ferric, and lead ferrocyanides. Copper ferrocyanide films gave patterns indicating crystals having no common direction or orientation. The average crystal size of copper ferrocyanide as determined by the breadth of the diffractions was between 100 and 150 Å, and in no case was there any evidence of halos, which indicate the presence of amorphous material. It was found that contact with excess potassium ferrocyanide resulted in a pattern indicating the presence of cupric hydroxide.

Weiser, Milligan, and Bates in 1938 measured the X-ray diffraction of copper ferrocyanide gels, prepared by mixing varying amounts of cupric chloride and potassium ferrocyanide. The supernatant liquor was analyzed for copper and ferrocyanide by the iodometric and permanganate methods, respectively. The unwashed moist gels obtained by centrifuging the samples were placed in thin tubes of Lindemann glass and examined by X ray. Their results showed that where excess ferrocyanide is carried down with a gel, it is not combined to form a definite compound (double salt). Copper ferrocyanide precipitated in the presence of excess cupric ion as shown by the distinct X-radiogram was practically pure $Cu_2Fe(CN)_6$.

Along similar lines Rigamonti (1937) reported results of extensive studies on the structures of the heavy bivalent metal ferrocyanides and concluded that they are isomorphous with mixed ferrocyanides of bivalent and univalent metals. He used electron-ray and X-ray examinations to ascertain whether $(M^{+}Fe^{++}(CN)_6)^{-2}$ rather than $(Fe^{++}(CN)_6)^{-4}$ ions are present. The compounds used were $Cu_2Fe(CN)_6$, $CuK_2Fe(CN)_6$, and $Cu_3K_2(Fe(CN)_6)_2$. Each was found to possess a cubic face-centered structure and with elementary cells containing four, four, and two molecules, respectively. Four Fe atoms and four Cu atoms are situated on two lattices with central faces, the origin of one being in the center of the other. The other Cu and K atoms are located in the interior of the lattice. These interpretations help to explain the equilibrium involving so-called mixed crystals as, for example, $(Cu_2Fe(CN)_6)_m \cdot (CuK_2Fe(CN)_6)_n$ where $m$ and $n$ can vary continuously. Rigamonti acknowledges the existence of the polynuclear complex $(CuFe(CN)_6)^{-2}$, which signifies that cupric ferrocyanide is in reality $Cu(CuFe(CN)_6)$ and that $H_2(CuFe(CN)_6)$ is the parent acid of the univalent mixed salts. Consistent with this formulation are the results of Keggin and Miles (1936) and Davidson (1937b). Thus, cupric ferrocyanide is portrayed as having a central Fe atom to which C atoms are attached. The N atoms of each CN can then coördinate with another heavy metal atom to fill one coördination position; hence, each $Fe(CN)_6$ becomes surrounded by six attached cations in consistency with a cubic lattice (Eméleus and Anderson, 1938).

Rollier and Arreghini (1939), using magnetic moment and susceptibility measurements, also agreed with the polynuclear configuration as applied to cupric ferrocyanide. They also confirmed the absence of any cuprous ion in $Cu_2Fe(CN)_6$ and $CuK_2Fe(CN)_6$.

Additional work by Rigamonti (1938) demonstrated that whereas the ferrocyanides of iron, cobalt, nickel, and aluminum have the basic cubic structure, others such as those of cadmium, zinc, calcium, barium, and strontium have a lower state of symmetry. Weiser, Milligan, and Bates (1938, 1942) completed similar work using X-ray analysis on the bivalent metal ferrocyanides and concluded that structures of the copper, cobalt, nickel, and manganese compounds were isomorphous, but the lead compound was not. They obtained two distinct X-ray diffraction patterns for zinc, cadmium, silver, and ferrous compounds, according to whether the anion or cation was in excess. It was assumed that one pattern represented the normal salt and the other a double salt involving potassium ions. On the other hand,

cupric ferrocyanide gave only one X-radiogram regardless of excess potassium ferrocyanide or cupric ion added, which indicates that only adsorption is involved here.

Also of interest is the observation of a mitogenetic effect (emission of ultraviolet light) accompanying the precipitation of cupric ferrocyanide (also demonstrated by $BaSO_4$ precipitation) by Rabinerson and Vladimirskaya (1939). The energy of precipitation must be of high order to raise an electron from the lattice to the required energy level to yield a 2000 Å radiation.

Weiser, Milligan, and Bates (1942) reported determinations of densities of dehydrated samples of Prussian and Turnbull's blues using a pycnometer with xylene as the liquid. First the adsorbed water was removed under a vacuum obtained with a Hyvac pump. The samples were exposed to the vacuum for 72 hours at a temperature of 67° C. The density values obtained were for Prussian blue, $d_{40}^{25°} = 1.785$; for Turnbull's blue, $d_{40}^{25°} = 1.790$. Apparently these values are sufficiently close to fit in with the popular concept that Prussian and Turnbull's blue are one and the same.

## Toxicology of Ferrocyanide and Possible Substitutes

**Ferrocyanide.** The experimental evidence on the toxicity of ferrocyanide had been limited largely to tests of acute toxicity when orally administered and investigations of chronic oral toxicity have not been reported. The oral toxicity of ferrocyanide ion is relatively low. Potassium ferrocyanide in alkaline solution has been reported as nontoxic (Anonymous, 1953a). Autenrieth (1893), for example, reported that ferrocyanide solutions are relatively nontoxic. In the older literature Prussian blue is referred to as "the well-known nonpoisonous pigment." The diuretic effect of sodium ferrocyanide is well established and it has been used in clinical tests of kidney function. There is evidence that sodium ferrocyanide is excreted by the kidney of the rabbit and the dog by a process of glomerular filtration (Van Slyke, Hiller, and Miller, 1935a). In man, however, tubular reabsorption of ferrocyanide occurs and is accompanied by renal irritation even at levels of serum ferrocyanide lower than those used in dog experiments (Miller and Winkler, 1936). Miller and Winkler found that when sodium ferrocyanide was injected into men at the rate of 20 mg per kg of body weight it led to marked albuminurea and appearance of casts, white cells, and epithelial cells in the urine. They concluded that sodium ferrocyanide exerts a definitely toxic action on the human kidney. Intravenously injected ferrocyanide circulates in the blood plasma without entering the erythrocytes (Van Slyke, Hiller, and Miller, 1935b).

Aqueous solutions of the alkali metal ferrocyanides are rather stable at room temperature but will decompose slowly when irradiated, with the production of alkali, formation of cyanide ion, and precipitation of ferrous ferrocyanide (Anonymous, 1953a). In lakes or streams under conditions such that ferrocyanide levels over 1 to 2 p.p.m. exist, irradiation by sunlight may produce cyanide ion or hydrogen cyanide in concentrations lethal to fish (Burdick and Lipschuetz, 1948). Strongly alkaline solutions are more stable towards heat than neutral solutions. Solutions of the fer-

rocyanide ion acidified with mineral or organic acids decompose slowly at room temperature and more rapidly when heated, with the formation of hydrogen cyanide and ferrous ferrocyanide. The rate and extent of decomposition increases with increase in acidity (Anonymous, 1953a).

There are no data on the chronic toxicity of ferrocyanide in the levels in which it may be present in overfined wines, nor on the extent of its possible hydrolysis to the extremely toxic hydrogen cyanide in wine.

A recent review of the chemistry of the ferrocyanides (Anonymous, 1953a) points out that the use of potassium ferrocyanide in beverage wines to remove sediment-forming heavy-metal ions is quite frequent in European wineries. Some European investigators reject this procedure, while a number approve but counsel caution and indicate limitations. Fontanelli (1947) reported that although hydrogen cyanide could be liberated from potassium ferrocyanide in mildly acid wine, the amount would not be detectable unless a large excess of ferrocyanide was used. Even then it probably would be oxidized rapidly by air or transformed into the relatively harmless thiocyanic acid by traces of sulfur compounds. Wines that have been overfined with potassium ferrocyanide are characterized by the presence of a blue deposit and also by a cyanide odor. The ability to detect hydrogen cyanide by odor, however, varies with individuals (Kirk and Stenhouse, 1953).

**Ethylenediaminetetraacetate.** Versene, sodium ethylenediaminetetraacetate manufactured under the F. C. Bersworth patents, was reported not to be toxic to humans in dosages up to 5 grams over an 8-hour period. At these levels and rates of administration, hypocalcium tetany did not occur (Anonymous, 1952a). Krum and Fellers (1952) conducted acute and chronic toxicity tests of Sequestrene, another trade name for the same chemical. With rats, they found no toxic effects either with single oral doses of 4 grams per kilogram of body weight or with long-time feeding of basal ration plus 0.5 per cent Sequestrene.

**Rubeanic Acid.** Acute and chronic toxicity tests of rubeanic acid are now under way. Preliminary results with rats indicate little or no toxicity during feeding periods of over 6 months. The tests on chronic toxicity have been carried out for over 6 months and are still being continued.

## ANALTYICAL METHODS

### Copper Content

In the experiments here reported, copper was determined by the sodium diethyl dithiocarbamate colorimetric method, using the modification of Marsh (1941). In this modification of the Coulson (1937) and Drabkin (1939) procedure, wet-ashing is not used; and the colored complex of copper, which is formed directly in the wine, is extracted with amyl acetate in the presence of methyl alcohol.

The standard copper solution was made by dissolving pure electrolytic copper in nitric acid according to the procedure outlined by Sandell (1944). Readings were taken on a Klett-Summerson photoelectric colorimeter using a green filter (540 m$\mu$).

Ten ml of wine were pipetted into a clean 25 by 150 mm Pyrex test tube. One ml of hydrochloric–citric acid reagent was added. (Prepared by dis-

solving 75 grams of citric acid in 350 ml of copper-free distilled water, adding 50 cc of copper-free concentrated hydrochloric acid, and making up to 500 ml in a volumetric flask.) Then, after shaking, 2 ml of 5 $N$ ammonium hydroxide (accurately prepared by diluting 333 ml of concentrated $NH_4OH$ to 1 liter) was added. The mixture was again shaken, and then 1 ml of 1 per cent aqueous solution of sodium diethyl dithiocarbamate was added. After shaking and waiting for a minute or so, 10 ml of copper-free amyl
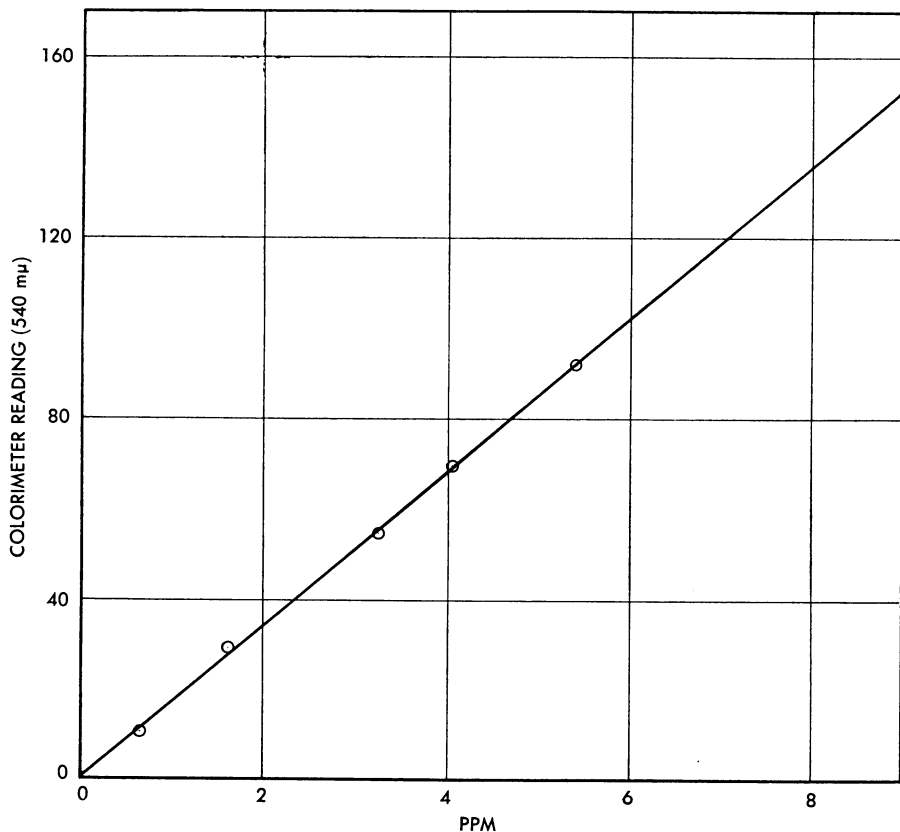


Fig. 2. Copper-determination standard graph of colorimeter reading of complex versus parts per million of copper.

acetate was added and then 5 ml of copper-free absolute methyl alcohol. The tube was next corked and shaken vigorously for at least 30 seconds. The bottom aqueous layer was drawn off by suction, and the organic phase was dried by adding anhydrous sodium sulfate. The dried organic phase was transferred to a clean colorimeter tube, the optical density was determined, and parts per million of copper were then read off the standard calibration curve prepared in the same way with known concentration of copper (fig. 2).

**Sources of Error.** The determination of copper must be carefully conducted to obtain reliable results. Berg (1953) has called attention to possible errors due to improper preparation of standards, introduction of copper

during the determination, inaccurate addition of solutions, change in color standards, and instrument errors. The standards must be prepared carefully from pure copper and accurately diluted by using standardized volumetric flasks and pipettes. It is best to prepare a standard curve shortly before use and to check the instrument by using a standard solution while running the unknowns to avoid possible changes in the photoelectric colorimeter since preparation of the standard curve. The sensitivity of a photometer of the Klett-Summerson type is least in the range of low concentrations of copper, and particular attention must be paid to selecting an aliquot of sufficient size in this range. The organic phase must be carefully and thoroughly dried before transferring to the colorimeter tube, and this tube must be clean and dry since the presence of moisture in the tube or in the amyl acetate extract will cause turbidity. The introduction of copper in the reagents used, in the distilled water, glassware, filter paper, or from hands should be avoided so as to reduce the magnitude of the blank correction. The filter paper used in filtering the solutions should be copper-free, such as E. & D. No. 613. The reagent solutions, especially methyl alcohol and amyl acetate, should be tested for copper. If copper is found they should be redistilled in an all-glass apparatus before use. The distilled water also should be redistilled from glass.

The dilution of the concentrated $NH_4OH$ to $5N$ should be accurately made since the pH of the solution in which the color is developed is very important. Drabkin (1939), who introduced the use of isoamyl acetate for the extraction of copper diethyl dithiocarbamate from weakly ammoniacal solutions (pH 8.5 to 9.0), pointed out that under these conditions even large quantities of iron (10 mg) would not interfere with the determination of as little as 0.02 mg of copper. Coulson (1937) reported that although ferric iron will also produce a brown carbamate at pH 8.0, this interference can be effectively overcome if the pH of the ammonium citrate solution is adjusted to about 8.5 before extraction with organic solvents. Coulson reported that moderate excesses of ammonium hydroxide do not greatly increase the pH of the solution and that from pH 5.7 to pH 8.5 the color of the copper carbamate extracted is the same, while above pH 8.5 there is a very slight decrease in color. Marsh (1941), however, found that the pH of the solution after addition of ammonium hydroxide should be between 6 and 9 and preferably at 8. He also found that the addition of methyl alcohol greatly reduced the pH effect; it permits good accuracy between pH 6 and 9 and avoids the necessity of adjusting to exactly pH 8.

**Effect of Methyl Alcohol on Copper Determination.** The addition of methyl alcohol was proposed by Marsh (1941) to reduce emulsification and to reduce variation in pH and thus in the color intensity of the complex formed. To check on this, data were obtained in the present experiments on the effect of methyl alcohol and pH on the color intensity of the complex formed in wine, vinegar, and apple juice whose copper concentration was built to 2.0 p.p.m. The pH was varied from 2.5 to 9.0 with various reagents. The methyl alcohol addition was varied from 0 to 10 ml. Fifty ml of liquid was pipetted into 100-ml beakers. The pH was systematically varied using $6 N$ HCl, $6 N$ acetic acid, $6 N$ citric acid and $6 N$ ammonium hydroxide.

To determine the effect of methyl alcohol on color intensity, two 10-ml samples were withdrawn from each pH sample and analyzed for copper, using the reagents called for in the accepted procedure except that the methyl alcohol content was varied. Change in pH after adding the prescribed reagents and 0 to 10 ml alcohol was determined on the Beckman pH meter.

When wines were adjusted to initial pH of 2.5 to 2.9 with acetic acid or hydrochloric acid, the addition of methyl alcohol increased the resulting



1—INITIAL pH 4.0–9.0 (NH$_4$OH)
2—INITIAL pH 2.5–3.0 (HCl)
3—INITIAL pH 3.0 (CITRIC ACID)
4—INITIAL pH 3.0 (ACETIC ACID)
5—INITIAL pH 2.5 (CITRIC ACID)
6—INITIAL pH 2.9 (ACETIC ACID)
7—INITIAL pH 2.8 (ACETIC ACID)
8—INITIAL pH 2.7 (ACETIC ACID)
9—INITIAL pH 2.6 (ACETIC ACID)
10—INITIAL pH 2.5 (ACETIC ACID)
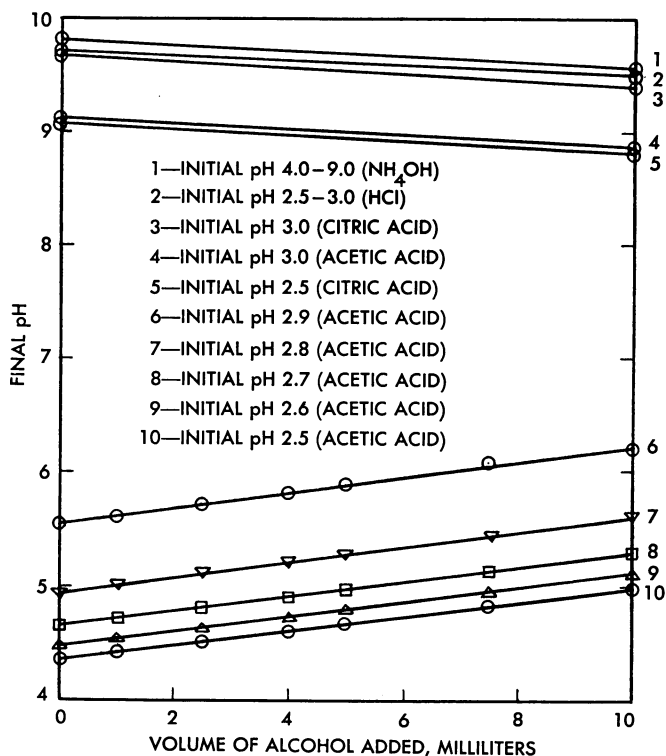
Fig. 3. Effect of methyl alcohol on pH or reagent mixture used in determination of copper.

pH only slightly. Wines made initially alkaline by ammonia were even less affected by methyl alcohol, as shown in figure 3. The effect of wide variation in initial pH value on copper content is shown in table 5. The addition of 5 ml of methyl alcohol changes the pH by about 0.3, and 10 ml changes it by only about 0.5. Below pH 7, methyl alcohol acted as a base and above pH 7 as an acid. Color development, however, was more intense with methyl alcohol and was optimum at pH 8 to 9. The minimum pH at which color formation occurred in wine acidified with 6 $N$ acetic acid was pH 3.0. Similar conditions were found for cider vinegar and apple juice.

## Iron Content

The total iron content was determined by a colorimetric method using ortho-phenanthroline (Hummel and Willard, 1938; Saywell and Cunningham, 1937). The standard iron solutions were prepared by dissolving 99.8

per cent pure iron wire in dilute nitric acid. All readings were made using the Klett-Summerson photoelectric colorimeter with a purple filter (420 m$\mu$).

Two ml of wine were pipetted into 25 by 150 mm Pyrex tubes previously marked at 10 ml. The wine was evaporated to dryness in a 100° C oven, after which 1 ml of concentrated sulfuric acid was added. To liquefy the contents, the mixture was heated gently under a hood. After cooling, 0.5 ml of 70 per cent perchloric acid ($HClO_4$) was added, and mixture was heated continuously under a shatterproof-glass-protected hood until the contents were clarified. (If this did not clarify, another 0.5 ml of $HClO_4$ was added and excess $HClO_4$ was completely evaporated off.)

After cooling, 2 ml of distilled water and a small piece (0.5 cm square) of Congo red paper were added. One ml of a 10 per cent aqueous solution of hydroxylamine hydrochloride was added and then 1 ml of a 0.1 per cent solution of $o$-phenathroline in 50 per cent alcohol. This solution was then titrated to the color change (blue to light red) of the Congo red paper with concentrated $NH_4OH$ and set aside to cool. The salmon-colored solution was then made up to the 10-ml mark with distilled water and was transferred to a colorimeter tube. The optical density was determined and the parts per million of iron were read off the standard iron calibration curve (fig. 4).

The total ferric plus ferrous iron was determined similarly after the addition of hydrogen peroxide to oxidize the ferrous to ferric iron. Hence, with the total iron already determined, the concentration of each constituent in the test wine was calculated as follows:

$$(Fe^{+++}) + (Fe^{++}) + (Fe\ complex) = 5.8\ p.p.m.$$
$$(Fe^{+++}) = 0.7\ p.p.m.$$
$$(Fe^{+++}) + (Fe^{++}) = 2.3\ p.p.m.$$

Therefore: $(Fe^{+++}) = 0.7$ p.p.m., $(Fe^{++}) = 1.6$ p.p.m., and $(Fe\ complex) = 3.5$ p.p.m.

### Phosphorus Content

Phosphorus was determined by the ammonium molybdate method (Sumner, 1944). One ml of wine was pipetted into 25 by 200 mm tubes marked at 50 ml. Five ml of 6.6 per cent ammonium molybdate solution was added, after which distilled water was added to about 40 ml final volume. Five ml of 7.5 $N$ sulfuric acid was added and then 4 ml of 5 per cent ferrous sulfate. Volume was then made up to 50 ml with distilled water, and optical density determined on a Klett-Summerson photoelectric colorimeter using the 660 m$\mu$ filter. The instrument was set at zero with a blank made up of all reagents except the wine. This gave reading $A$. This blank corrected for the color contribution of the various reagents. To determine the effect of the wine on optical density, another blank was run. In this case the wine and all reagents were used except the ferrous sulfate. (The control for this determination was prepared with all reagents except wine and ferrous sulfate.) This gave a reading $B$. The optical density for the wine was calculated as $A - B$, and the milligrams of phosphorus were read off a calibration curve for phosphorus prepared by using a standard solution of anhydrous dibasic sodium phosphate ($Na_2HPO_4$) (fig. 5).

**Comparison of Sumner and Beck and Pro Methods.** The Sumner method

<div align="center">TABLE 5</div>

## EFFECT OF VARYING pH AND METHYL ALCOHOL ON COLOR INTENSITY AND COPPER CONTENT, AS DETERMINED BY THE MARSH PROCEDURE

| Reagent and amount added, ml | Initial adjusted pH | Final pH | | | | Copper content from colorimetric readings, p.p.m. | |
|---|---|---|---|---|---|---|---|
| | | Without methyl alcohol | With 2.5 ml methyl alcohol | With 5.0 ml methyl alcohol | With 10 ml methyl alcohol | Without methyl alcohol | With 5.0 ml methyl alcohol |
| **White wine** | | | | | | | |
| HCl: | | | | | | | |
| 0.1 | 2.50 | 9.7 | 9.65 | 9.5 | .... | 2.2 | 2.1 |
| 0.05 | 3.00 | 9.75 | 9.65 | 9.45 | .... | 2.2 | 2.1 |
| Citric acid: | | | | | | | |
| 4.0 | 2.50 | 9.12 | 9.05 | 8.82 | .... | 2.2 | 2.1 |
| 0.50 | 3.00 | 9.70 | 9.50 | 9.4 | .... | 2.2 | 2.1 |
| Acetic acid: | | | | | | | |
| 23.0 | 2.50 | 4.35 | 4.52 | 4.68 | .... | | |
| 17.7 | 2.60 | 4.50 | 4.65 | 4.82 | .... | | |
| 12.9 | 2.70 | 4.66 | 4.82 | 4.99 | .... | No color formed | |
| 8.8 | 2.80 | 4.95 | 5.11 | 5.45 | .... | | |
| 5.7 | 2.90 | 5.55 | 5.73 | 5.90 | .... | | |
| 2.5 | 3.00 | 9.13 | 9.05 | 8.85 | .... | 2.2 | 2.2 |
| None (control) | 3.35 | 9.6 | 9.55 | 9.3 | .... | 2.2 | 2.1 |
| NH₄OH: | | | | | | | |
| 0.4 | 4.00 | 9.78 | 9.65 | 9.5 | .... | 2.2 | 2.2 |
| 0.65 | 5.00 | 9.80 | 9.70 | 9.5 | .... | 2.2 | 2.0 |
| 0.70 | 6.00 | 9.80 | 9.7 | 9.5 | .... | 2.2 | 2.0 |
| 0.71 | 7.00 | 9.80 | 9.7 | 9.5 | .... | 2.2 | 2.0 |
| 0.72 | 8.00 | 9.80 | 9.7 | 9.5 | .... | 2.2 | 2.0 |
| 0.74 | 9.00 | 9.8 | 9.7 | 9.5 | .... | 2.2 | 2.0 |
| **Cider vinegar** | | | | | | | |
| NH₄OH: | | | | | | | |
| 1.5 | 4.00 | 6.3 | .... | 6.6 | 6.8 | No color | No color |
| 2.1 | 4.10 | 7.83 | .... | 7.58 | 7.50 | 1.56 | No color |
| 2.3 | 4.20 | 8.1 | .... | 7.83 | 7.70 | 2.36 | 2.05 |
| 2.9 | 4.30 | 8.46 | .... | 8.20 | 8.03 | 0.64 | 1.96 |
| 3.3 | 4.40 | 8.60 | .... | 8.33 | 8.19 | 0.64 | 1.96 |
| 3.7 | 4.50 | 8.80 | .... | 8.57 | 8.44 | 0.96 | 1.98 |
| 5.7 | 5.00 | 8.90 | .... | 8.63 | 8.49 | 1.11 | 1.90 |
| 7.7 | 6.00 | 8.36 | .... | 9.13 | 8.98 | 0.98 | 1.90 |
| 8.0 | 7.00 | 9.40 | .... | 9.16 | 9.01 | 1.16 | 1.86 |
| 8.4 | 8.00 | 9.4 | .... | 9.13 | 8.98 | 1.17 | 1.81 |
| 10.6 | 9.00 | 9.5 | .... | 9.25 | 9.1 | 1.21 | 1.82 |
| **Apple juice** | | | | | | | |
| HCl: | | | | | | | |
| 0.25 | 2.50 | 9.48 | .... | 9.22 | 9.08 | 0.55 | 2.2 |
| 0.10 | 3.00 | 9.55 | .... | 9.29 | 9.15 | 0.65 | 2.2 |
| Citric acid: | | | | | | | |
| 4.30 | 2.50 | 8.95 | .... | 8.68 | 8.53 | 0.60 | 2.3 |
| 1.00 | 3.00 | 9.48 | .... | 9.19 | 9.05 | 0.50 | 2.2 |
| Acetic acid: | | | | | | | |
| 9.9 | 2.90 | 4.77 | .... | 5.12 | 5.38 | No color | No color |
| 6.80 | 3.00 | 5.20 | .... | 5.54 | 5.81 | No color | No color |
| None (control) | 3.57 | 9.6 | .... | 9.32 | 9.20 | 0.55 | 2.1 |
| NH₄OH: | | | | | | | |
| 0.10 | 4.00 | 9.6 | .... | 9.35 | 9.22 | 0.55 | 2.1 |
| 0.40 | 5.00 | 9.6 | .... | 9.40 | 9.27 | 0.55 | 2.1 |
| 0.50 | 6.00 | 9.6 | .... | 9.42 | 9.3 | 0.60 | 2.1 |
| 0.60 | 7.00 | 9.67 | .... | 9.43 | 9.3 | 0.55 | 2.1 |
| 0.70 | 8.00 | 9.66 | .... | 9.43 | 9.3 | 0.60 | 2.1 |
| 1.00 | 9.00 | 9.7 | .... | 9.47 | 9.34 | 0.65 | 2.1 |
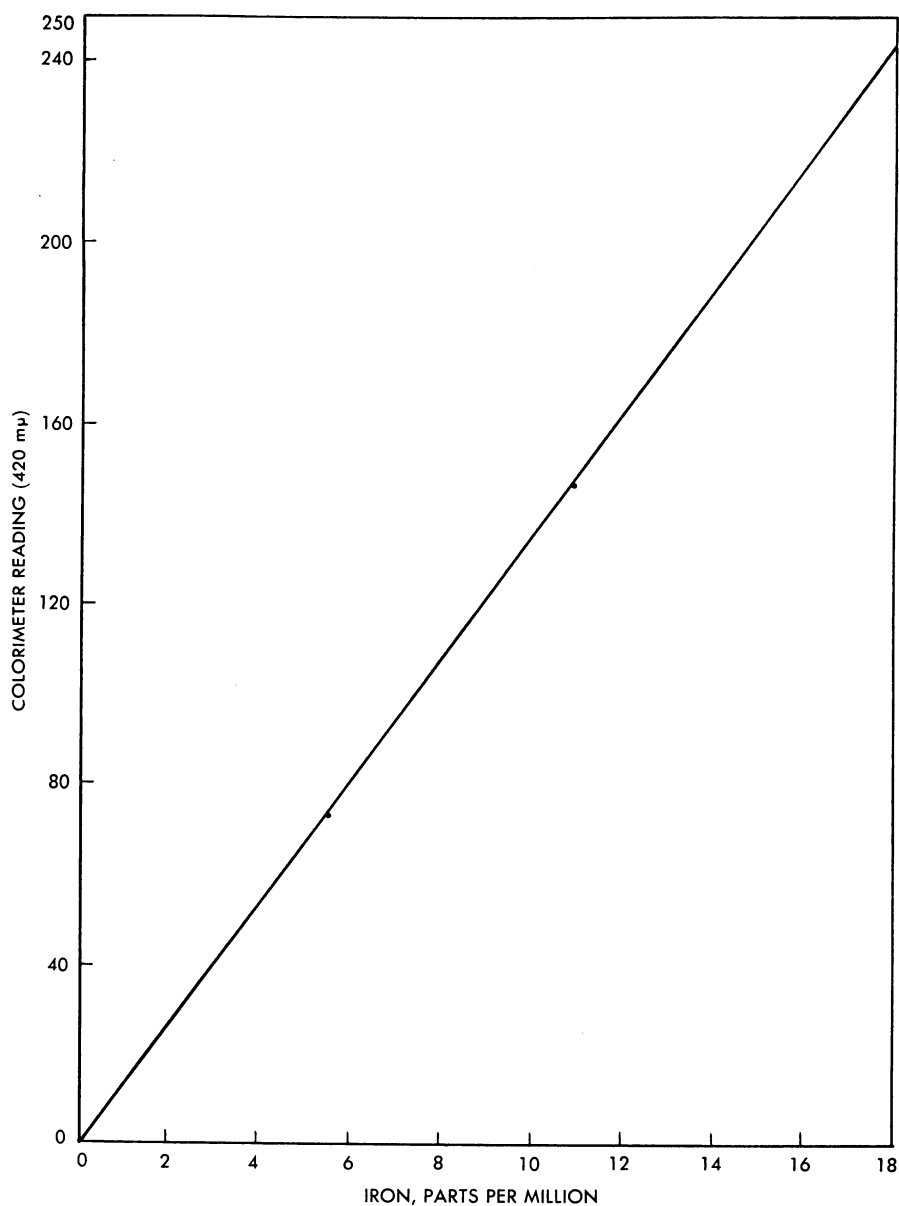
Fig. 4. Iron-determination standard graph of colorimeter reading of complex versus parts per million of iron.
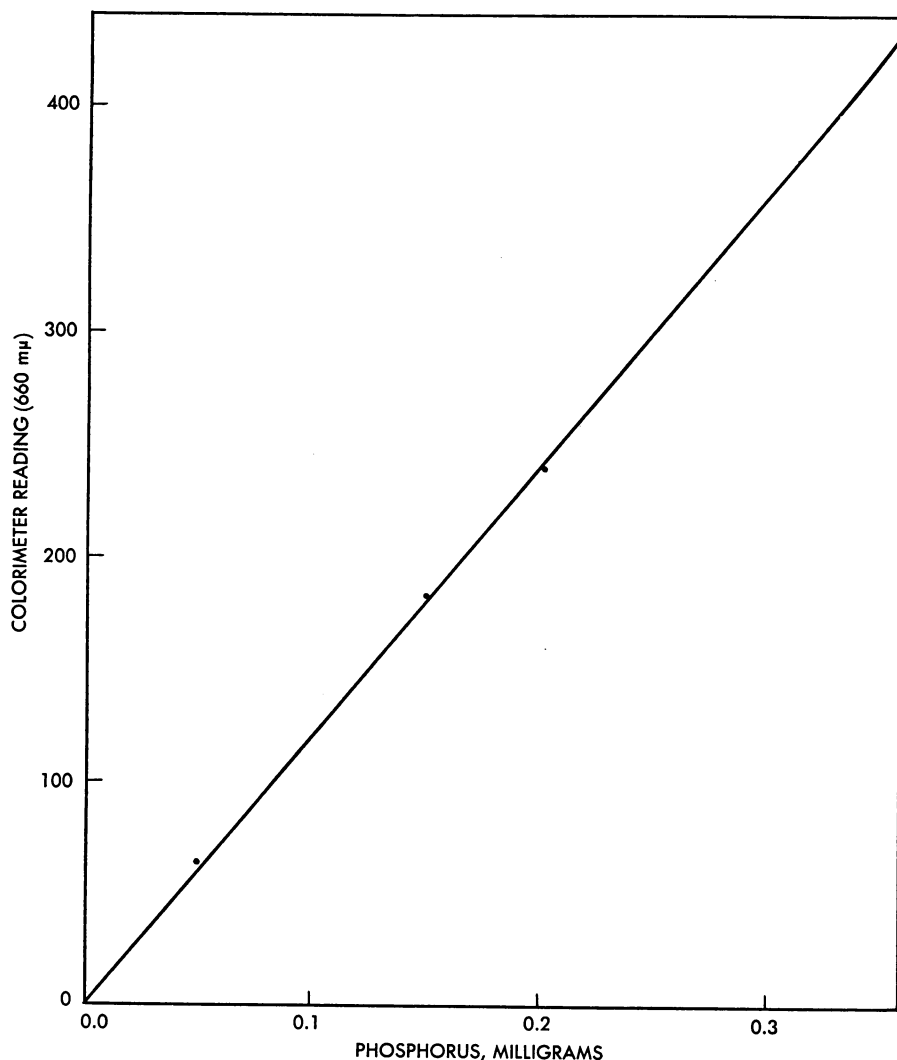
Fig. 5. Phosphate-determination standard graph of colorimeter reading of complex
versus milligrams of phosphate per 100 milliliters.

is simpler than the procedure of Beck and Pro (1952) and gives phosphate
recoveries and phosphate content values sufficiently close to the results
obtained by the latter method for our purposes.

Pro[13] carefully compared the Sumner method with the Beck and Pro
method on a wide variety of wines and found the former to give lower results,
particularly where organically combined phosphates were present (see table
6). He pointed out that ferrous sulfate is a very unstable reducing agent
requiring daily preparation, and that preparation and concentration of the

[13] Pro, M. J. Personal correspondence, March 15, 1953.

ferrous sulfate solution must be carefully controlled to obtain reproducible results. For the Sumner method Pro found the optimal wave length for measurements from spectral transmittance curves to be 830 m$\mu$, and at this wave length a plot of transmittancy against concentration in the range 0.0 to 1.0 mg of phosphorus was not linear. He concluded that the Sumner method could be used as a rapid control procedure if the nature of the material analyzed remains the same and if extreme accuracy is not essential.

TABLE 6

COMPARISON OF PHOSPHATE CONTENT IN VARIOUS
WINES BY SUMNER AND BY BECK-PRO METHODS*

| Wine | Phosphate content as mg $P_2O_5$ per 100 ml | |
|---|---|---|
| | Sumner method | Beck-Pro method |
| Blackberry | 11.9 | 10.2 |
| Blueberry | 4.6 | 10.6 |
| Cherry, Montmorency | 17.4 | 25.0 |
| Cherry, Morello | 30.9 | 33.1 |
| Elderberry | 17.2 | 22.0 |
| Grape concentrate, dilute | 49.2 | 63.2 |
| Grape concentrate, red | 117.5 | 65.0 |
| Loganberry | 6.4 | 20.1 |
| Youngberry | 7.3 | 12.2 |
| Vermouth, dry | 22.9 | 28.2 |
| Vermouth, sweet | 22.9 | 26.9 |

* Data furnished by Pro, personal communication, 1953.

Our determinations of phosphate content by the Sumner method for five wines[14] are shown below in comparison with determinations by the Beck and Pro method.

| Wine sample | Phosphate content, mg $P_2O_5$ per 100 ml of wine | |
|---|---|---|
| | Beck and Pro method | Sumner method |
| 1 | 29.3 | 31.3 |
| 2 | 26.0 | 26.0 |
| 3 | 16.4 | 12.6 |
| 4 | 12.0 | 11.3 |
| 5 | 18.0 | 16.5 |

With the exception of sample 3, our results agreed to within $\pm$ 2 mg with those obtained by the Beck and Pro method. Closer agreement here than Pro reported may be due to our use of the 660 m$\mu$ filter in the Klett-Summerson photoelectric colorimeter, instead of measurement of transmissivities at 830 m$\mu$ in a Beckman D. U. Spectrophotometer, as was done by Pro.

## Tannin Content

Tannin was determined by the modified A.O.A.C. tentative method of Pro (1952). Wine sample of tannin concentration up to 1 mg in final solu-

[14] The samples of wine used were those distributed by the A.O.A.C. referee on phosphate in wine to various collaborators. These were supplied by Professor M. A. Amerine, Department of Viticulture, University of California, Davis, who also supplied the analysis of phosphate content by Beck and Pro procedure.

tion (usually 1 ml), was pipetted into a 100-ml volumetric flask, and distilled water added to 85 ml final volume. Next 5 ml of Folin Denis reagent was added. (Reagent prepared by reflexing for 2 hours and making to 1 liter a mixture of 750 ml of water, 100 grams of sodium tungstate hydrate, 20 grams of phosphomolybdic acid, and 50 ml of phosphoric acid.) After 10 ml of a saturated sodium carbonate solution was added, the solution was shaken and allowed to stand for 30 minutes. Then optical density was measured. Tannin concentration was read from standard tannin curve prepared from a freshly prepared solution of tannic acid (fig. 6).
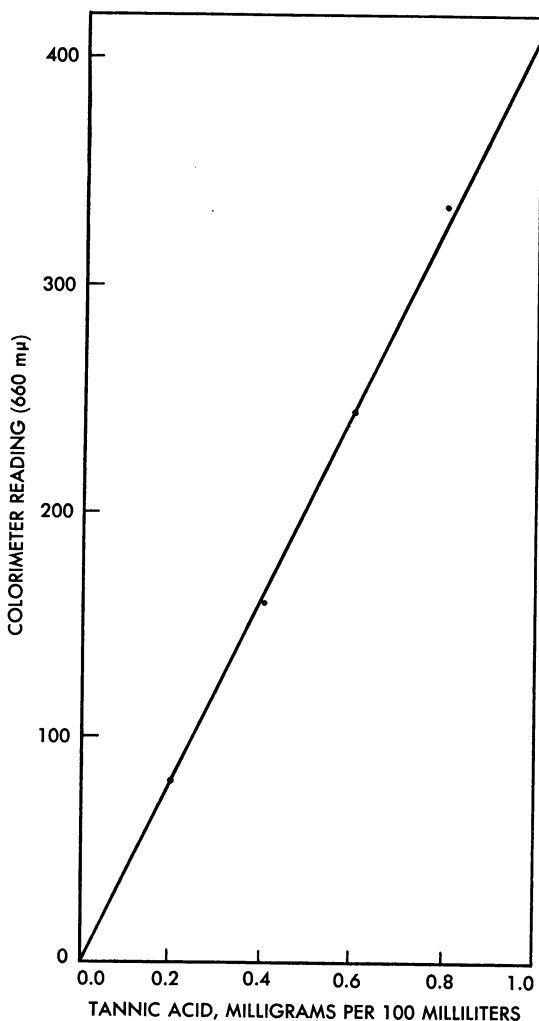


Fig. 6. Tannin-determination standard graph of colorimeter reading of complex versus milligrams of tannic acid per 100 milliliters.

## Turbidity

The turbidity of samples of wine was measured on a photoelectric turbidity meter constructed on the circuit described by Gucker, O'Konski, *et al.* (1947) and Gucker, Pickard, and O'Konski (1947).

## pH

The pH was measured by a Leeds and Northrup research-model glass electrode assembly No. 7661-A1 or a Beckman's industrial-model line-operated electrode assembly.

## REMOVAL OF COPPER AND IRON

This section describes the investigations on the removal of excess copper and iron by the addition of organic compounds likely to form insoluble copper and iron chelates, by special preparations of insoluble ferrocyanides, by naturally occurring nontoxic substances which would form precipitates with copper and iron, and by several ion-exchange resins.

### Survey of Synthetic Organic and Inorganic Compounds

In the preliminary survey of possible substitutes for ferrocyanides which might form insoluble metallic derivatives, a white table wine made of French Colombard grapes in Martinez was used. This wine, of the vintage of 1947, was racked in January, 1948, and gelatin-fined and filtered in August, 1948, when it was obtained for our investigations. It was stored in full 5-gallon carboys at 50° F until used, care being taken to avoid aeration. Its analysis, by the methods given in Amerine and Joslyn (1940), was as follows:

| | |
|---|---|
| Alcohol by volume, per cent | 12.28 |
| Total acidity as tartaric, grams per 100 ml | 0.66 |
| Volatile acidity as acetic, grams per 100 ml | 0.045 |
| Reducing sugar as glucose, grams per 100 ml | 0.14 |
| Total sulfur dioxide, p.p.m. | 167 |
| Tannin, grams per 100 ml | 0.050 |
| pH (at 21° C) | 3.55 |

This wine was used also in the calcium phytate, rubeanic acid, and ferrocyanide precipitation tests. (A wide variety of other commercial wines was also used in the latter tests.) Another lot, with a slightly different composition, was used in the ion-exchange resin tests.

Samples of this wine, whose initial copper content was 0.7 p.p.m. and whose iron content was 5.8 p.p.m. (distributed as 0.7 p.p.m. free ferric iron, 1.6 p.p.m. of free ferrous iron, and 3.5 p.p.m. of iron complexes) were adjusted to 1, 2, 4, and 6 p.p.m. of copper at the original iron-content level. Samples of the wine ranging from 25 to 40 ml were treated with the reagents shown in table 7, added either stoichiometrically or in definite excess.

In calculating the quantity of chelating agent to be added, the metal content of the wine in parts per million was converted into milliequivalents of metal per liter by the use of conversion chart shown in figure 7. After approximately 48 hours, Super-cel[15] was added with agitation and each sample filtered through No. 2 Whatman paper in glass funnels.

[15] Diatomaceous earth, Hyflo Super-cel grade, made by Johns Manville Company.

Appropriate samples for the iron and copper determinations were then withdrawn from the filtrates and the analyses carried out according to the procedures mentioned above.

Excesses of reagent were also checked for rubeanic acid, the Prussian blue potassium ferrocyanide, and ordinary potassium ferrocyanide treat-
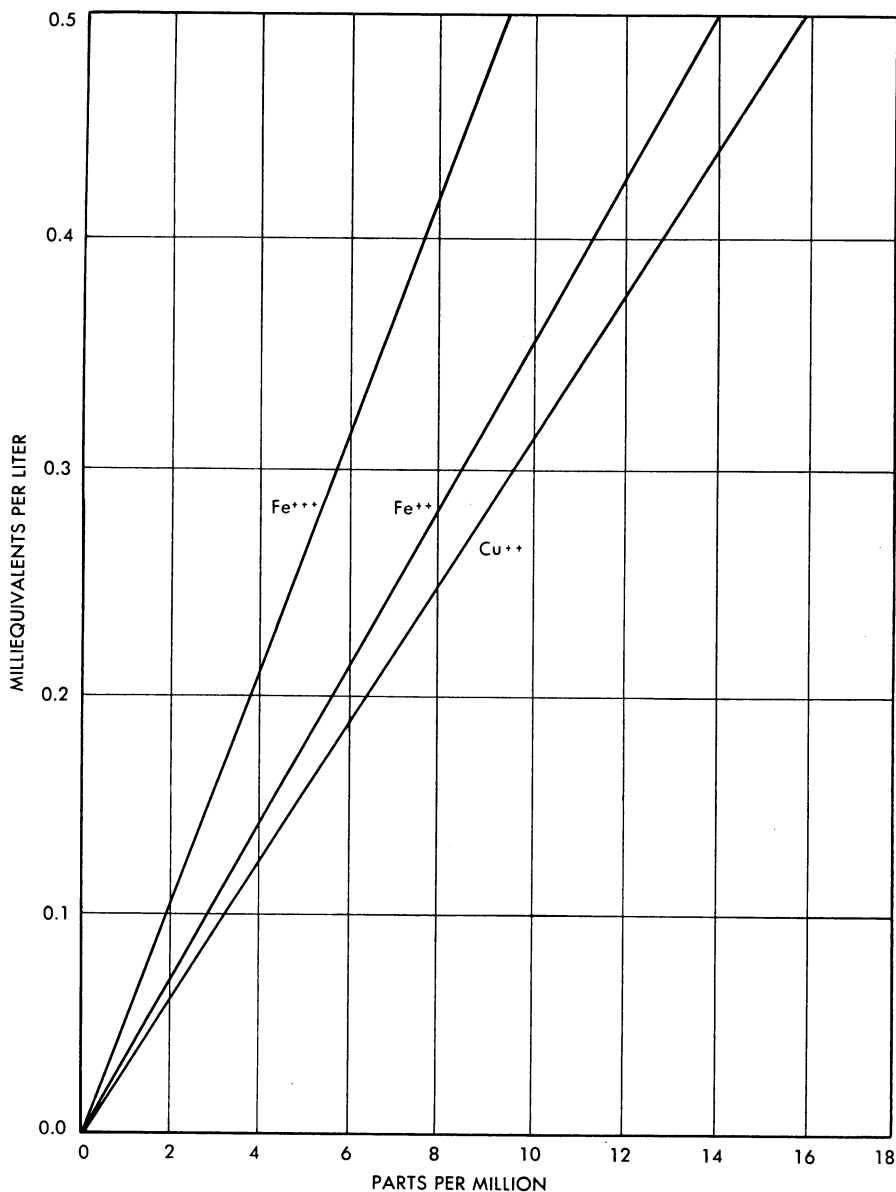
Fig. 7. Conversion chart for calculating milliequivalents of metal ion per liter from parts per million.

ments. This was accomplished by merely adding copper ion in the case of rubeanic acid and ferric ion in the latter two treatments, which involved possible ferrocyanide ion excess. The determination of excess ferrocyanide present in treated wine can be made after conversion of the ferrocyanide, by · cuprous-chloride-catalyzed hydrolysis with sulfuric acid into cyanide. The

TABLE 7

PRECIPITATION OF IRON AND COPPER IN WHITE TABLE WINE BY
VARIOUS CHELATING AGENTS

| Reagent, and solution in which it was added | Equivalents of metal ion chelated per mol | Residual metal content, p.p.m. | | |
|---|---|---|---|---|
| | | Copper | | Iron (5.8 p.p.m. initially) |
| | | 2 p.p.m. initially | 6 p.p.m. initially | |
| Alpha-benzoinoxime, 0.10 mM per ml 95% alcohol............ | 1 | 2.0 | 5.3–6.2 | 9.2 |
| Dithizone, 0.039 mM per ml 95% alcohol made alkaline with NH₃............................................................. | 1 | ...* | —*–3.8 | 7.1* |
| Schiff base of salicylaldehyde, 0.05 mM per ml 95% alcohol..... | 2 | 2.1 | 5.6–6.1 | 5.3 |
| Schiff base of sodium salicylaldehyde-5-sulfonate, 0.04 mM per ml 95% alcohol....................................... | 2 | 1.7 | 5.2–5.7 | 6.4 |
| Sodium ethylenediaminetetraacetate (analytical-grade Versene), 0.02 mM per ml water made alkaline with NH₃........ | 1 Atom | 1.7 | 5.4–5.5 | 5.9 |
| Thenoyl trifluoro acetone, 0.1 mM per ml 65% alcohol......... | 1 | 1.8 | 5.3 5.4 | 6.4 |
| Phthalocyanine, added as solid in conc. H₂SO₄ (0.2 gm for lower copper level, 0.25 gm for higher, per 20 ml wine)........ | 1 Atom | 2.0 | 5.1–5.8† | 7.2 |
| Rubeanic acid, 0.01 mM per ml absolute alcohol............... | 1 Atom | 0.1 | 0.0 | 4.7–5.9 |
| 8-Hydroxyquinoline, 0.01 mM per ml 95% alcohol............. | 1 | 1.4 | 5.4 | 5.0 |
| Quinaldinic acid, 0.01 mM per ml 95% alcohol................. | 1 | 1.4 | 5.3 | 5.8–8.4 |
| Salicylaldoxime, 0.01 mM per ml 95% alcohol................. | 1 | 1.4 | 5.3 | 5.6–7.7 |
| Alpha-nitroso-beta-naphthol, 0.01 mM per ml 95% alcohol..... | 1 | ... | 4.4 | 6.9 |
| Cupferron, 0.01 mM per ml 50% alcohol....................... | 1 | 1.5 | 5.8 | 2.9–5.3 |
| Tannic acid, 0.01 mM per ml 10% alcohol..................... | .. | ... | 5.4 | 7.8 |
| Prussian blue plus potassium ferrocyanide, added as solid (0.08–0.10 gm per 40 ml wine)................................ | .. | 0.0–0.1 | 0.0 | 0.0–5.9‡ |
| Potassium ferrocyanide, 0.025 mM per ml water.............. | .. | 0.1 | 0.0–0.1 | 2.6–3.1 |
| Diatomaceous earth, Hyflo Super-cel grade, added as solid (blank control)............................................. | .. | 1.5–1.8 | 5.5–5.7 | 5.4–8.3 |
| Bauxite, activated, added as solid, 0.16–0.19 gram per 40 ml of wine.................................................. | .. | ...§ | ... | 6.7–7.2 |

* Interfering coloration or cloudiness not removable by filtration.
† The first datum in the range from Dupont-manufactured reagent, the second from reagent supplied by Dr. M. Calvin.
‡ The higher datum in the range was obtained with 0.08 gram of reagent per 40-ml wine sample; another test with 0.1 gram of reagent per 40-ml wine sample gave a reading of 3.1 p.p.m. iron.
§ No tests run with 2 or 6 p.p.m. initial copper content; two tests with 4 p.p.m. initial copper content gave 3.7 and 4.8 p.p.m. copper, respectively.

determination may be made by the Hubach (1948) test, by recent modifications of the picramate test (Sinclair and Ramsey, 1944; Fisher and Brown, 1952), or the sensitive pyridine-pyrozol procedure of Kruse and Mellon (1953). This determination was not carried out since we were interested primarily in copper and iron removal.

The reagents used and the results obtained with wine samples containing 2 and 6 p.p.m. of copper initially are shown in table 7. The reagents were added usually in alcoholic solution and in equivalent amounts. Two samples of phthalocyanine were used, a powdered commercial preparation and a pure crystalline preparation. Each was added both as a solid and in con-

centrated sulfuric acid. This chemical was known to form metal chelates slowly, but the resulting compounds were quite stable.

Rubeanic acid, dithiooxamide, was selected because it was known to form insoluble compounds with copper and other metals (Rây and Rây, 1926; Rây and Bagchi, 1939; Rây and Shiddhanta, 1943), and its copper complex · was used in colorimetric determinations (Center and MacIntosh, 1945). It was prepared as a solution in absolute ethyl alcohol to avoid hydrolysis.

Crystalline potassium ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$, with a molecular weight of 422.4, was used as a basis for comparison. It was assumed that only 3 of the 4 K's are replaceable. On the berlinate concept, the reactions of potassium ferrocyanide with $Fe^{++}$, $Fe^{+++}$, and $Cu^{++}$ are as follows:

$$3Fe(CN)_6^{-4} + 4Fe^{++} + K^+ \rightarrow KFe[Fe(CN)_6Fe]_3$$
<div align="center">Potassium ferrous berlinate</div>

$$3Fe(CN)_6^{-4} + 4Fe^{+++} \rightarrow Fe[Fe(CN)_6Fe]_3$$
<div align="center">Ferric berlinate</div>

$$2Fe(CN)_6^{-4} + 2K^+ + 3Cu^{++} \rightarrow K_2Cu_3[Fe(CN)_6]_2$$
<div align="center">Potassium copper berlinate</div>
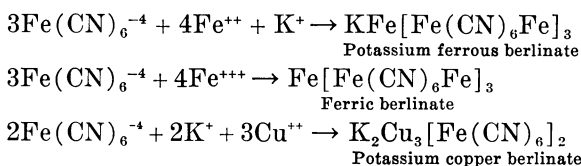
Super-Cel was used as a filter aid with all of the above reagents, as well as with the distilled water and wine blanks. Apparently (table 7) it neither removed nor added any copper, but there is an indication that it contributed a small amount of iron.

A test was also made with activated bauxite; 0.16 and 0.19 gram of a sample of this chemical was added to 40 ml of wine.

Except for the potassium ferrocyanide, the only materials that gave satisfactory results in removing excess copper were rubeanic acid and the Prussian blue–ferrocyanide complex (see table 7). These two materials will be discussed in more detail.

**Results with Rubeanic Acid.** Rubeanic acid, dithiooxamide, forms a copper chelate after conversion to the dithiol form, in alkaline solution:
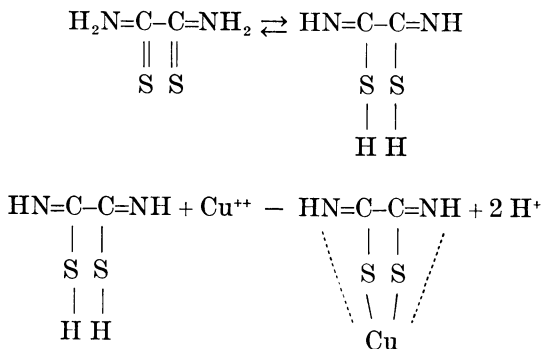


In alkaline solution one mol of rubeanic acid chelates one atom of copper. Our results indicate that this is true also in wine, although in some tests we have assumed that two mols of rubeanic acid chelate one atom of copper. Rubeanic acid effectively and almost quantitatively precipitates copper ion when used in stoichiometric proportion. (Both copper and iron were con-

sidered in calculating the theoretical quantity of reagent required.) The precipitation seems to proceed slowly enough to assume relatively large particle size (in contrast to the blue-fining process) and filtration or decantation of the black precipitate was accomplished readily.

The results indicate that the iron content is only slightly reduced by rubeanic acid. It is possible that rubeanic acid precipitates only the iron not bound in complex form or that the solubility of iron rubeanate is too high to reduce the concentration below the minimum value in table 7 (4 to 6 p.p.m.)

**Results with Prussian Blue–Ferrocyanide Complex.** Various preparations of ferrocyanides have been suggested for heavy-metal removal, such as soluble ferrocyanides followed by addition of zinc salts or of casein to remove excess of ferrocyanide from wine, or specially prepared ferrocyanide complexes. Ramage (1938a, 1941) proposed the use of a ferrous ferricyanide preparation precipitated in the presence of excess soluble ferricyanide, either alone or in combination with iron oxide gel, silica gel, activated carbon, or cellulose. Ramage (1938a) clearly recognized that ferrous ferricyanide and its related compounds contain combined alkali metals or alkaline earth metals which are readily replaceable by iron, especially if the ferrous ferricyanide is precipitated in the presence of excess soluble ferricyanide and not exposed to solutions containing excess iron. To increase the ease of subsequent clarification, he precipitated ferrous ferricyanide on acid-resistant zeolites, iron oxide gels, silica gels, activated carbon, and cellulose. The zeolite was first treated with an iron solution and then with a ferricyanide solution to form an adherent coating of insoluble ferricyanide. Subsequently (Ramage, 1941) he patented a modification of this process, in which the product is first partially neutralized to improve base exchange with zeolite preparations and then restored to its original acidity by treatment with hydrogen zeolite. In 1952, the use of a fining preparation containing ferrocyanide, developed by Fessler, was reported (Fessler, 1952; Marsh, 1952). It was introduced in 1952 as a readily dispersible suspension containing ferrocyanide and more recently was prepared in paste form. Its use was permitted by the California State Board of Health on a trial basis in 1952, when it was demonstrated to satisfactorily remove the excess of copper and most of the iron without altering the chemical composition or organoleptic qualities of the wine treated. Fessler's compound was distributed originally as a suspension containing bentonite. Recently it was prepared as a heavy concentrated cream and distributed under the trade name of Cufex. Cufex in recent tests (Anonymous, 1953b) was shown to reduce the copper content of a muscatel wine from 4 p.p.m. to 0 p.p.m. and the iron content from 6 p.p.m. to about 2 p.p.m.—that is, as effectively as blue-fining with or without bentonite.

As a result of our survey of the chemistry of heavy metal ferrocyanides, it appeared likely that an adsorption complex of potassium ferrocyanide would be useful, particularly because of the ease with which ferrocyanide ion as well as cations are adsorbed by Prussian blue (Weiser, 1949; McBain, 1950).

Our preparation was made by adding a 50 per cent excess of potassium ferrocyanide solution, over the stoichiometric requirement, to ferric chloride solution. After washing thoroughly with several batches of water and drying

in the vacuum oven, the resulting powder was ground in a mortar and mixed
with approximately 10 per cent by weight of Super-cel. The latter step pro-
vided nuclei for the relatively minute Prussian blue particles to adhere to
and thus increased the effective particle size. This was conducive to more
rapid settling and better filtration in the subsequent wine treatment.

The formula for the above powder was assumed for the purpose of calcu-
lation to be $KFe^{+++}Fe^{++}(CN)_6 \cdot \frac{1}{3} K_4Fe(CN)_6$, with a molecular weight of
430.

This reagent was added in proportions ranging from 0.2 to 1.0 per cent,
corresponding to excesses of ferrocyanide ranging from 5 to 1,000 per cent.
It was assumed that only 3 K's of $K_4Fe(CN)_6 \cdot 3 H_2O$ are replaceable.

This substance, based on the extremely high adsorptive power of Prussian
blue, reduced the copper content to practically zero in all cases and, in
addition, shows possibilities of eliminating iron also. In one case the iron
content was reduced to practically zero, but in other cases, in which decreased
amounts of the powder were used, the iron was removed only partially or
not at all.

Another difficulty encountered in the first runs was that a slight trace of
ferrocyanide ion was imparted to the wine; however, a sample of the Prussian
blue ferrocyanide powder, after rewashing thoroughly with warm water,
effected removal of copper and partial removal of iron without indication
that ferrocyanide was introduced.

The fact that this powder contains iron in its "inert carrier" (Prussian
blue) and also in its active component (ferrocyanide ion) and yet does not
increase the iron concentration—actually lowering it in some cases—is indic-
ative of a sound theoretical basis for this type of reagent. Theoretically it
amounts to an ion exchanger whereby traces of the heavy metal can be ex-
tracted from their solutions and replaced with potassium (or possibly
hydrogen) without the introduction of any other soluble constituent.

Another possibility along this line is the use of a coagulated protein such
as edestin or albumin as a carrier for ferrocyanide ion. It is known (Hendrix,
1928), that these amphoteric substances are capable of combining with anions
when on the acid side of the isoelectric point. The following equations illus-
trate, in general, what could be expected to take place:

$$R-CH-COO^- + H^+ \rightarrow R-CH-COOH$$
$$\underset{N^+H_3}{\big|} \qquad\qquad \underset{N^+H_3}{\big|}$$

$$4\ R-CH-COOH + Fe(CN)_6^{-4} \rightarrow \left(\begin{array}{c} R-CH-COOH \\ \big| \\ N^+H_3 \end{array}\right)_4 Fe(CN)_6^{-4}$$
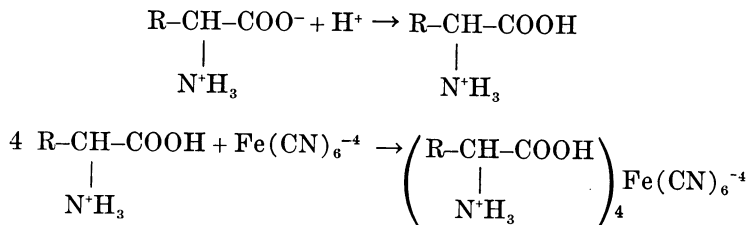
In this way the ferrocyanide becomes available in soluble form only in the
presence of heavy metal ions, and the danger of its contamination of wines
is considerably lessened.

It is also noteworthy that in the copper determinations, conducted without
preliminary ashing of the wine, the high copper recovery obtained with a

variety of chelating agents, including Versene, indicates that these do not bind cupric ion as effectively as diethyl dithiocarbamate.

## Removal of Iron and Copper by Calcium Phytate and Other Naturally Occurring Substances

Several naturally occurring substances are known to react with and thus possibly remove iron and copper salts. Lignic acid has been reported by Nelson, Levine, and Lynch (1939) to remove iron from water. Humic acid has long been known to bind iron and recently has been shown to react with salts of heavy metals (Kukharenko, 1946). Amino acids, peptides, and proteins are known to form chelates (Martell and Calvin, 1952), but this property has been used only in controlling certain biological reactions. The ability of ribonucleic acid to complex metals including copper, manganese, iron, and magnesium is well established (Neuberg and Roberts, 1949). Chlorophyll, hemoglobin, and other porphyrins will exchange magnesium and iron for copper and other cations under certain conditions. (The Mg in chlorophyll cannot be exchanged with $Mg^{++}$ in aqueous solutions; ferric hemoglobin will not exchange its Fe with $Fe^{+++}$ in aqueous solution, although ferric methemoglobin will rapidly exchange its Fe with $Fe^{+++}$ in solution.) Phytic acid and calcium phytate have been suggested for removal of heavy metals from vinegar (Cohee and Steffen, 1949).

Negre and Cordonnier (1952) and Cordonnier (1952) recently reported comparative tests of reduction of iron in wine by calcium phytate and potassium ferrocyanide. They reported that a French white dry wine containing 12.5 milligrams of iron per liter must be treated with 8.3 grams of potassium ferrocyanide per liter to eliminate all iron. According to them, any excess of ferrocyanide will slowly liberate hydrocyanic acid, and for this reason the treatment with ferrocyanide is prohibited in France. They found that calcium phytate was preferable to ferrocyanide for iron removal. From 4 to 5 milligrams of calcium phytate is necessary for each milligram of iron to be removed. To precipitate all of the phytate as the insoluble ferric phytate, it was necessary to aerate the wine strongly before addition of calcium phytate; otherwise the excess phytate will make the wine thick and viscous. They report no data on copper removal by phytate.

**Calcium Phytate.** Equivalent amounts of solid calcium phytate were added to the white wine used previously, adjusted to various concentrations of copper at a constant iron concentration of 8.1 p.p.m. Samples were allowed to stand at 45°C for 120 hours, during which 20-ml samples were removed at various intervals, filtered, and analyzed for copper and iron. The calcium phytate was added to 200-ml portions of wine in the following amounts:

| Copper content of wine, p.p.m. | Calcium phytate, grams |
|---|---|
| 0.6 | 0.007 |
| 5.0 | 0.010 |
| 10.0 | 0.015 |

The results obtained are shown in table 8. Under our conditions, this reagent was found to be effective only in reducing the iron content; it removed little or no copper.

TABLE 8

EFFECT OF CALCIUM PHYTATE ON IRON AND COPPER
CONTENT OF WINE

| Time, hours | Residual metal content, p.p.m. | | | |
|---|---|---|---|---|
| | Copper | | | Iron (8.1 p.p.m. initially) |
| | 0.6 p.p.m. initially | 4.8 p.p.m. initially | 9.2 p.p.m. initially | |
| 1 | 0.6 | 4.8 | 8.9 | 4.0–4.4 |
| 2 | 0.6 | ... | ... | 4.0 |
| 4 | 0.6 | ... | ... | 3.6 |
| 5 | 0.6 | ... | ... | 3.9 |
| 21 | ... | 4.7 | 9.2 | 2.4 |
| 24 | 0.6 | ... | ... | 2.6 |
| 45 | ... | 4.8 | 9.2 | 2.2–2.5 |
| 48 | 0.6 | ... | ... | 2.5 |
| 96 | 0.6 | ... | ... | 2.5 |
| 100 | ... | 4.5 | 9.0 | 2.4–2.6 |
| 120 | ... | 4.5 | 8.9 | 2.3–2.4 |

TABLE 9

EFFECT OF PHYTIC ACID ON COPPER AND IRON
CONTENT OF WINE

| Reagent used and amount per 200 ml of wine | Time, hours | Final copper content, p.p.m. | Final iron content, p.p.m. |
|---|---|---|---|
| 0.01 ml of 40% phytic acid | 0 | 5.6 | 14.0 |
| | 1 | 5.6 | 12.1 |
| | 6 | 5.6 | 5.7 |
| | 24 | 5.6 | 3.5 |
| | 48 | 5.4 | 3.1 |
| | 72 | 5.0 | 3.4 |
| 0.1 ml of 40% phytic acid | 0 | 5.5 | 12.8 |
| | 3 | 5.5 | 3.2 |
| | 18 | 5.5 | 2.3 |
| | 72 | 5.6 | 2.3 |
| 0.1 gram of calcium phytate | 0 | 5.5 | 12.8 |
| | 3 | 5.5 | 1.8 |
| | 18 | 5.5 | 2.0 |
| | 72 | 5.6 | 2.0 |
| 0.1 gram of calcium phytate + 0.1 ml of 40% phytic acid | 0 | 5.5 | 12.8 |
| | 3 | 5.1 | 3.4 |
| | 18 | 5.1 | 2.6 |
| | 72 | 5.6 | 2.6 |

**Phytic Acid.** To determine whether the poor results obtained with calcium phytate were due to the inability of copper and iron to replace the calcium because of the rather high pH of wine as compared with vinegar, the above tests were repeated using phytic acid. A 40 per cent aqueous solution of phytic acid was added to a sample of sauterne wine (no. 1 of those used in the

Versene tests; see table 23, p. 514), adjusted to 5.5 p.p.m. of copper and 12.8 to 14 p.p.m. of iron. The phytic acid was added at 0.01 ml and 0.1 ml levels to 200 ml of wine. After shaking samples were withdrawn periodically.

The results obtained (table 9) do not indicate that phytic acid was any more efficient than calcium phytate in removal of iron and copper, even when added in large excess.

It was observed that in aqueous solution phytic acid in excess formed a soluble complex with calcium or iron phytate. The effect of adding both calcium phytate and phytic acid to wine was determined as shown in table 9. At levels much higher than those used previously, calcium phytate had little effect on copper but rapidly reduced the iron content when added alone. When added with phytic acid essentially the same effect was observed.

**Chlorophyll.** Although magnesium in chlorophyll is readily replaced by copper when green vegetables are scalded in the presence of copper salts, Ruben, Frenkel, and Kamen (1942) found that unpurified extracts of mixed chlorophylls took up radioactive magnesium and copper whereas the separated highly purified components of chlorophyll did not exchange magnesium ion in 80 per cent acetone. For this reason our tests with chlorophyll were made with an old partially purified mixture of chlorophyll a and chlorophyll b. We added 0.390 gram of this chlorophyll preparation to 200 ml of the Rhine wine used in the Versene tests (table 23), adjusted to 5.5 p.p.m. of copper and 10 p.p.m. of iron. There was no detectable effect on the copper and iron content of the wine treated after storage up to 48 hours.

To determine whether these results were due to poor contact of the wine with chlorophyll, in another test 0.1857 gram of chlorophyll was dissolved in 2 ml of absolute alcohol and added to 200 ml of the same wine, adjusted to 5.5 p.p.m. of copper and 12.8 p.p.m. of iron. Even after storage for 72 hours no change in the copper or iron content was observed. In water solutions, however, an alcoholic solution of chlorophyll did reduce both copper and iron content. Apparently at the pH of the wine there is little tendency for the metal exchange to occur.

**Other Substances.** Preliminary experiments have demonstrated that dithio compounds such as cystine or proteins rich in sulfur amino acids, such as insulin, precipitate copper from wine in the presence of sulfur dioxide or ascorbic acid. Sulfhydryl compounds, such as cysteine and thioglycolic acid, precipitate copper directly in the absence of added reducing agents. Ascorbic acid added to wine containing dissolved oxygen also precipitates copper but does not do so if previously oxidized by hydrogen peroxide or activated carbon. Investigations with these compounds, however, have not been extensive enough to test their applicability under existing winery conditions.

### Removal of Metals by Ion-Exchange Resins

Several selected ion-exchange resins were investigated to determine their exchange capacity for iron and copper in wine. They were tested by the downflow displacement method and by direct addition to and mixing with wine. The cation resins were converted first into the hydrogen or potassium form and the anion resins into the tartrate form.

**Methods.** The resin was stored in distilled water overnight to allow for volume expansion. Thirty ml of the wet resin measured in a volumetric cylinder was used each time. The resin was transferred to cylinder $k$ of the system shown in figure 8. To convert the cation resin into the hydrogen form,
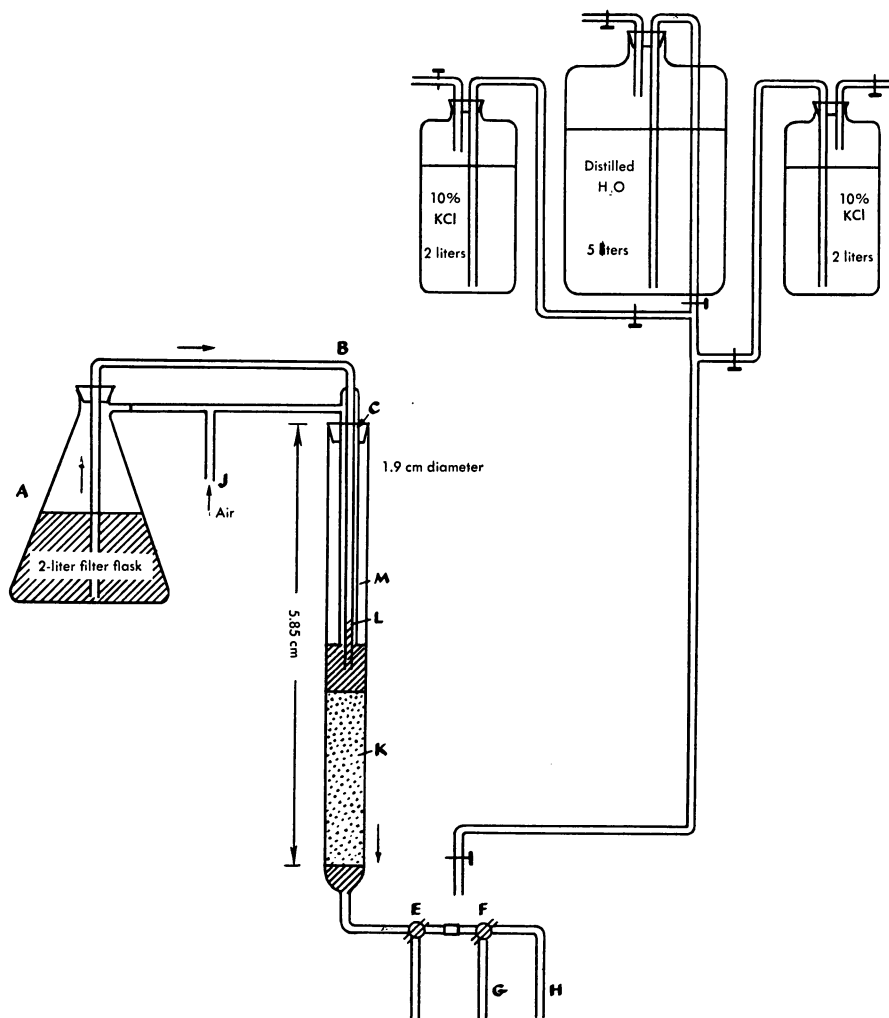


Fig. 8. Down-flow displacement resin bed assembly used for conducting ion-exchange resin tests.

a 10 per cent solution of HCl was passed through the column by upflow. A filter stick was used at $c$ to prevent resin particles from flowing out of the column. The volume of the HCl solution used varied with the resin, each requiring at least 200 to 300 per cent of the volume of resin at a flow rate of 1 to 3 ml per minute. The excess HCl was removed by washing thoroughly with distilled water. To regenerate resin from hydrogen form to potassium

form, a 10 per cent solution of KCl was passed through the column, which was then washed in the same fashion as above.

After the resin bed (*k* in fig. 8) was prepared, several volumes of water were passed through the column at *c* to pack down the bed and to insure constant rate of flow. To maintain a constant level of wine in the column and thus further insure a constant rate of flow, the flow system shown in figure 8 was used. To start the flow of wine, air was blown into tube *j* with the system open at *c*. When wine started to flow into the column through tube *l*, the system was closed at *c* and the wine was brought to the level of tube *m* to close the system to the atmosphere. Thus as wine flowed out of the system, the level in the column was constant at *m*.

The rate of flow was adjusted at stopcock *e* and samples were taken off at *g* and *h* via the three-way stopcock at *f*. The first 40 ml that came through the column was discarded, being mostly water. The next 25 ml constituted sample no. 1, taken off at *g*. The next 100 ml was taken off at *h* and discarded. Sample no. 2 was the next 25 ml, after which 100 ml was taken off at *h*. This alternating sampling continued until the pH of the sample indicated that saturation of the resin column had taken place. The rate of flow was measured by timing each sample taken off, and kept at 5 to 10 ml per minute. The flow rate is not a critical variable as long as it remains within a certain contact time limit (which varies with resin wash), within which maximum exchange occurs as the wine passes through the column. The pH of each 25-ml sample was taken and each sample was analyzed for copper and iron.

The anion-exchange resins were converted into tartrates by activation with ammonium hydroxide and then with potassium acid tartrate. First, 300 ml of $N$ $NH_4OH$ was passed through the column. During this treatment the resin particles changed from cream to yellow. Then 2 liters of saturated potassium acid bitartrate was passed through, and the particles become brownish cream. The pH of the final effluent tartrate wash was 3.53, approximately the same as in the original wine.

The wine used was another lot of French Colombard 1947 from Martinez, California. Its analysis was as follows:

| | |
|---|---|
| Alcohol by volume, per cent | 12.15 |
| Total acidity as tartaric, grams per 100 ml | 0.70 |
| Volatile acidity as acetic, grams per 100 ml | 0.039 |
| Reducing sugar as glucose, grams per 100 ml | 0.17 |
| Total sulfur dioxide, p.p.m. | 95 |

The wine was adjusted to approximately 2.2 p.p.m. of copper and 5.9 p.p.m. of iron.

Dowex-50, Duolite C-3, Zeo Rex, Permutit Q, Monsanto L 1189B, and Zeo Carb cation-exchange resins were used both in the hydrogen and in the potassium form. In addition, Duolite A-3 and Amberlite IR-4B anion-exchange resins were used in the tartrate form.

**Hydrogen Form.** The changes in pH, copper, and iron content for the resins used in the hydrogen and potassium forms are shown in tables 10 to 15 and in figure 9.

In the hydrogen form of all the resins tested, the pH, the copper content, and, with one exception, the iron content reached the minimum in the first

TABLE 10

## COPPER AND IRON REMOVAL FROM WHITE TABLE WINE BY DOWEX-50 RESIN

Flow rate 5.3 to 5.5 ml per minute; 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | H form | | | | | | K form | | | | | |
| | | Run 1 | | | Run 2 | | | Run 1 | | | Run 2 | | |
| | | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm |
| Original wine...... | 0 | 3.53 | 2.20 | 5.9 | 3.53 | 2.25 | 6.0 | 3.60 | 2.0 | 6.0 | 3.58 | 2.15 | 5.9 |
| 1................ | 25 | 1.94 | 0.0 | 5.0 | 1.95 | 0.0 | 0.45 | 3.88 | 1.0 | 0.4 | 3.96 | 1.0 | 0.45 |
| 2................ | 150 | 1.94 | — | — | 1.95 | — | — | 3.72 | — | — | 3.77 | — | — |
| 3................ | 275 | 1.94 | 0.0 | 2.85 | 1.96 | 0.20 | 1.50 | 3.70 | 1.20 | 0.7 | 3.71 | 2.0 | 0.82 |
| 4................ | 400 | 1.95 | — | — | 1.97 | — | — | 3.68 | — | — | 3.68 | — | — |
| 5................ | 525 | 2.00 | 0.25 | 4.25 | 2.00 | 0.60 | 2.50 | 3.66 | 1.65 | 2.2 | 3.67 | 2.0 | 1.3 |
| 6................ | 650 | 2.02 | — | — | 2.06 | — | — | 3.65 | — | — | 3.66 | — | — |
| 7................ | 775 | 2.10 | 0.70 | 5.03 | 2.20 | 1.10 | 3.15 | 3.65 | 1.80 | 2.0 | 3.66 | 2.0 | 2.1 |
| 8................ | 900 | 2.27 | 0.95 | — | 2.37 | — | — | 3.65 | — | — | 3.65 | — | — |
| 9................ | 1,025 | 2.50 | 1.43 | 5.2 | 2.61 | 1.70 | 5.40 | 3.64 | 2.0 | 2.6 | 3.64 | 2.25 | 3.0 |
| 10................ | 1,150 | 2.72 | 1.90 | — | 2.82 | — | — | 3.64 | — | — | 3.64 | — | — |
| 11................ | 1,275 | 2.92 | 2.97 | 4.75 | 3.02 | 2.30 | 4.75 | 3.64 | 2.0 | 2.9 | 3.63 | 2.15 | 3.0 |
| 12................ | 1,400 | 3.12 | 3.05 | — | 3.20 | — | — | 3.63 | — | — | 3.62 | — | — |
| 13................ | 1,575 | 3.28 | — | — | 3.31 | 2.70 | 4.75 | — | — | — | — | — | — |
| 14................ | 1,650 | 3.42 | 3.15 | 5.03 | 3.40 | 2.85 | 4.80 | — | — | — | — | — | — |

TABLE 11

## COPPER AND IRON REMOVAL FROM WHITE TABLE WINE BY DUOLITE C-3

Flow rate 5.1 to 5.5 ml per minute; 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | H form | | | | | | K form | | | | | |
| | | Run 1 | | | Run 2 | | | Run 1 | | | Run 2 | | |
| | | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm |
| Original wine...... | 0 | 3.56 | 1.53 | 5.80 | 3.56 | 1.53 | 5.80 | 3.56 | 1.53 | 5.80 | 3.56 | 1.53 | 5.80 |
| 1................ | 25 | 2.00 | 0.0 | 5.60 | 2.00 | 0.0 | 1.5 | 4.02 | 0.18 | 2.0 | 4.02 | 0.25 | 3.9 |
| 2................ | 150 | 1.97 | 0.18 | 2.26 | 1.97 | 0.0 | 1.9 | 3.72 | 0.54 | — | 3.72 | 0.30 | — |
| 3................ | 275 | 1.97 | 0.30 | 3.50 | 1.97 | 0.13 | 2.9 | 3.65 | 0.60 | 3.4 | 3.65 | 0.30 | 4.5 |
| 4................ | 400 | 2.07 | 0.48 | 3.50 | 2.12 | 0.30 | 3.1 | 3.63 | 0.90 | — | 3.63 | 0.65 | — |
| 5................ | 525 | 2.46 | 0.83 | 4.30 | 2.49 | 0.65 | 3.5 | 3.62 | — | 4.4 | 3.62 | 0.70 | 4.7 |
| 6................ | 650 | 2.98 | 0.89 | 4.75 | 2.90 | 0.90 | 2.6 | 3.60 | 0.95 | — | 3.60 | 0.90 | — |
| 7................ | 775 | 3.40 | 0.96 | 5.02 | 3.27 | 1.02 | 4.2 | 3.60 | 0.95 | 4.8 | 3.60 | 1.02 | 5.2 |
| 8................ | 900 | 3.52 | 1.02 | 5.02 | 3.46 | 1.02 | 5.0 | 3.60 | 1.02 | — | 3.60 | 0.95 | — |
| 9................ | 1,025 | 3.56 | 1.02 | 6.05 | 3.53 | 1.02 | 5.8 | 3.60 | 1.02 | 5.7 | 3.60 | 0.95 | 5.9 |
| 10................ | 1,150 | 3.56 | 1.02 | 6.30 | 3.56 | 1.02 | 5.5 | 3.59 | 1.02 | — | 3.59 | 0.90 | — |

TABLE 12

COPPER AND IRON REMOVAL FROM WHITE TABLE WINE
BY ZEO REX RESIN

Flow rate 10 ml per minute; 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | H form | | | | | | K form | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Run 1 | | | Run 2 | | | Run 1 | | | Run 2 | | |
| | | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm |
| Original wine | 0 | 3.57 | 1.80 | 5.85 | 3.57 | 1.80 | 5.85 | 3.54 | 1.65 | 5.85 | 3.57 | 1.65 | 5.85 |
| 1 | 25 | 1.92 | 0.20 | 0.72 | 1.93 | 0.10 | 0.50 | 4.02 | 0.83 | 0.5 | 4.02 | 0.95 | 0.4 |
| 2 | 150 | 1.92 | 0.10 | 1.7 | 1.92 | 0.10 | 0.72 | 3.69 | 1.05 | 0.6 | 3.71 | 1.13 | 0.6 |
| 3 | 275 | 2.00 | 0.30 | 2.0 | 1.98 | 0.25 | 2.16 | 3.64 | 1.13 | 0.6 | 3.67 | 1.13 | 0.7 |
| 4 | 400 | 2.40 | 0.65 | 4.17 | 2.41 | 0.65 | 4.5 | 3.63 | 1.20 | 0.72 | 3.63 | 1.25 | 0.9 |
| 5 | 525 | 3.01 | 0.75 | 4.4 | 3.18 | 0.90 | 4.75 | 3.60 | 1.20 | 1.1 | 3.62 | 1.25 | 1.5 |
| 6 | 650 | 3.42 | 0.83 | 4.7 | 3.46 | 1.00 | 4.9 | 3.58 | 1.20 | 2.1 | 3.60 | 1.25 | 2.2 |
| 7 | 775 | 3.51 | 0.83 | 5.0 | 3.51 | 1.00 | 5.1 | 3.57 | 1.20 | 2.25 | 3.58 | 1.25 | 2.3 |
| 8 | 900 | — | — | — | 3.54 | 0.95 | 5.1 | 3.56 | 1.20 | 2.25 | — | — | — |

TABLE 13

COPPER AND IRON REMOVAL FROM WHITE TABLE WINE
BY PERMUTIT Q

Flow rate 10 ml per minute; 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | H form | | | | | | K form | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Run 1 | | | Run 2 | | | Run 1 | | | Run 2 | | |
| | | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm |
| Original wine | 0 | 3.54 | 1.65 | 5.85 | 3.54 | 1.85 | 5.85 | 3.54 | 1.65 | 5.85 | 3.54 | 1.65 | 5.85 |
| 1 | 25 | 1.87 | 0.47 | 0.50 | 1.87 | 0.60 | 0.75 | 3.95 | 1.00 | 2.15 | 3.96 | 0.90 | 1.8 |
| 2 | 150 | 1.88 | 0.60 | 0.70 | 1.88 | 0.47 | 0.80 | 3.71 | 1.05 | 2.7 | 3.72 | 1.15 | 2.3 |
| 3 | 275 | 1.88 | 0.60 | 0.75 | 1.88 | 0.60 | 1.1 | 3.67 | 1.05 | 2.85 | 3.66 | 1.20 | 2.6 |
| 4 | 400 | 1.88 | 0.65 | 0.75 | 1.88 | 0.65 | 1.5 | 3.63 | 1.20 | 3.3 | 3.62 | 1.20 | 3.3 |
| 5 | 525 | 1.89 | 0.70 | 0.90 | 1.89 | 0.70 | 2.3 | 3.62 | 1.30 | 3.4 | 3.61 | 1.37 | 3.4 |
| 6 | 650 | 1.92 | 0.70 | 1.5 | 1.93 | 0.85 | 2.4 | 3.61 | 1.42 | 3.3 | 3.60 | 1.50 | 3.7 |
| 7 | 775 | 2.00 | 0.76 | 2.15 | 2.05 | 0.90 | 2.7 | 3.60 | 1.42 | 3.5 | 3.59 | 1.50 | 3.9 |
| 8 | 900 | 2.25 | 1.00 | 2.52 | — | — | — | — | — | — | — | — | — |

TABLE 14

## COPPER AND IRON REMOVAL FROM WHITE TABLE WINE BY RESEARCH SAMPLE L1189B (MONSANTO PLASTICS DIVISION)

Flow rate 5.8 to 6.0 ml per minute; 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | H form | | | | | | K form | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Run 1 | | | Run 2 | | | Run 1 | | | Run 2 | | |
| | | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm |
| Original wine...... | 0 | 3.55 | 2.5 | 5.78 | 3.57 | 2.5 | 5.78 | 3.60 | 1.75 | 5.85 | 3.57 | 1.75 | 5.78 |
| 1................ | 25 | 1.92 | 0.6 | 0.45 | 1.93 | 0.75 | 0.68 | 3.88 | 1.05 | 0.8 | 3.86 | 1.13 | 0.9 |
| 2................ | 150 | 1.93 | — | — | 1.92 | — | — | 3.75 | 1.05 | 1.2 | 3.74 | 1.13 | 1.1 |
| 3................ | 275 | 1.93 | 0.6 | 0.40 | 1.94 | 0.75 | 1.4 | 3.70 | 1.35 | 2.18 | 3.70 | 1.30 | 2.0 |
| 4................ | 400 | 1.94 | — | — | 1.96 | — | — | 3.68 | 1.50 | 2.4 | 3.69 | 1.42 | 2.3 |
| 5................ | 525 | 1.97 | 0.6 | 0.40 | 2.00 | 1.15 | 1.4 | 3.67 | 1.65 | 2.27 | 3.67 | 1.60 | 2.4 |
| 6................ | 650 . | 2.07 | — | — | 2.11 | — | — | 3.65 | — | — | 3.64 | — | — |
| 7................ | 725 | 2.32 | 0.95 | 0.72 | 2.27 | 1.35 | 2.2 | 3.64 | 1.65 | 2.5 | 3.64 | 1.72 | 2.3 |
| 8................ | 900 | 2.63 | — | — | 2.50 | — | — | 3.63 | — | — | 3.63 | — | — |
| 9................ | 1,025 | 2.87 | 1.75 | 1.1 | 2.71 | 1.9 | 2.4 | 3.62 | 1.72 | 2.18 | 3.62 | 1.75 | 2.3 |
| 10................ | 1,150 | 3.09 | — | — | 2.90 | — | — | 3.61 | — | — | 3.62 | — | — |
| 11................ | 1,275 | 3.30 | 2.5 | 1.3 | 5.08 | 2.3 | 2.9 | — | — | — | — | — | — |
| 12................ | 1,400 | 3.40 | — | — | 3.24 | — | — | — | — | — | — | — | — |
| 13................ | 1,515 | 3.44 | 2.5 | 2.8 | 3.40 | 2.5 | 3.1 | — | — | — | — | — | — |

TABLE 15

## COPPER AND IRON REMOVAL FROM WHITE TABLE WINE BY ZEO CARB

Flow rate 10 ml per minute; 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | H form | | | | | | K form | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Run 1 | | | Run 2 | | | Run 1 | | | Run 2 | | |
| | | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm | pH | Cu, ppm | Fe, ppm |
| Original wine...... | 0 | 3.57 | 1.85 | 5.85 | 3.57 | 1.90 | 5.85 | 3.58 | 1.90 | 5.85 | 3.58 | 1.90 | 5.85 |
| 1................ | 25 | 2.22 | 0.25 | 0.4 | 2.20 | 0.30 | 0.5 | 4.01 | 0.35 | 1.1 | 4.00 | 0.40 | 1.2 |
| 2................ | 150 | 2.28 | 0.25 | 1.2 | 2.25 | 0.30 | 1.05 | 3.78 | 0.65 | 2.9 | 3.80 | 0.50 | 2.8 |
| 3................ | 275 | 2.93 | 0.65 | 2.0 | 2.95 | 0.60 | 1.8 | 3.67 | 0.60 | 3.4 | 3.67 | 0.60 | 3.3 |
| 4................ | 400 | 3.40 | 0.75 | 3.1 | 3.41 | 0.70 | 3.13 | 3.63 | 0.65 | 3.6 | 3.65 | 0.70 | 3.8 |
| 5................ | 525 | 3.53 | 1.15 | 4.4 | 3.55 | 1.0 | 4.4 | 3.61 | 1.20 | 4.2 | 3.62 | 1.15 | 4.2 |
| 6................ | 650 | 3.56 | — | — | 3.56 | — | — | 3.60 | 1.05 | — | 3.60 | 1.20 | — |
| 7................ | 775 | 3.57 | 1.20 | — | 3.56 | 1.25 | 4.5 | 3.59 | 1.15 | 4.7 | 3.59 | 1.20 | 4.4 |
| 8................ | 900 | 3.57 | 1.75 | 4.4 | 3.57 | 1.30 | 4.5 | 3.59 | 1.15 | 4.4 | 3.59 | 1.30 | 4.8 |
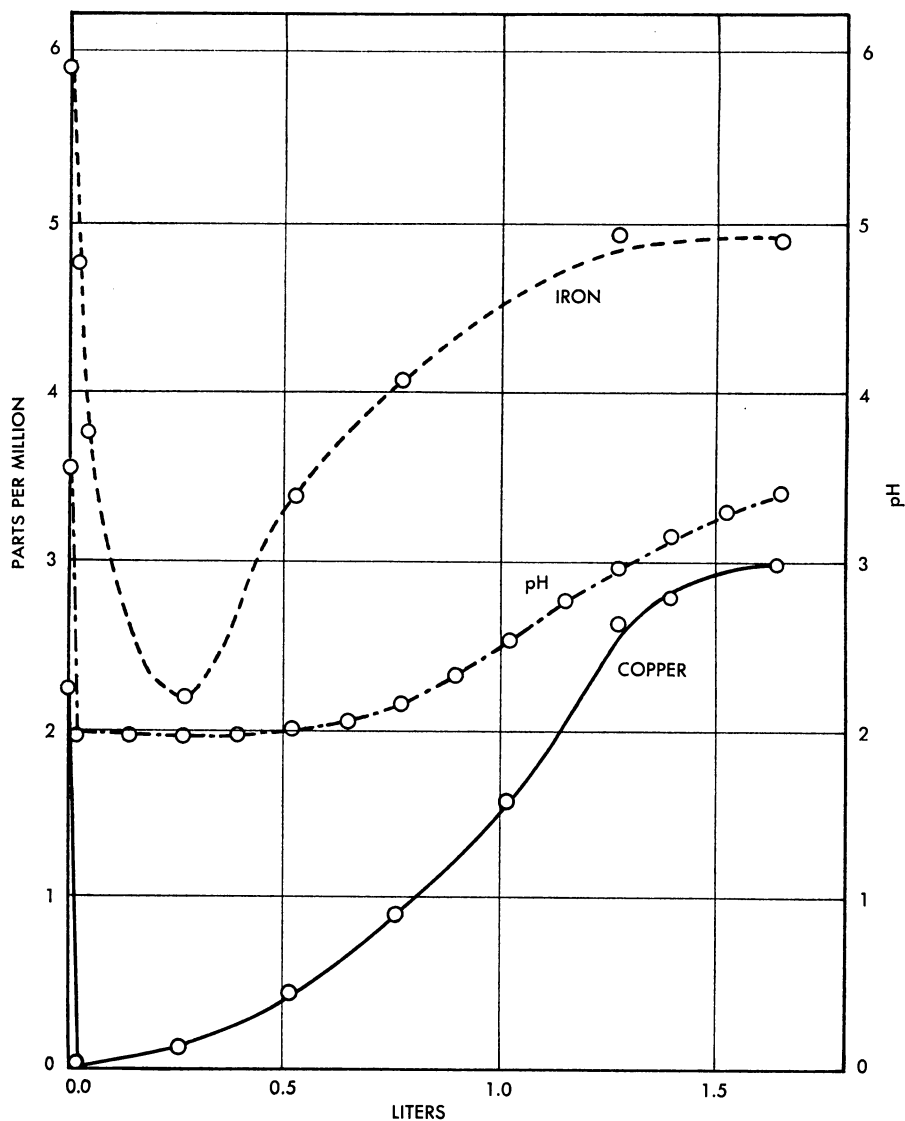
Fig. 9. Change in pH and copper and iron contents during flow through Dowex-50 cation exchange resin in the H form.
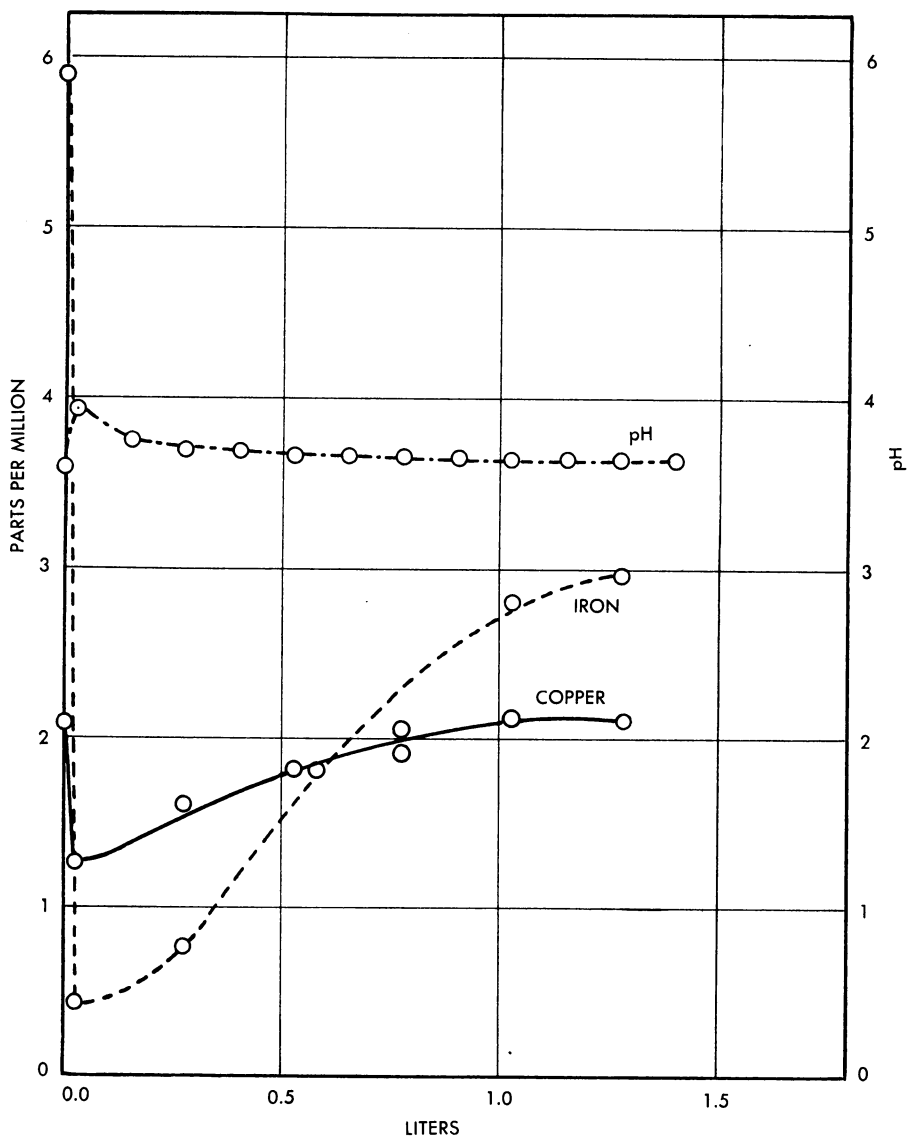
Fig. 10. Changes in pH and iron and copper contents during flow through Dowex-50 cation exchange resin in the potassium form.

sample, when a volume of wine approximately equal to the volume of resin had passed through. These values for the various resins were as follows:

| Resin | pH | Cu, p.p.m. | Fe, p.p.m. |
|---|---|---|---|
| Dowex-50 | 1.9 | 0.0 | 0.5–5.0 |
| Duolite C-3 | 2.0 | 0.0 | 1.5–5.6 |
| Zeo Rex | 1.9 | 0.1–0.2 | 0.5–0.7 |
| Permutit Q | 1.9 | 0.5–0.6 | 0.5–0.8 |
| Monsanto L 1189B | 1.9 | 0.6–0.8 | 0.5–0.7 |
| Zeo Carb | 2.2 | 0.3 | 0.4–0.5 |

In succeeding samples pH and copper content continued at approximately minimum levels until a volume of wine from five to seventeen times the volume of the resin had passed through. Then the resins apparently became saturated, and both pH and copper content of the wine began to increase. The ratios of volume of wine to volume of resin at the saturation point were as follows:

| Resin | Ratio |
|---|---|
| Dowex-50 | 10–17 |
| Duolite C-3 | 5 |
| Zeo Rex | 9 |
| Permutit Q | 13 |
| Monsanto L 1189B | 9–13 |
| Zeo Carb | 9 |

However, in the eighth sample, when a volume of wine thirty times that of the resin had run through, the copper and iron contents were still below that of the original wine with all six resins. Tests were continued longest with Dowex 50 and Monsanto L 1189B. With these, the copper content had reached a level at or above that of the original wine by the eleventh sample, when a volume of wine over forty times that of the resin had run through. Iron content was still below that of the original wine in the last samples taken.

The wine passed through the cation-exchange resins in the hydrogen form became objectionably sour to taste and lost most if not all of its original fruity bouquet.

**Potassium Form.** To reduce the changes in pH of wine during flow through the cation-exchange resins and to avoid exchange of potassium from the wine, the resins were converted into the potassium form. This was thought to be preferable to the use of both cation- and anion-exchange resins as proposed by Austerweil and Pêcheur (1951). (They suggested the use of a cation-exchange resin to remove iron, copper, calcium, and magnesium ions from wine and cider, followed by the use of an anion-exchange resin to restore any loss of pH.)

The changes in pH, copper, and iron content for the resins used in the potassium form are shown in tables 10 to 15, and figure 10. The minimum iron and copper levels were observed in the first sample, when a volume of wine approximately equal to the volume of the resin bed had passed through. During this period an initial rise in pH value occurred. The maximum pH and minimum copper and iron values for the various resins were as follows:

| Resin | pH | Copper, p.p.m. | Iron, p.p.m. |
|---|---|---|---|
| Dowex-50 | 3.88–3.96 | 1.0 | 0.4–0.45 |
| Duolite C-3 | 4.02 | 0.18–0.25 | 2.0–3.9 |
| Zeo Rex | 4.02 | 0.83–0.95 | 0.4–0.5 |
| Permutit Q | 3.95–3.96 | 0.90–1.00 | 1.8–2.15 |
| Monsanto L 1189B | 3.86–3.88 | 1.05–1.03 | 0.8–0.9 |
| Zeo Carb | 4.00–4.01 | 0.35–0.40 | 1.1–1.2 |

The resins in the potassium form had a much higher exchange capacity for iron than for copper. The minimum copper values were appreciably higher than for the resins in the hydrogen form, but the iron values, except for the last three resins listed, were somewhat lower than for the resins in the hydrogen form.

**Anion Resins.** The data obtained with Duolite A-3 anion-exchange resin in the tartrate form are shown in table 16 and figure 11 for a flow rate of 6 ml per minute through a resin bed of 30 ml wet volume. As the wine passed

TABLE 16

CHANGES IN pH, AND COPPER AND IRON CONTENT OF WHITE TABLE
WINE DURING FLOW THROUGH DUOLITE A-3 ANION-EXCHANGE
RESIN IN TARTRATE FORM

Flow rate 6 ml per minute, 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | Run 1 | | | Run 2 | | |
|---|---|---|---|---|---|---|---|
| | | pH | Cu, p.p.m. | Fe, p.p.m. | pH | Cu, p.p.m. | Fe, p.p.m. |
| Original wine.......... | 0 | 3.54 | 2.0 | 6.0 | 3.58 | 2.2 | 5.85 |
| 1..................... | 25 | 4.63 | 0.75 | 1.52 | 4.46 | 0.6 | 1.1 |
| 2..................... | 150 | 4.07 | 1.00 | 3.3 | 3.97 | 0.63 | 2.3 |
| 3..................... | 275 | 3.87 | 1.00 | 3.66 | 3.78 | 0.75 | 2.82 |
| 4..................... | 400 | 3.75 | 1.00 | 4.1 | 3.75 | 0.90 | 3.5 |
| 5..................... | 525 | 3.71 | 1.00 | 4.1 | 3.73 | 0.90 | — |
| 6..................... | 650 | 3.70 | 1.00 | 4.1 | 3.71 | — | — |
| 7..................... | 775 | 3.70 | 1.00 | 4.1 | 3.70 | 0.95 | 3.3 |
| 8..................... | 900 | 3.69 | 1.00 | 4.1 | 3.69 | — | — |
| 9..................... | 1,025 | 3.68 | 1.00 | 4.1 | 3.68 | 1.00 | 3.7 |

through the resin bed the resin changed to a light gray. The first sample of the wine removed was almost colorless and tasted much flatter than the original. That considerable pigment was removed from the wine was observed also in the color of the effluent liquid used in regenerating the resin before repeating the experiment.

Similar data for Amberlite IR-4B anion-exchange resin are shown in table 17. The Amberlite resin was prepared as was the Duolite resin. The resin changed from orange to dark brown during treatment with wine. The first sample of wine removed was partly decolorized but not so markedy as with Duolite A-3.

In both of the anion-exchange resins used, the pH of the wine increased at first, the rise in pH value being greater with Duolite A-3 than with Amberlite IR-4B. With the Duolite the copper content was reduced to 0.6 to 0.8 p.p.m. and the iron content to 1.1 to 1.5 p.p.m. when about 1 volume of wine had passed through; with the Amberlite resin the copper content was reduced to 0.3 to 0.5 p.p.m. and the iron content to 0.6 p.p.m. Both the anion-exchange resins used apparently had the ability to complex both copper and iron. They were as efficient in removing iron from the wine as the potassium cation-exchange resins, and the Amberlite was even more efficient in removing copper. After an initial increase, the pH value decreased and then remained

Fig. 11. Changes in pH and copper and iron contents of wine during flow through
Amberlite IR-4B anion exchange resin in the tartrate form.

constant. Accompanying this decrease in pH value was an increase in residual iron and copper content. The wines treated changed in flavor.

**Mixing Cation-Exchange Resins with Wine.** In addition to treatment of wine with ion-exchange resins by allowing the wine to flow through a prepared resin bed, cation-exchange resins were added to and mixed with the wine itself. Zeo Carb W and Zeo Carb WD resins, as finely divided activated particles, were added directly to the wine. The rate specified by the manu-

facturer was 0.5 gram of resin per 10 mg of metal ion per liter. For the French Colombard wine on the basis of its iron content this was 0.073 gram of resin per 250 ml of wine. The resins were added to wine at this rate and at two, ten, and twenty times this rate. The mixture of resin and wine was agitated by stirring mechanically under an atmosphere of nitrogen for 1 hour. The wine was then filtered and analyzed.
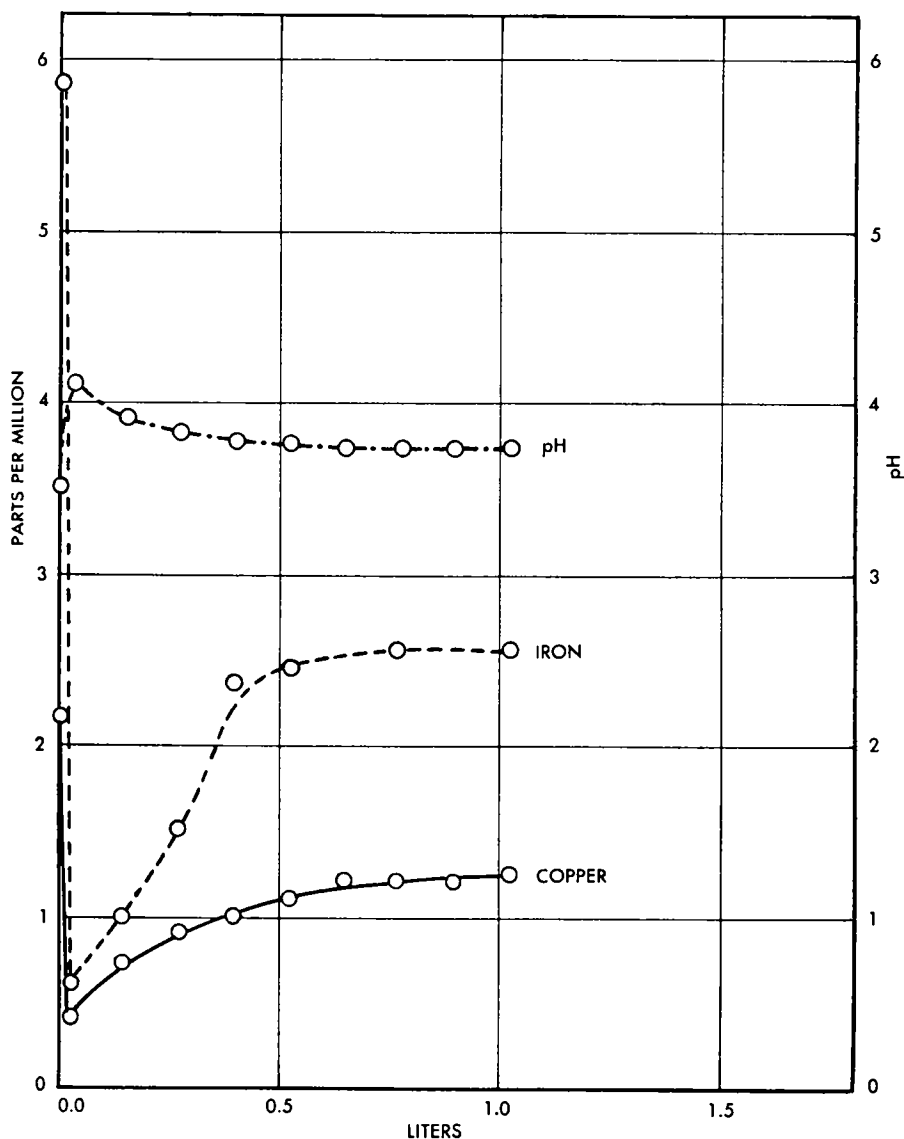
TABLE 17

CHANGES IN pH AND COPPER AND IRON CONTENT OF WHITE TABLE WINE DURING FLOW THROUGH AMBERLITE IR-4B ANION-EXCHANGE RESIN IN THE TARTRATE CYCLE

Flow rate 6 ml per minute, 30 ml wet resin used

| Sample no. (each 25 ml) | Total volume through resin, ml | Run 1 | | | Run 2 | | |
|---|---|---|---|---|---|---|---|
| | | pH | Cu, p.p.m. | Fe, p.p.m. | pH | Cu, p.p.m. | Fe, p.p.m. |
| Original wine......... | 0 | 3.59 | 2.15 | 5.85 | 3.57 | 2.20 | 5.85 |
| 1..................... | 25 | 3.99 | 0.48 | 0.6 | 4.20 | 0.30 | 0.6 |
| 2..................... | 150 | 3.83 | 0.83 | 1.0 | 3.98 | 0.60 | 1.1 |
| 3..................... | 275 | 3.77 | 1.05 | 1.0 | 3.87 | 0.75 | 1.5 |
| 4..................... | 400 | 3.74 | 1.20 | 2.52 | 3.80 | 0.83 | 2.15 |
| 5..................... | 525 | 3.72 | 1.30 | 2.7 | 3.77 | 0.90 | 2.15 |
| 6..................... | 650 | 3.71 | 1.42 | — | 3.74 | — | — |
| 7..................... | 775 | 3.71 | 1.42 | 2.52 | 3.74 | 1.00 | 2.4 |
| 8..................... | 900 | 3.71 | — | — | 3.74 | — | — |
| 9..................... | 1,025 | 3.70 | 1.42 | 2.52 | 3.73 | 1.1 | 2.4 |

TABLE 18

CHANGES IN COPPER AND IRON CONTENT OF WHITE TABLE WINE* TREATED WITH ZEO CARB RESINS

| Resin | Amount added, grams per 250 ml of wine | Copper content, p.p.m. | | Iron content, p.p.m. | |
|---|---|---|---|---|---|
| | | Final | Reduction | Final | Reduction |
| Zeo Carb W | 0.073 | 1.90 | 0.00 | 5.85 | 0.0 |
| | 0.30 | 1.90 | 0.00 | 4.60 | 1.25 |
| | 0.73 | 1.55 | 0.35 | 3.3 | 2.55 |
| | 1.50 | 1.50 | 0.40 | 3.0 | 2.85 |
| Zeo Carb WD | 0.073 | 1.90 | 0.00 | 5.85 | 0.0 |
| | 0.30 | 1.90 | 0.00 | 2.87 | 3.0 |
| | 0.73 | 1.50 | 0.40 | 2.60 | 3.25 |
| | 1.50 | 1.30 | 0.60 | 1.50 | 4.35 |

* With adjusted copper and iron contents of 1.9 p.p.m. and 5.9 p.p.m., respectively.

With both the resins used the pH and color and taste of the wine were not altered by the treatment.

As shown in table 18, this treatment had but little effect on the copper content although it reduced the iron content, particularly when Zeo Carb WD was used in large amounts. The initial copper and iron contents of the French Colombard wine used here were 1.9 and 5.9 p.p.m., respectively.

# RATE OF FORMATION AND PRECIPITATION OF INSOLUBLE FERROCYANIDES AND RUBEANATE

To obtain comparative data on the rate of formation and precipitation of copper and iron ferrocyanides and copper rubeanate, cloudy and clarified samples of wine were mixed with these reagents in various amounts, both with and without aeration. The rate of change in transmission was determined in a Klett-Summerson colorimeter using a 660 m$\mu$ filter.

## Ferrocyanide

**Preparation of Wine Samples.** Three sets of French Colombard wine (same lot as in the survey; see p. 487) were prepared as follows:

1. Slightly clouded wine, that is, not brilliantly clear. Wine was pipetted (50 ml) into ½-pint bottles and potassium ferrocyanide equivalent to 8.1

TABLE 19

VOLUME OF FERROCYANIDE SOLUTION ADDED TO FRENCH COLOMBARD WINE TESTED

| Cu$^{++}$ | | Total Cu plus: | | ml of 0.01 meq/l ferrocyanide added to 50 ml of wine | | | |
| | | | | Theoretical | | Used | |
| p.p.m. | meq/l | Fe$^{+++}$ | Fe$^{++}$ | For Fe$^{+++}$ | For Fe$^{++}$ | Assuming 1 equiv. | Assuming 2 equiv. |
|---|---|---|---|---|---|---|---|
| 0.6 | 0.016 | 0.454 | 0.306 | 2.27 | 1.53 | 2.2 | 4.4 |
| 2.0 | .063 | .499 | .353 | 2.49 | 1.77 | 2.3 | 4.6 |
| 5.0 | .157 | .595 | .447 | 2.98 | 2.24 | 2.6 | 5.2 |
| 8.0 | .251 | .689 | .541 | 3.45 | 2.70 | 3.2 | 6.4 |
| 10.0 | 0.314 | 0.752 | 0.604 | 3.76 | 3.02 | 3.6 | 7.2 |

p.p.m. iron plus 0.6, 2.0, 5.0, 8.0, or 10.0 p.p.m. copper was added to each, in one series. Two equivalents of ferrocyanide were added to the corresponding samples of a second series. The volumes of ferrocyanide solution added are shown in table 19. The samples were mixed by bubbling in nitrogen.

2. Wine samples were clarified with a 5 per cent bentonite solution and aerated by filtration, and then treated as above.

3. Wine samples pipetted into colorimetric tubes were placed in a vacuum desiccator and pressure was reduced until wine had boiled for a few minutes. Vacuum was then released with nitrogen gas, ferrocyanide added to wines, and mixed by bubbling in nitrogen gas.

**Rate of Formation and Precipitation.** Typical of the data obtained are those shown in figure 12 for clarified, aerated wine adjusted to varying levels of copper content and treated with one equivalent of ferrocyanide. The optical density first increases rapidly as particles of metal ferrocyanide form and then decreases as these grow in size and precipitate.

In all cases the samples became turbid and blue in color immediately after the addition of potassium ferrocyanide. A blue precipitate formed shortly thereafter and precipitation was complete in all samples within 2 hours. The time required to reach maximum cloudiness after addition of ferrocyanide
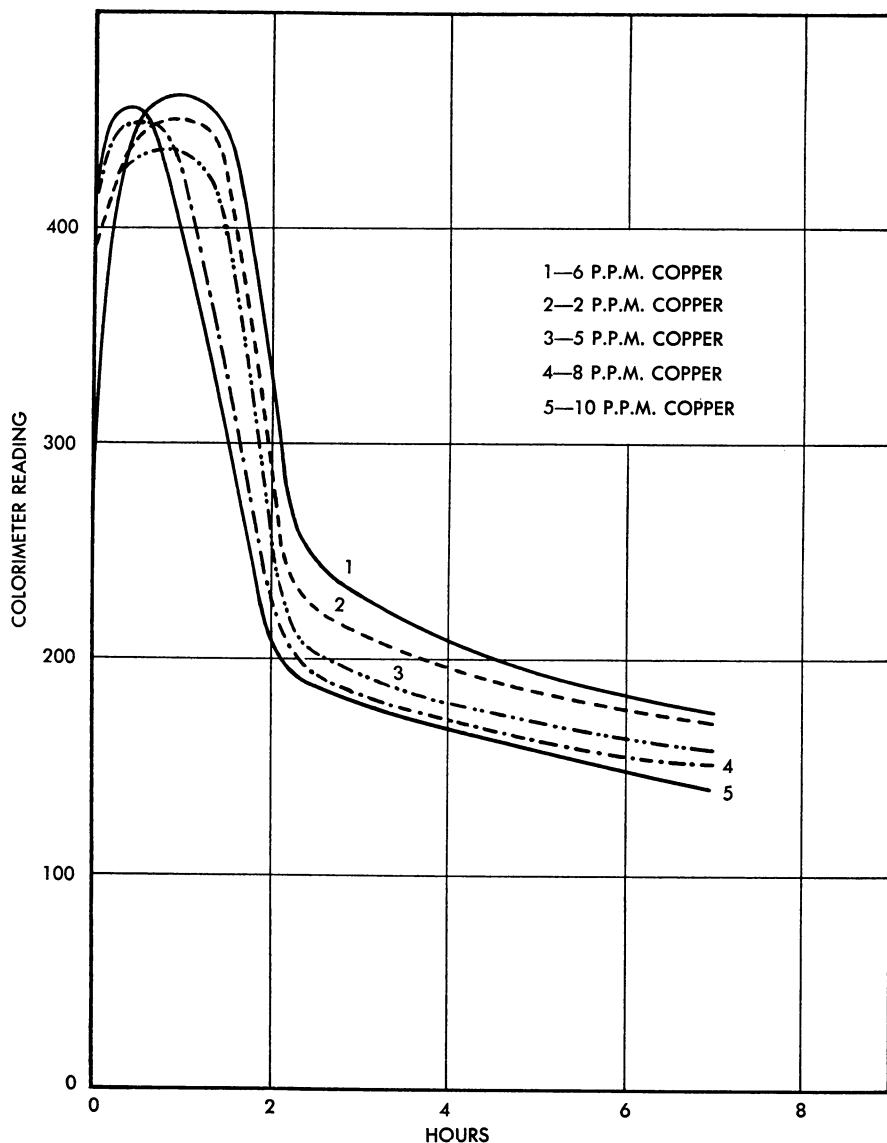
Fig. 12. Rate of formation and precipitation of metal ferrocyanide from white table wine.

as shown in table 20 varied from 45 minutes for wine containing 0.6 p.p.m. of copper to 25 minutes for that containing 10 p.p.m. of copper. The formation of maximum cloudiness was slower in clarified and aerated wine, somewhat faster in deaerated wine, and fastest in the cloudy wines. Difference in concentration of copper had more effect than difference in treatment of wine. Doubling the concentration of ferrocyanide added had little or no effect on the rate of precipitation. In all cases the copper was more completely and effectively removed than was the iron.

## Rubeanic Acid

The rate of formation and precipitation of copper rubeanate in wine and vinegar at various levels of copper was investigated by a procedure similar to that described in the ferrocyanide tests. Fifteen different wines and two vinegars were used in these tests; these are listed in table 21. The wines and vinegars were analyzed for copper and the calculated stoichiometric amount of rubeanic acid (dithiooxamide), one half, and twice this amount were added on the basis that two equivalents of rubeanic acid react with one of copper.

TABLE 20

RATE OF GROWTH OF FERROCYANIDE PRECIPITATE AS MEASURED BY
TIME TO ATTAIN MAXIMUM COLORIMETER READING

Under various conditions of clarification and aeration of the wine

| Copper content, p.p.m. | Time of maximum reading, minutes | | | | | |
|---|---|---|---|---|---|---|
| | With one equivalent of ferrocyanide | | | With two equivalents of ferrocyanide | | |
| | Not clarified or aerated | Clarified and aerated | Deaerated | Not clarified or aerated | Clarified and aerated | Deaerated |
| 0.6 | 45 | 105 | 75 | 65 | 90 | 75 |
| 2.0 | 40 | 60 | 60 | 50 | 55 | 75 |
| 5.0 | 35 | 45 | 45 | 30 | 45 | 60 |
| 8.0 | 30 | 30 | 30 | 30 | 40 | 45 |
| 10.0 | 25 | 25 | 30 | 30 | 40 | 45 |

TABLE 21

TIME TO REACH MAXIMUM COLORIMETER READING AND FINAL
COPPER CONTENT AFTER ADDING RUBEANIC ACID

| Initial copper content, p.p.m. | Type of wine or vinegar | With ½ equivalent rubeanic acid added | | With 1 equivalent rubeanic acid added | | With 2 equivalents rubeanic acid added | |
|---|---|---|---|---|---|---|---|
| | | Hours to reach maximum reading | Final copper content, p.p.m. | Hours to reach maximum reading | Final copper content, p.p.m. | Hours to reach maximum reading | Final copper content, p.p.m. |
| 0.54 | Concord | No change | 0.54 | No change | 0.54 | No change | 0.54 |
| 0.83 | Dry sauterne | >120* | 0.60 | >120* | 0.18 | <120† | .00 |
| 1.00 | Champagne stock | No change | 1.00 | >130* | 0.83 | >130* | .18 |
| 1.06 | Angelica | No change | 0.87 | 30 | 0.40 | 20 | .18 |
| 1.06 | Pale dry sherry | No change | 0.87 | >120* | 0.48 | <120† | .18 |
| 1.07 | Dry sauterne | No change | 1.00 | No change | 0.65 | >120* | .48 |
| 1.13 | Sherry | No change | 0.90 | <130† | 0.48 | 70 | .25 |
| 1.20 | Dry sauterne | >120* | 0.83 | >120* | 0.12 | 25 | .00 |
| 1.54 | Vermouth | >120* | 0.75 | 30 | 0.23 | 25 | .00 |
| 1.60 | Sherry | <120† | 1.30 | <120† | 0.72 | 20 | .23 |
| 2.00 | Dry sauterne | <120† | 0.83 | 15 | 0.18 | 15 | .00 |
| 2.14 | Angelica | 30 | 1.14 | 20 | 0.60 | 10 | .18 |
| 3.63 | Red wine vinegar | 1 | 2.30 | 5 | 1.25 | 10 | .55 |
| 5.33 | Sweet Semillon | 3 | 1.50 | 3 | 0.12 | 3 | .00 |
| 5.40 | Dry sauterne | 10 | 1.84 | 7 | 0.06 | 5 | .00 |
| 8.17 | Burgundy | 4 | 3.30 | 4 | 0.35 | 3 | .12 |
| 11.50 | White wine vinegar | <120† | 3.45 | <120† | 0.95 | <120† | 0.83 |

* Density still increasing when final reading was taken at 120 or 130 hours.
† Constant at 120 hours, but no reading taken between 70 or 72 hours and 120 or 130 hours.

Fifty ml of wine was pipetted into four ¼-pint bottles; ½, 1, and 2 equivalents of rubeanic acid were added to three bottles, and the fourth bottle was used as a blank. The reaction mixture was agitated by bubbling in nitrogen gas, after which 7 ml was pipetted into colorimetric tubes. The wine remained stoppered at room temperature without further mixing for 120 hours. The rate was observed by noting changes in color and turbidity in a Klett-Summerson colorimeter. After 120 hours had elapsed, the wine samples were filtered and then analyzed for copper.

**Rate of Formation.** The time required to reach maximum optical density, taken from rate graphs, is shown in table 21. It varied with the type of wine, the initial copper concentration, and the equivalents of rubeanic acid added.

TABLE 22

THE TIME TO REACH MAXIMUM COLORIMETER READING
AND FINAL COPPER CONTENT AFTER ADDING 1
EQUIVALENT OF RUBEANIC ACID

French Colombard wine adjusted to from 0.2 to 10.0 p.p.m. copper;
and 1 equivalent of rubeanic acid

| Initial copper content, p.p.m. | Time of maximum reading, hours | Final copper content, p.p.m. |
|---|---|---|
| 0.2 | No change | 0.2 |
| 1.0 | Increasing at 95 | .48 |
| 2.0 | 20 | .23 |
| 4.0 | 5 | .20 |
| 6.0 | 5 | .10 |
| 8.0 | 5 | .10 |
| 10.0 | 5 | 0.10 |

With the addition of ½ equivalent of rubeanic acid the optical density usually increased slowly with time. With 1 equivalent of rubeanic acid the optical density increased rapidly at first and then either remained constant, increased slowly, or decreased, according to the type of wine and its initial copper content. With two equivalents of rubeanic acid the optical density increased to a maximum and then remained constant, or decreased rapidly or slowly depending on the wine and its initial copper content.

A similar experiment was performed with French Colombard wine (same lot as was used in the survey; see p. 487), adjusted to from 0.2 to 10 p.p.m. of copper, to which 1 equivalent of rubeanic acid was added. The data obtained are shown in table 22 and figure 13. With this wine no change in optical density was observed for 0.2 and little change for 1 p.p.m. of copper. Maximum clouding occurred at 20 hours for 2 p.p.m. and at 5 hours for 4 to 10 p.p.m. As in the case of ferrocyanide fining, after the peak of maximum optical density there was a rapid decrease owing to flocculation and precipitation of the copper rubeanate.

**Rate of Precipitation.** In general, the rate of precipitation varied with the kind of wine and the initial copper concentration, the rate increasing with increase in copper content.

The French Colombard wine adjusted to varying copper content behaved similarly, particularly at higher copper concentrations—that is, 2.0 to 10.0

p.p.m. At lower copper concentrations, 0.2 and 1.0 p.p.m., the degree of cor-relation was less. This indicates that the copper concentration is just as decisive as type of wine used in determining rate of precipitation, if not more so.

**Final Copper Content.** The effect of equivalents of rubeanic acid added on the final copper content of several wines is shown in figure 14. The data indi-cate that both the type of wine and its initial copper content affect the
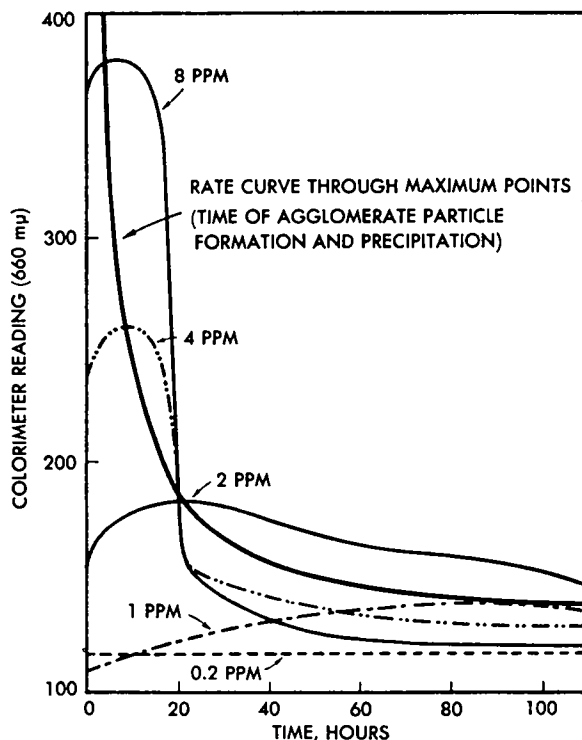


Fig. 13. Rate of formation and precipitation of rubeanates from white table wine.

removal of copper by rubeanic acid. While there was no exact correlation between the amounts of rubeanic acid added and copper removed, two equivalents of rubeanic acid removed more copper than one equivalent, and one equivalent removed more than one-half equivalent, except in one wine sample (table 21). In six wine samples two equivalents reduced the copper content to zero, and in several other samples nearly that low.

## SEQUESTERING OF COPPER AND IRON BY ETHYLENEDIAMINETETRAACETATE

The tetravalent anion of ethylenediaminetetraacetic acid (Versene)[16] forms a very stable soluble calcium complex, upon which is based its use as a water

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[16] The sodium salts of ethylenediaminetetraacetic acid are distributed under the trade-mark Versene by Bersworth Chemical Company, and under the trademark Sequestrene by Alrose Chemical Company.

softener. In this reaction the calcium ion is so firmly bound or chelated in solution that it no longer is available to react with soap or other substances. The complexing reaction which occurs is as follows:
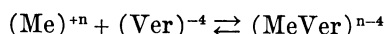
$$
\begin{array}{ccc}
O & & O \\
\| & & \| \\
{}^{-}O - C - CH_2 & & CH_2 - C - O^{-} \\
\quad\quad\quad\quad N - CH_2 - CH_2 - N & \\
{}^{-}O - C - CH_2 & & CH_2 - C - O^{-} \quad + Ca^{++} \\
\| & & \| \\
O & \downarrow & O
\end{array}
$$

$$
\begin{array}{c}
{}^{-}OOC\ CH_2 \quad\quad CH_2 - CH_2 \quad\quad CH_2COO^{-} \\
\diagdown \quad \diagup \quad\quad \diagdown \quad \diagup \\
N \quad\quad\quad\quad N \\
\diagup \quad \diagdown \quad \diagup \quad \diagdown \\
H_2C \quad\quad Ca \quad\quad CH_2 \\
| \quad\quad \diagup \quad \diagdown \quad\quad | \\
CO - O \quad\quad O - CO
\end{array}
$$
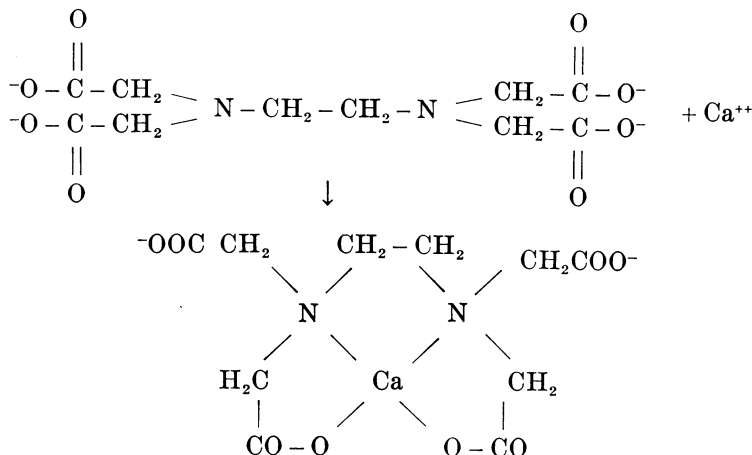
In a similar way this compound can react with $Fe^{++}$, $Fe^{+++}$, and $Cu^{++}$. The equilibrium constants for these ions for the reaction

$$(Me)^{+n} + (Ver)^{-4} \rightleftarrows (MeVer)^{n-4}$$

as log $K$ at 20° C, are 14.4, 25.1, 18.3, respectively (Martell and Calvin, 1952), in comparison with 3.5 for calcium. The order of chelation of these metals by Versene should be $Fe^{+++}>Cu^{++}>Fe^{++}>Ca^{++}$ if only the metal ion and the Versene were involved. In a product like wine, where the existence of metals in complexes with organic acids as well as the free ions would be expected, the pH and composition would affect the sequestering power of Versene added. Krum and Fellers (1952) reported on the clarification of wines containing both iron- and copper-induced cloudiness after addition of equivalent amounts of ethylenediaminetetraacetate distributed under the trade name Sequestrene. Their data indicate that addition of 8 parts of Sequestrene per part of metal present produced clarification but do not indicate whether or not the clarification of copper casse was caused by added Sequestrene or by exposure to air during observation. As is shown in table 7 (p. 489), wines to which Versene was added apparently do not bind copper as firmly as it is bound by the diethyl dithiocarbamate used in the colorimetric determination; but there is some evidence that the iron is firmly chelated so that it will no longer react either with added ferrocyanide or thiocyanate. Wines suspected of containing Versene have been found to continue to form copper casse and no longer respond to ferrocyanide fining.[17]

**Procedure.** In order to determine whether Versene by chelating copper or iron could prevent formation of metallic hazes and deposits, several white wines were adjusted with added copper, iron, phosphate, and tannic acid and stored under conditions favorable to formation of blue ferric tannate, white ferric phosphate, and the white "cuprous sulfide" casse.

[17] Fessler, J. H. Personal communication, 1951.

For the copper casse test, four wines having the composition shown in table 23 were adjusted to 1 and 5 p.p.m. of Cu, and 5.7 to 11.8 p.p.m. of iron, with and without added Versene (1 and 5 equivalents). One series of samples was put cold in crown-capped bottles. Another series was heated to 140° F before filling. Both series were stored in completely filled bottles in light to promote copper casse formation.
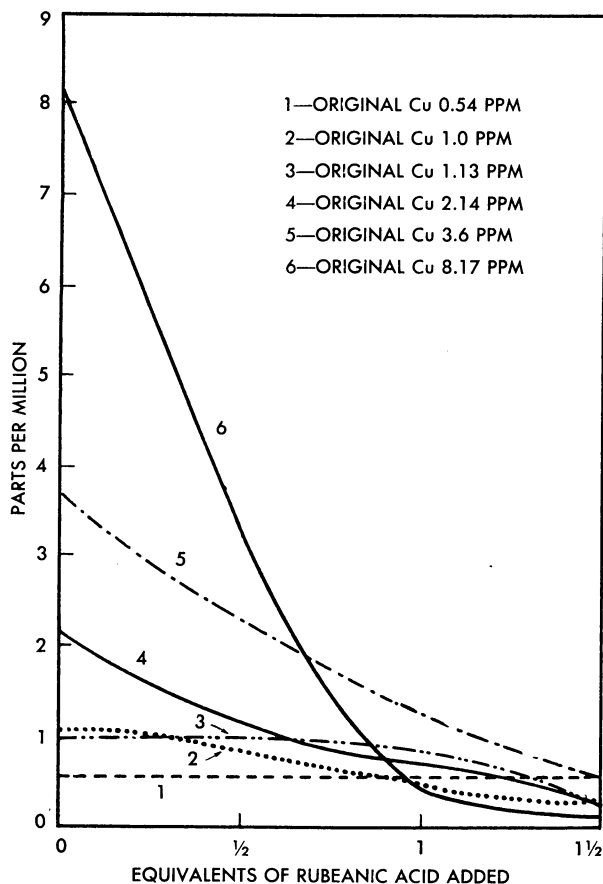


Fig. 14. Effect of equivalent of rubeanic acid added on the final copper concentration of various wines.

For the ferric tannate tests, samples of the adjusted wines were further adjusted to 0.125 gram tannic acid per 100 ml and stored, along with check samples, under oxidizing conditions in partly filled, cork-stoppered bottles.

For the ferric phosphate tests, samples of the adjusted wines containing added 0.1 per cent of dibasic sodium phosphate were stored under oxidizing conditions as for the ferric tannate test. The samples were examined periodically for turbidity. After storage for 2 and 16 to 18 weeks, turbidity was measured quantitatively by determination of light scattered in the tur-

bidimeter described previously and by measurement of optical density in a Klett-Summerson colorimeter using filters at 420, 540, 600, and 660 m$\mu$. At the end of 16 weeks all samples were analyzed for copper, iron, phosphate, tannin, and total weight of precipitate formed.

**Effect on Ferric Phosphate Casse.** The results obtained on the ferric phosphate casse samples are shown in table 24. Within 2 weeks the ferric phosphate samples without added Versene were cloudy, and the cloudiness increased on storage. Those containing added Versene either did not cloud or did not cloud as much, indicating suppression of ferric phosphate casse by Versene added in equivalent amount.

TABLE 23
COMPOSITION OF WINES USED IN VERSENE TESTS

| Wine | Alcohol, per cent by volume | Total acid, grams/100 cc as tartaric | Volatile acid, grams/100 cc as acetic | Reducing sugar, grams/100 cc | SO₂, p.p.m. | Iron, p.p.m. | Copper, p.p.m. | Tannin, mg/100 cc | PO₄⁻⁻⁻, mg per 100 cc |
|---|---|---|---|---|---|---|---|---|---|
| White Pinot.... | 12.05 | 0.600 | 0.048 | 0.150 | 200 | 1.0 | 0.0 | 38 | 36.5 |
| Sauterne no. 1.. | 11.8 | .600 | .039 | .150 | 190 | 8.1 | .32 | 42 | 46 |
| Sauterne no. 2.. | 12.4 | .600 | .048 | .810 | 333 | 6.2 | .52 | 48 | 49.5 |
| Rhine.......... | 12.0 | 0.615 | 0.048 | 0.085 | 190 | 5.7 | 0.57 | 42 | 44 |

In comparing precipitation data for samples with and without Versene it is apparent that Versene is effective in preventing phosphate casse formation. Only in one sample at a Versene level of 1 equivalent did a precipitate appear, whereas in all samples without added Versene, a white precipitate appeared. The relative turbidity readings would seem to belie this fact. But two opposing factors are involved here: (1) amount of precipitate and its particle size, and (2) the color of the wine. A precipitate of small particles may give greater light scattering than one of large particles, and a dark wine transmits less of the scattered light than a light wine. These factors explain some of the anomalous data appearing in tables 24 to 26.

Oxidative changes in wine (as evidenced by a darkening of its color) that are catalyzed by copper and iron appear to be inhibited by Versene. The change in light absorption was most pronounced at shorter wave lengths and in general the increase was less with Versene than without. Yet there were a number of instances in which wine with added Versene showed definite change (darkening) in light absorption. This would seem to indicate that (excluding other oxidation factors) these Versene-bound metals are still effective in catalyzing oxidation reaction in the wine.

The change in iron and phosphate concentrations was within the experimental errors of their methods of determination.

**Effect on Ferric Tannate Casse.** The results obtained on the ferric tannate casse samples are shown in table 25. As in the phosphate casse samples, the samples without added Versene were cloudy in 2 weeks and the cloudiness increased on further storage. The addition of Versene reduced the tendency to cloud and sediment, but it was most effective with White Pinot wine and least effective with sauterne no. 2. The tannate casse in general was not suppressed as much as was the phosphate casse.

TABLE 24

## EFFECT OF VERSENE ON FERRIC PHOSPHATE CASSE FORMATION

| Phosphate and Versene treatments, and initial iron content | Turbidity (H₂O=0) after: | | Color values (H₂O=100), with a filter of: | | | | Wt. of precipitate, mg per 125 cc | Final iron content p.p.m. | PO₄⁻⁻⁻, mg per 100 cc | Tannin, mg per 100 cc |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 wks. | 16 wks. | 420 mμ | 540 mμ | 600 mμ | 660 mμ | | | | |
| **White Pinot** | | | | | | | | | | |
| **Untreated wine, 1 p.p.m. Fe.** | 2.2 | 2.2 | 171 | 122 | 111 | 107 | .... | 1.0 | 36.5 | 36 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 6.6 p.p.m. Fe............ | 2.2 | 14.0 | 246 | 150 | 115 | 112 | 4.2 | 6.6 | 40 | 36 |
| 10.5 p.p.m. Fe............ | 2.2 | 1.5 | 303 | 172 | 123 | 118 | 10.0 | 10.2 | 408 | 33 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 6.6 p.p.m. Fe............ | 2.2 | 37.5 | 210 | 134 | 110 | 109 | 4.0 | 6.4 | 140 | 36 |
| 10.5 p.p.m. Fe............ | >100 | 18.5 | 260 | 155 | 117 | 114 | 8.6 | 10.8 | 142 | 37 |
| 1 eq. Versene: | | | | | | | | | | |
| 6.6 p.p.m. Fe............ | 2.2 | <0 | 198 | 129 | 109 | 109 | .... | 6.6 | 140 | 36 |
| 10.5 p.p.m. Fe............ | 2.2 | <0 | 190 | 125 | 108 | 107 | .... | 10.5 | 140 | 36 |
| 5 eq. Versene: | | | | | | | | | | |
| 6.6 p.p.m. Fe............ | 2.2 | <0 | 195 | 129 | 110 | 110 | .... | 6.6 | 140 | 36 |
| 10.5 p.p.m. Fe............ | 2.2 | <0 | 190 | 126 | 105 | 105 | .... | 10.5 | 140 | 36 |
| **Sauterne no. 1** | | | | | | | | | | |
| **Untreated wine, 8.1 p.p.m.Fe** | 7.8 | 7.8 | 182 | 126 | 112 | 110 | .... | 8.1 | 46 | 36 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 8.1 p.p.m. Fe............ | 6.6 | 33.0 | 206 | 132 | 111 | 112 | 4.5 | 8.0 | 47.5 | 39 |
| 11.8 p.p.m. Fe............ | 7.5 | 16.5 | 300 | 160 | 120 | 119 | 0.6 | 12.1 | 49.6 | 36 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 8.1 p.p.m. Fe............ | 8.0 | 41.0 | 204 | 130 | 108 | 110 | 4.1 | 8.6 | 146 | 39 |
| 11.8 p.p.m. Fe............ | 7.5 | 21.5 | 236 | 143 | 114 | 115 | 0.7 | 10.9 | 146 | 38 |
| 1 eq. Versene: | | | | | | | | | | |
| 8.1 p.p.m. Fe............ | 2.5 | 12.0 | 214 | 130 | 108 | 110 | .... | 8.1 | 146 | 36 |
| 11.8 p.p.m. Fe............ | 6.0 | 12.0 | 255 | 145 | 112 | 112 | .... | 11.8 | 146 | 36 |
| 5 eq. Versene: | | | | | | | | | | |
| 8.1 p.p.m. Fe............ | 2.0 | <0 | 228 | 138 | 110 | 112 | .... | 8.1 | 146 | 36 |
| 11.8 p.p.m. Fe............ | 4.8 | 1.5 | 256 | 146 | 109 | 110 | .... | 11.8 | 146 | 36 |
| **Sauterne no. 2** | | | | | | | | | | |
| **Untreated wine, 6.2 p.p.m.Fe** | 2.7 | 2.7 | 206 | 129 | 112 | 109 | 7.3 | 6.2 | 49.5 | 56 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 6.2 p.p.m. Fe............ | 2.7 | 6.5 | 355 | 189 | 123 | 115 | 5.4 | 6.4 | 51 | 48 |
| 11.6 p.p.m. Fe............ | 2.7 | 39.0 | 370 | 189 | 125 | 117 | 4.9 | 11.5 | 53.2 | 47 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 6.2 p.p.m. Fe............ | 2.7 | 9.5 | 345 | 178 | 120 | 114 | 3.5 | 6.4 | 146 | 43 |
| 11.6 p.p.m. Fe............ | 60.2 | 54.5 | 365 | 190 | 124 | 115 | .... | 10.2 | 157 | 45 |
| 1 eq. Versene: | | | | | | | | | | |
| 6.2 p.p.m. Fe............ | 2.7 | 1.0 | 261 | 145 | 112 | 109 | .... | 6.2 | 150 | 56 |
| 11.6 p.p.m. Fe............ | 2.7 | 4.5 | 280 | 148 | 112 | 108 | .... | 11.6 | 150 | 54 |
| 5 eq. Versene: | | | | | | | | | | |
| 6.2 p.p.m. Fe............ | 2.7 | <0 | 258 | 143 | 109 | 106 | .... | 6.2 | 150 | 53 |
| 11.6 p.p.m. Fe............ | 2.7 | 9.5 | 292 | 160 | 116 | 112 | .... | 11.6 | 150 | 50 |
| **Rhine** | | | | | | | | | | |
| **Untreated wine, 5.7 p.p.m.Fe** | 4.0 | 4.0 | 172 | 122 | 111 | 107 | .... | 5.7 | 44 | 42 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 5.7 p.p.m. Fe............ | >100 | 41.0 | 208 | 132 | 111 | 110 | 3.6 | 5.4 | 44.5 | 40 |
| 10.8 p.p.m. Fe............ | 4.4 | 34.5 | 202 | 130 | 110 | 108 | 3.0 | 11.0 | 46.7 | 43 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 5.7 p.p.m. Fe............ | >100 | 47.0 | 218 | 135 | 109 | 109 | 3.6 | 6.1 | 139 | 39 |
| 10.8 p.p.m. Fe............ | 4.5 | 18.0 | 263 | 152 | 113 | 110 | 3.4 | 10.8 | 146 | 39 |
| 1 eq. Versene: | | | | | | | | | | |
| 5.7 p.p.m. Fe............ | 4.3 | 22.5 | 205 | 132 | 110 | 109 | 6.6 | 6.4 | 145 | 40 |
| 10.8 p.p.m. Fe............ | 4.0 | 17.5 | 229 | 136 | 108 | 108 | .... | 10.8 | 145 | 40 |
| 5 eq. Versene: | | | | | | | | | | |
| 5.7 p.p.m. Fe............ | 4.5 | <0 | 232 | 137 | 107 | 107 | .... | 5.7 | 145 | 40 |
| 10.8 p.p.m. Fe............ | 4.5 | <0 | 242 | 143 | 111 | 112 | .... | 10.8 | 145 | 40 |

TABLE 25

## EFFECT OF VERSENE ON FERRIC TANNIN CASSE FORMATION

| Phosphate and Versene treatments, and initial iron content | Turbidity (H₂O=0) after: | | Color values (H₂O=100), with a filter of: | | | | Wt. of precipitate, mg per 125 cc | Final iron content p.p.m. | PO₄⁻⁻⁻, mg per 100 cc | Tannin, mg per 100 cc |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 wks. | 18 wks. | 420 mμ | 540 mμ | 600 mμ | 660 mμ | | | | |
| **White Pinot** | | | | | | | | | | |
| **Untreated wine, 1.0 p.p.m.Fe** | 2.2 | 7.2 | 171 | 122 | 111 | 107 | .... | 6.6 | 36.5 | 36 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 6.6 p.p.m. Fe | 2.2 | 20.0 | 264 | 156 | 118 | 113 | 17.8 | 6.0 | 35.4 | 34 |
| 10.5 p.p.m. Fe | 2.2 | 2.2 | 320 | 178 | 128 | 119 | .... | 10.5 | 36.5 | 36 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 6.6 p.p.m. Fe | 2.2 | 2.5 | 282 | 166 | 126 | 119 | 24.4 | 5.2 | 36.5 | 138 |
| 10.5 p.p.m. Fe | 2.2 | <0 | 395 | 225 | 159 | 142 | .... | 10.5 | 36.5 | 136 |
| 1 eq. Versene: | | | | | | | | | | |
| 6.6 p.p.m. Fe | 2.2 | 2.2 | 218 | 137 | 117 | 114 | .... | 6.1 | 36.5 | 136 |
| 10.5 p.p.m. Fe | 2.2 | 2.2 | 214 | 133 | 113 | 110 | .... | 10.5 | 36.5 | 136 |
| 5 eq. Versene: | | | | | | | | | | |
| 6.6 p.p.m. Fe | 2.2 | 2.5 | 206 | 132 | 112 | 110 | .... | 6.6 | 36.5 | 136 |
| 10.5 p.p.m. Fe | 2.2 | 2.4 | 200 | 130 | 111 | 108 | .... | 10.5 | 36.5 | 136 |
| **Sauterne no. 1** | | | | | | | | | | |
| **Untreated wine, 8.1 p.p.m.Fe** | 7.8 | 7.8 | 182 | 126 | 112 | 110 | .... | 8.1 | 46 | 36 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 8.1 p.p.m. Fe | 10.5 | 26.5 | 264 | 148 | 115 | 112 | 21 | 6.9 | 45.2 | 37 |
| 11.8 p.p.m. Fe | 5.8 | 25.0 | 256 | 148 | 114 | 112 | 14.3 | 10.8 | 43.1 | 37 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 8.1 p.p.m. Fe | 2.2 | 13.0 | 290 | 168 | 130 | 123 | 17.1 | 7.3 | 46 | 140 |
| 11.8 p.p.m. Fe | 2.2 | 0 | 353 | 195 | 140 | 129 | .... | 11.8 | 46 | 140 |
| 1 eq. Versene: | | | | | | | | | | |
| 8.1 p.p.m. Fe | 3.3 | 15.5 | 221 | 130 | 105 | 102 | 26.6 | 7.6 | 47.4 | 154 |
| 11.8 p.p.m. Fe | 5.5 | 5.5 | 274 | 163 | 125 | 119 | .... | 11.8 | 46 | 140 |
| 5 eq. Versene: | | | | | | | | | | |
| 8.1 p.p.m. Fe | 3.3 | 3.2 | 241 | 142 | 110 | 107 | .... | 8.1 | 46 | 140 |
| 11.8 p.p.m. Fe | 5.5 | 5.5 | 246 | 143 | 110 | 106 | .... | 11.8 | 46 | 140 |
| **Sauterne no. 2** | | | | | | | | | | |
| **Untreated wine, 6.2 p.p.m.Fe** | 2.7 | 2.7 | 206 | 129 | 112 | 109 | .... | 6.2 | 49.5 | 56 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 6.2 p.p.m. Fe | 1.7 | 8.0 | 350 | 178 | 124 | 116 | .... | 6.2 | 49.3 | 44 |
| 11.6 p.p.m. Fe | 1.4 | 17.5 | 390 | 195 | 129 | 121 | 20.4 | 10.2 | 49.3 | 43 |
| **Phosphate added:** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 6.2 p.p.m. Fe | >100 | 68.0 | 385 | 204 | 139 | 130 | 25.7 | 6.2 | 49.6 | 145 |
| 11.6 p.p.m. Fe | >100 | 44.0 | 470 | 238 | 150 | 135 | 19.2 | 9.1 | 49.6 | 155 |
| 1 eq. Versene: | | | | | | | | | | |
| 6.2 p.p.m. Fe | >100 | >100 | 266 | 149 | 113 | 112 | 21.7 | 5.9 | 47.8 | 165 |
| 11.6 p.p.m. Fe | >100 | >100 | 292 | 162 | 121 | 117 | 33.3 | 10.5 | 47.8 | 160 |
| 5 eq. Versene: | | | | | | | | | | |
| 6.2 p.p.m. Fe | >100 | >100 | 250 | 142 | 111 | 110 | 16.8 | 6.7 | 47.8 | 160 |
| 11.6 p.p.m. Fe | >100 | >100 | 263 | 147 | 112 | 110 | 23.5 | 11.4 | 46.0 | 145 |
| **Rhine** | | | | | | | | | | |
| **Untreated wine, 5.7 p.p.m.Fe** | 4.0 | 4.0 | 172 | 122 | 111 | 107 | .... | 5.7 | 44 | 42 |
| **No phosphate or Versene:** | | | | | | | | | | |
| 5.7 p.p.m. Fe | 20.0 | >100 | 195 | 126 | 109 | 107 | 14.8 | 5.9 | 43.8 | 40 |
| 10.8 p.p.m. Fe | 7.0 | >100 | 256 | 147 | 112 | 108 | 17.6 | 11.0 | 41 | 39 |
| **Phosphate added:,** | | | | | | | | | | |
| No Versene: | | | | | | | | | | |
| 5.7 p.p.m. Fe | 2.6 | <0 | 395 | 209 | 142 | 130 | .... | 5.7 | 44 | 140 |
| 10.8 p.p.m. Fe | 2.8 | <0 | 445 | 233 | 150 | 136 | .... | 10.8 | 44 | 140 |
| 1 eq. Versene: | | | | | | | | | | |
| 5.7 p.p.m. Fe | 6.4 | 5.5 | 243 | 142 | 112 | 109 | .... | 5.7 | 44 | 140 |
| 10.8 p.p.m. Fe | 5.2 | 0 | 278 | 162 | 120 | 115 | .... | 10.8 | 44 | 140 |
| 5 eq. Versene: | | | | | | | | | | |
| 5.7 p.p.m. Fe | 5.5 | 3.0 | 254 | 147 | 110 | 107 | .... | 5.7 | 44 | 140 |
| 10.8 p.p.m. Fe | 5.6 | 4.0 | 264 | 150 | 111 | 107 | .... | 10.8 | 44 | 140 |

TABLE 26

EFFECT OF VERSENE ON COPPER CASSE FORMATION

| Initial copper and iron contents, and Versene treatment | Heated samples | | Unheated samples | |
|---|---|---|---|---|
| | Extent of pptn.* | Color of ppt.† | Extent of pptn.* | Color of ppt.† |
| Sauterne no. 1 | | | | |
| 1 p.p.m. Cu, 8.1 p.p.m. Fe: | | | | |
| No Versene......................................... | l | rb | vl | rb |
| 1 eq. Versene...................................... | vl | w | vl | rb |
| 5 eq. Versene...................................... | None | — | vl | rb |
| 5 p.p.m. Cu, 11.8 p.p.m. Fe: | | | | |
| No Versene......................................... | l | rb | h | rb |
| 1 eq. Versene...................................... | vl | w | h | rb |
| 5 eq. Versene...................................... | vl | w | vl | rb |
| Sauterne no. 2 | | | | |
| 1 p.p.m. Cu, 6.2 p.p.m. Fe: | | | | |
| No Versene......................................... | l | w | vl | rb |
| 1 eq. Versene...................................... | l | w | vl | w |
| 5 eq. Versene...................................... | l | w | None | — |
| 5 p.p.m. Cu, 11.6 p.p.m. Fe: | | | | |
| No Versene......................................... | l | w | h | rb |
| 1 eq. Versene...................................... | l | w | h | rb |
| 5 eq. Versene...................................... | None | — | h | rb |
| Rhine | | | | |
| 1 p.p.m. Cu, 5.7 p.p.m. Fe: | | | | |
| No Versene......................................... | None | — | l | rb |
| 1 eq. Versene...................................... | None | — | l | rb |
| 5 eq. Versene...................................... | None | — | vl | — |
| 5 p.p.m. Cu, 10.8 p.p.m. Fe: | | | | |
| No Versene......................................... | vl | rb | h | rb |
| 1 eq. Versene...................................... | vl | w | h | rb |
| 5 eq. Versene...................................... | None | — | vl | rb |
| White Pinot | | | | |
| 1 p.p.m. Cu, 6.6 p.p.m. Fe: | | | | |
| No Versene......................................... | None | — | vl | rb |
| 1 eq. Versene...................................... | None | — | vl | rb |
| 5 eq. Versene...................................... | None | — | — | — |
| 5 p.p.m. Cu, 10.5 p.p.m. Fe: | | | | |
| No Versene......................................... | None | — | vl | rb |
| 1 eq. Versene...................................... | None | — | vl | rb |
| 5 eq. Versene...... .................. | None | — | — | — |

\* vl = very light; l = light: h = heavy.
† rb = red-brown; w = white.

**TURBIDITY INDEX**

**OBSERVED REDOX POTENTIAL, VOLTS**

REDOX POTENTIAL

TURBIDITY

**TIME, MINUTES**
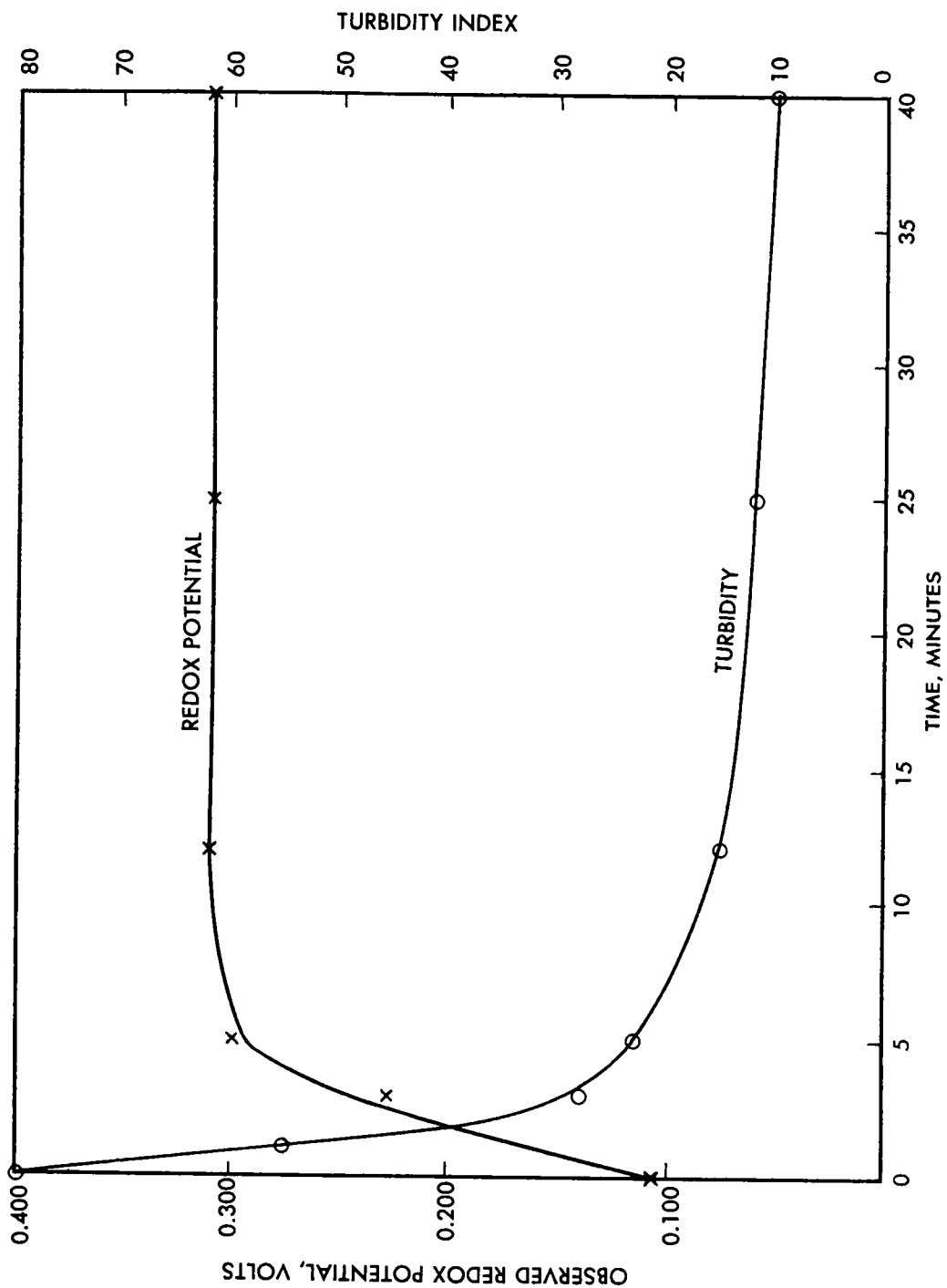
Fig. 15. Changes in redox potential and turbidity of wine showing copper.

**Effect on Copper Casse.** In the copper casse samples (table 26) the wines which were filled hot did not show any casse after storage for a month either with or without Versene. Unheated wines showed more tendency to cloud than heated ones.

In general, but not in all cases, Versene was effective in preventing copper casse formation. The color of the copper precipitate appears to be governed by its state of aggregation. When in suspension or when the precipitate is light, it appears white; when heavy, it appears reddish brown. The test for the presence of actual copper casse—which forms under reducing conditions and disappears under oxidizing conditions—was made first by shaking in air and then, if it did not dissolve, adding 2 drops of 3 per cent $H_2O_2$. Figure 15 shows the rate of decrease in turbidity with increase in redox potential in a typical test. Immediately on shaking, the $E_h$ increased to 0.345 volts, but within an hour there was no change in turbidity. Addition of $H_2O_2$ caused an immediate and rapid drop in turbidity with a corresponding increase in $E_h$, which leveled off at 0.555 volts, while turbidity asymptotically approached the limiting value of that of the untreated wine.

## HARD CHROME-PLATING

To obtain data on the applicability of hard chrome-plating to winery processing machinery, several exploratory investigations were carried out. These included comparisons of chrome-plated and unplated copper, bronze, brass, iron, and steel samples stored in wine under oxidizing and anaerobic conditions, and the effect of thickness of chrome plate.

**Metals Used.** Four sets of metal samples were obtained for the tests on the effect of type of metal and conditions of storage. Two sets were chrome-plated to a thickness of 0.001 to 0.002 inches while two corresponding control sets were not plated. All samples were uniform in shape and size, 1.5 by 3 inches by ⅛ inch.

To obtain data on effect of thickness of chrome plate, two sets of various metal plates with different thicknesses of chrome were obtained. A 0.001 to 0.002 inch chrome plate was compared with 0.003 to 0.004 inch plate.

**Wine and Conditions of Storage.** Two lots of French Colombard wine having the following composition were used.

|  | Lot 1 | Lot 2 |
|---|---|---|
| Alcohol by volume, per cent | 12.28 | 11.4 |
| Total acidity as tartaric, grams per 100 ml | 0.66 | 0.62 |
| Volatile acidity as acetic, grams per 100 ml | 0.045 | 0.052 |
| Reducing sugar as glucose, grams per 100 ml | 0.14 | 0.14 |
| Total sulfur dioxide, p.p.m. | 167 | 390 |
| Free sulfur dioxide, p.p.m. |  | 198 |
| Tannin, grams per 100 ml | 0.050 | 0.022 |
| Extract, grams per 100 ml |  | 2.0 |
| pH | 3.55 | 3.35 |
| Copper, p.p.m. | 0.20 | 0.12 |
| Total iron, p.p.m. | 5.85 | 11.80 |

Lot 1 was used in comparison of storage conditions and lot 2 for determining effect of plate thickness.

Pint mason jars were prepared to store wine. The caps were paraffined

TABLE 27

## EFFECT OF CHROME-PLATING IN PREVENTING METAL PICKUP IN WINE

| Sample | Color values, H₂O = 100 | | | | Final pH | Cu, p.p.m. | Fe, p.p.m. | Wt. of ppt., gms | Volume, ml | Color of wine | Turbid + or − |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 420 mμ | 540 mμ | 600 mμ | 660 mμ | | | | | | | |
| Series A, oxidizing condition | | | | | | | | | | | |
| Untreated wine | 256 | 131 | 116 | 111 | 3.51 | 0.20 | 5.85 | — | 175 | Amber | − |
| Brass: | | | | | | | | | | | |
| Plated | 215 | 145 | 117 | 112 | 3.51 | 0.20 | 5.85 | — | 181 | Amber | − |
| Not plated | 340 | 175 | 139 | 133 | 3.51 | 87.5 | 5.85 | — | 165 | Amber | + |
| Bronze: | | | | | | | | | | | |
| Plated | 263 | 145 | 116 | 106 | 3.51 | 0.40 | 5.85 | — | 179 | Amber | − |
| Not plated | 137 | 174 | 134 | 126 | 3.51 | 87.5 | 5.85 | — | 195 | DarkAmber | + |
| Copper: | | | | | | | | | | | |
| Plated | 259 | 145 | 116 | 104 | 3.51 | 0.75 | 5.85 | — | 183 | Amber | + |
| Not plated | 337 | 173 | 136 | 131 | 3.51 | 87.5 | 5.85 | — | 190 | DarkAmber | + |
| Stainless steel: | | | | | | | | | | | |
| Plated | 256 | 142 | 115 | 101 | 3.51 | 0.20 | 7.1 | — | 190 | Amber | − |
| Not plated | 254 | 137 | 113 | 106 | 3.51 | 0.20 | 7.8 | — | 165 | Amber | − |
| Hot-rolled steel: | | | | | | | | | | | |
| Plated | 379 | 168 | 137 | 127 | 3.60 | 0.20 | 47.8 | 0.16 | 170 | Green Tint | + |
| Not plated | 439 | 197 | 168 | 151 | 4.23 | 0.20 | 45.3 | 0.23 | 207 | Green Tint | + |
| Cast iron: | | | | | | | | | | | |
| Plated | 349 | 159 | 135 | 124 | 3.56 | 0.20 | 50.4 | 0.18 | 203 | Green Tint | + |
| Not plated | >900 | >900 | >900 | >900 | 5.28 | 0.20 | 43.9 | 0.35 | 203 | DarkBrown | − |
| Cold-rolled steel: | | | | | | | | | | | |
| Plated | 322 | 114 | 129 | 116 | 3.51 | 0.20 | 47.8 | 0.03 | 205 | Amber | + |
| Not plated | 847 | 412 | 317 | 267 | 4.60 | 0.20 | 52.5 | 0.22 | 205 | Green Tint | + |
| Series B, nonoxidizing condition | | | | | | | | | | | |
| Untreated wine | 244 | 135 | 118 | 112 | 3.56 | 0.20 | 5.85 | — | 455 | Amber | − |
| Brass: | | | | | | | | | | | |
| Plated | 240 | 134 | 117 | 109 | 3.56 | 0.20 | 5.85 | — | 425 | Amber | − |
| Not plated | 239 | 132 | 118 | 111 | 3.56 | 0.75 | 5.85 | ·· | 475 | Amber | − |
| Bronze: | | | | | | | | | | | |
| Plated | 240 | 133 | 115 | 105 | 3.56 | 0.20 | 5.85 | ·· | 430 | Green Tint | − |
| Not plated | 253 | 152 | 118 | 107 | 3.56 | 3.60 | 5.85 | ·· | 410 | Green Tint | + |
| Copper: | | | | | | | | | | | |
| Plated | 239 | 133 | 116 | 107 | 3.56 | 0.20 | 5.85 | — | 520 | Green Tint | − |
| Not plated | 271 | 150 | 122 | 113 | 3.56 | 5.20 | 5.85 | — | 500 | Green Tint | + |
| Stainless steel: | | | | | | | | | | | |
| Plated | 240 | 134 | 115 | 107 | 3.56 | 0.20 | 10.2 | ·· | 440 | Amber | − |
| Not plated | 243 | 136 | 118 | 111 | 3.56 | 0.20 | 7.0 | — | 453 | Amber | − |
| Hot-rolled steel: | | | | | | | | | | | |
| Plated | 230 | 130 | 119 | 109 | 3.56 | 0.20 | 42.8 | ·· | 450 | Amber | − |
| Not plated | 247 | 131 | 148 | 138 | 3.77 | 0.20 | 45.2 | 0.013 | 445 | Green Tint | + |
| Cast iron: | | | | | | | | | | | |
| Plated | 236 | 134 | 124 | 117 | 3.60 | 0.20 | 41.0 | — | 460 | Green Tint | − |
| Not plated | >900 | 392 | 230 | 662 | 4.60 | 0.20 | 44.8 | 0.19 | 450 | Green Tint | + |
| Cold-rolled steel: | | | | | | | | | | | |
| Plated | 229 | 126 | 117 | 107 | 3.56 | 0.20 | 37.6 | — | 435 | Green Tint | − |
| Not plated | 245 | 128 | 165 | 152 | 3.91 | 0.20 | 42.0 | 0.40 | 460 | Green Tint | + |

and strings were suspended from the center of the cap to which the metal strips were attached. Two series of tests were run to observe the effect of plating under oxidizing and nonoxidizing conditions. For the series under oxiding conditions, the jars were half filled with wine and metal strips were freely suspended so that half was exposed to air and half was submerged in the wine (table 27, series A). To create nonoxidizing conditions, the other series was prepared by suspending metal strips in completely filled, tightly capped bottles (table 27, series B). Each series included the chrome-plated samples and the corresponding nonchrome-plated samples, with both types of samples being tested under oxidizing and under nonoxidizing conditions.

All samples from both the thin- and thick-plated sets were suspended in wine under oxidizing conditions—that is, pint mason bottles were half filled with wine, with one half the sample exposed to the atmosphere and the other half immersed in the wine.

The metal samples were washed with soap and water then with distilled water. The nonplated cast iron samples (table 27), which were rusted when received, were first washed with dilute hydrochloric acid. Wine was siphoned into the volume-marked (250 ml) bottles and samples attached to the bottle caps were introduced. Caps were allowed to rest lightly on bottles and all bottles were exposed to light during the course of the experiment.

After standing undisturbed for one month, samples of the wine were removed and analyzed for copper, iron, pH, color, and weight of precipitate. For color values, wine was pipetted into colorimeter tubes with the minimum of agitation.

**Effect of Chrome-Plating on Various Metals.** The results obtained in the two series of lightly chrome-plated metals stored in wine under oxidizing and nonoxidizing conditions are shown in table 27. All samples under oxidizing conditions (series A) showed a slight sediment formation, even the copper samples and the untreated wine. Since the wine was initially high in iron (5.85 p.p.m.), this was presumably an iron casse formation. The precipitate was finely divided and could not be filtered out. The nonplated samples of hot- and cold-rolled steel and cast iron, especially the last, emitted a strong $H_2S$ odor on opening. The pH increase in some samples was due to ferric oxide formation, which neutralized the acids of the wine. There was no chromium pickup in any of the samples.

The color values in table 27 readily show the protective effect of chrome-plating. In every case, particularly in the oxidized series, the color values were higher in the nonplated than in the corresponding plated sample; this effect is most manifest at the lower wave lengths.

It is readily apparent from the comparative figures of copper and iron pickup that chrome-plating is effective in preventing metal solution and that it is more effective under nonoxidizing conditions than under oxidizing ones. The results show further that chrome-plating was more effective on copper than on iron. The chrome-plated iron samples at the outset showed extensive pitting, which gives rise to local cell action and consequent greater iron solution. (See figs. 16 to 18.)

**Effect of Thickness of Chrome Plate.** The effect of thickness of chrome

plate in reducing metal pickup by wine is shown in table 28. The cast-iron samples showed rust on air-exposed surfaces after 3 days, the cold-rolled steel samples after 7 days. After 17 days all iron-containing samples showed rust on air surfaces. Lower surfaces showed no corrosion. After 24 days both hot-rolled steel samples, both cast-iron samples, and the thin-plated cold-rolled steel sample showed clouding and precipitate. The iron samples
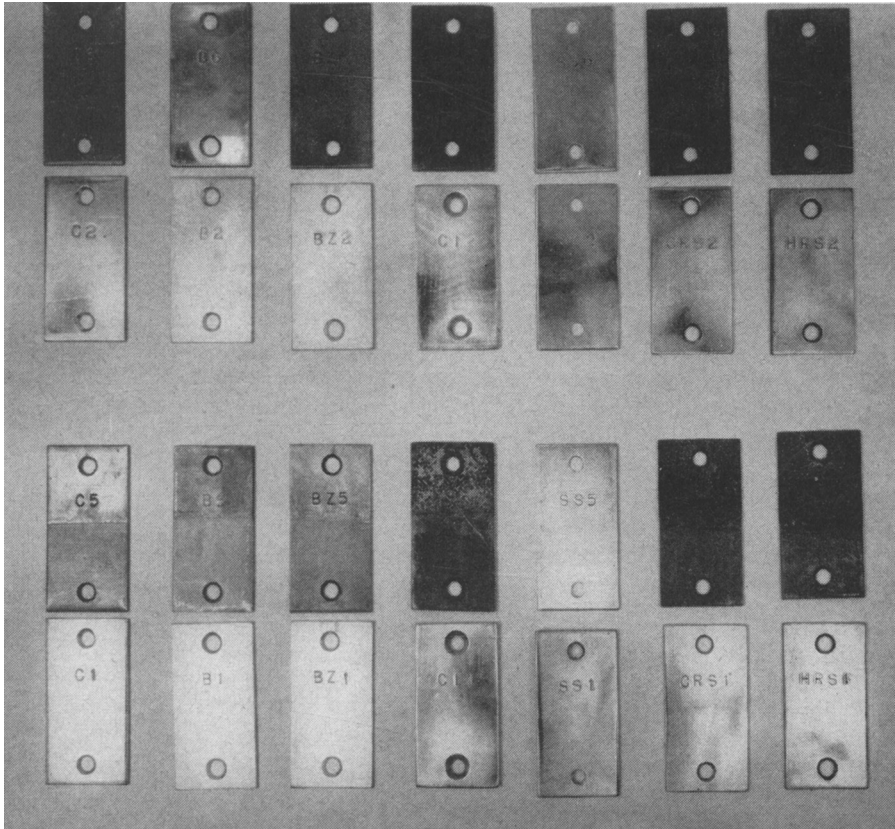


Fig. 16. Appearance of the metal strips used after removal from the wine. The two top rows were exposed under oxidizing conditions and the bottom two under anaerobic conditions. From left to right, in each row: copper, brass, bronze, cast iron, stainless steel, cold-rolled steel, and hot-rolled steel. The second and fourth rows are the chrome-plated strips.

corroded in the following order: thick-plated cast iron > thin-plated cast iron > thick-plated hot-rolled steel = thin-plated hot-rolled steel > thick-plated cold-rolled steel = thin-plated cold-rolled steel > thick-plated stainless steel > thin-plated stainless steel.

On upper surfaces of copper-containing samples, green and blue droplets and deposits were visible. The samples corroded in the following order: thin-plated bronze > thick-plated copper > thick-plated bronze > thick-

plated brass > thin-plated brass > thin-plated copper. Although thin-plated copper and brass showed corrosion on the exposed surface, the wine in contact with them showed no dissolved copper.

The wine analyses for these samples (table 28) are similar to those ob-
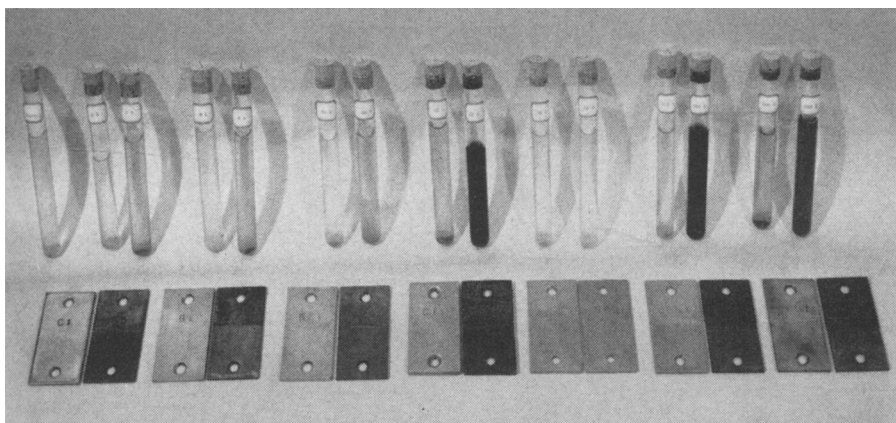


Fig. 17. Appearance of the wine and the metal strips after attack by the wine under oxidizing conditions. The first tube on the left is the control, wine containing no metal strip during the test. The chrome-plated metal strip is on the left in each pair, and the successive pairs of strips are copper, brass, bronze, cast iron, stainless steel, cold-rolled steel, and hot-rolled steel, with the corresponding wine samples in the same order above them.
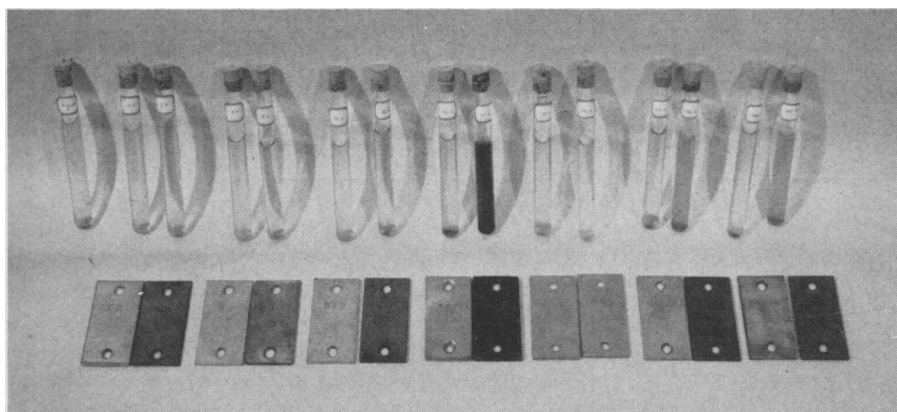


Fig. 18. Appearance of the wine and the metal strips after attack by the wine under anaerobic conditions. The arrangement of tubes and strips is the same as in figure 17.

tained previously (table 27). There was no correlation between chrome-plate thickness and the observed results. Some thick-plated samples had less and others more metal pickup than the corresponding thin-plated samples. The effectiveness of chrome-plating must be related to such factors as evenness and coverage of plate and to the absence of pitting. Again, in no case was there chromium pickup.

TABLE 28

EFFECT OF THIN AND THICK CHROME-PLATING IN PREVENTING
METAL PICKUP IN WINE

| Metal sample and thickness of chrome-plating | Color values (H₂O = 100) | | | | Copper, p.p.m. | Iron, p.p.m. |
|---|---|---|---|---|---|---|
| | 660 mμ | 600 mμ | 540 mμ | 420 mμ | | |
| Before filtration | | | | | | |
| Untreated wine | 107 | 107 | 121 | 179 | 0.05 | 11.8 |
| Brass: | | | | | | |
| Thick plating | 106 | 106 | 120 | 181 | 0.40 | — |
| Thin plating | 110 | 110 | 125 | 181 | 0.05 | — |
| Bronze: | | | | | | |
| Thick plating | 110 | 110 | 128 | 190 | 0.20 | — |
| Thin plating | 111 | 111 | 128 | 192 | 3.85 | — |
| Copper: | | | | | | |
| Thick plating | 107 | 107 | 125 | 188 | 3.70 | — |
| Thin plating | 106 | 105 | 120 | 177 | 0.05 | — |
| Hot-rolled steel: | | | | | | |
| Thick plating | 290 | 290 | 340 | 515 | — | 110.0 |
| Thin plating | 108 | 107 | 122 | 187 | — | 29.9 |
| Cast iron: | | | | | | |
| Thick plating | 260 | 260 | 298 | 455 | — | 108.0 |
| Thin plating | 242 | 244 | 288 | 445 | — | 108.0 |
| Cold-rolled steel: | | | | | | |
| Thick plating | 110 | 110 | 130 | 210 | — | 80.0 |
| Thin plating | 110 | 110 | 127 | 197 | — | 47.0 |
| Stainless steel: | | | | | | |
| Thick plating | 108 | 109 | 124 | 181 | — | 13.2 |
| Thin plating | 110 | 110 | 126 | 182 | — | 14.0 |
| After filtration | | | | | | |
| Hot-rolled steel, thick plating | 108 | 108 | 123 | 224 | — | 110.0 |
| Cast iron, thick plating | 107 | 107 | 123 | 234 | — | 108.0 |
| Cast iron, thin plating | 110 | 110 | 127 | 240 | — | 106.0 |

## DISCUSSION AND CONCLUSIONS

The formation of metallic turbidities and sediments in wines is not limited to copper and iron casse but may be due also to introduction of calcium, aluminum, and other metal ions. The tolerance of wines to heavy metals is known only in general because only recently have methods been introduced for the objective measurement of turbidities.[18] Measurements of particle size and shape and of density of suspensions, although they have been developed for protein and polysaccharide suspensions (Bender, 1952; Doty and Edsall, 1951; Greenwood, 1952; and Oster, 1948) have not been applied as yet to wines. The mechanism of the formation of suspensions of insoluble metal

[18] In recent years several photoelectric photometers employing a phototube of the multiplier type have been designed for measurement of light scattering. Oster (1953) described a high-sensitivity photometer employing a photomultiplier whose current is amplified by an electrometer tube. This instrument, applicable to a wide variety of accurate photometric measurements, is particularly useful in quantitative measurement of

compounds, the nature of the loci or nuclei about which particles form, the factors influencing supersaturation and formation and growth of particles, and the chemical changes involved are still not known. Until this information is obtained the susceptibility of a particular wine to casse formation and the conditions favoring casse can be obtained only empirically. Even this information for all the various wines produced in California and for the various cellar practices used is too meager to state definitely the minimum level of copper and iron at which a particular wine will remain clear under all conditions of storage.

Wines prepared completely out of contact with metal surfaces have not been available in sufficient quantity nor stored under carefully enough controlled conditions to determine whether casse would form from the naturally present mineral constituents of the grapes used.

On the assumption that a metal-stable wine under California conditions must contain less than 0.2 to 0.3 p.p.m. of copper and 3 to 4 p.p.m. of iron when bottled—conditions that can be achieved by carefully conducted blue-fining—possible substitutes for blue-fining can be evaluated. Of the methods investigated, the use of an adsorption complex of Prussian blue and potassium ferrocyanide appears to be the most promising. Prussian blue adsorption complexes on various inert carriers, such as diatomaceous filter-aid, bentonite, cellulose, and protein, can be and are now being prepared. When properly prepared they can be added in excess without the danger of contamination of the wine with soluble ferrocyanides. While they have the disadvantage in not removing iron as effectively as soluble ferrocyanides, wines sufficiently low in phosphate and tannic acid content may be stabilized by the addition of citric acid.

Rubeanic acid, because it effectively—usually completely—precipitates copper when used in the proper proportions, is also of value. The precipitation with rubeanic acid appears to proceed slowly enough to insure formation of particles of relatively large size so that separation of the black copper rubeanate can be readily accomplished by filtration or decantation. Rubeanic acid, like the ferrocyanide complex, can be used also with a coagulated protein carrier which would facilitate its application. One limiting factor is the need to dissolve rubeanic acid in alcohol to avoid hydrolysis, so that small amounts of alcohol would be added to the wine with it; this must be overcome before it can be used industrially. Its relatively high cost is another limiting factor; but if a demand for its use can be established the price can no doubt be reduced.

While none of the ion-exchange resins tested was a suitable substitute for ferrocyanide either in efficiency of copper and iron removal or in effects on the organoleptic qualities of wine, it is still possible that a synthetic ion-exchange resin with specific high exchange capacity for copper in wine can be developed. The ferrocyanide complexes, in effect, are such exchange resins.

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light scattering to determine total turbidity, particle numbers, and dissymetry of particles. Oster describes its use for measuring absolute turbidity of a light-scattering sample by comparing its intensity of scattering with that of an aqueous suspension of vitrous silica (Ludox).

There is evidence now that proteins and polypeptides, particularly those rich in sulfur-containing amino acids, as well as copper and other constituents, are involved in copper casse formation. It is possible that the removal of these substances, particularly by avoiding yeast autolysis during fermentation, would render even a wine high in copper content immune to clouding. Or it may be that cysteine and related compounds could be used to remove copper without introducing public-health hazards.

Hard chrome-plating, while it appears to have limited possibilities in the wine industry today, can be improved. Better preparation of the surface and better electroplating are needed to achieve more uniform and complete coating. Improvement in hardness to avoid uncovering of the metal surface under the plate by mechanical abrasion has been solved in other industries and can be solved for the wine industry. It offers at present the most economical recovery of equipment now in use. Chemical deposition of nickel has been developed recently and found superior to electroplating by providing more uniform and adherent coatings. Chemical deposition of chromimum if developed may have possibilities. Our investigations in the use of chrome-plating were only preliminary.

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