find harmful poisons in a horse's mouth after a race, you are questioning the honor of the men who own and train the horses: "The work Dr. Munch is doing is a credit to him and to the country, because all decent racing men will appreciate the fact that it is only the 'rotters' who are doping their horses and the good men don't want it. I would like to suggest that the perspiration from under the saddle would be a more handy substance to use than the gooey saliva. The favorite mixture for doping horses is nitroglycerin, strychnine, digitalis and heroin. Is it possible to get an accurate result between the time the horse comes back from weighing in and the time he returns to the stable?"

The author stated that the chemical methods developed by Dr. Penniman are much more sensitive than those published in the literature, and he deserves to be commended for his studies on this subject: "We are uncertain whether the chemical or biological methods are the most delicate, as well as the most specific, for testing the horse's saliva. With the animal test you get definite symptoms more rapidly than with the chemical: with the chemical method you can often isolate the specific alkaloids and identify them. The combination of both methods appears highly desirable. I have had no experience with perspiration from under the saddle, but believe that it might be contaminated with too much dirt. It is hoped that the application of these methods of chemical and biological testing will stop the doping of race-horses."

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# THE STABILIZATION OF SYRUP OF FERROUS IODIDE, U. S. P. X.

### BY WILLIAM J. HUSA AND LYELL J. KLOTZ.
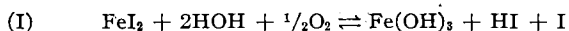
*(Concluded from page 683, July Journal.)*

As indicated in Table II, the hydrolysis of aqueous solutions of ferrous iodide corresponds predominantly to the equation:
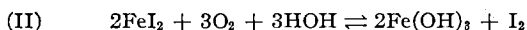
$$FeI_2 + HOH \rightleftharpoons Fe(OH)I + HI$$
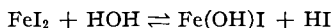
and apparently, two equilibria exist since reasonably constant values of $K_1$ were obtained for solutions having either a $p_H$ of 3.2 or 4.1. In the former case, the degree of hydrolysis is approximately 0.30% at the concentration of the U. S. P. Syrup; in the latter instance, it is approximately 0.027%. Solutions of ferrous iodide of $p_H$ 4.1 are hereafter designated as solutions at primary equilibrium; those at $p_H$ 3.2 are considered to be at a condition of secondary equilibrium.

*The Mechanism of Iodine Formation.*—The decomposition of aqueous solutions of ferrous iodide consists in the formation of free iodine and ferric hydroxide. If sucrose, or other peptizing agent is present, however, ferric hydroxide does not precipitate and the appearance of iodine is dependent upon the rate at which the peptizing agent reacts with free iodine as well as upon the rate of auto-oxidation. The presence of soluble ferric ion is prohibited by the presence of iodide ion which reduces it to the ferrous state.
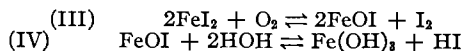
Several equations to account for the decomposition of ferrous iodide preparations have been advanced. Salzer (9) and Sadtler and Coblentz (10) formulated the equation

$$(I) \qquad FeI_2 + 2HOH + \tfrac{1}{2}O_2 \rightleftharpoons Fe(OH)_3 + HI + I$$

Mylius (11) suggested

$$(II) \qquad 2FeI_2 + 3O_2 + 3HOH \rightleftharpoons 2Fe(OH)_3 + I_2$$

and Bentley and Driver (12) account for the decomposition by two reactions:

$$\text{(III)} \qquad 2FeI_2 + O_2 \rightleftharpoons 2FeOI + I_2$$
$$\text{(IV)} \qquad FeOI + 2HOH \rightleftharpoons Fe(OH)_2 + HI$$

On the other hand, Phillips (13) believed the decomposition to result from deterioration of the hydriodic acid formed by hydrolysis of the salt; no experimental evidence was offered in support of this hypothesis.

The above equations require, from a stoichiometric standpoint, the loss of two mols of ferrous iron for each mol of free iodine formed. Equations (I) and (IV) indicate, however, that this ratio would decrease slightly as the result of subsequent deterioration of the hydriodic acid formed. Thus, the validity of these equations may be established by analyses of partially decomposed solutions of ferrous iodide, a 2:1 ratio or smaller indicating that such formulations are acceptable.

In order to determine the validity of these theories of decomposition, samples of aqueous solutions of ferrous iodide were stored under conditions of excess air, in completely filled, tightly stoppered bottles and in sealed pycnometers and the loss of ferrous iron in mols per liter compared with the free iodine present. The result of such a series of analyses upon a sample stored in the presence of air and prepared from Reagent grade chemicals follows in Table III.

TABLE III.—RATIO OF MOLARITY OF IRON AND IODINE IN AQUEOUS SOLUTION OF FERROUS IODIDE STORED AT 30° C. IN THE PRESENCE OF EXCESS AIR.

| Hours. | Loss $Fe^{++}$ Mols/L. | $I_2$ Mols/L. | $Fe^{++}/I_2$. |
|---|---|---|---|
| 0 | . . . . . | . . . . . | . . |
| 172$^1$/$_2$ | 0.00772 | 0.00144 | 5.36 |
| 240 | 0.00799 | 0.00177 | 4.52 |
| 292 | 0.00705 | 0.00197 | 3.58 |
| 340 | 0.00705 | 0.00220 | 3.20 |
| 384 | 0.00705 | 0.00313 | 2.26 |
| 436 | 0.01089 | 0.00337 | 3.21 |
| 557 | 0.01614 | 0.00418 | 3.86 |
| 774 | 0.02094 | 0.00561 | 3.72 |
| 918 | 0.02093 | 0.00666 | 3.14 |
| 1178 | 0.01983 | 0.00897 | 2.21 |
| 1538 | 0.03675 | 0.01300 | 2.82 |

As indicated by the data in Table III, no evidence exists for the disappearance of 2 mols of ferrous iron per mol of iodine formed. Moreover, similar results were obtained in other samples stored in the presence of air. Samples stored in completely filled, tightly stoppered bottles apparently remained near the condition exemplified by the early readings in Table III, and ranged in ratio between 8.64 and 2.21 as determined from 11 analyses. Samples stored in sealed pycnometers, showed no loss of ferrous iron over a period of 1127 hours although they contained traces of free iodine. It was concluded that the equations previously advanced were not acceptable as representing the mechanism of decomposition.

An examination of the precipitates contained in the samples of solution stored in completely filled, tightly stoppered bottles gave some indication of basic salt formation during the early stages of decomposition. In general, however, the precipitate proved to be ferric hydroxide.

In order to test the effect of a common ion on the rate of decomposition, a solution containing 1 mol per liter of potassium iodide in addition to its ferrous

iodide content and another solution containing 0.22 mol per liter of excess ferrous sulphate were stored together with a control. Analyses for free iodine showed that the presence of either iodide or ferrous ion in excess results in an increased rate of decomposition.

*The Rate of Decomposition of Ferrous Iodide Solutions.*—In order to elucidate further the mechanism of decomposition of aqueous solutions of ferrous iodide, the rate and order of reaction were determined. A solution of ferrous iodide was prepared from Reagent chemicals and stored in the thermostat at 30° C. in darkness in a container with an excess of air. The progress of the decomposition was followed by titration of the liberated iodine. Results were treated mathematically according to equations (I) and (II) below, in order to determine the order of reaction (14):

$$\text{(I)} \qquad K_m = 2.303/t \log a/a - x$$
$$\text{(II)} \qquad K_b = 1/t \cdot x/a(a - x)$$

where $K_m$ represents the specific reaction rate as calculated for a first order reaction and $K_b$ represents that for a second order reaction. The fraction decomposed in time $t$ is represented by $x$, and the initial concentration by $a$. All calculations are based upon the total iodide content of the preparation, thus regarding the oxygen content as sufficiently large to be considered constant. Data follow in Table IV.

TABLE IV.—RATE OF DECOMPOSITION OF FERROUS IODIDE SOLUTIONS PREPARED FROM REAGENT IRON AND REAGENT IODINE AND STORED IN THE THERMOSTAT AT 30° C. IN DARKNESS.

| Total Hours. | Segment Hours. | Iodide Mols/L. | Ferrous Iron Mols/L. | Iodine Mols/L. $\times 10^3$. | $K_m \times 10^5$. | $K_b \times 10^4$. |
|---|---|---|---|---|---|---|
| 0 | 0 | 0.22710 | 0.2348 | . . . | . . . | . . |
| 95 | 95 | 0.22672 | 0.2354 | 0.87 | 4.05 | 1.79 |
| 148½ | 53½ | 0.22648 | 0.2348 | 1.22 | 1.51 | 4.46 |
| 215 | 66½ | 0.22710 | 0.2334 | 1.43 | 1.06 | 4.20 |
| 263 | 48 | 0.22680 | 0.2244 | 1.63 | 0.98 | 6.65 |
| 315½ | 82½ | 0.22672 | 0.2263 | 1.83 | 1.74 | 6.86 |
| 359 | 43½ | 0.22680 | 0.2263 | 1.99 | 1.74 | 8.98 |
| 411 | 52 | 0.22651 | 0.2263 | 2.72 | 1.80 | 10.29 |
| 532 | 121 | 0.22694 | 0.2244 | 3.41 | 1.32 | 5.57 |
| 719 | 187 | 0.22643 | 0.2159 | 4.46 | 1.29 | 4.73 |
| 863 | 144 | 0.22643 | 0.2165 | 5.03 | 2.76 | 6.95 |
| 1123 | 260 | 0.22620 | 0.2155 | 6.38 | 2.38 | 4.92 |
| 1483 | 360 | 0.22679 | 0.2094 | 7.89 | 1.89 | 4.42 |
| 2400 | 917 | 0.22683 | 0.2052 | 11.55 | 1.84 | 2.60 |
| 3120 | 720 | 0.22659 | 0.2003 | 14.35 | 1.82 | 1.14 |

Mean 1.79

As indicated in Table IV, the values of $K_m$ are in good agreement while those of $K_b$ show considerable variation. The iodide content, including free iodine present, remained practically constant. For the 14 values of $K_m$ given, the standard deviation (S. D.) is $0.72 \times 10^{-5}$, the standard deviation of the mean is $0.19 \times 10^{-5}$, the probable error (PE), is $0.48 \times 10^{-5}$ and the probable error of the mean is $0.13 \times 10^{-5}$.

As further verification of the order of reaction, three samples of aqueous solu-

tion of ferrous iodide, prepared from card teeth and Reagent iodine and differing in concentration were stored under the conditions outlined above and the rate of reaction determined.   The data are shown in Table V.

TABLE V.—RATE OF REACTION OF AQUEOUS SOLUTIONS OF FERROUS IODIDE PREPARED FROM CARD TEETH AND REAGENT IODINE AND STORED IN THE THERMOSTAT AT 30° C. IN DARKNESS.

| Conc. FeI$_2$ Mols/L. | No. of Det'ns. | Total Hours. | $K_m \times 10^5$ Mean. | S. D. of $K_m \times 10^5$. | $K_b \times 10^5$ Mean. | S. D. of $K_b \times 10^4$. |
|---|---|---|---|---|---|---|
| 0.47935 | 9 | 3048 | 3.21 | 0.27 | 23.91 | 11.43 |
| 0.22594 | 17 | 3007 | 3.22 | 0.76 | 6.86 | 3.36 |
| 0.11770 | 6 | 2086 | 3.88 | 0.25 | 63.30 | 15.21 |

It was concluded from the data shown in Table V that the first order reaction equation is applicable to this decomposition.   The standard deviation of the mean at the concentration of the U. S. P. Syrup being $0.18 \times 10^{-5}$, the mean may be stated as $3.22 \pm 0.18 \times 10^{-5}$.

The rate of decomposition for card teeth solutions is greater than for solutions prepared from Reagent iron, nearly twice as much iodine being formed per hour in terms of per cent decomposition per unit time.

*The Effect of Type of Glass upon the Rate of Decomposition.*—Containers used for determination of the rate of reaction as shown in Tables IV and V, imparted alkalinity to distilled water upon standing.   To ascertain the effect of a container imparting no alkalinity to glass, similar determinations were made upon an aqueous solution of ferrous iodide stored under conditions identical with the above, in such a container (Pyrex bottle).   Ten analyses were made upon the solution which assayed 0.22693 mols/L. of ferrous iodide.   These determinations were made over a period of 1675 hours.   The mean value for $K_m$ was $6.96 \times 10^{-5}$; the standard deviation was $0.57 \times 10^{-5}$; the standard deviation of the mean was $0.18 \times 10^{-5}$.   Thus $K_m$ may be stated as $6.96 \pm 0.18 \times 10^{-5}$.

It was concluded that the type of glass in the container exerted a marked effect upon the rate of decomposition, alkaline glass exerting a retarding effect.

During the progress of this investigation, alkaline glass was employed in order to obtain results directly applicable to common pharmaceutical containers.

*The Effect of Surface Area upon the Rate of Decomposition.*—Since decomposition of aqueous solutions of ferrous iodide requires oxygen, it is probable that the rate of reaction would depend directly upon the rate of oxygen absorption, provided auto-oxidation is appreciably more rapid than absorption.   This rate of absorption would in turn depend to some extent upon the surface exposed, *i. e.,* the reaction would be of the zero order.

In order to extend the previous determinations of reaction rates to include conditions of minimal practical surface area exposed, an aqueous solution of ferrous iodide was stored under conditions identical with those of previous reaction rate determinations.   In this case, however, a container was chosen of such shape that only about $1/5$ of the previous surface exposure occurred.   This container was typical of common prescription bottles.   Ten determinations of the rate of reaction over a period of 2986 hours gave a mean value for $K_m$ of $3.15 \times 10^{-5}$.   The standard deviation was $0.37 \times 10^{-5}$ and the standard deviation of the mean was $0.11 \times 10^{-5}$.   Thus, $K_m$ may be stated as $3.15 \pm 0.11 \times 10^{-5}$.

It was concluded that under ordinary conditions, the absorption of oxygen is more rapid than auto-oxidation and that, therefore, the reaction is not one of the zero order.

*The Effect of Limited Availability of Oxygen.*—Fifty samples of aqueous solution of ferrous iodide were stored in darkness in the thermostat at 30° C., in completely filled, tightly stoppered bottles. Stoppers were protected from the action of iodine and the containers sealed with paraffin. A sample was removed and assayed for iodine content at intervals over a period of 3122 hours. Variable results, ranging from $1.90 \times 10^{-4}$ mols/L. to $9.51 \times 10^{-4}$ mols/L. of free iodine, were found present in 14 determinations. It is significant that low values were impartially distributed over the duration of the experiment.

It was concluded from the distribution and magnitude of the results, that upon expiration of the contained oxygen, little decomposition occurs in aqueous solutions of ferrous iodide stored without access to atmospheric oxygen.

A series of samples of aqueous solution of ferrous iodide was next stored in sealed pycnometers. All water used in the preparation was vigorously boiled before use and allowed to cool in completely filled, tightly stoppered bottles. No precipitate appeared in these samples during 792 hours. The value of $K_m$ was $2.59 \times 10^{-6}$. Bubbles of gas appeared at the surface of the liquid in these preparations but did not appear in solutions of ferrous sulphate similarly prepared and stored. It appears probable that a slow secondary decomposition occurs in the absence of oxygen according to the equation:

$$2HI \rightleftharpoons H_2 + I_2$$
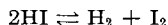
*The Effect of Viscosity upon the Rate of Decomposition.*—As a means of determining the effect of viscosity upon the rate of auto-oxidation of solutions of ferrous iodide, aqueous solutions of the salt were rendered viscous with tragacanth and the rate of reaction evaluated in terms of the specific reaction rate, $K_m$. Tragacanth was chosen as being a neutral non-reactant carbohydrate. Viscosities were determined by means of viscosimeter tube and stop-watch (15). Preliminary experiments showed that tragacanth in the concentrations used was without effect upon free iodine during the duration of the experiments. Table VI shows the data obtained. Values of a solution without added tragacanth are from Table V.

TABLE VI.—RATE OF DECOMPOSITION OF AQUEOUS SOLUTIONS OF FERROUS IODIDE RENDERED VISCOUS WITH TRAGACANTH AT 30° C.

| Conc. of FeI₂ in Mols/L. | Relative Viscosity (30°/30°). | No. of Det'ns. | Time Hours. | $K_m \times 10^5$ Mean. | S. D. $\times 10^5$. | $K_m \times 10^5$. |
|---|---|---|---|---|---|---|
| 0.22594 | 1.072 | (See Table V) | | 3.22 | 0.25 | 3.22 ± 0.18 |
| 0.22811 | 2.507 | 5 | 1227 | 1.73 | 0.10 | 1.73 ± 0.04 |
| 0.22783 | 6.296 | 5 | 1227 | 1.01 | 0.01 | 1.01 ± 0.01 |
| 0.21323 | 19.8 | 5 | 1848 | 1.05 | 0.07 | 1.05 ± 0.03 |
| 0.21490 | 539.7 | 5 | 1848 | 0.79 | 0.02 | 0.79 ± 0.01 |

As indicated in Table VI, increases above a relative viscosity of approximately 6 have little effect upon the specific reaction rate. From these data it was concluded that the specific reaction rate is a hyperbolic function of the viscosity or of some factor proportional to viscosity.

*The Effect of Variations in Temperature upon the Rate of Decomposition of Aqueous Solutions of Ferrous Iodide.*—In order to determine the effect of variations in temperature upon the auto-oxidation rate of aqueous ferrous iodide solutions, samples of various viscosities were stored in the oven at 50° C. $\pm$ 2° and in the refrigerator at 6° C. $\pm$ 1°. All samples were stored in containers with an excess of air. Reaction rates were determined in the usual manner, viscosities are stated relative to water at 30° C. although determined at the reaction temperature, and tragacanth was used as the substance imparting viscosity. Data in Table VII referring to solutions at 50° C. were determined after short intervals of exposure since the solutions increased greatly in viscosity with time. These results, therefore, are only approximate.

TABLE VII.—THE EFFECT OF VARIATION IN TEMPERATURE UPON THE SPECIFIC REACTION RATE OF AQUEOUS SOLUTIONS OF FERROUS IODIDE OF VARIOUS VISCOSITIES.

| Conc. FeI₂ Mols/L. | Relative Viscosity. | Temp. ° C. | Total Hours. | No. of Det'ns. | Mean $K_m \times 10^4$. | S. D. $\times 10^4$. | $K_m \times 10^4$. |
|---|---|---|---|---|---|---|---|
| 0.22690 | 1.062 | 50 | 575 | 5 | 3.21 | 0.27 | 3.21 $\pm$ 0.12 |
| 0.22525 | 1.200 | 50 | 68 | 1 | 1.25 | .. | ........ |
| 0.22811 | 2.26 | 50 | 336 | 1 | 0.60 | .. | ........ |
| 0.22383 | 5.13 | 50 | 336 | 1 | 0.59 | .. | ........ |
| 0.21225 | 26.74 | 50 | 1126 | 5 | 0.61 | 0.19 | 0.61 $\pm$ 0.01 |
| 0.22690 | 1.075 | 6 | 1012 | 5 | 0.38 | 0.05 | 0.38 $\pm$ 0.02 |
| 0.22810 | 64.32 | 6 | 840 | 3 | 0.22 | 0.005 | 0.22 $\pm$ 0.003 |
| 0.22380 | 89.68 | 6 | 840 | 3 | 0.10 | 0.04 | 0.10 $\pm$ 0.002 |
| 0.21007 | 525.0 | 6 | 1152 | 3 | 0.35 | 0.02 | 0.35 $\pm$ 0.01 |

It was concluded that solutions of ferrous iodide at 50° C. have a temperature coefficient of approximately 3 for each ten degree rise in temperature. The curve of viscosity against reaction rate is hyperbolic and the greatest variation is between relative viscosities of 1 and 26. At 6° C., however, the reaction is more rapid than at 30° C., and the curve of viscosity against specific reaction rate is nearly linear for relative viscosities below 100.

*The Effect of Light upon the Rate of Decomposition of Aqueous Solutions of Ferrous Iodide.*—That light catalyzes the auto-oxidation of iodide ion has long been known and Berthoud and Nicolet (16) have shown that all light from the visible spectrum is active. It has not previously been shown, either that the decomposition of ferrous iodide is typical of iodide ion, with respect to the catalytic effect of light, or is sensitive to certain spectrum bands. To evaluate this factor, aqueous solutions of ferrous iodide were stored in containers with excess air, behind a series of light filters in direct sunlight, and a control run with the experiment. No significant difference in specific reaction rate could be found in any sample, although the auto-oxidation proved to be more rapid than in solutions stored in darkness under similar conditions of heat and exposure. It was concluded that the auto-oxidation of ferrous iodide is catalyzed by all rays of the visible spectrum and is thus typical of iodide ion oxidation.

The action of ultraviolet light was evaluated indirectly. The specific reaction rate of solutions stored in sunlight in quartz flasks was compared with that for solutions similarly stored in pyrex. Since results were identical, it is probable that ultraviolet light has no catalytic effect upon this decomposition.

*The Appearance of Permanent Dark Coloration in Syrup of Ferrous Iodide.*—Obviously, the liberation of free iodine in Syrup of Ferrous Iodide results in dark coloration; this, however, seldom occurs due to the hypophosphorous acid content of the preparation. Nevertheless, the Syrup, upon standing, assumes a very dark brown or black color rendering it unfit for use. Since neither aqueous solutions of ferrous iodide nor aqueous solutions of ferrous iodide containing hypophosphorous acid show this phenomenon, the discoloration must be ascribed to the presence of sucrose or its decomposition products. The change has commonly been misnamed caramelization.

Preliminary experiments showed that either ferrous ion as ferrous sulphate or hypophosphorous acid was capable, the latter much more efficiently than the former, of producing a dark coloration in simple syrup if present in the proportions found in the U. S. P. Syrup of Ferrous Iodide. Phosphoric acid also possesses this property, but free iodine was slowly and completely decolorized.

Samples of Syrup of Ferrous Iodide with the hypophosphorous acid omitted, stored in direct sunlight or in the oven at 50° C. rapidly became black in color and contained a black precipitate. Thus hypophosphorous acid does not cause this change to occur although it may act as a catalyst.

Sucrose, upon inversion, forms equimolecular quantities of dextrose and levulose, and Krantz (17) has shown that inversion is 96.2% complete in Syrup of Ferrous Iodide in 20 days. Accordingly, samples of Syrup of Ferrous Iodide were prepared, substituting in one case 2.484 mols/L. of Merck's C.P. dextrose for the sucrose, and in the other, 2.484 mols/L. of Cenco pure levulose. In one month's exposure in the oven at 50° C., in the presence of air, the sample containing levulose was black; that containing dextrose was yellow, but no darker than a sample of simple syrup containing an equivalent quantity of ferrous sulphate and stored under the same conditions. Similarly, samples of U. S. P. X Syrup of Hydriodic Acid also turn black in color under these conditions. This Syrup contains hypophosphorous acid.

It was concluded that levulose, formed by hydrolysis of the sucrose present in the official Syrup, is the cause of the dark coloration appearing in Syrup of Ferrous Iodide.

*The Effect of Various Preservatives upon Free Iodine.*—The action of any preservative or stabilizer in Syrup of Ferrous Iodide may be twofold: it may decrease the rate of auto-oxidation and it may cause the disappearance of free iodine. Adding the stipulation that it must not cause side reactions to occur, gives three criteria for the evaluation of preservatives from a chemical standpoint. Thus a substance may act as a stabilizer under one of these criteria, yet be rejected on the basis of another. Sucrose is typical of this class.

In order to evaluate various stabilizers on the basis of their action on free iodine, solutions containing approximately 0.01 mols/L. of free iodine as Compound Solution of Iodine, U. S. P. X, and 2.484 mols/L. of the preservative were stored in darkness with excess air in the thermostat at 30° C. Free iodine was determined at intervals. A control showed no loss of iodine during the duration of the experiment. The value 2.484 mols/L. was used to give results directly comparable with sucrose, this figure representing the sucrose content of Syrup of Ferrous Iodide.

Merck's Lactose, Merck's C.P. dextrose, Merck's U. S. P. citric acid, Merck's

U. S. P. tartaric acid, Colgate's C.P. glycerin, U. S. P. sucrose, U. S. P. alcohol and U. S. P. honey were tested in this manner. Only 170 Gm./L. of lactose were used due to its low solubility and honey was weighed as dextrose. It was found that sucrose, dextrose, lactose, honey, and to a lesser degree, citric acid, exert a slow chemical action upon free iodine in the presence of iodide ion in aqueous solution.

To determine the effect of ferrous ion upon this reaction, solutions were prepared containing approximately 0.236 mol/L. of ferrous iodide, 2.484 mols/L. of either honey, sucrose or dextrose and approximately 0.01 mol/L. of free iodine. They were stored either at 30° C. or at 50° C. and free iodine frequently determined. The quantity of iodine reacting with the preservative is the difference between that originally present and the quantity present at the time of analysis plus free iodine formed at that temperature and viscosity by decomposition. The latter values were obtained from the equation:

$$K_m = 2.303/t \log a/a - x$$

where $x$ is the quantity of iodine formed in time $t$ from a solution of original concentration $a$. Values of $K_m$ were obtained from Tables VI and VII by interpolation to the proper viscosities.

It was found that heat accelerated the carbohydrate reaction to a greater degree than the auto-oxidation since samples at 50° C. rapidly became free from iodine. Samples at 30° C. were decolorized slowly in the case of sucrose and more rapidly in the case of honey; although honey was more efficient in this respect at 30° C., sucrose was more rapid than honey at 50° C. The action of dextrose was not so rapid, but at room temperature, about 22° C., none of these sugars reduced iodine as rapidly as it was formed. Apparently, the ferrous ion present exerted a catalytic effect since reduction of iodine was more rapid than in simple aqueous solution. The presence of some relatively rapid reducing agent in honey is indicated by the rate of reaction which is much greater than with sugars alone.

TABLE VIII.—THE EFFECT OF PRESERVATIVES UPON AQUEOUS SOLUTIONS OF FERROUS IODIDE AT 30° C. STORED IN EXCESS AIR IN DARKNESS.

| Preservative. | Hours. | No. of Det'ns. | $K_m \times 10^5$ Mean. | S. D. $\times 10^5$. | $K_m \times 10^5$. | Rate of Decomp. $\times 10^5$. |
|---|---|---|---|---|---|---|
| Sucrose (Card teeth) | 3003 | 6 | .. | .. | ........ | 0..85 |
| Sucrose (Reag. iron) | 2824 | 5 | .. | .. | ........ | 0.85 |
| Honey-calc. as dextrose | 2520 | 4 | .. | .. | ........ | 1.56 |
| Dextrose | 2956 | 5 | 0.53 | 0.23 | 0.53 ± 0.10 | 1.05 |
| Lactose 170 Gm./L. | 2525 | 4 | 1.77 | 0.61 | 1.77 ± 0.30 | 2.5 |
| Glycerin | 2478 | 4 | 1.25 | 0.64 | 1.25 ± 0.32 | 2.1 |
| Citric acid | 2775 | 5 | 1.75 | 0.36 | 1.75 ± 0.16 | 1.5 |
| Tartaric acid | 2394 | 5 | 2.71 | 0.95 | 2.71 ± 0.44 | 1.65 |
| Alcohol | 2374 | 4 | 1.95 | 0.43 | 1.95 ± 0.19 | 2.07 |

*The Chemical Evaluation of Preservatives.*—To verify conclusions reached above, samples of aqueous solutions of ferrous iodide containing 2.484 mols/L. of preservative were placed in the thermostat at 30° C. in darkness with an excess of air in the container. Iodine was determined at frequent intervals. The data are summarized in Table VIII. The quality of chemicals has been noted above. Column 7 shows the rate of decomposition to be expected from viscosity readings performed upon these solutions. These data are interpolated from Table VI.

As indicated in Table VIII, true preservative effect beyond the effect of the viscosity imparted to the solution is shown by sucrose, honey, dextrose, lactose and glycerin. Alcohol is apparently without effect and citric and tartaric acids increase the rate of decomposition. This is probably due to the increased hydrogen-ion concentration of these acid solutions; the $p_H$ was 0.4.

*The Pharmaceutical Evaluation of Preservatives.*—Solutions of ferrous iodide, approximately 5% except as noted, were prepared, except as noted, from card teeth and U. S. P. iodine and contained the following: honey, 500 cc./L.; honey, 250 cc./L.; sucrose, 425 Gm./L. and honey, 425 cc./L.; sucrose, 800 Gm./L. and honey, 50 cc./L.; 10% $FeI_2$ and 800 Gm./L. of dextrose; sucrose, 850 Gm./L. and $H_3PO_2$, 5 cc./L.; sucrose, 850 Gm./L. and $Na_2S_2O_3$, 1 Gm./L.; 10% $FeI_2$ and sucrose, 850 Gm./L.; sucrose, 850 Gm./L. and NaOH, 0.1975, 0.2949 and 0.6908 Gm./L. in separate samples; sucrose, 850 Gm./L., prepared with Reagent iron; sucrose, 850 Gm./L. and hydroquinone, 5 Gm./L.; glycerin, 800 cc./L.; sucrose, 850 Gm./L. and $H_3PO_2$, 5 cc./L. (control); and sucrose, 850 Gm./L. with $H_3PO_2$, 5 cc./L., prepared from Reagent iron. Samples of these solutions were stored both in half-filled and in completely filled, tightly stoppered bottles in diffused light and in darkness at room temperature, in direct sunlight and in the refrigerator at about 6° C.

At the end of 3 months, those samples stored in direct sunlight in the presence of air were black in color with the exception of those containing dextrose or glycerin; the dextrose sample alone contained free iodine. Free iodine was contained in all samples stored in the refrigerator except those containing $H_3PO_2$ which had, however, darkened somewhat.

In general, after 9 months' exposure, the following observations were made: storage in completely filled bottles is desirable; sunlight hastens the appearance of black coloration although the increased temperature accelerates the reduction of iodine to a greater degree than the auto-oxidation of iodide; the addition of NaOH decreases the rate of iodine formation but is undesirable due to the coloration produced in the freshly made solution; Reagent iron samples show less free iodine than those prepared from card teeth; honey alone is valueless as a preservative; glycerin is ideal in sunlight but valueless in darkness or diffused light; $Na_2S_2O_3$ gives a cloudy preparation by liberation of free sulphur; hydroquinone is a valuable anti-oxidant except at low temperatures; and free iodine added to blackened preparations of ferrous iodide containing sucrose or honey, rapidly disappears.

It was concluded that none of the combinations used was entirely satisfactory as offering a stable preparation.

*The Stabilization of Syrup of Ferrous Iodide.*—It has been shown that sucrose is incompatible with aqueous solutions of ferrous iodide. It has likewise been shown that dextrose is compatible with both ferrous iodide and hypophosphorous acid. Thus, it would appear that the ideal preparation would contain dextrose to give the properties of a syrup and hypophosphorous acid to prevent the liberation of iodine.

Solutions of ferrous iodide containing the official quantity of $H_3PO_2$ and either 700 Gm./L. of C.P. dextrose, commercial dextrose[1] or 400 Gm./L. of U. S. P. Glu-

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[1] A brand of dextrose, "Penford Crystal Sugar," made by Penick and Ford, Cedar Rapids, Iowa, was employed.

cose were subjected to a temperature of 50° C. for 3 months both in half-filled and in completely filled, tightly stoppered bottles. A U. S. P. control was run in conjunction. The freshly prepared solutions varied in color with the purity of the dextrose used; C.P. dextrose gave a perfectly colorless preparation but that from U. S. P. Glucose was definitely yellow.

At the end of one month, the control was black but all other samples remained unchanged. In 4 months, the syrups containing dextrose were pale yellow and contained a slight sediment; the change was more pronounced in samples stored in the presence of air and varied in direct proportion to the purity of the dextrose. Samples containing Glucose, U. S. P. X, had become very dark.

One month's exposure in the oven at 50° C. is roughly equivalent to 1 year under ordinary conditions, hence it may be assumed that dextrose samples would be acceptable for 4 years at ordinary temperatures.

It was concluded that Syrup of Ferrous Iodide may be stabilized by substituting 700 Gm. of dextrose for the sucrose contained in the official formula, either C.P. dextrose or the commercial grade being satisfactory. The resulting preparation is very palatable. The syrup so prepared is best kept at room temperature as some of the dextrose may crystallize at low temperatures.

### DISCUSSION OF RESULTS.

It can be shown by solubility product data and $p_H$ values that precipitation of ferric hydroxide cannot occur in Syrup of Ferrous Iodide. [*] On the other hand, similar calculations in conjunction with the quantity of air present, indicate that precipitation occurs in the reaction mixture of preparations at secondary equilibrium before dilution, but does not take place until after dilution in preparations from Reagent iron at primary equilibrium.

It has been shown that no stoichiometric expression is applicable to the decomposition of aqueous ferrous iodide solutions. On the other hand, hydrolysis has been shown to occur predominantly as:

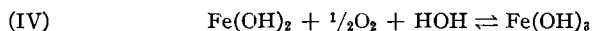$$(I) \qquad FeI_2 + HOH \rightleftharpoons Fe(OH)I + HI$$
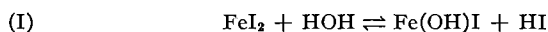
and therefore, the solution contains iodide and ferrous ions in the presence of hydrogen ions. Iodide ion is oxidizable by oxygen according to the equation:

$$(II) \qquad 2I^- + \frac{1}{2}O_2 + 2H^+ \rightleftharpoons I_2 + HOH$$
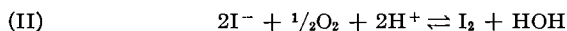
Secondary hydrolysis of the basic ferrous iodide formed as shown in equation (I) furnishes small quantities of ferrous hydroxide:

$$(III) \qquad Fe(OH)I + HOH \rightleftharpoons Fe(OH)_2 + HI$$
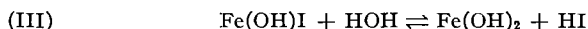
which may then be oxidized,

$$(IV) \qquad Fe(OH)_2 + \frac{1}{2}O_2 + HOH \rightleftharpoons Fe(OH)_3$$

This reaction may occur even in faintly acid media. Thus the relative amounts of ferric hydroxide and iodine formed will depend entirely upon the relative ease of oxidation of ferrous hydroxide and iodide ion. The normal oxidation potentials being:

$$(Fe^{+++}, Fe^{++})Pt = .743$$
$$(\frac{1}{2}I_2, I^-\quad)Pt = .538$$
$$(O_2, OH^-\quad\quad) = .396$$
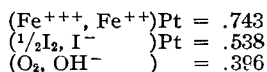
it is evident that when both are present in the reduced form, ferrous iron will be oxidized more readily than iodide ion. This is in accord with the experimental results.

The oxidation of iodide ion in this preparation has been shown to be a first order reaction and dependent in rate upon the purity of the iron, the temperature, the action of light, access to air and viscosity or of some factor proportional to viscosity. Its dependence also upon the hydrogen-ion concentration has been shown by Husa and Magid (18). Consequently, aside from slight increases in viscosity, changes in the ferrous iodide content of the preparation can have no effect upon the rate of iodine formation and increases in concentration of ferrous iodide will increase the quantity of iodine formed.

It would appear, from data on preservatives, that levulose is a very efficient reducing agent for iodine in faintly acid media. The action of honey is very rapid as regards the reduction of iodine, dextrose is much slower than sucrose and free iodine added to blackened ferrous iodide syrups is rapidly reduced; all these factors point to the action of levulose in this respect. Thus it appears highly probable that the true decomposition of Syrup of Ferrous Iodide is the darkening caused by the oxidation of levulose by iodine or oxygen. Darkening due to free iodine is not a factor in this decomposition since the appearance of free iodine is effectively prevented by hypophosphorous acid.

The reducing action of glycerin in sunlight may be due to the formation of glyceric aldehyde which in turn is oxidized. This aldehyde is formed only in light (19).

Obviously, Syrup of Ferrous Iodide may be stabilized by omitting sucrose from the formula. Dextrose serves to give the characteristic properties of a syrup to the preparation and by its use the darkening is avoided.

### SUMMARY.

A study has been made of the preparation, deterioration and stabilization of Syrup of Ferrous Iodide. Impurities present in card teeth were found to cause variations in the progress of the reaction.

Equations previously advanced to represent the decomposition of ferrous iodide preparations are shown to be unsatisfactory. The present study indicates that the deterioration involves several different changes which occur simultaneously, but at different rates according to the conditions.

The darkening of Syrup of Ferrous Iodide, U. S. P. X, has been shown to be due chiefly to decomposition of levulose formed by hydrolysis of sucrose. This darkening can be avoided by using dextrose in place of sucrose.

The most practical means for the preservation of Syrup of Ferrous Iodide is the use of hypophosphorous acid to prevent the liberation of free iodine and the addition of dextrose rather than sucrose to give the properties of a syrup. The use of 700 Gm./L. of either C.P. or commercial dextrose yields a satisfactory product.

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# THE PREPARATION AND BACTERIOLOGICAL STUDY OF CERTAIN THIAZOLE AZO DYES.*
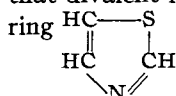
BY W. A. LOTT AND W. G. CHRISTIANSEN.

In view of the recent demonstrations of the germicidal activity of divalent sulphur compounds such as *p*-hydroxy diphenyl sulphide, I,



(I)
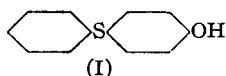
prepared by Hilbert and Johnson (1), and 3-methyl 4-hydroxy diphenyl sulphide, II, and 3-ethyl 4-hydroxy diphenyl sulphide, III,



(II)        (III)
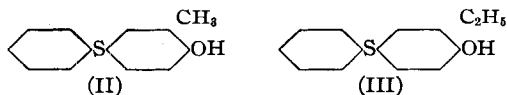
prepared by E. Moness, W. Braker and W. G. Christiansen (2), it seemed possible that divalent ring sulphur, as it appears in, for example, the thiazole heterocyclic ring  might also carry bactericidal activity. The most readily available starting material of this type was the easily synthesized amino thiazole, IV,

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* Scientific Section, A. PH. A., Madison meeting, 1933.