

Preparation of Iron(III)-Sugar Complexes

Tsuneaki IMAMURA and Hidekazu KAWAMOTO

*Department of Food Chemistry and Technology,
Faculty of Fisheries and Animal Husbandry,
Hiroshima University*

(Table 1, Figs. 1-3)

Iron-sugar complexes have called up interest in pharmaceutical research circles in view of the development of a new therapy for anemia. Numerous papers^{1)~20)} have already published in this line and several of them have dealt with the method for preparing the complexes^{1)~6)}. However, some questionable points such as the performance of complex-formation reaction under an alkaline condition are found. Although the manner convinces us from the standpoint of acid dissociation of sugars, yet the possibility of alkaline-oxidation of sugars has not been sufficiently taken into account. Accordingly, further experiments on the preparation of iron(III)-sugar complexes were carried out and the results obtained are reported in this paper.

MATERIALS AND METHODS

Reagents

The commercial lactose preparation contains a considerable amount of nitrogen compounds which contribute such impurities as coordinate to iron competitively with sugars. Accordingly, the previous removing of them was performed as follows: the treatments made with the precipitation of lactose by adding ten-fold the volume of ethanol to the saturated lactose solution and the washing the precipitate with ethanol, were repeated several times.

Ferric hydroxide was made up fresh for each preparation of the complexes as follows. An excess volume of 1N-NaOH was added to 0.5M-FeCl₃ solution and the precipitate formed in the mixed solution was separated by a low speed centrifugation. The wet sediment was used.

Amount of iron

The concentration of iron in samples was determined by means of the Hitachi's Atomic Absorption Spectrophotometer Type-207.

Absorbance

Changes of absorption maximum (λ_{\max}) and absorbancy during reaction time were determined by means of the Hitachi's Autorecording Spectrophotometer Type-124.

Paper electrophoresis

In order to confirm the formation of a desired complex, the paper electrophoretic experiment was conducted using Toyo No. 50 filter paper strips (2 × 40 cm) and bicarbonate buffer (pH 9.0, $\mu=0.1$) at an applied voltage of 450 V. After 2 hrs of phoresis at 20°C, the position of the sugar band was identified with an aniliphthalic acid solution and that of iron band with 5% potassium ferrocyanide solution, respectively.

RESULTS AND DISCUSSION

1. Effects of pH on the formation of iron-sugar complexes

Iron in aqueous solution exists as aquo-ion under high acidic (below pH 1) conditions. With the rise of pH in the solution, the aquo-ion is hydrolysed at first to form hydroxo-ion, then the hydroxo-ion olates to form polymers, and finally ferric hydroxide is produced as precipitate. When sugar exists in a mixed solution, a ligand exchange reaction between the sugar and the hydroxo-group of the above mentioned hydroxo-complex will occur and a soluble iron(III)-sugar complex will be formed instead of the $\text{Fe}(\text{OH})_3$ -precipitate. Therefore, the fact whether the $\text{Fe}(\text{OH})_3$ -precipitate is formed or not in the reaction mixture is regarded as an index of the formation of the iron(III)-sugar complex.

Thirty reaction solutions with different Fe/sugar rations were prepared by mixing, each one of the following series of iron solution and of sugar solution, that is, 10^{-3} , 2.5×10^{-3} , 10^{-2} , 2×10^{-2} , 4×10^{-2} M FeCl_3 solutions and 5×10^{-3} , 10^{-2} , 2×10^{-2} , 5×10^{-2} , 10^{-1} M sugar solutions. After the pH of all 30 reaction solutions was adjusted to a range of 7-13 with 0.1 or 1N-NaOH, all the samples were kept at 20°C for 24 hrs and then the formation of $\text{Fe}(\text{OH})_3$ -precipitate was examined. The experiment was carried out on five different kinds of sugar, i. e., glucose, galactose, fructose, lactose, and sucrose. Frequencies (%) of the $\text{Fe}(\text{OH})_3$ -precipitate formation have been visualized in Table 1.

Table 1. Effects of pH on Fe(OH)₃-Precipitate Formation.

Complexes	Ranges of pH of Reaction Solution			
	pH < 9	9 ≤ pH < 10	10 ≤ pH < 11	11 ≤ pH
Fe(III)-Glucose	100	67	22	19
Fe(III)-Fructose	100	80	0	11
Fe(III)-Galactose	100	23	12	31
Fe(III)-Lactose	100	—	31	35
Fe(III)-Sucrose	100	—	39	53

The numbers represents % of reaction solutions on which Fe(OH)₃-precipitate were observed.

On the first group in which pH was below 9, the precipitate formed in all the samples. Accordingly, the pH region is considered to be an unsuitable condition for preparing iron(III)-sugar complexes. A significant decrease of the frequencies was observed with the rise of pH of reaction solutions. The reason may be that the acid dissociation of sugars results in a ligand exchange reaction between the hydroxo-group and sugar. Therefore, the reaction solution is found to be adjusted to high alkaline conditions.

2. Effects of Fe/sugar ratio

As described above, iron-sugar complexes are products of the ligand exchange reaction between the hydroxo-group of iron-hydroxo complex and sugars. Accordingly, both the ratio of Fe/sugar and the concentration of the chemical species in the reaction solution are the main factors affecting to formation of the iron-sugar complexes. In a series of reaction solutions similar to the previous experiment, —except that their pH were all adjusted to 12—, the Fe(OH)₃-precipitate formation has been examined and the results obtained are as described in Fig. 1. The mark indicated the formation of Fe(OH)₃-precipitate distributed remarkably on the left-upper site where samples with high ratio of Fe/sugar were located. On the contrary, no precipitate was detected in the reaction solutions whose Fe/sugar ratio were under 1/10.

Among the five kinds of sugar, lactose is the most difficult to dissolve in water and the concentration in saturated solution state is about 0.56 M. Therefore, equal volumes of 0.05 M-iron solution and 0.5 M-sugar solution were mixed on the subsequent experiments.

3. Effects of reaction temperature

On the basis of the above two experiments, it was found that iron-sugar complexes did not form in the reaction solution whose pH was near 7. According to several reports^{1)~4)}, however, the preparation of stable iron-sugar complexes is

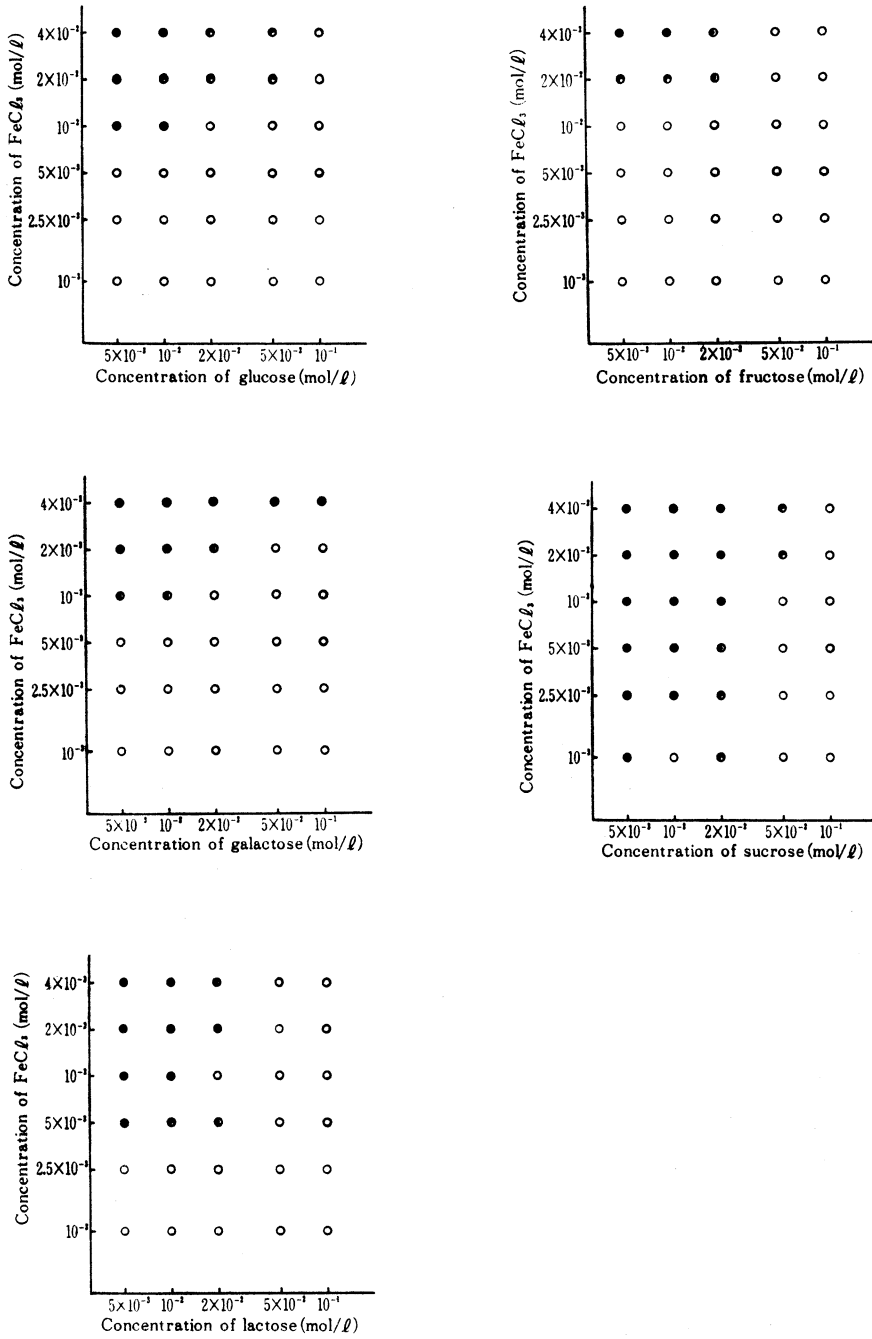


Fig. 1. Effects of Fe/sugar Ratio on $\text{Fe}(\text{OH})_3$ -Precipitate Formation in Various Reaction Solutions.

possible even in neutral solution by heating reaction solutions at about 100°C. It is doubtful that the products appear to be real iron-sugar complexes because the heat treatment induces an alkaline oxidation of sugars. The effects of reaction temperature on the decomposition of sugars have been examined according to this finding.

Two kinds of samples were prepared, one was a reaction solution composed of $\text{Fe}(\text{OH})_3$, lactose, and NaOH on the mole ratio of 1 : 10 : 20 and the other a control solution composed of lactose and NaOH on the mole ratio of 1 : 2. Both samples were divided into four portions, respectively, and four couples of each one of the above samples were kept at 20, 40, 60, and 80°C during 24 hrs. After the reaction time, the changes of their absorption spectra were examined. The results obtained are represented in Fig. 2.

The λ_{max} of the control solution was 290 $\text{m}\mu$ at the beginning of the reaction, and during the reaction time the λ_{max} shifted toward a short wave length reaching 270 $\text{m}\mu$ after 24 hrs. The time required for the shifting was short in the case of higher reaction temperature. On the other hands, the initial λ_{max} of the reaction solution was 300 $\text{m}\mu$, and the changes during reaction time were similar to those of the above control solution.

Referring to the literature by WILT *et al.*²¹⁾, the shifting of λ_{max} is

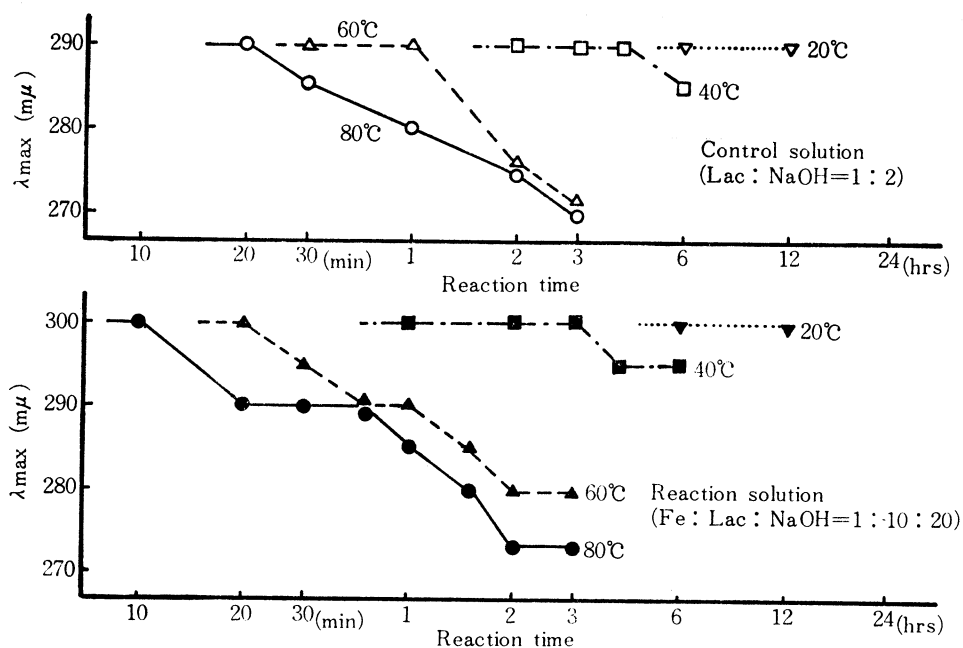


Fig. 2. Effects of Reaction Temperature on Shifting of λ_{max} .

considered to be resulted from the production of endiols and peroxides by alkaline oxidation of sugars. Accordingly, the existence of the endiols was confirmed by the following two experiments, (1) the reaction solution after 24 hrs of reaction time made the color of indophenol solution fade away and (2) the endiol fraction isolated from the reaction solution by paper electrophoresis reacted to the BTB solution. Because the oxidative product of sugars can form iron-complexes competitively with sugars, the reaction must be terminated prior to the shifting of λ_{\max} will start.

4. Final method for preparing iron(III)-sugar complexes

As a conclusion of the above description, the most appropriate conditions for preparing iron(III)-sugar complexes may be defined as follows: (1) fresh $\text{Fe}(\text{OH})_3$ must be used because its reactivity will decrease gradually, (2) complex formation reaction must be performed in high alkaline solution because acid dissociation of sugars occurs easily, (3) the Fe/sugar ratio desired is 1/10, and (4) complex formation reaction must be terminated before the alkaline oxidative products accumulate, i. e., within 3 hrs at 35°C .

The iron(III)-sugar complex formed in reaction solutions under the above mentioned conditions was precipitated by adding ten-fold volume of ethanol and then was separated by decantation. Finally, the separated preparation was dried in vacuo. Proof that the final preparation contained the required iron(III)-sugar complex was performed by paper electrophoresis, as shown in Fig. 3.

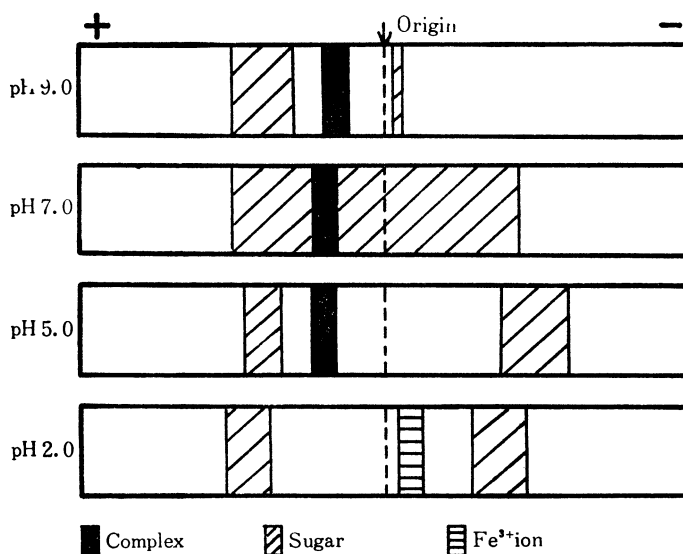


Fig. 3. Paper-electrogram of Iron(III)-Sugar Complex.

5. Significances of iron-sugar complexes

CHARLEY *et al.*⁶⁾ have studied the iron(III)-fructose complex and came to the conclusion that metal-sugar complexes may play an important biological role in the transport of mineral elements across cell membranes. The problem is of interest when considering a new iron-fortifier, because iron-citrate or iron-lactate employed on the manufacturing of modified milk powder has been chosen only for the convenience of manufacturing engineering. Accordingly, the absorption of the above iron(III)-sugar complex was compared with that of ferric citrate. By means of an everted sac procedure¹⁶⁾ with the small intestines of the USHIGAERU (a Japanese species of frog), the permeability of the complexes was found to be lower than that of ferric citrate. Similar results were obtained by dialysis through a cellophane tube. Moreover similar tendencies showed up in the comparison of the complex with potassium ferrocyanide. Such experimental results fell short of our expectation which intended only to apply the iron-sugar complexes for an iron-fortifier. The cause is supposed to be polymerization of the complexes.

SUMMARY

With the aim of developing a new iron-fortifier, methods for preparing iron-sugar complexes were investigated. The sugars examined were glucose, galactose, fructose, lactose, and sucrose. The most appropriate requirements for the experiment can be summarized as follows:

- (1) Fresh $\text{Fe}(\text{OH})_3$ must be used.
- (2) The complex formation reaction must be terminated before the alkaline oxidative products get.
- (3) Fe/sugar ratio desired is 1/10.
- (4) The complex formation reaction should be performed in high alkaline solution.

The complexes formed in reaction solution are precipitated by adding ten fold volume of ethanol and drying the precipitate *in vacuo*. The product contains surely iron(III)-sugar complex, but the complex is assumed to be polymerized on the basis of permeability through a cellophane tube.

ACKNOWLEDGEMENT

The authors feel greatly indebted to Mr. Tsukuru HATANO to whom has been allotted a part of this work.

REFERENCES

- 1) BERSIN, T., et al.: *Ann.*, **623**, 138 (1959).
- 2) NAQVI ALI, S. T., et al.: *Arzneimittel-Forsch.*, **9**, 720 (1959); *C. A.*, **54**, 6036c (1960).
- 3) ZAIDI, S., ALI, H., et al.: *Pakistan J. Sci. Ind. Res.*, **5**, 43 (1962); *C. A.*, **57**, 8169f (1962).
- 4) ZAIDI, S., ALI, H., et al.: *ibid.*, **6**, 114 (1963); *C. A.*, **63**, 13378c (1965).
- 5) SALTMAN, P., et al.: *U. S. Appl. Apr.*, **12**, 42pp (1960).
- 6) CHARLEY, P. J., et al.: *Biochim. Biophys. Acta*, **69**, 313 (1963).
- 7) OERIW, S., et al.: *Farmacia*, **9**, 655 (1961).
- 8) SHITT, C., et al.: *Proc. Soc. Exptl. Biol. Med.*, **110**, 70 (1962).
- 9) CHALEY, P. J., et al.: *J. Lab. Med.*: **61**, 397 (1963).
- 10) HELBOCK, H., et al.: *Biochim. Biophys. Acta*, **135**, 979 (1967).
- 11) LINKENHEIMER, W. H.: *Toxicol Appl. Pharmacol.*, **6**, 669 (1964).
- 12) ENERBACK, L., et al.: *ibid.*, **7**, 523 (1965).
- 13) SARKER, B., et al.: *J. Inorg. Nucl. Chem.*, **26**, 1551 (1964).
- 14) 玉置弥栄, 中沢利勝: *工業化学雑誌*, **67**, 1271 (1964).
- 15) ROLAND, A., et al.: *Biochim. Biophys. Acta*, **80**, 430 (1964).
- 16) SALTMAN, P.: *J. Chem. Educ.*, **42**, 682 (1965).
- 17) ZAIDI, S., ALI, H., et al.: *Arzneimittel-Forsch.*, **15**, 636 (1965); *C. A.*, **63**, 11257f (1965).
- 18) 藤田 孟: *日化*, **88**, 162 (1967).
- 19) 寺戸国昭, *薬学雑誌*, **92**, 1247 (1972).
- 20) BATES, G., et al.: *Bioinorg. Chem.*, **2**, 311 (1973).
- 21) DE WILT, H. G. J., et al.: *Carbohydr. Res.*, **19**, 5 (1971).

鉄(Ⅲ)-糖錯体の調製

今村 経明, 河本 秀一

鉄-糖錯体の調製に関する従来の報文は、アルカリ条件下で加熱するものが多い。これは糖の酸解離には望ましいが、反面糖のアルカリ酸化が進行し、生成した錯体はこのアルカリ酸化生成物が配位したものかも知れない。この点を重視して従来の調製法を再検討し、次いで得られた鉄(Ⅲ)-糖錯体を一部食品の鉄強化剤として使用することの可能性を検討した。

望む鉄(Ⅲ)糖錯体を調製するためには、(1)鉄/糖比を1/10にして、(2)pH10以上のアルカリ条件で、(3)酸化生成物が蓄積する以前(35°Cなら3時間以内)に反応を打切るのがよい。生成した錯体は、10倍量のエタノールを添加して沈澱させ、傾斜法で分別後減圧乾燥して保存した。

この標品の吸収性を、ウンガエルの腸管並びにセロハン膜の透過性によって調べたが、クエン酸鉄、乳酸鉄などより低かった。この理由は、鉄(Ⅲ)-糖錯体が重合し、かなり高分子のポリマーとして存在するためであろうと考えた。