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## PREPARATION AND CHARACTERIZATION OF MULTIMINERAL IRON-SACCHARATE

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A preparation is described in which iron saccharate has been supplemented with manganese, copper and cobalt. The preparation is stable between pH 6 to 8.

### INTRODUCTION

Iron therapy would remain a permanent feature of medical treatment since it manifests a deficiency state which could only be cured by taking iron. Iron-saccharate first described by Nissim[1] in 1947 for intravenous administration is a non-ionic, colloidal preparation. It is relatively less toxic[2] LD<sub>50</sub> 300 mg/kg. as compared with other iron salts like ferrous sulphate LD<sub>50</sub> 11 mg/kg. Iron-saccharate is included in pharmacopias of Austria, Germany and Switzerland for oral[3] use as well. We reported its preparation earlier[4]. Certain trace elements like manganese[5], copper[6], and cobalt[7] has been reported to accelerate the regeneration of haemoglobin and correction of anaemia. The available multi-mineral oral iron preparation like ferrous sulphate, gluconate and fumarate contains Mn, Co, Cu, in the form of their salts such as sulphate. The sulphate of manganese, copper and cobalt can not be added to iron saccharate because it effects adversely its stability. We[4] found that sulphate ions have much adverse effect on quality of the finished product. Iron saccharate made from ferric sulphate instead of ferric chloride, observing exactly same conditions for its preparation, became unstable. It has also been observed that when manganese sulphate (0.062 g) equivalent to 0.02 g, copper sulphate (0.0784 g) equivalent to 0.02 g and cobalt sulphate (0.0096 g) equivalent to 0.002 g elemental cobalt are added to 1% iron-saccharate (100 ml) and then it is boiled for one hr, gel formation takes place. Similarly excess of chloride ions also effects adversely the stability of iron saccharate. Obviously, a multi-mineral iron saccharate preparation should not contain chloride or sulphate ions.

In this paper we report the preparation and characterization of a multiminerall iron-saccharate in which manganese citrate, copper-ethylene diamine complex[6] and cobalt-saccharate[7] have been added. For a developing country like Pakistan injectable preparations could not prove useful, since trained medical practitioners are almost

non-existent in villages, where anaemia is more common. The preparation is, therefore, made for oral use. Further work on making a preparation for intravenous administration is in progress.

#### *Methods and Characterization of the Preparation*

**Iron Saccharate.** Ferric hydroxide prepared from FeCl<sub>3</sub> 6H<sub>2</sub>O and anhydrous sodium carbonate solution (20%) was freed from electrolytes by washing with distilled water. Sucrose, dissolved in minimum quantity of water and sodium hydroxide (15% solution), was added to the precipitate in the ratio of Fe: Sucrose: NaOH (1:5:0.3) in a dish. The contents heated at 130° for 6-8 hrs forming a dark brown cake which gave a clear and stable solution when dissolved in water. Iron was then estimated in the solution. Its isoelectric point ranged between pH 4.8-5.2. The viscosity and density of solution containing 2% iron was 13.84 millipoise and 1.073 respectively.

**Manganese Citrate.** Manganese carbonate (MnCO<sub>3</sub>) equivalent to 1g. manganese was taken in a 100 ml. conical flask with citric acid (4g) and water (about 20 ml). The contents were heated on water bath, adding water in small portions till the greyish manganese carbonate was completely changed into clear white precipitate. The precipitate was filtered under vacuum, air dried and weighed (4.1g). Manganese citrate contained 21-22% elemental manganese. Manganese citrate (1g) was then dissolved in 5% citric acid solution (100 ml) by heating on water bath.

**Copper Ethylene Diamine Complex.** Copper complex of ethylene diamine was prepared by adding slowly and with vigorous stirring, ethylene diamine (2.1ml) to electrolyte free precipitate of Cu(OH)<sub>2</sub>, prepared from 2.66g CuCl<sub>2</sub> 6H<sub>2</sub>O. The homogeneous royal blue solution so obtained was then diluted and estimated to contain 1% elemental copper. It did not give precipitate with hydrochloric acid, but its colour began to change at a pH 4.0. Its density and viscosity was 1.015 and 13.499 millipoise respectively.

**Cobalt-Saccharate.** Cobaltous hydroxide prepared from  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (4g) was oxidized to cobaltic hydroxide by hydrogen peroxide (about 10ml) of 6% V/V). The black precipitate so obtained was washed free of electrolyte and transferred to a porcelain dish. Sucrose (7g), dissolved in the minimum quantity of water and sodium hydroxide (1.65g as 15% solution) were mixed with the precipitate. The contents were heated at  $170^\circ$  for three hrs to form cobalt-saccharate as darkish cake. The cake was dissolved in known volume of water and cobalt concentration was then determined in the solution. Its isoelectric point ranged between pH 3.9-5.3. The viscosity and density of a 2% solution was 20.3 millipoise and 1.1008 respectively.

**Multimineral Preparation.** Iron-saccharate (334ml of 3% equivalent to 10g elemental iron) manganese citrate (100 ml of 1% Mn citrate equivalent to 0.21g Mn), copper ethylene diamine complex (20 ml of 1% equivalent to 0.2g Cu) and cobalt saccharate (2.0ml of 1.00 per cent equivalent to 0.02g Co) were adjusted to pH 6.5-7.5. All the solutions were then mixed and volume made upto 1000 ml with distilled water to get a multimineral preparation with Fe 1%, Mn = .02%; Cu = 0.02%. and Co = 0.002%. The preparation was stored in dark brown bottle with 0.1% methyl-p-hydroxy benzoate. Its isoelectric point ranged between pH 4.0-4.5, the viscosity and density was 14.419 millipoise and 1.0375 respectively.

## ESTIMATION

**Wet Oxidation for the Determination of iron, Copper Manganese and Cobalt** Multimineral preparation (5-10 ml) was digested with concentrated nitric acid and sulphuric acid (5 ml and 2 ml) in a 250 ml Kjeldahl flask adding nitric acid in small portions (0.5-1 ml) until on evaporation it gives off white fumes of sulphuric acid and colourless or pale yellow solution was obtained. After cooling, water (2-3ml) was added and the solution evaporated to white fumes. The same process repeated twice to destroy nitrosyl sulphuric acid. Finally the solution boiled with little water and after cooling made upto a definite volume (50 or 100 ml) in a volumetric flask.

**Determination of Iron.** Iron was determined volumetrically by standard potassium dichromate solution (0.025 N) after reducing ferric iron to ferrous form by stannous chloride. Diphenyl amine was used as internal indicator.

**Determination of Manganese.** Digested solution (2-5ml) was taken in a 100 ml conical flask with a little water (10 ml). Phosphoric acid (1-2ml of 85%) was added to the solution and mixed. The solution should be practically colour-

less. Potassium periodate (0.1g) was added and the mixture heated to boiling and kept at or near this temperature for 10-20 mints to convert completely manganese to potassium permanganate. The solution was diluted to a suitable volume (usually 20ml) depending upon the colour intensity and absorbance noted at 525 nm. For making standard manganese solution good grade potassium permanganate (28.8mg) in 10ml water, equivalent to 10mg manganese, was reduced to manganous state with a little sodium sulphite after the addition of sulphuric acid. Sulphur dioxide was boiled out and the solution made upto 100 ml to make 0.01% manganese solution.

## Copper and Cobalt Estimation

**Separation of Iron from Cobalt and Copper.** Digested solution (10ml) was shaken repeatedly with ether (20 ml) each time adding concentrated hydrochloric acid (5 ml) to aqueous layer after each extraction till ether layer gave no blue colouration with potassium ferrocyanide (negative test of  $\text{Fe}^{++}$ ). The acid layer now contained whole of copper and cobalt free of iron.

**Separation of Copper and Cobalt.** The pH of the acid layer brought to 1-1.2 with sodium hydroxide and then adjusted to 3.8-4 with sodium acetate. The solution now extracted twice with dithiazone (50 ml and 10ml of 0.2 per cent in chloroform). The dithiazone layer contained copper while cobalt remained in the aqueous phase.

**Estimation of Copper** The combined dithiazone extracts were evaporated to dryness on water bath, concentrated sulphuric acid (2ml) and perchloric acid (1ml) added to it and the mixture heated on flame till a colourless solution was obtained. The volume of the solution was then made upto 50ml and copper in the solution estimated colorimetrically by means of dithiazone (10ml of 0.0015 per cent).

**Estimation of Cobalt** The aqueous layer made upto a definite volume and the cobalt in solution estimated colorimetrically by means of sodium-1-nitroso-2-hydroxy-naphthalene-3,6-disulphonate. Absorbance was noted at 500 nm. to cancel the effect of traces of iron if still present in the solution.

## STABILITY

The aqueous solution of multimineral preparation containing Fe:Mn:Cu:Co in the ratio 1:0.02:0.02:0.002 was taken in a sealed ampoule and heated at  $100^\circ$  for 1 hr. The preparation remained clear. The stability of iron saccharate containing manganese, copper and cobalt in the ratios (2:0.02:0.02:0.002) and (1.5:0.05:0.05:0.005) was also

tested in the same way. There was no visual indication of any deterioration in the stability of iron saccharate.

*Stability at Different pH.* The pH of the multimineral preparation in aqueous solution was regulated within the range of 1-7 and 7 to 12, pH 1-7 with 0.1 to 1N HCl and pH 7-12 with 1% NaOH in accordance with the method of Nissim [8] and Robson. The concentration of iron in all the solution was Fe 1 mg/ml. After the solution had been standing for 24 hrs. at room temperature, the precipitate was removed by centrifugation and Fe, Mn, Cu and Co content and pH in the supernatant was determined. The results from these studies showed that the preparation precipitated with in the pH range of 4-5 and was also unstable above pH 9.0. The preparation was stable between pH 6-8.

### DISCUSSION

Required quantities of cobalt and copper chelates could be added in iron saccharate without difficulty since all of these are alkaline. In case of manganese citrate which is insoluble in water or alkaline medium it is first dissolved in 5% citric acid solution. The pH of the solution of iron saccharate, cobalt-saccharate, and copper ethylene diamine is adjusted at 6.5 to 7.5 and then manganese citrite solution which is also brought to pH 6.5-7.5 is added slowly with vigorous stirring to get multimineral iron saccharate. Stability of the preparation is determined by boiling its solution in a sealed ampule at 100° for 1 hr. However, the isoelectric point or the pH at which precipitation takes place is more reliable measure of the stability. This is due to the fact that iron saccharate, cobalt saccharate and copper ethylene diamine are alkaline and binding of these metals with sucrose and ethylene diamine is more firm if the pH of precipitation is lower towards acidic side. We found in case of

iron saccharate that a preparation with precipitation pH of 6.5-7.5 showed gel formation on keeping for a few days while that with a precipitation pH of 4.9-5.2 remained stable for years. Same phenomenon was observed with iron [9] sorbitol-dextrin and citric acid complex. Copper-ethylene diamine complex does not precipitate with hydrochloric acid but changed its colour from royal blue to green at pH 4. The original pH of this complex is 13.2-13.8. When its pH is lowered to 6.7 or below with hydrochloric acid and potassium ferrocyanide solution is added a brown precipitate is obtained indicating that the complex got partially ionized.

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